Sudhir Sopory *Editor*

Sensory Biology of Plants

Springer

Sensory Biology of Plants

Sudhir Sopory
Editor

Sensory Biology of Plants

Springer

Editor
Sudhir Sopory
International Centre for Genetic Engineering
and Biotechnology
New Delhi, India

ISBN 978-981-13-8921-4 ISBN 978-981-13-8922-1 (eBook)
https://doi.org/10.1007/978-981-13-8922-1

© Springer Nature Singapore Pte Ltd. 2019
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

It is a great pleasure to write this Foreword for this volume edited by my former pupil Professor Sudhir Sopory, who came to my laboratory for work toward his doctoral degree back in the late 1960s. Not all teachers have the longevity and good fortune of seeing their former pupils grow for such a long period – I am 86 now. Sudhir Sopory was among the founding group after I returned from Caltech in the early 1960s. I was member of the faculty of a department at the University of Delhi whose beginning is intimately linked with the British Raj and the Empire and which greatly benefited by the direct attention given by the imperial government during the 1930s and 1940s. (If I can expand a bit, it so happens the main building of the University of Delhi, an iconic structure, was indeed originally the Viceroy's residence and the surrounding campus the Viceregal Estate – but later as the new capital, New Delhi got ready in the early 1930s, the Majesty's Government decided to give over the old lodge and the surrounding estate to start the University of Delhi.) Thus, it has been a privilege to pass most of my active life in this historic spot not only for me but also of Sudhir while he finished his Ph.D. But around the time he got his degree, University of Delhi had already gotten too big and crowded and the Government of India took the decision to set up the new Jawaharlal Nehru University (JNU). Sudhir was recruited as a member of the new Life Sciences School. Being in the same metropolis, it was possible for me to maintain regular contact and watch him gradually become one of India's topmost plant biologists.

After my own superannuation, I moved to the International Centre for Genetic Engineering and Biotechnology (ICGEB) in New Delhi as an Honorary Guest Scientist. By a happy coincidence, Sudhir too moved from JNU to the ICGEB as Head of the Plant Molecular Biology group, giving us an opportunity to maintain even closer contact for nearly a decade. Sudhir has not only been a keen researcher but also a great scholar. For many years, we took our lunch together. Certainly in his younger days, I may have taught him a few things, but I think I benefited more by our daily meetings. I have been in Jaipur for a decade now, but happily Sopory has maintained a regular contact. It was my good fortune to have had many talented students. But he has been unique in many ways, both academically and as a person.

Sudhir had returned to JNU for a brief span as a Vice Chancellor. But I am happy that the higher authorities in Trieste decided to bring him back to ICGEB as an Arturo Falaschi Emeritus Scientist and utilize his knowledge and experience. I think

ICGEB made a wise choice in commemorating the memory of this outstanding investigator, Prof. Falaschi, late former Director General of ICGEB. Sudhir Sopory has had wide interests. In his lectures and writings, he always had a new way of looking at things. Turning to this book, the truth is in a sense that all of modern physiology biochemistry is signaling. Though the widespread use of this word is recent, research on signaling has been going on for a long time. In the last century, when Charles and Francis Darwin were doing their classic studies on phototropism, they were in fact studying signaling. Such was also the case with Boysen Jensen, Frits Went, and two of my own gurus and grand gurus, namely, James Bonner and Kenneth Thimann (both began their careers at Caltech but Thimann had moved to Harvard in 1935). However, signaling came to have special focus and meaning when Earl Sutherland discovered the first second messenger, cyclic AMP, for which he was awarded a Nobel Prize in 1971. In my view, the work by F Jacob and J Monod also propelled research in the area greatly through development of key concepts of allostery and bringing in a new area of biochemistry of regulation. Many other key discoveries followed, such as those of transmembrane receptors, protein kinases, and G proteins and even signaling cascades were found that penetrated the nucleus and turned genes on or off. Indeed through an entire century, a string of Nobel Prizes were awarded (from Bayliss and Starling and F Banting and McLeod in the early period to more modern investigators like Cohen and Levi-Montalcini, Fischer and Krebs, and Lifkowitz) resulting in the establishment, so to say in its own right, of the new discipline in the 1980s. It is indeed then that the first reviews with the term "signaling" or "signal transduction" in their titles were published. Signaling had come to age and in 1982 came the first exclusive volume on the subject published by Elsevier.

Prof. Sopory has been interested in signaling for a long time. In 2002, he organized the first international symposium in India on *Signal Transduction in Plants* (the contributions are already published in a volume, of which, I and Ralf Oelmuller were Co-editors with him). By editing this new volume, he has brought to focus a lot of advances that have taken place since then and reaffirmed the centrality of signaling in plant biology. Largely, this volume is a product of contributions of his many collaborators and mentors, with whom he worked in India and abroad, and his students who had worked in foreign laboratories and are now working in various Institutes in India and the USA. He had a talented group of researchers, and leading the list of contributions (by his former associates) is an article by Rameshwar Sharma, who has made many outstanding contributions to photobiology of plants. Many other contributions come from the alumni or members of the Departments of Botany and Plant Molecular Biology of University of Delhi, National Institute of Plant Genome Research, JNU, and ICGEB. But there are also articles from other investigators from institutions in India and abroad (four articles are from the USA, one each from Israel, Canada, Korea, and Germany). Professor Sopory has had excellent links with all of them, and to my mind, his meticulous planning is bringing

to light the influence of a whole variety of factors affecting plants in a coherent manner. The volume ends with two intriguing titles (the last one with an Indian view on plant life). I am sure there will be some surprise for us all. Once again, my sincere admiration for this valuable enterprise.

Honorary Visiting Professor,
Biotechnology Laboratories,
Centre for Converging Technologies,
University of Rajasthan,
Jaipur, India

Satish C. Maheshwari[1]

[1] Prof. S.C. Maheshwari passed away on June 12, 2019

Preface

All life on this planet is dependent on plants for their survival. The life story of a plant in the form of a poem by a class 7 student (my granddaughter) is given in Box I, and the views of Nobel laureate Rabindranath Tagore on life of trees and on Sir J.C. Bose, who first showed the sensory nature of plants, are given in Box II. Since the time of Bose, amazing advancements have been made to understand the physiological, biochemical, and molecular aspects of plant growth and development and responses of plants to external environment. During the course of evolution, as new plant forms evolved, they also developed sensory perceptions and mechanisms to decide the best ecosystem for them to adapt to their new home and accordingly developed good relationships with soil, climate, insects, and other plants around. One thing that is becoming clear from lot of new research is that plants respond efficiently to the changes in the environment, regulate necessary biochemical and molecular machinery, and process the input information for their development and survival. It is this aspect of plant sensory biology that we are partly covering in this volume. Each chapter presents scientific evidence and knowledge that have accumulated, with cited references, to communicate the sentient nature of plants and to reveal how plants perceive physical and biological environment around them and respond accordingly.

Chapter 1 deals with plant diversity and adaptation during the evolution of plant life, as it moved from the aquatic to the terrestrial environments. Following this broad overview, the chapters in this volume have been categorized under three parts.

Part I is on the awareness of plants to the external environment. There are six chapters in this section which deal with the present state of knowledge on perception and responses of plants to light and darkness, to various nutrients, and to water. Other aspects such as how plants respond to gravity, sound, and touch, and also about variations in conditions that are perceived by plants as stress environment, are also covered in this section as separate chapters.

Part II discusses about the plant cellular machinery, both chemical and molecular, and the mechanisms thereof, for decoding and transmitting external information and cues. The broader questions are the following: What molecular machinery is functioning in plants? What are the various chemicals and hormones that are used by plants to regulate their inner self following perception of changes in the environment? This is needed for their proper growth and development both under normal

Box I Excerpts from Poem "Plant"
by Dhriti Medigeshi Class VII

It all started as a sapling
Every plant, every flower, every tree
But before it was a sapling
It was a tiny little seed

Tucked into the soil
Living on water and sunlight
Waiting to see the world
With tremendous delight

Summer went
And then came monsoon
It rained all day
All night and afternoon

The little seed
Quenched its thirst
And felt like
It would burst

The next day
Popped a tiny root
The day after that
You could see the shoot

Days after that
The stem could be seen
With leaves peeking
Out From between

A plant is a Mathematician
And a scientist altogether
Well, you just don't know
A plant is very clever

It knows chemistry
Biology and physics
It can also perform
Magic tricks

(continued)

It knows many
Complicated processes
The one it performs
Is photosynthesis

The leaves take up
The energy of the sun
And then their job
Has just begun

After doing
A lot of chores
It makes food
Called glucose

The stem acts
As a transporter
And takes the food
From one part to another

A plant stores its food
In leaves, stems and roots
And sometimes it's also
Present in a fruit

A fruit comes from
A colourful flower
That's what you call
Flower power

Box II Tagore on Trees
Sushanta Dattagupta

Rabindranath Tagore – though universally acclaimed as a poet, philosopher, and lyricist – was an avid lover of science. In a book in Bengali on science, called *Visva Parichay* (*Introduction to the Universe*) [1], he had written in 1934:

Any educated person must enter the arena of science if not the core of science, and in this regard, it is no shame to take the help of literature…. I am not a serious student of science but I had this endless temptation for tasting the nectar of science from my very childhood….

(continued)

Tagore's views on science are completely enmeshed in nature and natural phenomena, as revealed in the famous dialogue with Albert Einstein, through the years of 1926–1930 [2]. In this context, trees and forests occupied a significantly large space in his mind. On this, Tagore had written in a letter to C. F. Andrews in April 1921: "…The environment in which the Aryan immigrants found themselves in India was that of the forest. The forest, unlike the desert or rock or the sea, is living, it gives shelter and nourishment to life. In such a surrounding the ancient forest dwellers of India realised the spirit of harmony with the universe, and emphasized in their mind the monastic aspect of truth…." [3]. On his concern for the environment Tagore had written in details in *Visva Parichay*.

On the importance of the tree and its relevance to the climate, Tagore wrote:

As the earth began the process of freezing into a solid lump from a liquid mass at the time of its inception its surroundings were filled with humid vapour and carbon-related gases. Further cooling led to nitrogen and other gases. It is surprising at first sight that so much oxygen had survived even though the latter is highly reactive and prone to form compounds. The reason is the abundance of trees and vegetation. The trees help imbibe carbon from atmospheric carbon dioxide to form cells and release oxygen. The resultant loss of carbon dioxide is replenished from the exhaled air of living and nonliving ones. It is surmised that life began from the semblance of oxygen left behind in ancient vegetation. The growth of the latter released further oxygen gas in the atmosphere at the expense of carbon dioxide…. The molecule called chlorophyll is present in green leaves which store sunlight in the form of energy. This energy helps create food in the form of fruits, crops, etc. On the other hand, the tiny presence of carbon dioxide in the air penetrates as carbon in vegetables, from which coal is produced, thereby aiding sustenance to life. It is the tree that is central to the food production in the form of rice and wheat through the process of mixing carbon dioxide with water with the aid of chlorophyll that draws energy from the sunlight.

It is no wonder then that Rabindranath wholeheartedly embraced and lauded the scientific achievements of his close friend Jagadish Chandra Bose in the area of plants and plant physiology. These two great sons of India were similar in age: Bose was born on 30 November 1858 and Tagore on 7 May 1861. They had other common threads – both were inheritors of emancipated and affluent "Brahmo" families of what is known as Bengal Renaissance.

In a tribute to Bose, Tagore had said [4]: "… in the prime of my youth I was strangely attracted by the personality of this remarkable man and found his mind sensitively alert in the poetical atmosphere of enjoyment which

(continued)

belonged to me. At that time he was busy detecting in the behaviour of the non-living some hidden impulses of life. This aroused a keen enthusiasm in me who had ever been familiar with the utterance of Upanishad which proclaims that whatever there is in nature vibrates with life. He had then shifted his enquiries from physics to the biological realm of plants. With the marvellously sensitive instruments that he had invented he magnified the inaudible whisperings of vegetable life, which seemed to him similar in language to the message of our own nerves. My mind was overcome with joy in the idea of the unity of the heartbeats of the universe, and I felt sure that the pulsating light that palpitates in the stars has its electric kinship in the life that throbs in my own veins…."

On 30 November 1928, Tagore had dedicated a remarkable poem in Bengali, "Vano-Vani" (The Voice of the Forest), to J. C. Bose, on his 17th birthday, which aptly captured Bose's scientific discovery [5]. We translate that poem in parts, separately highlighting the scientific content.

On photosynthesis:

Day in and day out light strikes the leaves,
to arouse the excited molecules into a
silent, rhythmic and melodious vibration;
The trees sing muted paeans to the Sun at dawn.

On the evolution of trees and Bose's path-breaking contribution:

Years and years ago our mother earth was an
arid, dreary and inert desert;
Slowly and apprehensively tree made its
appearance bringing-in the joy of life;
It had to expectantly wait through ages
to hear the footsteps of man;
Came human beings whom the tree
provided shelter and nourishment;
Primitive life was hidden in its interior that
did not find ample expression through its
pulsating leaves.
It is YOU who delightfully awakened yourself
to align your creative mind to the unravelling
of the secret of life within plants.
The primordial message of life was aroused in
grassy fields and forests but stayed unspoken.
It is YOU the great sage endowed the mute with speech,
heard the pathos of the jungle from your solitude.

(continued)

In one of the numerous letters that Tagore wrote to Bose [4], he had lightheartedly referred to Bose's experiments on plant response to external pulses – on 21 May 1901 – from Shelaidaha, thus:

I feel proud to read about the method you have discovered to pinch every aspect of nature. Until now, inanimate objects were troubling us – now I can contemplate revenge on them, thanks to your discovery. Go ahead and administer unending pinches and poisons to them – don't leave them alone. From now on Judges can pronounce 'Pinching Punishments' for inanimate objects if they ever come up for courtroom trials….

∗Senior Scientist of the Indian National Science Academy at the Bose Institute, Kolkata; also at the Tagore Centre for Natural Sciences and Philosophy, Rabindratirtha, New Town, Kolkata; (electronic address: sushantad@gmail.com); all entries in italics are author's translation from Tagore's Bengali writings, some of which are reproduced from [2], cited below

[1] Rabindranath Tagore, *Visva Parichay*, 1934, Visva-Bharati Publications, Granthan Vibhag, Kolkata
[2] Sushanta Dattagupta, *A Random Walk in Santiniketan Ashram*, 2016, Niyogi Books, New Delhi
[3] The Archives of Rabindra Bhavan, Visva-Bharati, Santiniketan
[4] Acharya J. C. Bose – A Scientist and a Dreamer 1997, Bose Institute Publication Section, Kolkata
[5] Rabindranath Tagore, in "Chitthi-Patra," Republished by Granthan Vibhag, Visva-Bharati, Kolkata, 2015

situation and also when plants face stress conditions. There are nine chapters under this section. Two of these deal with membrane-associated transducers, namely trimeric G-proteins, two-component systems, and others, and describe the role of chemical signalling. For this latter part, we have chosen to discuss about the involvement of plant hormones, calcium, nitric oxide, and reactive oxygen species (ROS). Furthermore, plants have also developed ways to sense sugars and use them to transduce signals in consonance with hormones, which is covered in one chapter. In addition, the role of an energy molecule ATP in signalling has also been discussed in another chapter where a comparison of this has been made with animal signalling. Interestingly, plants have even been shown to produce neurotransmitters which can also monitor changes in the environment and accordingly regulate plant development. This aspect is also covered in one of the chapters included in this section.

Part III deals with various plant communication systems and also how plants integrate various signals. Plants, unlike animal systems, have a cell wall. The role of

cell wall in mediating external cues and regulating internal cell communications is presented in one chapter. In plants, the genetic information resides, other than in nucleus, in chloroplasts as well. A chapter deals with this aspect of communication and signalling among different organelles, especially plastids, to define how retrograde signalling between chloroplast and nucleus regulates gene expression. Furthermore, two chapters in this section deal with the communication systems. One is about the electrical signalling and long-distance communication, and the other is on how plants respond to attack from pathogens. Finally, a chapter summarizes, with a few case studies, the concept of how different cues are integrated in a coherent manner within plant cells to take decisions about their growth and survival under ever-changing environmental conditions of light, temperature, nutrients, etc.

Part IV deals with the end of the plant life and a few views on plant cognitions. There is a chapter that deals with plant cell death. Like all living organisms, plant life also comes to an end, though there are large variations in the life span in plants. From a few days, like in *Arabidopsis*, for this reason and also due to its small genome size, it has become the most sought-after model plant to trees which live for hundreds of years. The mechanism of cell and organ death as compared to the death of the plant itself is also presented.

One of the philosophical questions which have been discussed by some is as follows: Do plants have "consciousness"? A non-human type! We have attempted to compile a chapter on this with views and logic of different authors, as also the views of various theologists and spiritualists on plant life. Experiments of J.C. Bose, and those of other recent workers on the use of anaesthesia and also the work on plant memory, more specifically stress memory, are covered in this chapter.

Lastly, a commentary of a young artist, a dancer who takes inspiration from plants and innovates her dance choreography, has been included as a separate chapter on the Indian view of the natural and plant world.

One of the reasons for me to edit this book, rather than writing it solely by myself, is to acknowledge the support of my students, colleagues, and all those in whose laboratory I had worked at some time or the other during my career, coauthoring publications with them. It was nice to share my ideas and literature with some of them. This enabled me to learn a lot during the process of compilation and editing. I am also thankful to many other students who could not be a part of this project. In addition, there are a few chapters which are authored by those whom I have known but have not had any direct collaboration with them. The topics that they have covered were important for this volume, and hence, I extended an invitation to them which they kindly accepted.

I am aware that the topic of this book is rather vast. Moreover, a lot of new information is also pouring in, on daily basis, especially on various modes of plant communication, both above and below grounds and with other organisms (see some suggested readings). Nevertheless, I am hopeful that this volume will be useful to the students of plant biology and will encourage them to unravel the mysteries of plant life and further investigate how plants interact with environment and other biological species and survive successfully in their ecosystem.

Suggested Readings

Chamovitz D (2012) What a plant knows. One world Publ. Oxford ISBN 978-1-85168-910-1
Elhakeem A, Markovic D, Broberg A, Anten NPR, Ninkovic V (2018) Above ground mechanical stimuli affect belowground plant-plant communication. PLoS One 13:e0195646
Falk O, Mordoch Y, Quansah L, Fait A, Novaplansky A (2011) Rumor has it…Relay communication of stress cues in plants. PLos One 6:e23625
Falk O, Mordoch Y, Ben-Natan D, Vanunu M, Goldstein O, Novaplansky A (2012) Plant responsiveness to root -root communication of stress cues. Ann Bot 110:271–280
Gorzelak MA, Asay AK, Pickles BJ, Simard SW (2015) Interplant communication through mycorrhizal networks mediates complex adaptive behaviour in plant communities. AoB PLANTS 7:plv050
Haskell DG (2017) The songs of trees. Penguin Books. ISBN 97805-2542-7520
Karben R (2015) Plant sensing and communication. The University of Chicago Press. ISBN 978-0-26-26467-7
Kimmer RW (2013) Braiding sweet grass. Milkweed Eds. ISBN 978-1-57131-356-0
Mabey R (2015) The cabaret of plants. W.W. Norton and Co. ISBN 978-0-393-35386-0 pbk
Mancuso S (2017) The revolutionary genius of plants. Atria Books. ISBN 978-1-5011-8785-8
Mancuso S, Viola A (2015) (Eng. Ed). Brilliant green. Island Press, Washington Lib. Of congress catl. Number: 2014956813
Mescher MC, Pearse IS (2016) Communicative interactions involving plants: information, evolution and ecology. Curr Opinion Plant Biol 32: 69–76
Nevo O, Razaflimandimby D, Feffrey JAJ, Schultz S, Ayasse, M (2018) Fruit scent as an evolved signal to primate seed dispersal. Sci Adv 4eaat4817
Tomkins P, Bird C (2004) The secret life of plants. Rupa Publ. India (Pvt) Ltd.
Wohllebeen P (2016) The hidden life of trees. Greystone Books Ltd., Vancouver. ISBN 978-1-77164-248-4

Acknowledgement

I acknowledge my Ph.D. Supervisor Prof. Satish Maheshwari for encouraging me to take up research in the area of photomorphogenesis, biochemistry, and molecular biology in the early 1970s when not many plant laboratories had initiated any work in these directions in India. All through my career, I received support from my colleagues at Jawaharlal Nehru University (JNU) and at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi. I am thankful to my students and collaborators in India and abroad for enriching my knowledge and expertise and agreeing to participate in this project by contributing their chapter.

My heart goes to my family, especially my wife, Meena Sopory, who in all these 45 years of our married life never demanded even an hour for herself and let me spend my time in the laboratory and library or even to undertake long sabbaticals for going abroad. I remain indebted to her for the encouragement and support all through.

A word of thanks to my aunt Prabha Devi, disciple of Swami Laxman joo, a Shaivite saint of Kashmir, India, for her blessings. She said, "While we have taken the path to understand the nature of the creator of this universe, you as scientists are in quest toward understanding the nature of the creation, both physical and biological, and hence there is a bonding between science and spiritualism."

My thanks are due to Prof. Mauro Giacca, former Director General, ICGEB, and Dr. Dinkar Salunke, Director, ICGEB, New Delhi Component, for offering me the Arturo Falaschi Emeritus Scientist position at ICGEB and providing me an office and lab space post retirement.

I am also thankful to the Government of India for their recognition and selecting me as Science and Engineering Research Board Distinguished Fellow and providing me personal and grant support to carry on with my research interests.

Thanks are due to the staff of Springer Nature, Ms Mamta Kapila, Raman Shukla, Vaishnavi Venkatesh and Metilda Nancy Marie Rayan. T for their support and understanding.

My special thanks to Dr Charanpreet Kaur for her untiring assistance in broadly going through the book, in its editorial organization, and also helping me upload the manuscript on the Springer site.

Contents

About the Editor

Sudhir Sopory completed his Ph.D. degree from University of Delhi, and later worked at the Jawaharlal Nehru University, New Delhi. He got trained at the Max Planck Institute in Cologne, at the University of Munich in Germany, at the University of Texas, Austin, and at the United States Department of Agriculture, Beltsville, USA. He served as a Group Leader, Director, and later as Arturo Falaschi Emeritus Scientist at the International Centre of Genetic Engineering and Biotechnology. He was also the Vice Chancellor of Jawaharlal Nehru University, New Delhi.

He is an elected fellow of the major Indian science academies and The World Academy of Sciences (TWAS) and has received numerous honours, including the 1987 Shanti Swarup Bhatangar Prize, the highest Indian award in science and technology. The Government of India awarded him the fourth highest civilian honour of the Padma Shri, in 2007, for his contributions to science and technology.

He is currently a Science and Engineering Research Board, Government of India Distinguished Fellow at the International Centre for Genetic Engineering & Biotechnology, New Delhi, India.

Abbreviations

12-OPDA	12-oxo-10, 15-phytodienoic acid
2,4-D	2,4-dichlorophenoxyacetic acid
5HT	5 hydroxytryptamine
7TM	Seven transmembrane
ABA	Abscisic Acid
ABA2	*ABA DEFICIENT 2*
ABCB19	ATP-Binding Cassette B 19 protein
ABI	Abscisic acid insensitive
ABI4/5	*ABSCISIC ACID INSENSITIVE 4/5*
ABRE	ABA responsive promoter element
ACA	Auto-inhibited Ca^{2+}-ATPases
ACC	1-Aminocyclopropane-1-Carboxylic Acid
AChE	Acetylcholinesterase
ADH1	Alcohol dehydrogenase 1
AG	Arabinogalactan
AGO1	Argonaute
AGP	Arabinogalactan protein
AHK	Arabidopsis histidine kinase
AHK1	Arabidopsis histidine kinase 1
AHP	Arabidopsis histidine phosphotransfer protein
AMF	Arbuscular mycorrhizal fungi
AMPK	AMP-ACTIVATED PROTEIN KINASE
AMTs	Ammonium transporters
AOA	Aminooxyacetic acid
AP	Action Potentials
AP2	Apetella 2
AP2/ERF	APETALA 2/ERE binding factor
AP3	APETALA3
APX	Ascorbate Peroxidase
AqPs	Aquaporins
Ara	Arabinose
AREB/ABF	ABA responsive element (ABRE) binding factors
ADP-RF	ADP-ribosylation factor
ARF	Auxin response factor

ARFs	AUXIN RESPONSE FACTORs
ARR	Arabidopsis response regulator
ASA	Ascorbic Acid
ASMT	Acetylserotonin-*O*-methyltransferase
Asp	Aspartate
ATP	Adenosine triphosphate
AUX1	Auxin transporter 1
BABA	β-amino butyric acid
BetP	Glycine betaine transporter
bHLH	Basic Helix Loop Helix
BIC	Blue-Light Inhibitor of Cryptochrome
BLUS1	Blue Light Signalling 1
BR	Brassinosteroid
BRI1	BRASSINOSTEROID-INSENSITIVE 1
bZIP	Basic leucine zipper
BZR1	BRASSINAZOLE RESISTANT1
CA	Carbonic anhydrase
Ca^{2+}	Calcium Ions
CaCA	Ca^{2+}/cation antiporters
cADPR	cyclic ADP ribose
CaM	Calmodulin
CaMK	CaM-activated kinases
cAMP	Cyclic adenosine monophosphate
CAMTA	CaM-binding transcription activator
CAT	Catalase
CBF1	C-repeat Binding Factor 1
CBL	Calcineurin B-like
CC	Companion Cells
CCA	Circadian cock-associated
CCaMK	Ca^{2+} and CaM activated kinases
CCT	Cryptochrome Carboxyl Terminus
CDF	Cycling DOF Factor
CDPKs	Ca^{2+} dependent protein kinases
CesA	Cellulose synthase
CEZ	Central elongation zone
cGMP	Cyclic Guanosine monophosphate
CHASE	Cyclases/Histidine kinases associated sensory extracellular
ChAT	Choline acetyltransferase
CHK	CHASE domain containing histidine kinase
CIB1	Cryptochrome-Interacting Basic-Helix-Loop-Helix Protein
Cis-OPDA	Cis-(+)-12-Oxo-Phytodienoic Acid
CK	Cytokinin
CKI	Cytokinin insensitive
Cl^-	Chloride Ions
CLC	Chloride channel family

CMF	Cellulose microfibril
CML	CAM-Like
CNGC	Cyclic nucleotide gated channel
Cnr1	*cytokinin resistant 1*
CO	CONSTANS
CoI1	Coronatine Insensitive 1
COP	Constitutive Photomorphogenic
COP1	Constitutive Photomorphogenic 1
CPK	Calcium-dependent protein kinase
CRAC	Ca^{2+} release activated Ca^{2+}
CRC	Central Columella Root Cap
CRF	Cytokinin response factor
CRK	CDPK-related protein kinases
CrRLK1	*Catharanthus roseus* Receptor-Like Kinase 1
Cry	Cryptochrome
CSC	Cellulose-synthesizing complex or cellulose synthase complex
CTR	Constitutive response
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
CWD	Cell wall damage
CWDEs	Cell wall-degrading enzymes
CWI	Cell wall integral/integrity
DACC	Depolarization activated Ca^{2+} permeable channels
DAMP	Damage-associated molecular pattern
DBH	Dopamine-β-hydroxylase
DCL1	Dicer Like 1
DCMU	3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea
DD	Dopamine decarboxylase
DEK1	Defective Kernel 1
DET	De-Etiolated
DEZ	Distal elongation zone
DHA	Dehydroascorbate
DHAR	Dehydroascorbate Reductase
DNA	Deoxyribonucleic acid
DRMs	Detergent-resistant membranes
DTI	DAMP-triggered immunity
E2F	E2 FACTOR
eATP	Extracellular ATP
ECA	ER-type Ca^{2+}-ATPases
ECM	Extracellular matrix
ECM	Extracellular matrix
EPR	Electron paramagnetic resonance
ED	Ectodomain
EDRF	Endothelium-derived relaxing factor
EGF	Epidermal growth factor

EGTA	Ethylene glycol-bis (*b*-amino ethylether)-*N*, *N*, *N′*, *N′*-tetra acetic acid
Ehd1	Early Heading Date 1
EIL	Ethylene insensitive 3-like
EIN	Ethylene insensitive
EIN3/4	ETHYLENE-INSENSITIVE 3/4
EMF	Earth's Magnetic Field
ER	Endoplasmic reticulum
ERF	Ethylene response factor
ERMK	Elicitor responsive MAPK
ERS	Ethylene response sensor
ET	Ethylene
Eth	Ethylene
ETR	Ethylene response
ETR1/2	ETHYLENE RESPONSE factor 1/2
EXT	Extensin
EZ	Elongation zone
FAD	Flavin Adenine Di-Nucleotide
FER	FERONIA
FHL	FHY1 Like
FHY	Far Red Elongated Hypocotyl
FKF1	Flavin-Binding Kelch Repeat F-Box 1
FLC	Flowering locus c
FMN	Flavin Mononucleotide
FPI	Floral Pathway Integrator
FLT	Flowering Locus T
FT	Flowering Time
Fuc	Fucose
FUS	FUSCA
GA	Gibberellic acid
GABA	Gamma-Aminobutyric Acid
GAF	c**G**MP-specific phosphodiesterases, **A**denylyl cyclases, and **F**hlA domain
Gal	Galactose
GalA	Galacturonic acid
GAP	GTPase activity accelerating protein
GCN2	General amino acid control non-derepressible 2
GDI	Guanine nucleotide dissociation inhibitor
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
Ghd7	Grain Number Plant Height and Heading Date 7
GI	GIGANTEA
GID1	GIBBERELLIN INSENSITIVE DWARF1
gin2	*glucose insensitive 2*
GlcA	Glucuronic acid

GLRs	Glutamate-like receptors
GPCR	G-protein coupled receptor
G-proteins	Guanine nucleotide-binding proteins
GPX	Glutathione peroxidase
GRP	Glycine-rich protein
GS	Gravistimulation
GSA	Gravity set point angle
GSG	Glutathione
GSNOR	S-nitroglutathione reductase
GSSH	Oxidized glutathione dimer
GTL1–SDD1	GT-2 LIKE 1 -STOMATAL DENSITY AND DISTRIBUTION1
GTP	Guanine triphosphate
GUN	Genome uncoupled
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen Sulfide
HACC	Hyperpolarization- activated Ca^{2+} permeable channels
HAMPs	Herbivore Associated Molecular Patterns
HATS	High-affinity transport systems
Hd3a	Heading Date 3a
HFR1	Long Hypocotyl in Far-Red 1
HG	Homogalacturonan
HHK	Hybrid histidine kinase
HIR	High Irradiance Response
His	Histidine
HK	Histidine kinase
HKRD	Histidine Kinase-Related Domain
HKT	$H^{+/}K^{+}$ transporter
HLS1	HOOKLESS 1
HMA1	Heavy metal ATPase 1
HOG	High-osmolarity glycerol response
HPT	Histidine phosphotransfer protein
HR	Hypersensitive Response
HRGP	Hydroxyproline-rich glycoprotein
HSP	Heat shock protein
HXK1	HEXOKINASE 1
HY	Long Hypocotyl
HY5	Long Hypocotyl 5
HYH	HY5-Homolog
IAA	Indole acetic acid/auxin
IDP	Inherently Disordered Hydrophilic Protein
Ile	Isoluceine
InsP3	Inositol 1,4,5-trisphosphate
IP_3	Inositol-1,4,5-triphosphate
IP_6	Inositol hexakis phosphate
JA	Jasmonic Acid

JAZ	Jasmonate Zim-Domain
K^+	Potassium Ions
LAF1	Long After Far-Red Light 1
LATS	Low-affinity transport systems
LAX3	Aux1-Like Protein 3
L-DOPA	Dihydroxyphenylalanine
LFR	Low Fluence Response
LFY	Leafy
LHY	Late elongated hypocotyl
LKP2	Lov Kelch Protein 2
LOV	Light, Oxygen or Voltage Sensing Domain
LPR1	Low-phosphate root 1
LRC	Lateral Root Cap
LRR	Leucine-rich repeat
LTP	Lipid Transfer Proteins
MADS	Minichromosome Maintenance1, Agamous, Deficiens and Serum Response Factor
MAMP	Microbe-associated molecular pattern
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MAPKK	Mitogen activated protein kinase kinase
MAPKKK	Mitogen activated protein kinase kinase kinase
MCA	Mid1-Complementing Activity
MCA1/MCA2	MID1-complementing activity 1/ MID1-complementing activity 2
MCUC	Mitochondrial Ca^{2+} uniporter complexes
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate Reductase
MEcPP	Methylerythritol cyclodiphosphate
MED	Mediator
meJA	Methyl jasmonate
Mg^+	Magnesium
MH	Monophenol hydroxylase
MID1	Mating pheromone-Induced Death 1
MIPs	Major intrinsic proteins
miRNA	Micro RNA
MKK	MAPK kinase
mLST8	mammalian LETHAL WITH SEC13 PROTEIN 8
MScL	Large conductance mechanosensitive ion channel
MscS	Mechanosensitive channels of small conductance
MSL	MscS-Like
MSL1/MSL3	MScS-like 1/MScS-like 3
MYB	Myeblastosis
Na^+	Sodium ions
NAADP	Nicotinic acid adenine dinucleotide phosphate

NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NAE	*N*-Arachidonylethanolamine
NAGK	*N*-acetyl-L-glutamate kinase
NAMPs	Nematode Associated Molecular Patterns
NAS	*N*-acetylserotonin
NB	Nuclear Bodies
NBS-LRR	Nucleotide-binding site Leucine Rich Repeat
NGR	Negative gravitropic response of roots
NHX	Na^+/H^+ exchanger
NIR	Nitrite reductase
NLP7	Nodule inception-like protein 7
NO	Nitric oxide
NPF	Nitrate transporter 1/ peptide transporter family
NPH3	Non-Phototropic Hypocotyl 3
NR	Nitrate reductase
NRT2.1	Nitrate Transporter 2.1
NUE	Nitrogen use efficiency
NutUE	Nutrient use efficiency
OG	Oligogalacturonide
OGA	Oligogalacturonide
OmpR	Outer membrane protein R
OPDA	12-oxo-phytodienoic acid
OpuA	Osmoregulatory ATP-binding cassette transporter
OSCA	Reduced hyperosmolality-induced Ca^{2+} increase
PAE	Pectin acetylesterase
PAFT	Plant Acoustic Frequency Technology
PAMP	Pathogen-associated molecular pattern
PAMPs/MAMPs	Pathogen/Microbe Associated Molecular Patterns
PAP	3′-Phosphoadenosine 5′-phosphate
PAR	Photosynthetically Active Radiation
PAS	Per (period circadian protein)-Arnt (aryl hydrocarbon receptor nuclear translocator protein)-Sim (single-minded protein)
PCD	Programmed Cell Death
PCIB	p-chlorophenoxyacetic acid
PDR2	Phosphate deficiency response 2
PERKs	Proline-rich extensin-like receptor kinases
Pfr	Phytochrome-far red absorbing form
PGs	Polygalacturonases
PhANG	Photosynthesis Associated Nuclear Genes
PHF1	Phosphate transporter traffic facilitator 1
Phot	Phototropin
PHR	Photolyase Homology Region
Phy	Phytochrome
PHY	Phytochrome

PhyB	Phytochrome B
PI	PISTILLATA
PIF	Phytochrome Interacting Factor
PIN	PIN-formed
PKS4	Phytochrome Kinase Substrate 4
PLC	Phospholipase C
PLD	Phospholipase D
PLP	PAS/LOV protein
PM	Plasma membrane
PM	plasma membrane
PME	Pectin methylesterase
PNMT	Phenylethanolamine-*N*-methyltransferase
POD	Peroxidase
PR	Pathogenesis Related
Pr	Phytochrome-red light absorbing form
ProP	Proline/betaine transporter
PRR	Pattern recognition receptor
PsRR	Pseudo response regulator
PRSL1	Protein Phosphatase1 regulatory subunit2-like protein1.
PRX	Peroxiredoxin
PS	Photosystem
PSI	Phosphate starvation induced
PSRs	Phosphate stress responses
PTI	Pathogen Triggered Immunity
PTM	Post-translational modifications
PΦB	Phytochromobilin Chromophore
QTL	Quantitative trait loci
RALF	Rapid alkalization factor
RAPTOR	REGULATORY-ASSOCIATED PROTEIN OF mTOR
RBCS	Ribulose-1,5-bisphosphate carboxylase
RBOH	Respiratory burst oxidase homologue
RD	Receiver domain
RGA	Repressor of gibberellic acid
RG-I	Rhamnogalacturonan I
RGS	Regulator of G-protein signaling
Rha	Rhamnose
RHP1	RGS1-HXK1 INTERACTING PROTEIN 1
RICTOR	RAPAMYCIN-INSENSITIVE COMPANION OF MTOR
RLCK	Receptor-like cytoplasmic kinase
RLK	Receptor like kinase
RLP	Receptor-like protein
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROP	Rho of plants
ROP2	Rho-related protein 2

ROS	Reactive Oxygen Species
RR	Response regulator
RSA	Root system architecture
RSS1	*REGULATED BY SUGAR AND SHADE1*
RUP	Repressor of UV-B Photomorphogenesis
S1P	Spingosine-1-phosphate c
S6K1	RIBOSOMAL PROTEIN S6 KINASE 1
SA	Salicylic acid
SAG	Senescence Associated Genes
SAR	Systemic Acquired Resistance
SAvR	Shade Avoidance Response
SAUR	Small auxin up RNA
SCF	SKP1/CULLIN1/F-BOX
SE	Sieve Elements
SEP	SEPALLATA
Ser	Serine
Sho1	High osmolarity signaling protein1
SHY2	*SHORT HYPOCOTYL 2*
SIPK	Salicylic acid induced protein kinase
siRNA	Small Interfering RNA
Sln1	Synthetic lethal of N-end rule 1
SLs	Strigolactones
SNAT	Serotonin-*N*-acetyltransferase
SNF1	SUCROSE NON-FERMENTING 1
SNO	*S*-nitrothiol
SnRK1	SNF1-RELATED PROTEIN KINASE 1
SOC1	Suppressor of Overexpression of Constans
SOD	Superoxide Dismutase
SOS	Salt-overly Sensitive
SP	Systemic Potentials
SPA	Suppressor of PHYA
SPL	SQUAMOSA promoter binding protein like
STIM	stromal interaction molecules
SV	slow vacuolar type
T-5-H	Tryptophan-5-hydrolyase
T6P	trehalose-6-phosphate
tasiR	Trans acting siRNA
TCH	Touch-inducible
TCL	Thin cell layer
TCS	Two-component system
TD	Transmitter domain
TDC	Tryptophan decarboxylase
TF	Transcription factor
TH	Tyrosine hydroxylase
THE1	THESEUS 1

Thr	Threonine
TIBA	2,3,5-triiodobenzoic acid
TLR	Toll-like Receptor
TML	Too Much Love, a Kelch-Repeat F-Box Protein
TOC	Translocon on the outer chloroplast membrane
TOR	TARGET OF RAPAMYCIN
TPC1	Two-pore channel1
TPK	Two-pore K^+ channel
TRPV4	Transient receptor potential cation channel subfamily V member 4
TRX	Thioredoxin
Tyr	Tyrosine
uORF	Upstream Open Reading Frame
UPS	Ubiquitin Proteosome System
UVR8	UV-B Resistance 8 *aka* Ultraviolet-B Receptor
VDAC	Voltage-gated anion channel
VICCs	Voltage-Independent Ca^{2+} Channels
VLFR	Very Low Fluence Response
VM	Vacuolar membrane
VOC	Volatile Organic Compound
VP	Variation Potentials
WAK	Wall-associated kinase
WAKLs	WAK-like kinases
WGD	Whole genome duplication
WIPK	Wound induced protein kinase
XG	Xyloglucan
XTH	Xyloglucan endotransglucosylase/hydrolase
Ypd1	Tyrosine phosphatase dependent 1
YUCCA	Flavin Monooxygenase-Like Enzyme
ZTL	ZEITLUPE
Ψ_p	Hydrostatic potential
Ψw	Water potential
Ψ_Π	Osmotic potential
Ψ_g	Gravitational potential

Plant Diversity and Adaptation

1

Sudhir Sopory and Charanpreet Kaur

Abstract

Ancestors of modern land plants evolved in aquatic environments, with the first land plants appearing around 470–700 million years ago. Terrestrial colonization has been credited to a series of major revolutions in the body plan, anatomy and biochemistry of plants which is required for their survival and reproduction. Plant adaptations to life on land encompassed development of many specialized structures such as water-repellent cuticles, stomata for regulating water evaporation, structures for collecting sunlight, a vascular transport system and many more. In addition, intricate signalling mechanisms regulated by hormones for the perception of the environment have also come into place in higher plants. How these features have evolved in modern-day plants and how these have contributed to diversity are fascinating. In this chapter, we aim to shed light on a few interesting facets of plant functions with a bearing on evolution, which have not only contributed to their establishment on land but also allowed their enormous expansion leading to huge diversity. We believe that plants have a remarkable ability to adapt themselves in the ever-changing environments, despite being rooted to ground.

Keywords

Angiosperms · Evolution · Gymnosperms · Parasitism · Perennial plants · Plant survival · Polyploidy

S. Sopory (✉)
International Centre for Genetic Engineering and Biotechnology, New Delhi, India
e-mail: sopory@icgeb.res.in

C. Kaur
Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_1

1.1 Introduction

According to Christenhusz and Byng (2016), there are a total of about 374,000 plant species, which include algae (44,000), liverworts and hornworts (9225), mosses (12,700), lycopods (1290), ferns (10,560) and gymnosperms (1079). The rest, which is roughly 90% of the earth's population, constitute flowering plants (monocots, 74,273; dicots, 210,008). In fact, many more are added every year as per *Phytotaxa*; hence, the total number of species is not yet fixed.

It is not clear as to how these plant species have evolved and what changes in their physiology, anatomy and perception mechanisms have led to their colonization, adaptation and spread across different zones of temperature and altitude on land. Comparative genetic studies between simple multicellular organisms and their single-celled relatives suggest that much of the molecular apparatus required for cells to group together and coordinate their activities may have existed even before multicellularity evolved. For instance, in *Chlamydomonas*, the unicellular relative of Volvox, centrioles perform dual functions. They not only anchor flagella but also help in reproduction, allowing *Chlamydomonas* to both swim and reproduce, but not at the same time. However, multicellular Volvox because of cell specialization can do both at once (Richter et al. 2018).

The first plants that appeared on land were most similar to what are known today as bryophytes and descended from early water-dwelling alga. From these seedless non-vascular bryophytes and mosses arose seedless vascular plants like horsetails (*Equisetum*), and of these, ferns became the most advanced seedless vascular plants with more than 10,000 species and distribution ranging from tropics to temperate forests. Later, evolution led to the appearance of gymnosperms. However, the appearance of angiosperms seems to be sudden, which could not be explained by Darwin as he was unable to find any paleontological data. He suggested a very fast diversification of flowering plants in the mid-Cretaceous period. For example, some families of flowering plants such as Orchidaceae and Astraceae possess a large number of genera and species, probably as a result of fast diversifications. Precisely, Orchidaceae has 736 genera and about 28,000 species, and Astraceae possesses 1623 genera and 24,700 species. This could be due to a much better and accurate sensing of the overall environment and strategic reproductive behaviour, throwing wider variations and adapting to different habitats. Further, there are some species that have very restricted ecological niches, whereas the presence of many others can be located from low to high altitudes. *Ferula jaeschkeana* is a monocarpic herbaceous perennial plant that has a wide distribution, and the species of the genus mostly grow in mountainous regions, but some are distributed in desert areas. However, the widespread arctic alpine species, mountain sorrel (*Oxyria digyna*), along with other hygrophilous snow-bed species, such as *Cerastium cerastoides* and *Ranunculus pygmaeus*, have more restricted habitat demands. Further, another plant species, the blue heath (*Phyllodoce caerulea*), is in danger of disappearing in Scotland due to a lack of winter snow.

Plant adaptation seems to depend directly on the availability of water, light duration and quality, and nutrients. For example, pine trees adapt to places where there is more nitrogen than phosphorus, whereas a desert plant like cactus needs lot more

phosphorus but not much nitrogen. The cause of natural selection and, thus, of adaptive evolution is therefore the environmental factor that results in differential fitness among phenotypes. Kokko et al. (2017) discuss if evolution can fulfil the demands of ecology. They, however, state that adaptation to a changing environment is far from simple as evolutionary 'supply' and ecological 'demands' can interact and alter evolutionary trajectories.

With more and more plant genomes getting sequenced, our understanding of the factors that influence plant adaptation is improving. Further, more clarity has been achieved regarding the sensory systems and the signalling mechanisms that have evolved to monitor and respond to the changes in the environment for plant survival. In this chapter, we will discuss some of the important plant functions that have significant bearing on evolution and adaptation and also a few interesting plant families which have developed very unique characteristics of perception and existence.

1.2 Conservation and Evolution of Light Perception Systems

Plant development across kingdoms seems to be influenced by light, perceived via different photoreceptors, thereby shaping adaptive strategies under different ecological niches. Many studies pertaining to the characterization and functioning of these photoreceptors have been carried out in higher plants, and the same has been covered in Chap. 2. Major light sensing in higher plants is done via red and far-red light receptors called phytochromes and UV/blue light receptors called cryptochromes and phototropins. However, it is still not known whether similar light-sensing mechanisms and receptors operate in lower plants as well and is indeed a subject of intense research in different labs. In their study, Li et al. (2014) have shown that ferns, which grow under low light intensity, possess a novel photoreceptor called neochrome that contains modules of both phytochrome and phototropin in a single molecule. The fused molecule is seen in some algae like *Mougeotia* and is present in only some family of ferns, indicating that the early origin of such fused molecules may have two independent routes, evolving in shade-loving or less light-requiring ferns.

Unlike ferns, which prefer shade, we generally see a shade avoidance response in higher plants. Plants, in fact, use photoreceptor proteins to detect their closeness with other plants and to even activate adaptive responses. Of the number of phytochromes which have been reported, phytochrome B (phyB), which is sensitive to changes in the red to far-red ratio of sunlight, seems to play an important role, along with some other receptors in regulating growth and development in response to competition cues (Ballaré and Pierik 2017).

Prof. Clark Lagarias, at the UC Davis, has been studying photoreception in aquatic algae that can sense a wide range of colours across the whole light spectrum. Since red light does not penetrate too deep into water, it has been generally presumed that red/far-red wavelengths do not have any effect in aquatic environments. There are some algae which lack phytochromes. However, those that possess

phytochromes are able to perceive blue, green, yellow, orange, red and far-red light, unlike phytochromes from higher plants. In fact, it has been shown that some of the diatoms do have phytochromes that use biliverdin as a chromophore, and their absorbance characteristics are somewhat like algal and plant phytochromes (Fortunato et al. 2016). Further, Fortunato et al. reported that though far-red wavelengths from sunlight are detectable at only the ocean surface, chlorophyll fluorescence and Raman scattering can generate red/far-red photons in deeper water layers. This is indeed an adaptive strategy of aquatic plants to use whatever light that they can receive under aquatic conditions for light sensing and light harvesting. Based on genome sequencing data, a phytochrome was also predicted in a marine alga, *Micromonas pusilla*, which showed similar domain architectures except for the lack of a C-terminal response regulator domain. Duanmu et al. (2014) suggested that green alga and land plants have a common progenitor of phytochromes. Further, the nuclear movement of phytochromes upon perception of light, as a mechanism of its action to regulate gene expression in higher plants, also seems to have its origin in algal systems.

Red and blue light regimes have also been studied to regulate stomatal development and movements during evolution in plants. In early vascular plants, stomatal opening has been found to respond to both red and blue lights; however, blue light responses were not seen in true fern polypodiopsida, whereas it was an absolute requirement in a gymnosperm, *C. revolute*, and the ferns, *Equisetum hyemale* and *Psilotum nudum* (Doi et al. 2015). The acquisition of stomatal responsiveness to blue light might have evolved to confer the ability to rapidly open and close the stomata, thereby providing competitive benefits in both uptake of CO_2 and prevention of water loss.

1.3 Gymnosperms to Angiosperms: Flower Origin and Diversity

The evolution of land plants from green algae via bryophytes, ferns and gymnosperms to the angiosperms has occurred by shifting the life cycle from the gametophyte as a dominant form to the sporophyte form and from non-vascular structures to the development of vascular structures for water and nutrient management and, finally, the development of seeds as protective structures in angiosperms from the naked seeds in gymnosperms.

In a correspondence published in *Nature Ecology and Evolution* (2017), Richard Buggs writes: ‘In 1879, in a private letter to Joseph Hooker, Charles Darwin grumbled, the rapid development as far as we can judge of all the higher plants within recent geological times is an abominable mystery’. Though some monocots were present from the Devonian to the Cretaceous period, the sudden appearance of dicots was most perplexing to Darwin.

The origin of flowers and the diversification of angiosperms in various habitats are not well understood. Douglas Soltis from the University of Florida, who is part of the Floral Genome Project Research Group, says that extensive data on flowering

genes and their expression are needed not only from model flowering plants but also from gymnosperms to get clues on the origin of modern flowers and the diversity in floral architecture that we see today in all the ecological niches on this planet. This group will be working on 15 selected species of gymnosperms and early angiosperms, monocots and dicots and will look at 100,000 ESTs in early flower development. The development of flowers and consequently the pollination mechanisms must have played a major role in the production of seeds, their dispersal and their spread, thereby creating variations in survival under different environmental conditions ranging from sea level to mountains. Frohlich (2003) discussed some of these issues in a review entitled An evolutionary scenario for the origin of flowers.

The origin of the angiosperm flower basically entails that the male and female reproductive organs that are spatially separated in gymnosperms, get combined together in most of the angiosperms, into a perfect flower where both self- and cross-fertilization mechanisms give rise to the formation of triploid endosperms within the seeds. Despite these major differences, it seems that the basic genetic tools used by gymnosperms and angiosperms may be somewhat similar. Studies on various homeotic mutants of MADS box-like transcription factors show that these combine in quaternary complexes to bind some specific cis elements, regulating downstream target genes that then result in the development of sepals, petals, stamens and carpels. Comparison of expression and co-localization of some of these genes revealed that while AP3, PI, AG (AGAMOUS) and SEP3 regulate male identity, AG3 and SEP3 control female identity in Arabidopsis and AP3/PI (B) genes are expressed only in male cones in a gymnosperm, *Gnetum gnemon*. This suggests that though the complexes leading to organ identity may be different in gymnosperms and angiosperms, the basic building blocks are similar (see Ruelens et al. 2017 and references therein). Further, the work of Chen et al. (2017) has identified 14 monophyletic clades of the MIKC^c-type MADS box genes by comparing genomes of all orders of gymnosperms and basal angiosperms. In addition to identifying previously characterized orthologs, a novel family of MADS box genes, GMADS, was also found in gymnosperms. In addition, ABCE model prototype genes were found to be conserved, whereas others like SVP, SOC1 and GMADS expanded in gymnosperms. Collectively, gymnosperms were found to possess near-complete set of MIKC^c genes, which harbour a K-box at the C-terminal of type II TFs, suggesting that genome duplication along with expressional transition of MIKC^c genes in the ancestors of angiosperms is the major contribution to the first flower.

One of the major changes that occur in the flowering plants is the conversion of a vegetative shoot meristem into a floral meristem. In the mid-1940s, Chailakhyan had put forward the concept of florigen as an active signalling component produced in the leaves but acting on the meristem to induce flowering. Recent work of George Coupland and others shows that it is the flowering locus T (FT) protein which serves as the mobile signal responsible for floral transition. Whether similar signals are present in gymnosperms is not clear, but currently it is believed that FT-like signals are restricted to angiosperms and may have an important role in their adaptation, and regulated flowering behaviour under different light and dark conditions. In fact,

FT has now been shown to have implications in diversity, adaptation and domestication (Pin and Nilsson, 2012).

Variations in flower traits such as, size, shape, odour and colour can be viewed as cues of adaptation to attract pollinators, ensuring reproduction, seed development and dispersal. Among all flower parts, major variations can be seen in petals, with plants having three, five or even more petals. Further, variations in the types of flowers have also been observed, ranging from the presence of simple flowers in families like Orchidaceae to compound flowers in the sunflower family. It can be said that plants have tuned visual signals of their flowers to the sensory system of pollinators in order to look as conspicuous and attractive as possible to them.

Different groups have been working on the evolution and molecular mechanisms underlying the unique flower architecture of orchids, which constitute about 10% of the flowering plants and colonize diverse habitats on earth. Comparative transcriptome studies of representative members of various orchid families along with genome data of a couple of species have helped in identifying the ancestral orchid gene kit (Zhang et al. 2017). Analysis of new gene families, gene family expansions and contractions and changes in MADS box gene classes, is revealing mechanisms that control a diverse suite of developmental processes such as those involved in the formation of flowers with labellum and gynostemium, pollinia and seeds without endosperm during orchid evolution. These studies are also revealing the evolution of the epiphytic nature of some of the orchids.

Why and how traits like flower pigmentation, pigment intensity and flower symmetry have evolved are some of the other questions that are being addressed in different laboratories. In a recent study, genetic differences in *Clarkia* flowers that are responsible for evolutionary changes in the spot colour position were investigated (Jiang and Rausher, 2018). A shift in the position of *cis*-regulatory elements in the promoter of the R2R3 MYB gene resulted in the activation of the MYB gene by a different transcription factor that is expressed in different positions in the petal and, thus, led to a shift in the position of colour spots in the petals. This work thus showed the importance of regulatory elements in the evolution of flower patterns.

Further, as the survival of plants depends on efficient pollination and seed set, plants act to avoid pollen robbers and attract those helping in successful transfer of pollen to the stigmas. But what do flowers do to attract the insect pollinators? Moyroud et al. (2017) have shown the presence of ‘messy’ microscopic structures on the petals of some flowers that can manipulate light to produce a blue colour effect in order to attract bumblebees. These nanostructured motifs in petals of different flowers show apparent ‘disorder’ in dimensions and spacing, but despite huge variations in anatomies, all possess convergent optical properties, that is, all petals produce a similar ‘blue halo’ effect. Bees have an innate preference for colours in the violet-blue range, but as many flowers lack the ability to produce such pigments, the presence of such blue halo structures provides an alternative pathway to produce signals that attract insects. These studies, thereby, reflect ecological implications of plant-insect co-evolution, species survival and diversification.

1.4 Hormonal Regulation: From Non-vascular to Vascular Plants

The transition of plant life from an aquatic environment to terrestrial grounds probably occurred over 450 million years ago. However, it is not clear whether plants were already equipped with the necessary biochemical machinery required for adaptation to this new drought-type environment or was this acquired during colonization. Further, what new innovations might have occurred to help plants settle in the new environment?

Different stages of growth and development of land plants and their tolerance to different biotic and abiotic environments are controlled by many growth regulators, of which, about 10 have been termed as plant hormones. These include auxins (indole acetic acid, IAA), cytokinins, gibberellins (GA), abscisic acid (ABA), ethylene, brassinosteroids, jasmonates, nitic oxide, salicylic acid and strigolactones. It is believed that the emergence of hormone signalling pathways might have potentially contributed to the emergence of land plants. An account of their modulation and mechanism of action is covered in Chap. 9. Here, we will only discuss few facts related to the evolution of hormone machinery and their role in adaptation.

Hormones like IAA, GA3, zeatin and ABA were found in early land plants including some species of mosses and lichens (Ergün et al. 2002). Auxins were even detected in marine algae (Van Overbeek 1940) and could stimulate cell division and enlargement along with affecting rhizoid development in red and green algae. Analysis of transcriptomics data of five representative charophyte species, which are considered as important intermediates in the transition of aquatic freshwater plants to land, revealed the presence of putative homologs of genes involved in the biosynthesis, transport, perception and signalling of major plant hormones. *Spirogyra pratensis*, for example, produces ethylene and even shows cell elongation response to this hormone, similar to land plants. These studies of Ju et al. (2015) suggest that some of the hormone machinery existed even before the transition of plants to land. However, hormone signalling mechanisms in algae, mosses and liverworts are still not well worked out.

In higher plants, auxin is produced in apical regions and is transported down to the roots via auxin efflux carriers and PIN-FORMED (PIN)-like proteins. While auxin carriers were not found in the unicellular simple algae, like *Chlorella vulgaris*, a naphthylphthalamic acid (NPA, a phytotropin)-sensitive carrier was found in the branched multicellular green alga, *Chara*. Dibb-Fuller and Morris (1992) write that 'the appearance of specific auxin carrier systems in the charophyta may have been fundamentally associated with the evolution of multi-cellularity rather than with the evolution of plant body, which is characterized by different morphological regions'. This may be true, since after the development of multicellular organisms, simple diffusion of IAA would not be efficient enough; hence, movement across cell membranes may be required for polar transport of IAA. Though PIN is associated only with land plants, some endoplasmic reticulum-localized PINs,

like PIN5 and PIN8, seem to have their origins in Streptophyta algae. In fact, the EST database reveals that partial PIN sequences may be present in many algae, like *Spirogyra* and *Penium*.

Auxins have been shown to control gene expression in different organs via Aux/IAA family of transcription factor proteins, which act as either positive or negative regulators of gene expression and, thus, control plant development. Goldfarb et al. (2003) showed that auxin signalling and Aux/IAA family of proteins existed in gymnosperms as well and that some of the classes of these proteins are more close to angiosperms. In this study, five members of the Aux/IAA gene family were isolated from loblolly pine, of which *PTIAA2* exhibited lesser sequence similarity to other four genes but was found to be most closely related to the angiosperm genes. Further, Remington et al. (2004) proposed that the origin of the Aux/IAA gene could be correlated with the origin of land plants and further suggested that the major Aux/IAA and ARF lineages originated before the monocot-eudicot divergence. In fact, Aux/IAA-domain-containing genes could not be found in green algae and some charophytes.

The hormone ABA, though not a plant-specific compound as it is present across kingdoms from bacteria to animals, is considered to be associated with plant adaptation under terrestrial conditions, especially stress environments like drought or low water and dry conditions. The phase of seed development in plants, when the water is being removed from the developing seeds, is correlated with the appearance of ABA. Hence, the evolution of this hormone can be considered as an adaptation of plants to land. However, despite the fact that there has been a movement of plants towards life on dry areas, many mosses, ferns and some flowering plants have returned to an aquatic environment, especially, to fresh water. Likewise, many plants can grow in water-logged areas or even under flood. Under these conditions, flowering plants need to specifically keep their flowers above the water surface for pollination. However, in others, the leaf is kept above the water level under such conditions. Overall, ABA seems to be one of the key hormones to let the plants adapt under 'submersed and emersed' life styles (Wanke 2011).

From an evolutionary perspective, ABA is not only associated with the adaptation of vascular plants but is also found in non-vascular bryophytes. Work on *Physcomitrella patens*, the genome of which has been sequenced, reveals that ABA has an important role in dehydration stress in mosses as well (Takezawa et al. 2011). Further, signalling machinery for ABA responses is also preserved in liverworts, representing the most basal members of existing land plants.

In fact, a recent comparative genomic and phylogenetic study undertaken by Wang et al. (2015) provides important insights into the origin and evolution of various plant hormones. Auxin, cytokinin and strigolactone signalling pathways were predicted to originate in charophytes, while ABA, jasmonate and salicylic acid signalling pathways probably originated in the last common ancestor of land plants. Further, gibberellin signalling was proposed to evolve after the divergence of bryophytes from land plants, and brassinosteroid signalling originated before the emergence of angiosperms but most likely after the split of gymnosperms and angiosperms. Lastly, the origin of the ethylene signalling pathway was anticipated to occur shortly after the emergence of angiosperms. These signalling pathways

have probably emerged and evolved into their current forms as a result of selection pressures exerted by the biotic or abiotic stresses encountered in the terrestrial environments.

1.5 Survival Under Cold Climate

It is thought that the early adaptation of plants on land was restricted to warmer climates, and under these conditions, new life forms evolved and spread across different environmental niches by modifying their physiology and morphological features. In desert regions, the plants developed mechanisms to function with low water by reducing transpiration and those that occupied the sea coasts developed their physiology and anatomy to survive under high salinity. Constant changes over millions of years created a vast variation in plant life forms, both in vegetative and reproductive structures. One of the interesting questions is how and why plants moved into cold or freezing conditions and what adaptive changes they had to acquire for their survival in temperatures which normally would inflict frost bite on other species.

To answer the above question, research teams in USA and Australia recently assembled a large species-level database of growth habit of 49,064 woody or herbaceous species. The parameters they looked for were leaf phenology, diameter of xylem vessels and tracheids and time of exposure to freezing. The data was combined with that of molecular phylogeny for 32,223 species of land plants (Zanne et al. 2014). Using a time tree, the data obtained could be correlated with the geological events. The authors found three major changes that could have helped plants withstand extreme cold conditions. First, plants learnt to sense the arrival of cold and, hence, dropped their leaves annually and simultaneously and also slowed down the movement of water between roots and leaves. Second, genetic changes occurred which led to alterations in the anatomy of water-conducting channels, and third, plants learned to avoid cold altogether, by developing as herbs, losing above-ground stems as in annual species, retreating as seeds or storing organs underground as in potato or tulips. Further, the authors also identified the order of evolutionary events. They suggested that woody plants became herbs or developed skinnier water-conducting pipes before moving into freezing climates but began dropping their leaves after confronting freezing climates. Since the transition of plants from an aquatic environment to land had prepared plants for life in less water conditions, it is possible that such a drought-type of environmental pressure might have caused these plants to evolve this way, which happened to work well for freezing tolerance too. As also, one of the authors, Solitis, said ‘sometimes the trait evolves for some other purpose, and then the organism is able to adapt and use it for something new’.

However, it is not clear as to how frequently adaptation arises, which conditions promote or hamper it, and whether different species exhibit similar adaptive responses to similar selection pressures. Elevation gradients have been used to study climatic effects on adaptation and suggest that differentiation in phenotypic traits like height and phenology along elevation gradients has a genetic basis. Common garden and

reciprocal transplant experiments indicate that genetically based trait differentiation along elevation gradients is common in plants and is, in fact, associated with variation in morphological and phenological traits (Halbritter et al. 2018). Interestingly, tree seedlings and natives of alpine or high-latitude ecosystems have been found to migrate to higher elevations and latitudes as a result of changing winter climates, so that they can stay within their original cooler climate niches. But to enable this migration, seedlings need to establish and interact with the existing vegetation. In this context, Lett et al. (2018) studied plant-plant interactions during winter climate changes. They investigated whether bryophytes facilitate tree seedling survival in a changing winter climate and whether these effects are consistent with the stress-gradient hypothesis (SGH) along elevational gradients and under contrasting snow conditions. Their studies suggested that the generally observed negative or neutral effects of bryophytes on seedlings were enhanced under conditions caused by increased snow cover immediately after winters. Bryophytes exerted a largely negative effect on overwinter seedling survival relative to the bryophyte-free soils. Overall, it was concluded that interactions from bryophytes can modify the impacts of winter climate change on tree seedlings but not always consistent with the SGH.

1.6 Plant Survival with Enemy and Friends Around

For millions of years, plants have survived the presence of microbial pathogens and insects that feed on them and also of the grazing animal species, suggesting that they have developed defence systems despite being sessile in nature. These systems act as morphological, biochemical and molecular blocks, allowing survival of species even in the presence of such predators. Recent research in angiosperms has elucidated various defence mechanisms which provide 'immunity' to plants. This aspect has been dealt in Chap. 20, focussed on plant-microbe interactions.

How plants acquired immunity against invaders is intriguing. Were the early plants inherently equipped with the defence toolkit against existing microbes, resulting in their swift transition to land, or whether evolution and spread of plants led to the emergence of different defence mechanisms? It is possible that the population of species which could not defend themselves from the opponents perished over a time span. Another possibility is that the plants entered into mutual cooperation with their opponents in order to establish themselves on land. To this end, plants can be said to have adopted several ways to develop friendship with microbes, such as providing them with nutrition and safe homes (as for endophytes) or some like mycorrhizal fungi were allowed to colonize on leaves and roots and in turn helped plants get nutrition. However, to keep animals away from feeding, some plants have developed spines or produced toxic compounds, while others developed efficient reproductive mechanisms producing large number of seeds, which could be dispersed far off to ensure species survival even if few plants died due to these predators.

To check if the defence toolkit did exist in early land plants, Ponce de Leon and Montesano (2017) studied defence systems in the non-vascular moss, *Physcomitrella patens*. Analysing gene expression profiles and functions via a targeted gene

disruption approach revealed defence mechanisms to be conserved in moss and higher plants. Perception by PAMP (pathogen-affected molecular patterns) proteins and signal transduction via the MAPK (mitogen-activated protein kinase) pathway, which activates plant resistance to pathogens, were found to exist in mosses as well. It seems, therefore, that the early invaders on land brought their defence artillery with them, to meet the challenges of the existing pathogenic microbes.

During the evolution of plant herbivores and microbes, one finds that plant-enemy interactions remain more generalized in nature, while in some cases there is a specificity of plant-enemy interactions. However, transitions from specialist to generalist strategies are common and that genomic plasticity and rapid evolution of the mechanisms underlying specialization are responsible for changes in interaction specificities. Mobility of plant communities in different niches, probably to escape, may have resulted in their encountering different pathogens. Hence, the development of new defence strategies is a prerequisite for their survival. Studying associations between the tree genus *Inga* and its lepidopteran herbivores in the Amazon, Endara et al. (2017) suggest that plant defences might be more evolutionarily labile than the herbivore traits linked to host association. While plants may have evolved under selection by herbivores, these herbivores do not show co-evolutionary adaptations and instead 'chase' hosts based on their own traits at the time of encountering a new host. *Inga* shows high local diversity with as high as 45 closely related species co-existing at a single site. This high local diversity is believed to be shaped by herbivores, preventing any particular species from domination. It is believed that herbivore-based selection causes strong divergence for defensive traits.

1.7 The Perennial Life Style

Plant life styles vary greatly with respect to their reproductive behaviours. The annuals, flower once in their life whereas perennials, flower repeatedly during their short or long life spans. Of these, there are some known as bi-annuals, which flower once in 2 years, while others like those belonging to the bamboo family may flower once in a decade or once in three to four decades and then perish. Many trees which live for decades or centuries keep on flowering every year, produce seeds and establish new progenies simultaneously, ensuring their own survival and growth.

How did these different flowering behaviours evolve, and does this have any relationship to the sensing of environmental conditions? Theoretical and empirical studies suggest that the unpredictable weather and climate conditions have probably led to the evolution of annual habits in otherwise perennial plants. This has happened especially under extreme temperatures and arid conditions, where perennials would have perished without having gone through the reproductive phase (see Friedman and Rubin 2015 and references therein).

The sensing and signalling in perennials is an interesting topic of study. Annuals die after flowering as also some bamboos which have long vegetative lives. The cycle of vegetative phase and flowering is repeated in perennials. Which ones and how many vegetative meristems need to be converted to flowering meristems is a

decision which the plant has to make depending on the environment and the need of the system. In aspen trees which show perennial behaviour, day length controls flowering through CONSTANS (CO) and FLOWERING LOCUS T (FT) genes, similar to that observed in annuals (Böhlenius et al. 2006). However, in the fall season, the CO/FT regulon inhibit growth and budding, indicating their role in controlling a highly adaptive trait for forest trees.

In a recent study, Coupland's group (Kiefer et al. 2017) has compared the expression of flowering locus C (FLC) orthologs from three annual and two perennial species of *Arabis* (of Brassicaceae family) and found differences in their expression patterns. FLC, an inhibitor, is stably repressed in cold in annuals. On the other hand, in perennials, it is repressed by winter cold but is reactivated in spring, conferring seasonal flowering patterns. Sequence comparisons of FT genes from perennials and annuals revealed that variations in the two regulatory regions of the first intron correlated with the divergence of expression patterns between annuals and perennials. Further, an earlier study on *Arabis* by Wingler et al. (2015) showed that sugars and hormones are involved in the adaptation of some perennial *Arabis* species to different altitudes. Authors showed that the senescence-inducing effect of sugars, otherwise observed at warmer temperatures, was abolished at cold temperatures as sugar accumulation was required for protection. In fact, a positive correlation between sucrose and jasmonic acid (JA) contents was observed only at warmer temperatures, and JA exhibited an overall negative correlation with chlorophyll content, thereby, promoting stress-dependent decline in chlorophyll at warm but not cold temperatures. The details of sugar signaling ad its crosstalk with other hormones is covered in Chap. 13.

1.8 Polyploidy in Plant Evolution

G. Ledyard Stebbins Jr was one of the first to develop a model of polyploid evolution. He published extensively on evolution, with his first paper in 1929 till his last paper in 1999. He and many others later suggested that genome duplications result in speciation and increasing biodiversity (see Soltis et al. 2014).

Across angiosperms, plants can be grouped into diploids and polyploids. In addition, there are some plants which are triploid in nature. These triploids, although flowering, generally multiply through vegetative propagation. Polyploidy represents whole-genome duplications (WGD), and it has been suggested that at least two ancestral WGD must have occurred before the origin of flowering and seed plants. However, Ruprecht et al. (2017) suggest that the phylogenomic dating studies indicate little evidence for two ancient WGDs in plants and that it is too early to conclude the exact number, timing and phylogenetic position of these ancient duplications.

Following the analysis of the spruce genome, the first conifer genome to be published, it was suggested that conifers lack WGD. However, Li et al. (2015) presented evidence for three ancient genome duplications during the evolution of gymnosperms. They came to this conclusion following phylogenomic analysis of transcriptomes from 24 gymnosperms and stated that 2 duplication events occurred in the ancestry of *pinaceous* and *cuppressophyte* conifers and one in *Welwitschia*

belonging to Gnetales. Thus, a role of polyploidy in the evolution of conifers and gymnosperms was determined. Wood et al. (2009) showed that vascular plant species with a polyploid origin are ubiquitous and, in fact, represent a high proportion (35%) of plant diversity, with 15% of angiosperms and 31% of ferns being polyploid in origin. Polyploidy is thought to be an ancient phenomenon, and probably, all extant angiosperms have polyploid ancestors. However, there are examples of newly formed polyploids (or neopolyploids) too. This has led to the differences in the opinion on whether diploids or polyploids speciate at higher rates. As against the previous notion of polyploid flowering plants generating more diversity than their diploid counterparts, Scarpino et al. (2014) propose that diploids speciate at higher rates than polyploids. Further, Mayrose et al. (2011) also feel that 'polyploids are evolutionary dead ends', but they explain it in terms of polyploids being more likely to go extinct than diploids, a concept not accepted by Soltis and others.

What are the advantages of polyploids vs diploids in terms of environment perception, adaptation and survival? Working on wild yarrow (*Achillea borealis*), Ramsey (2011) found that hexaploids exhibit greater advantages than tetraploids for survival under dune habitats. Increase in ploidy also builds invasive character in plants to succeed under fluctuating environmental conditions and help them to colonize new ecological niches (see te Beest et al. 2011). There are many other studies on polyploidy and adaptations (Moghe and Shiu 2014). However, even with new genomic-based and other related studies, Madlung (2013) feels that the cause-and-effect relationship of polyploidy with its distinct advantage in successful adaptation or on its evolutionary significance is not yet fully established. Maherali et al. (2009) compared natural diploid, tetraploid and colchicine-induced neotetraploids of *Chamerion angustifolium* to investigate the effect of genome doubling on water relations. Though the authors reported larger stomata, increased stem and vessel diameter and decreased specific hydraulic conductivity in both types of tetraploids over the diploids, they found that the established tetraploids showed significantly greater drought tolerance than the diploids and neotetraploids, suggesting that the tolerance to drought likely evolved after and independently of genome duplication in the fireweed.

Polyploids are generally bigger in size compared to their diploid counterparts and are reported to be more frequent in extreme environments, including the subarctic regions and high elevations. How did WGD bring about morphological changes? Comparing phenotypes and transcriptomes of diploid and autotetraploid mulberry plants, Dai et al. (2015) found that, of about 21,229 genes, around 609 were differentially expressed, and of these, 30 were those belonging to biosynthesis and signal transduction of plant hormones and about 41 were those involved in photosynthesis. This was in agreement to previously known increase in the rate of photosynthesis and chloroplast numbers with an increase in ploidy levels. Polyploidy can also induce phenotypic modifications in reproductive traits with reproductive organs being larger than their diploid counterparts, having more flowers per inflorescence and exhibiting increased selfing, probably due to loss in incompatibility. It is proposed that the evolutionary advantages of polyploids may stem from increased heterozygosity, genomic rearrangements, gene redundancy, variations in gene expression or epigenetic reprogramming. Further, to assess

whether trait covariation associated with polyploidy and genome size occurs at the microevolutionary scale, i.e. within species, Balao et al. (2011) studied 22 populations of *Dianthus broteri* s.l., a perennial herb with several cytotypes (2x, 4x, 6x and 12x). Highest-order neopolyploids (12x, 6x) were found to possess larger flowers and stomata, occupied very specific habitats and were served by an extremely narrow pollinator fauna. The authors reported that the ploidy levels covaried with organ dimensions, causing multivariate features to increase, remain unaffected or decrease with the DNA amount and concluded that polyploidy contributes to decouple variation among traits.

1.9 Evolution of Parasitism and Insectivorous Behaviour: Perception and Adaptation in Non-photosynthetic Plants

Plants are autotrophic, be these the algae, bryophytes or others, and this nature has been retained all through their evolution and diversification. Why then some plants had evolved to become parasites and be dependent on hosts or others acquired a heterotrophic mode of life becoming carnivorous or insectivorous in nature? A possible reason may be that, in order to dominate the land, plants have undergone manipulations and developed tricks for survival in every possible niche.

During different stages of evolution, certain plants may have found it difficult to survive due to their inability to use available light for efficient photosynthesis or due to their poor roots not suited for water and nutrient uptake in somewhat difficult and competitive environmental situations. Taking up parasitism as a mode of life must have been a successful strategy for their survival, reproduction and diversification. For this, the selection of proper host and development of the haustoria to feed on the host must have resulted in a heterotrophic mode of life. It is believed that the ancestral plant lineages possessed the developmental flexibility to meet the requirements of a parasitic life style. There are different kinds of parasitic plants. Some are able to carry out low levels of photosynthesis, whereas others are totally dependent on the specific host. According to some estimates, there are about 1% of angiosperms that lead a parasitic life and that parasitism has evolved independently at 12–13 different times in angiosperms. Some parasitic plants which can live on different hosts can even be very destructive (see Westwood et al. 2010 and references therein). *Striga* (witchweeds), which parasitize mostly grasses, and *Orobanche* (broomrapes), which feed on important food legumes, are among the most agronomically destructive parasitic plants.

Some of the parasitic plants like *Cuscuta campestri* (dodder plant) can communicate signals via their vines to the neighbouring plants they intertwine. By studying transcriptomic changes occurring in the leaves of insect-attacked and -unattacked host plants, it was found that if one of the *Cuscuta*-connected host plant is attacked by an insect, the systemic signal JA is sent to the next plant for inducing its defence response. This way, a dodder tries to save other consecutively *Cuscuta*-connected host plants over long distances by increasing their resistance to insects (Hettenhausen et al. 2017). Ian Baldwin, from the Max-Planck Institute of Chemical Ecology, Jena,

and one of the authors of this study says 'Ecological interactions in nature are extremely complex. A parasite reveals valuable nutrients from its host, but at the same time functions as an important link to warn neighbours. Whether this warning is unselfish after all, needs further study to clarify'. A recent study suggested the existence of even more intricate communications between the dodder plant and it hosts. The authors found that, in addition to the previously known movement of viruses, proteins and mRNAs between host and the parasite, bidirectional movement of microRNAs (~22 nt in length) also occurs across the haustoria. These *C. campestris* miRNAs were proposed to act as trans-species regulators of host-gene expression and may even act as virulence factors during parasitism (Shahid et al. 2018).

Other than the parasitic mode of life that many plants have adopted, the evolution and adaptation of insectivorous or carnivorous plants are even more intriguing. Darwin in 1875 wrote a treatise on insectivorous plants. Since then these groups of plants have intrigued scientists and fascinated the general public. Why did plants have to adopt this mode of nutrition? The adaptation of carnivory in plants probably resulted from occupying areas that were infertile and nutrient deficient. In order to survive on such harsh soils, developing morphological structures to catch prey along with the mechanisms for their digestion and nutrient uptake was much required. Large variations are generally seen in the structures and mechanism of trapping and digestion. Accordingly, over 583 species of carnivorous plants have been classified in 20 genera, 12 families and 5 orders (Givnish, 2015). As per the data obtained from the studies on the first fossilized trap of a carnivorous plant that allied to the modern-day *Roridula*, carnivory seems to have appeared between 8 and 72 million years ago (Sadowski et al. 2015).

What is the molecular basis of and what genetic changes would have occurred for the evolution of carnivory-related traits? In a study undertaken by different groups working in Japan, China, USA and Europe, the genome of the pitcher plant, *Cephalotus follicularis*, was sequenced. They took this plant as it has both types of leaves, those that get converted into pitchers and the non-carnivorous flat leaves. A transcriptome comparison of the two types of leaves showed that genetic changes did occur which could be related to prey attraction such as, those involved in producing nectar to lure insects, prey capture such as, genes encoding waxy substances that may make it hard for the insects to escape from the pitcher and, digestion and nutrient absorption (Fukushima et al. 2017). Further, analysis of digestive fluid proteins from *C. follicularis* and three other carnivorous plants with independent carnivorous origins revealed orthologous genes were repeatedly co-opted for digestive functions as well as for preventing microbial colonization of the digestive fluid.

1.10 Concluding Remarks

Land plants are remarkably diverse as a result of 475–700 million years of evolution and adaptation to the terrestrial environment. They owe their leap out of water to the hereditary assistance received from the green algae, their closest living relatives. Transition to the terrestrial environment has been advantageous for plants, but it

required them to evolve rigorously in order to survive the desiccated land environment. Besides adaptations needed for life on land, plants have also unveiled adaptations responsible for their diversity and predominance in the terrestrial ecosystems. Here, after reviewing various aspects of plant life related to their struggle for survival through the transition of times, we feel that plants have a far greater ability to sense their world than their appearances might suggest. Though our knowledge pertaining to plant evolution and adaptation has improved with the advent of advanced genomic approaches, much more is yet to be discovered regarding their amazing adaptation capabilities.

Acknowledgements SKS acknowledges SERB Distinguished Fellowship received from Science and Engineering Research Board (SERB). CK acknowledges DST-INSPIRE Faculty Award (IFA-14/LSPA-24) received from the Department of Science and Technology (DST), Government of India.

References

Balao F, Herrera J, Talavera S (2011) Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. New Phytol 192:256–265

Ballaré CL, Pierik R (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. Plant Cell Environ 40:2530–2543

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043

Buggs RJ (2017) The deepening of Darwin's abominable mystery. Nat Ecol Evol 1:0169

Chen F, Zhang X, Liu X, Zhang L (2017) Evolutionary analysis of MIKC^{c}-type MADS-box genes in gymnosperms and angiosperms. Front Plant Sci 8:895

Christenhusz MJ, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261:201–217

Dai F, Wang Z, Luo G, Tang C (2015) Phenotypic and transcriptomic analyses of autotetraploid and diploid mulberry (*Morus alba* L.). Int J Mol Sci 16:22938–22956

Dibb-Fuller JE, Morris DA (1992) Studies on the evolution of auxin carriers and phytotropin receptors: transmembrane auxin transport in unicellular and multicellular *Chlorophyta*. Planta 186:219–226

Doi M, Kitagawa Y, Shimazaki KI (2015) Stomatal blue light response is present in early vascular plants. Plant Physiol 169:1205–1213

Duanmu D, Bachy C, Sudek S, Wong CH, Jiménez V, Rockwell NC, Martin SS, Ngan CY, Reistetter EN, van Baren MJ, Price DC (2014) Marine algae and land plants share conserved phytochrome signaling systems. Proc Natl Acad Sci U S A 111:15827–15832

Endara MJ, Coley PD, Ghabash G, Nicholls JA, Dexter KG, Donoso DA, Stone GN, Pennington RT, Kursar TA (2017) Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. Proc Natl Acad Sci U S A 114:E7499–E7505

Ergün N, Topcuoğlu ŞF, Yildiz A (2002) Auxin (Indole-3-acetic acid), gibberellic acid (GA_3), abscisic acid (ABA) and cytokinin (Zeatin) production by some species of mosses and lichens. Turk J Bot 26:13–18

Fortunato AE, Jaubert M, Enomoto G, Bouly JP, Raniello R, Thaler M, Malviya S, Bernardes JS, Rappaport F, Gentili B, Carbone A (2016) Diatom phytochromes reveal the existence of far-red light based sensing in the ocean. Plant Cell 28:616–628

Friedman J, Rubin MJ (2015) All in good time: understanding annual and perennial strategies in plants. Am J Bot 102:497–499

Frohlich MW (2003) An evolutionary scenario for the origin of flowers. Nat Rev Genet 4:559–566
Fukushima K, Fang X, Alvarez-Ponce D, Cai H, Carretero-Paulet L, Chen C, Chang TH, Farr KM, Fujita T, Hiwatashi Y, Hoshi Y (2017) Genome of the pitcher plant *Cephalotus* reveals genetic changes associated with carnivory. Nat Ecol Evol 1:59
Givnish TJ (2015) New evidence on the origin of carnivorous plants. Proc Natl Acad Sci U S A 112:10–11
Goldfarb B, Lanz-Garcia C, Lian Z, Whetten R (2003) Aux/IAA gene family is conserved in the gymnosperm, loblolly pine (*Pinus taeda*). Tree Physiol 23:1181–1192
Halbritter AH, Fior S, Keller I, Billeter R, Edwards PJ, Holderegger R, Karrenberg S, Pluess AR, Widmer A, Alexander JM (2018) Trait differentiation and adaptation of plants along elevation gradients. J Evol Biol 31:784–800
Hettenhausen C, Li J, Zhuang H, Sun H, Xu Y, Qi J, Zhang J, Lei Y, Qin Y, Sun G, Wang L (2017) Stem parasitic plant *Cuscuta australis* (dodder) transfers herbivory-induced signals among plants. Proc Natl Acad Sci U S A 114:E6703–E6709
Jiang P, Rausher M (2018) Two genetic changes in cis-regulatory elements caused evolution of petal spot position in Clarkia. Nat Plants 4:14–22
Ju C, Van de Poel B, Cooper ED, Thierer JH, Gibbons TR, Delwiche CF, Chang C (2015) Conservation of ethylene as a plant hormone over 450 million years of evolution. Nat Plants 1:14004
Kiefer C, Severing E, Karl R, Bergonzi S, Koch M, Tresch A, Coupland G (2017) Divergence of annual and perennial species in the *Brassicaceae* and the contribution of cis-acting variation at FLC orthologues. Mol Ecol 26:3437–3457
Kokko H, Chaturvedi A, Croll D, Fischer MC, Guillaume F, Karrenberg S, Kerr B, Rolshausen G, Stapley J (2017) Can evolution supply what ecology demands? Trends Ecol Evol 32:187–197
Lett S, Wardle DA, Nilsson MC, Teuber LM, Dorrepaal E (2018) The role of bryophytes for tree seedling responses to winter climate change: implications for the stress gradient hypothesis. J Ecol 106:1142–1155
Li FW, Villarreal JC, Kelly S, Rothfels CJ, Melkonian M, Frangedakis E, Ruhsam M, Sigel EM, Der JP, Pittermann J, Burge DO (2014) Horizontal transfer of an adaptive chimeric photoreceptor from bryophytes to ferns. Proc Natl Acad Sci U S A 111:6672–6677
Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS (2015) Early genome duplications in conifers and other seed plants. Sci Adv 1:e1501084
Madlung A (2013) Polyploidy and its effect on evolutionary success: old questions revisited with new tools. Heredity 110:99
Maherali H, Walden AE, Husband BC (2009) Genome duplication and the evolution of physiological responses to water stress. New Phytol 184:721–731
Mayrose I, Zhan SH, Rothfels CJ, Magnuson-Ford K, Barker MS, Rieseberg LH, Otto SP (2011) Recently formed polyploid plants diversify at lower rates. Science 333:1257
Moghe GD, Shiu SH (2014) The causes and molecular consequences of polyploidy in flowering plants. Ann N Y Acad Sci 1320:16–34
Moyroud E, Wenzel T, Middleton R, Rudall PJ, Banks H, Reed A, Mellers G, Killoran P, Westwood MM, Steiner U, Vignolini S (2017) Disorder in convergent floral nanostructures enhances signalling to bees. Nature 550:469–474
Pin PA, Nilsson O (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ 35:1742–1755
Ponce de León I, Montesano M (2017) Adaptation mechanisms in the evolution of moss defenses to microbes. Front Plant Sci 8:366
Ramsey J (2011) Polyploidy and ecological adaptation in wild yarrow. Proc Natl Acad Sci U S A 108:7096–7101
Remington DL, Vision TJ, Guilfoyle TJ, Reed JW (2004) Contrasting modes of diversification in the Aux/IAA and ARF gene families. Plant Physiol 135:1738–1752
Richter DJ, Fozouni P, Eisen MB, King N (2018) Gene family innovation, conservation and loss on the animal stem lineage. elife 7:e34226

Ruelens P, Zhang Z, Van Mourik H, Maere S, Kaufmann K, Geuten K (2017) The origin of floral organ identity quartets. Plant Cell 29:229–242
Ruprecht C, Lohaus R, Vanneste K, Mutwil M, Nikoloski Z, Van de Peer Y, Persson S (2017) Revisiting ancestral polyploidy in plants. Sci Adv 3:e1603195
Sadowski EM, Seyfullah LJ, Sadowski F, Fleischmann A, Behling H, Schmidt AR (2015) Carnivorous leaves from Baltic amber. Proc Natl Acad Sci U S A 112:190–195
Scarpino SV, Levin DA, Meyers LA (2014) Polyploid formation shapes flowering plant diversity. Am Nat 184:456–465
Shahid S, Kim G, Johnson NR, Wafula E, Wang F, Coruh C, Bernal-Galeano V, Phifer T, Westwood JH, Axtell MJ (2018) MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs. Nature 553:82–85
Soltis DE, Visger CJ, Soltis PS (2014) The polyploidy revolution then… and now: Stebbins revisited. Am J Bot 101:1057–1078
Takezawa D, Komatsu K, Sakata Y (2011) ABA in bryophytes: how a universal growth regulator in life became a plant hormone? J Plant Res 124:437–453
te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P (2011) The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot 109:19–45
Van Overbeek J (1940) Auxin in marine algae. Plant Physiol 15:291–299
Wang C, Liu Y, Li SS, Han GZ (2015) Insights into the origin and evolution of the plant hormone signaling machinery. Plant Physiol 167:872–886
Wanke D (2011) The ABA-mediated switch between submersed and emersed life-styles in aquatic macrophytes. J Plant Res 124:467–475
Westwood JH, Yoder JI, Timko MP (2010) The evolution of parasitism in plants. Trends Plant Sci 15:227–235
Wingler A, Juvany M, Cuthbert C, Munné-Bosch S (2015) Adaptation to altitude affects the senescence response to chilling in the perennial plant *Arabis alpina*. J Exp Bot 66:355–367
Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH (2009) The frequency of polyploid speciation in vascular plants. Proc Natl Acad Sci U S A 106:13875–13879
Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlinn DJ, O'Meara BC, Moles AT, Reich PB, Royer DL (2014) Three keys to the radiation of angiosperms into freezing environments. Nature 506:89–92
Zhang GQ, Liu KW, Li Z, Lohaus R, Hsiao YY, Niu SC, Wang JY, Lin YC, Xu Q, Chen LJ, Yoshida K (2017) The *Apostasia* genome and the evolution of orchids. Nature 549:379–383

Sudhir Sopory did his Ph.D. from the University of Delhi with Prof. SC Maheshwari. He did his postdoc at the Max Planck Institute, Cologne, and worked at the University of Texas, Austin; USDA-ARS, Beltsville, Maryland; and at the University of Munich, Germany. He was a Professor at the Jawaharlal Nehru University, New Delhi, where he was also the Vice Chancellor for 5 years. He worked at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, as a Group Leader of Plant Biology and also as the Director. He worked at ICGEB as an Arturo Falaschi Emeritus Scientist and is currently working as a SERB Distinguished Fellow (Govt. of India) at ICGEB.

Charanpreet Kaur did her Ph.D. at ICGEB, New Delhi, with the Editor and is currently working as a DST-INSPIRE Faculty (Govt. of India) at the Jawaharlal Nehru University, New Delhi. She did a short postdoctoral training at the University of Melbourne, Australia, on metabolomics. She is a recipient of INSA medal for Young Scientist for her work on understanding the role of glyoxalases in stress physiology of rice. Her interests are in the area of stress biology and plant-microbe interactions.

Part I

Awareness of Plant to the External Environment

"The unexamined life is not worth living"

Socrates

"Won't you come to my garden? I would like my roses to see you"

Richard Brinsley Sheridan

The Light Awakens! Sensing Light and Darkness

2

Eros Kharshiing, Yellamaraju Sreelakshmi, and Rameshwar Sharma

Abstract

In the late nineteenth century, Charles Darwin observed that 'light exerts a powerful influence on most vegetable tissues, and there can be no doubt that it generally tends to check their growth' (*The Power of Movement in Plants*, 1880). Subsequent to this seminal work, light has been recognised as an important regulator of plant growth. Over the next 150 years, research on light regulation of plant growth and development by immensely imaginative and talented researchers in various laboratories across the globe has given us tremendous insights into how light governs plant growth both at the organismal and molecular levels. The discovery of light-responsive photoreceptor proteins that are activated by red, far-red, blue/UV-A and UV-B light has helped further our understanding of how plants respond to the light that falls on the surface of the earth. This chapter brings together the recent developments in our understanding of how plants sense light by using photoreceptors and the various molecular mechanisms involved in light perception and transmission of the light signal within the plant. Furthermore, the chapter discusses recently ascribed functions of photoreceptors such as the ability of plants to distinguish their kin from non-kin through the action of phytochrome, the role(s) of cryptochrome as a magnetoreceptor and the role of phytochrome and phototropin as temperature sensors. The chapter also rekindles the debate about whether plants can have vision despite the lack of optical or light-sensitive organs such as eyes.

E. Kharshiing
Department of Botany, St. Edmund's College, Meghalaya, India

Y. Sreelakshmi · R. Sharma (✉)
Repository of Tomato Genomics Resources, University of Hyderabad, Hyderabad, Telangana, India

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_2

Keywords
Cryptochrome · Light sensing · Photomorphogenesis · Phototropism · Phytochrome · Skotomorphogenesis · Shade avoidance · UVR8

2.1 Introduction

Darkness by definition is regarded as the lack of illumination or the absence of visible light and is therefore considered as the polar opposite of brightness. In organisms that have evolved optical or light-sensitive organs such as eyes, light enables them to respond to visual cues from the immediate surrounding area. During their course of evolution from simple unicellular algae to the highly complex phanerogams, plants have not evolved any tissue/organ specifically dedicated to perceiving light. However, despite lack of complex organs for perceiving light, plants surprisingly are extremely sensitive to the presence or absence of light. This ability to sense light is a very important factor for regulating the growth and development of plants.

The morphology of plants grown in the darkness is in stark contrast to those grown in the light, which is amply manifested during seedling development in nature. When seeds germinate in the soil, the shoots of growing seedlings are spindly and elongated with very little organ differentiation. Once these shoots emerge out of the soil and encounter light, there is a radical shift in the growth pattern resulting in organ differentiation and development of photosynthetic competence (Fig. 2.1). These multiple developmental changes stimulated by light are termed as photomorphogenesis (*photos* meaning 'light'), while developmental changes associated with darkness are termed skotomorphogenesis (*skotos* meaning 'dark'). Skotomorphogenesis is an adaptive mechanism, which increases the probability of germinating seedlings, buried too deep in the soil, to reach the light. The seedlings that fail to reach light before the exhaustion of food reserves would perish.

Once the seedlings emerge out of the soil, the plant continually monitors its environment to ensure optimal light availability for its growth throughout the life cycle. This continual monitoring allows plants to adapt to the environment by optimising its physiological responses and growth. For short-term changes in light duration/quality, plants adapt by changing their physiology, while for long-term changes, they respond by modulating growth and development. The sensing of light by the plant is not limited to detection of the presence or absence of light. Plants are endowed with the capacity to detect all facets of light such as quality, quantity, direction and duration. The capacity of plants to sense light direction is elegantly manifested by the directional growth of potted plants in room towards the window. Similarly, plants distinguish the onset of day and night as leaves as well as flowers of several plants close at dusk and reopen at dawn.

The sensing of light by plants is similar to the sensing of heat/cold by the human body. The entire plant body can detect variations in light, similar to how we sense temperature. Plants have evolved specialised light sensory molecules termed as

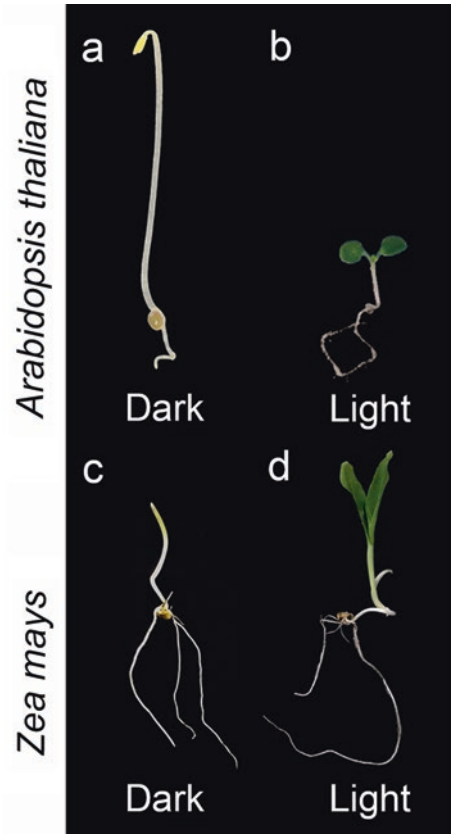

Fig. 2.1 Dark- and light-grown dicotyledonous (**a, b**) and monocotyledonous (**c, d**) seedlings. Seedlings grown in the absence of light develop an apical hook and unopened cotyledons (in dicots) or a coleoptile (in monocots) that serve to protect the cotyledons and young leaves and help push the hypocotyl or mesocotyl out from the soil into light (skotomorphogenesis). In the presence of light, the seedling develops a shortened hypocotyl and mesocotyl and well-developed open cotyledons. The presence of light also stimulates the differentiation of chloroplasts and production of chlorophyll from protochlorophyllide, which is required for establishing seedling autotrophy (photomorphogenesis)

photoreceptors, enabling them to perceive different spectral regions of light. The photoactivation of photoreceptors sets in motion signal transduction chains, leading to final adaptive and/or growth responses. For example, plants experiencing reduced light intensity under shade either grow tall to break out of shade or develop more chlorophyll to increase photosynthesis.

2.2 How Plants Sense Light

2.2.1 Plants Sense Light of Different Wavelengths Using Multiple Photoreceptors

The sunlight impinging on earth consists of ultraviolet light (200–400 nm), visible light (400–740 nm) and infrared radiation (>740 nm) which contributes to heat. Studies involving effects of narrow spectral bands of light on different photoresponses indicated that UV-B (280–320 nm), blue/UV-A (320–500 nm), red (600–700 nm) and far-red (700–750 nm) light are the most effective spectral regions

perceived by plants. The initial clue for the existence of a photoreceptor came from the discovery that a short pulse of far-red light can reverse induction of germination of lettuce seeds by a short pulse of red light. Based on this discovery, it was predicted that plants contain a red/far-red light reversible photoreceptor influencing such photoresponses. This was further confirmed with the purification of the photoreceptor from plants, which was named as phytochrome.

The molecular nature of blue/UV-A and UV-B photoreceptors, however, was identified several decades later. Nevertheless, the elegant studies carried out using the action spectra, which varied in intensity, duration and direction of blue/UV-A light, predicted that the photoreceptor mediating blue-light responses would most likely be a flavoprotein. The action spectrum of phototropic responses also strongly resembled the action spectrum of a flavoprotein with a maximum absorbance at 450 nm. The isolation of mutants defective in their responses to different intensity, duration or direction of blue-light finally enabled the molecular characterisation of these elusive blue-light photoreceptors. The mapping of mutant loci and cloning of genes encoding these receptors revealed the existence of three different blue/UV-A photoreceptors later named as cryptochrome, phototropin and zeitlupe (Christie et al. 2015). The molecular identity of a UV-B photoreceptor was uncovered recently when the characterisation of a UV-hypersensitive mutant of Arabidopsis led to the identification of UVR8 as a photoreceptor for UV-B (Rizzini et al. 2011, Christie et al. 2012).

Except for UVR8, which is a single copy gene, distinct multi-gene families encode other photoreceptor proteins. Though a different gene encodes each individual photoreceptor, the photoreceptors of the same family share a high degree of similarity. The number of the photoreceptors in each group may vary in a species-specific fashion. Arabidopsis has a repertoire of 13 photoreceptors consisting of 5 phytochromes (PhyA to PhyF), 2 cryptochromes (Cry1 and Cry2), 2 phototropins (Phot1 and Phot2) and a single UVR8 photoreceptor (Fig. 2.2). In addition, it has a family of three blue-light absorbing proteins referred to as ZTL/FKF1/LKP2 (ZEITLUPE/FLAVIN-BINDING KELCH REPEAT F-BOX 1/LOV KELCH PROTEIN 2) having a combination of photoreceptor and F-box protein activities within the same protein (Ito et al. 2012). The physiological-genetic analysis of these 13 photoreceptors indicates a complex interrelationship involving synergistic, antagonistic and redundant interactions.

2.2.2 Phytochromes Detect Relative Levels of Red/Far-Red Light

Though plants are exposed to the entire spectrum of light, they most efficiently utilise the red region of the light spectra for fuelling photosynthesis. Interestingly the chromophore of phytochrome utilises red and far-red light for detecting the light environment. In vivo phytochrome exists in the red light-absorbing Pr form (λ_{max} 660 nm) and far-red light-absorbing Pfr form (λ_{max} 730 nm) (Li et al. 2011). In dark-grown plants, phytochrome accumulates in Pr form, which on exposure to red light gets photoconverted to a physiologically active Pfr form. On the perception of

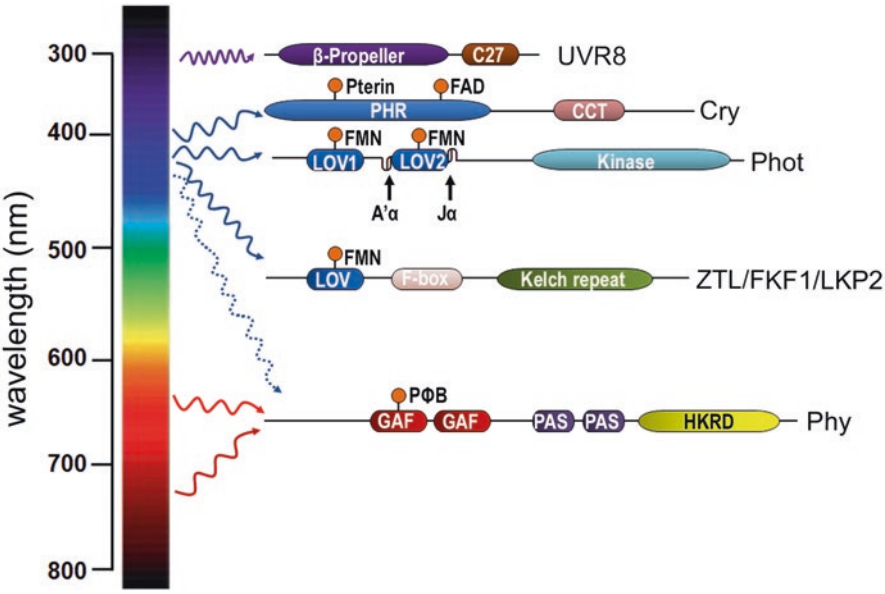

Fig. 2.2 The visible light spectrum showing excitation wavelengths of phytochrome (Phy), cryptochrome (Cry), phototropin (Phot), ZTL/FKF1/LKP2 family (ZTL, Zeitlupe; FKF1, flavin-binding Kelch repeat F-Box-1; LKP2, LOV Kelch protein 2) and UV resistance locus 8 (UVR8). The conserved protein domains are highlighted for each photoreceptor. Phytochrome characteristically has two GAF (cGMP-specific phosphodiesterases, **a**denylyl cyclases, and FhlA), two PAS (Per-Arnt-Sim) in the N-terminal region and an HKRD (histidine kinase-related domain) domain in the C-terminal region of the protein. Phytochrome has phytochromobilin (PϕB) as chromophore covalently attached to a cysteine in the first GAF domain. While ZTL/FKF1/LKP2 has a single LOV domain, phototropin has two LOV domains. ZTL/FKF1/LKP2 also has a single F-box domain (a protein-protein interaction motif) and a Kelch repeat (involved in protein-protein interactions) in the C-terminal region and has single FMN (flavin adenine mononucleotide) as the cofactor. Phototropin has two FMNs as cofactors and a kinase domain in the C-terminal region. The LOV2 domain of phototropin is flanked by characteristics A′α helix and Jα helix, which are important for its photoactivity. Cryptochrome in contrast to other two blue-absorbing photoreceptors uses a single FAD (flavin adenine mononucleotide) and pterin as the cofactors interacting with the N-terminal PHR (photolyase homology region) domain. The C-terminal CCT (cryptochrome carboxy terminus) domain is responsible for signal transmission and interacts with COP1. The light perception in UVR8 is mediated by tryptophan residues located in its β-propeller domain. The C27 refers to 27 amino acids from the C-terminus of UVR8 that mediates its interaction with COP1

far-red light, the active Pfr form reverts to the red light-absorbing Pr form. This ability of phytochrome to respond to two spectral wavelengths allows plants to more precisely detect the relative intensity of both red and far-red light by measuring the extent of the relative levels of Pr and Pfr forms of phytochrome.

The photoconversion of Pr to Pfr form, or vice versa, is effected by rotation of the linear tetrapyrrole phytochromobilin chromophore (PΦB) covalently attached to a cysteine residue on phytochrome. The exposure to red light induces

photoisomerisation of the C15-C16 double bond between the C and D rings of the chromophore. The photoisomerisation of PΦB triggers conformational changes in the protein, resulting in the active Pfr form, which characteristically absorbs far-red light (Burgie et al. 2014). Under ambient light conditions that consist of both red and far-red light, the phytochrome molecule undergoes cycling between both Pr and Pfr forms. The extent of Pfr/Pr ratio depends on the relative proportions of red/far-red light. In absence of any light, i.e. in darkness, phytochrome reverts to Pr form by a slow thermal reversion. The Pfr form of the phytochrome is also more sensitive to proteolytic degradation. Therefore, the final level of Pr/Pfr forms in vivo reflects a balance between photoconversion, its degradation and synthesis.

In Arabidopsis, five individual members make up the phytochrome family, viz. PhyA, PhyB, PhyC, PhyD and PhyF. These multiple copies of phytochrome may have evolved to ensure plant survival as the light environment is critical for completion of its life cycle. The multiple copies offset the damaging effect of any undesired mutations in these photoreceptors that could compromise the plant's ability to sense light. In addition, the individual photoreceptors and their combinations can perform different light sensory functions. Consistent with this view, except for PhyA, which exists as a homodimer, the other four phytochromes can form both heterodimers and homodimers. The formation of heterodimers further adds to the diversity of the photoresponses induced by phytochromes. PhyA also differs from the other four phytochromes in terms of its stability in light-grown plants. PhyA is also referred as light-labile phytochrome, as the Pfr form of PhyA is prone to ubiquitin-mediated proteolytic degradation. In contrast, Pfr forms of PhyB, PhyC, PhyD and PhyF are light stable. It is believed that while PhyA has a more prominent role in the early emergence of seedlings from the soil, other four species of phytochrome specifically function in green tissues of plants (Li et al. 2011; Sharma et al. 1993).

The red/far-red photoconversion ability naturally endows phytochrome with the capacity to monitor the spectral quality of light. However, its action is not limited to the detection of spectral quality. Remarkably, phytochromes can also detect a very broad range of ambient light. The seeds that require light for sprouting (photoblastic seeds) under soil cover is initiated by the perception of very low intensities of light indicating a break in the soil cover. This very low-intensity light perceived by phytochrome is termed 'very low fluence response' (VLFR) and lacks the typical red/far-red reversion of the associated photoresponses. The VLFR is a manifestation of ecological adaptation where plants ensure that germinating seeds have a chance to attain photoautotrophy after emergence from the soil. The VLFR response is believed to be mediated by PhyA. On the other extreme, the light of high intensity activates a range of phytochrome-mediated responses, which proportionally increase with the intensity of far-red light.

Interestingly the 'high irradiance response' (HIR) is also mediated by PhyA. At intermediate light intensity, phytochrome induces responses with the characteristic red/far-red reversibility also called as 'low fluence response' (LFR). The LFR is likely mediated by light-stable phytochromes consisting of PhyB and other species.

In addition to these three responses, phytochromes particularly PhyB, also detect the canopy shade and elicit responses in the plants termed as 'shade-avoidance response' (SAR). In addition to PhyB, PhyA also plays an important role in mediating SAR in higher plants particularly under deep shade (Devlin et al. 2003; Martínez-García et al. 2014).

2.2.3 Cryptochromes Sense Omnidirectional Blue-Light

In contrast to red/far-red light, plants respond to blue-light by the action of three distinct classes of photoreceptors. Unlike other plant photoreceptors, the blue-light sensing cryptochromes are present both in plants and in animals including humans. Cryptochromes may have evolved by re-duplication of a DNA photolyase gene family, which gained an ability to detect blue-light but lost its photolyase activity. Structurally, cryptochromes contain FAD (flavin adenine di-nucleotide), which is non-covalently bound to the photolyase homology region (PHR) and functions as the primary chromophore (Lin et al. 1995; Banerjee et al. 2007). Additionally, cryptochromes also contain a pterin that functions as a second chromophore and a cryptochrome carboxyl terminus (CCT), which enables the light-activated photoreceptor to interact with signalling components downstream of light perception.

Arabidopsis has two cryptochromes Cry1 and Cry2 that have partially overlapping as well as distinct functions in photomorphogenesis. Cry1 primarily functions in blue-light-triggered seedling de-etiolation, while Cry2 is responsible for flowering induction under long-day photoperiods. Activation of cryptochromes by blue-light results in the reduction of the FAD cofactor, which supposedly occurs via conserved flavin-reducing tryptophan residues (Giovani et al. 2003; Zeugner et al. 2005), resulting in subsequent phosphorylation of the CCT region. Phosphorylation of the CCT region alters the structural conformation of the photoreceptor, thereby allowing it to bind to downstream signalling components. Phosphorylation of the CCT correlates closely with the photoactivation and biological function of plant cryptochromes (Fig. 2.3).

Phosphorylated cryptochromes engage several pathways for signal transduction. The foremost of these involve the interaction of Cry1 and Cry2 with SPA (SUPPRESSOR OF PHYA) proteins, which in turn inactivate COP1/SPA (CONSTITUTIVE PHOTOMORPHOGENIC 1/SUPPRESSOR OF PHYA) E3 ubiquitin ligase. In the dark, the COP1-SPA1 complex degrades transcription factors such as HY5 (LONG HYPOCOTYL 5) responsible for initiating photomorphogenesis thereby resulting in skotomorphogenesis (Lian et al. 2011; Yang et al. 2001; Wang et al. 2001). The association of light-activated cryptochromes with the COP1/SPA ligase complex initiates a signalling pathway involving a number of components. Many of these signalling components are common with the signalling pathways of other photoreceptors, indicating redundancy and overlaps in photoreceptor signalling in plants.

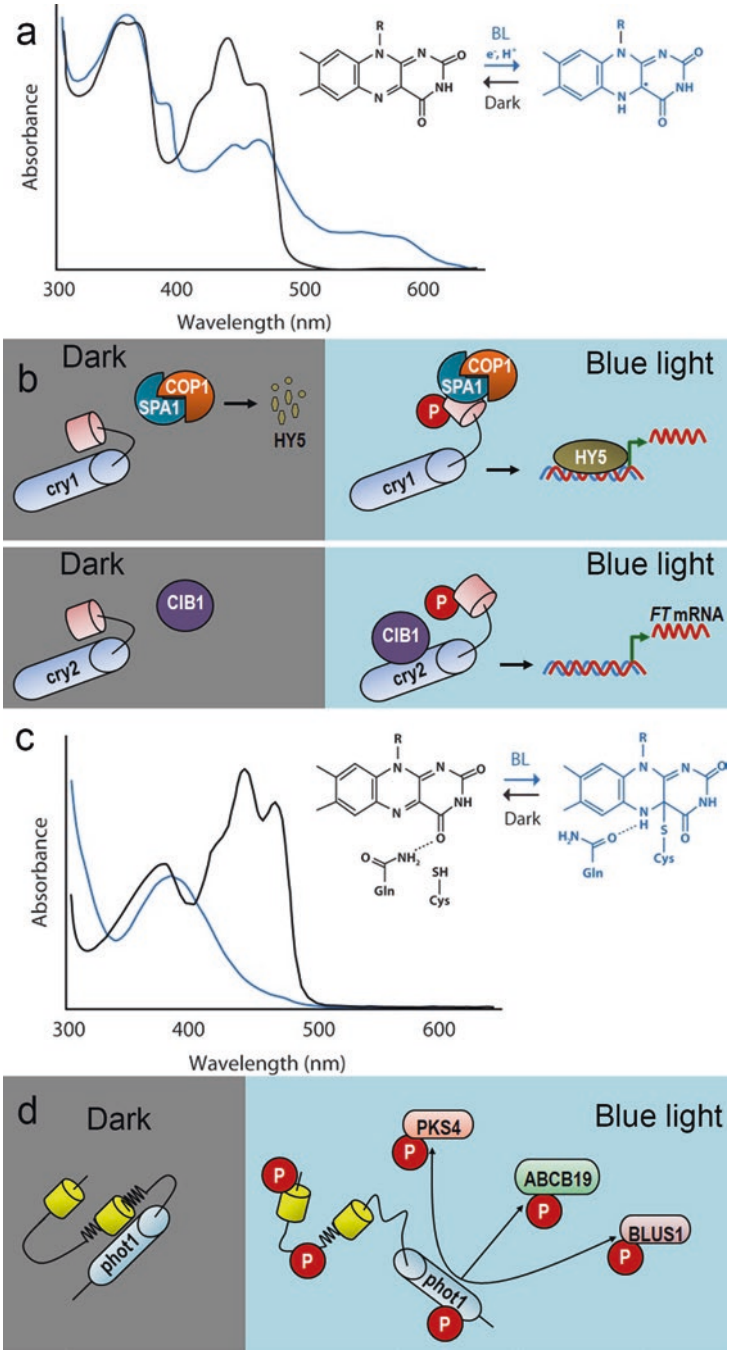

Fig. 2.3 Absorption spectra and initial signalling events of cryptochrome and phototropin (**a**) Blue-light activation of cryptochrome (Cry2) leads to reduction of the FAD cofactor (inset) resulting in changes in the absorption spectrum of the PHR domain (blue line) compared to the dark state

2.2.4 Unidirectional Blue-Light Is Sensed by Phototropins

Plants can distinguish not only light quantity or quality but can also decipher the direction of light. Under a dense canopy or in a crowded stand where the available light may not be optimal for the growth, plants can orient their growth towards more light. The capacity to detect the light direction and orientation of growth towards it provides plants with a competitive advantage to maximise the availability of light for photosynthesis. Plants perceive unidirectional light by sensing the blue/UV-A light component of the visible spectrum by phototropins (Phot) which have two members, Phot1 and Phot2. These two photoreceptors similar to phytochromes can sense different intensities of blue-light. Phot1 detects light of upto 0.1 μmole/m^2, and Phot2 mainly detects light intensities beyond 1 μmole/m^2. However, at a light intensity higher than 1 μmole/m^2, both Phot1 and Phot2 can redundantly detect light intensity (Briggs and Christie 2002; Kagawa et al. 2001; Sakai et al. 2001).

In addition to sensing directional light and orienting growth of plants, phototropins also regulate leaf expansion and leaf positioning. Additionally, phototropins also partly contribute to the light-induced opening of the stomata. At the cellular level, phototropins regulate chloroplast positioning in the mesophyll cells of leaves. Under low-light intensities, the chloroplasts redistribute themselves to the top of the cell and spread horizontally to optimise light capture. Conversely, under high-light intensities, the chloroplasts re-align to the vertical walls of the illuminated cells to avoid photo-damage. Similar to the chloroplast position, the nuclear localisation too in the cell is regulated by phototropins (Iwabuchi et al. 2010).

The molecular characterisation of phototropins indicates that these photoreceptors comprise of a serine/threonine kinase domain at their C-terminus and two specialised light, oxygen or voltage sensing (LOV) domains, designated LOV1 and LOV2, at their N-terminus. Each of these LOV domains non-covalently binds oxidised FMN (flavin adenine mononucleotide) as a blue-light sensitive chromophore (Christie et al. 1999). Although both LOV1 and LOV2 are photochemically active, kinase activity and function are predominantly controlled by LOV2 (Cho et al. 2007; Oide et al. 2018). Upon activation by blue-light, phototropins undergo autophosphorylation at multiple sites within the kinase domain, the linker region

Fig. 2.3 (continued) (black line) (Banerjee et al. 2007). (**b**) Blue-light-triggered conformational change in Cry1 enables it to bind and sequester the COP1-SPA1 proteolytic complex leading to HY5 accumulation, which in turn promotes gene expression. In the dark, the COP1-SPA1 complex degrades HY5, resulting in the suppression of HY5-mediated gene expression. Cryptochromes can also directly regulate gene expression by binding CIB proteins to the PHR domain of light-activated cryptochrome (Cry2), which leads to increased expression of genes such as *FT* for promoting flowering. (**c**) Blue-light activation of phototropin (Phot1) forms a covalent adduct between the FMN cofactor and a conserved cysteine residue within LOV2 (inset), resulting in a shift in the absorption spectrum of the LOV2 domain (blue line) as compared to the dark state (black line) (Jones et al. 2007). (**d**) Blue-light induces autophosphorylation of phototropin (Phot1) at multiple residues resulting in conformation change, which moves the LOV2 domain away from the kinase domain. This relieves the dark-state inhibition of the kinase domain, which allows the activated protein to phosphorylate substrate targets including PKS4, ABCB19 and BLUS1

between the LOV1 and LOV2 domains and sequences upstream of LOV1 (Christie et al. 2015). These phosphorylation events are initiated when blue-light activation of the photosensitive LOV2 domain relieves its action as a repressor of the kinase domain in the dark. The opening of the kinase domain promotes binding of ATP followed by autophosphorylation of the photoreceptor and later the phosphorylation of target substrates (Pfeifer et al. 2010, Fig. 2.3).

It remains to be biochemically established whether phototropins like other photoreceptors act as a dimeric molecule. Among the two LOV domains, the LOV1 domain acts as a dimerisation site in phototropin (Nakasako et al. 2008, 2013). In tomato, a dominant negative mutation in Phot1 strongly suppresses phototropic responses in F1 plants, suggesting that the mutated Phot1 protein likely hinders Phot1-mediated signal transduction by interacting with wild-type Phot1. Since this dominant mutation in Phot1 also suppressed Phot2 responses, it suggests the possibility of a close interaction between Phot1 and Phot2 proteins (Sharma et al. 2014).

Studies on grass coleoptiles have revealed that exposure to unidirectional blue-light initiates differential phosphorylation of phototropins with more phosphorylation in the illuminated side and less phosphorylation in the shaded side (Salomon et al. 1997). It is believed that the above phosphorylation gradient of phototropin within an organ/tissue is perceived as an early signal leading to the phototropic curvature of coleoptiles (Salomon et al. 1997). This view is supported by evidence chiefly derived from studies on grass coleoptiles, where phototropins and early signalling components are highly expressed in the upper region of coleoptiles, which is the most sensitive region to the light (Matsuda et al. 2011). After the initial phosphorylation event, the phototropin signal is then transmitted via other signalling partners. The signal finally culminates in the phototropic bending of organs, which is most likely mediated by the differential distribution of the plant hormone auxin. So far, only a few components have been identified in the downstream signal pathway of the phototropins and are discussed in Sect. 2.3.3.

2.2.5 UVR8 Protects Plants Against UV-B Radiation Damage

While light is essential for the survival of green plants, uninhibited exposure to strong sunlight throughout the day has hazardous consequences even for plants. Cellular DNA and proteins can be damaged by ultraviolet (UV) radiation from the sun, resulting in poor growth and even death. As a consequence, plants have evolved a highly specific adaptive response to UV radiation, especially to UV-B, which includes a suite of protective responses orchestrated by the UV-B photoreceptor, UVR8. Unlike all other photoreceptors known till date, which have prosthetic chromophores as light sensors, UVR8 utilises pyramids of several residues of the amino acid tryptophan present within the photoreceptor itself, for absorbing light (Christie et al. 2012).

Functional UVR8 exists as a dimer of two identical protein subunits, which undergo monomerisation after activation by UV-B and initiates transmission of light responses via interaction with COP1. In Arabidopsis, when the UV-B light is perceived by UVR8, it activates a range of protective responses involving several genes for DNA repair enzymes and other protective proteins, resulting in elevated levels of flavonoid sunscreen pigments (Demkura and Ballaré 2012). In a sense, plant responses to UV-B light via the action of UVR8 can, therefore, be considered equivalent to plants putting on sunscreen. The action of UVR8 also involves interaction with other photoreceptors and shared signalling intermediates as discussed below.

2.2.6 Photoreceptors with Single LOV Domain Sense Photoperiod

In addition to the phototropins, Arabidopsis has two additional types of proteins that possess LOV domains. Among these, three proteins of a family consisting of a single LOV domain with flavin as the light-sensitive chromophore have a light sensory function in Arabidopsis. These proteins, ZTL/FKF1/LKP2, play roles in the circadian clock and photoperiodic flowering. Another protein class named PAS/LOV protein (PLP) contains two LOV domains, although the physiological function of PLP is largely unknown (Kasahara et al. 2010; Ito et al. 2012; Song et al. 2012).

2.3 Mechanisms of Light Perception

2.3.1 Photoresponses Are Effected by Independent and Interdependent Signalling from Photoreceptors

It is believed that photoreceptors function in a cell-autonomous fashion, but the resulting signalling chain is not necessarily confined only to the concerned cells. The light triggered signalling can also involve the long-distance transmission of information through movement of the regulatory molecules. In addition to their respective actions, a given photoreceptor may contribute to the action of another photoreceptor by sensitivity amplification of a particular response. Therefore, the action of photoreceptors at the plant/organ level has to be considered in a broader context involving the independent and interdependent actions of the photoreceptors. However, to have a comprehensive view, it is first essential to understand the photoresponses that can be specifically ascribed to individual photoreceptors.

The spectral variance of ambient light sensed by different photoreceptors invokes a signalling cascade, which involves unique and/or common signalling partners. Hence, there is often an overlap of the various photoreceptor signalling pathways. This overlap makes it difficult to distinguish signalling cascades emanating from a specific photoreceptor family or the members thereof. However, genetic studies of

light signalling involving characterisation of photomorphogenic mutants and the encoding loci have significantly contributed to our understanding of how individual photoreceptors transmit light signals. A combination of physiological and genetic studies where photoresponses of mutant lines were compared under specific wavelengths of light with variation in intensity and duration has further advanced the identification of various components of light signalling and their interaction with different photoreceptors. Such studies have revealed that plant responses to light signals in natural conditions cannot be attributed to any single photoreceptor but rather reflects a concerted action of multiple photoreceptors.

2.3.2 Phytochrome and Cryptochrome Light Signalling Share Common Components

In accordance with the wide-ranging role of photoreceptors in regulating morphogenic responses, the initiation of morphogenic responses is closely linked with the modulation of nuclear gene expression. Consistent with this, all photoreceptors except phototropins show some degree of nuclear localisation. In dark-adapted plants, phytochromes in Pr form are predominantly localised in the cytosol; however, photoconversion to Pfr form stimulates the translocation of phytochromes to the nucleus within few minutes. The nuclear translocation of PhyA is observed only under the blue and far-red light, as under red light its level rapidly declines due to proteolysis. The PhyA protein per se does not have a nuclear localisation signal (NLS); therefore its translocation to the nucleus is strictly dependent on two chaperones, FHY1 (FAR RED ELONGATED HYPOCOTYL 1) and FHL (FHY1 LIKE), which interact with the active Pfr form of PhyA and transport it into the nucleus. In contrast, PhyB nuclear localisation is observed only after red-light exposure, and unlike PhyA, it is translocated via its own NLS or binding to transcription factors involved in phytochrome signalling. This nuclear translocation is a pivotal step in phytochrome signalling involving all phytochromes (Nagatani 2004; Wang and Wang 2015).

Once imported to the nucleus, both PhyA and PhyB interact with multiple partners. Their interaction is manifested as the appearance of discrete subnuclear structures called nuclear bodies (NB) containing phytochromes and the interacting partners. One of the key phytochrome interacting partners is a class of basic helix-loop-helix transcription factor, PIF (PHYTOCHROME INTERACTING FACTOR). PIFs function as negative regulators of photoresponses as they maintain the skotomorphogenic state of dark-grown seedlings (Leivar and Monte 2014). Consistent with this, Arabidopsis dark-grown *pif* mutants display light-grown phenotypes (Leivar et al. 2008). The binding of active Pfr to PIFs promotes phosphorylation of these transcription factors (Bauer et al. 2004, Shen et al. 2007), leading to subsequent polyubiquitination and degradation of these proteins (Al-Sady et al. 2006)

and transcription of genes regulating photomorphogenic development (Fig. 2.4). In addition to inducing degradation of PIFs, phytochrome also sequesters them preventing them to bind to DNA (Park et al. 2012). Current evidence indicates that PIFs get degraded both under darkness and light conditions; therefore, the transition from skotomorphogenesis to photomorphogenesis is modulated by an optimum level of PIFs (Pham et al. 2018).

Apart from sequestering and degradation of transcription inhibitors such as PIFs, it is assumed that photoactivated phytochromes rapidly inactivate another class of proteins, COP/DET/FUS (CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA) which also act as repressors of photomorphogenesis. The loss of these proteins leads to the accumulation of photomorphogenesis-promoting transcription factors, resulting in activation of genes regulating photomorphogenic development. At the same time, phytochrome is not inactive in the cytosol; the conversion to Pfr form also regulates the translation of mRNA in the cytosol (Paik et al. 2012).

Similar to Phys, Crys also appears to transduce light signals to downstream signalling components primarily in the nucleus, as these too undergo rapid nuclear relocalisation upon activation by light. As mentioned earlier, light activation of cryptochromes results in their interaction with COP1-SPA1 proteins, resulting in transcriptional control of gene expression in light. Blue-light triggers phosphorylation of both Cry1 and Cry2 thereby initiating their homodimerisation, which is needed for subsequent signal transmission. However, this dimerisation is suppressed by BLUE-LIGHT INHIBITOR OF CRYPTOCHROME 1 and 2 (BIC1 and BIC2), which act as negative regulators of cryptochromes. It is interesting to note here that transcription of BICs is induced by light, which is in turn mediated by phytochromes, indicating co-action of Phys and Crys in regulating plant responses to light (Wang et al. 2016, 2017).

The nuclear-localised Cry2 on photoactivation form nuclear bodies or photobodies similar to phytochrome, which also partially overlaps with PhyB photobodies, indicating a likely cross-talk between Cry2 and PhyB (Yu et al. 2009; Chen and Chory 2011). The sharing of signalling partners between phytochromes and cryptochromes is also indicated by the fact that both bind to the SPA complex, and both cryptochromes bind to a different subset of PIFs. On photoactivation, both cryptochromes and phytochromes inactivate the COP1/SPA complex. The inactivation of the COP1/SPA complex, in turn, leads to the accumulation of HY5 protein in the nucleus. HY5, a bZIP transcription factor, acts as the positive regulator of photomorphogenesis, thereby regulating the transcription of a number of light-responsive genes (Fig. 2.3).

As mentioned above, the signalling pathway activated by cryptochromes and phytochromes involves several common components shared with other signalling pathways. Some of these common components include positive regulators of photomorphogenesis, such as HFR1 (LONG HYPOCOTYL IN FAR-RED 1), HYH (HY5 HOMOLOG), LAF1 (LONG AFTER FAR-RED LIGHT 1) and CO

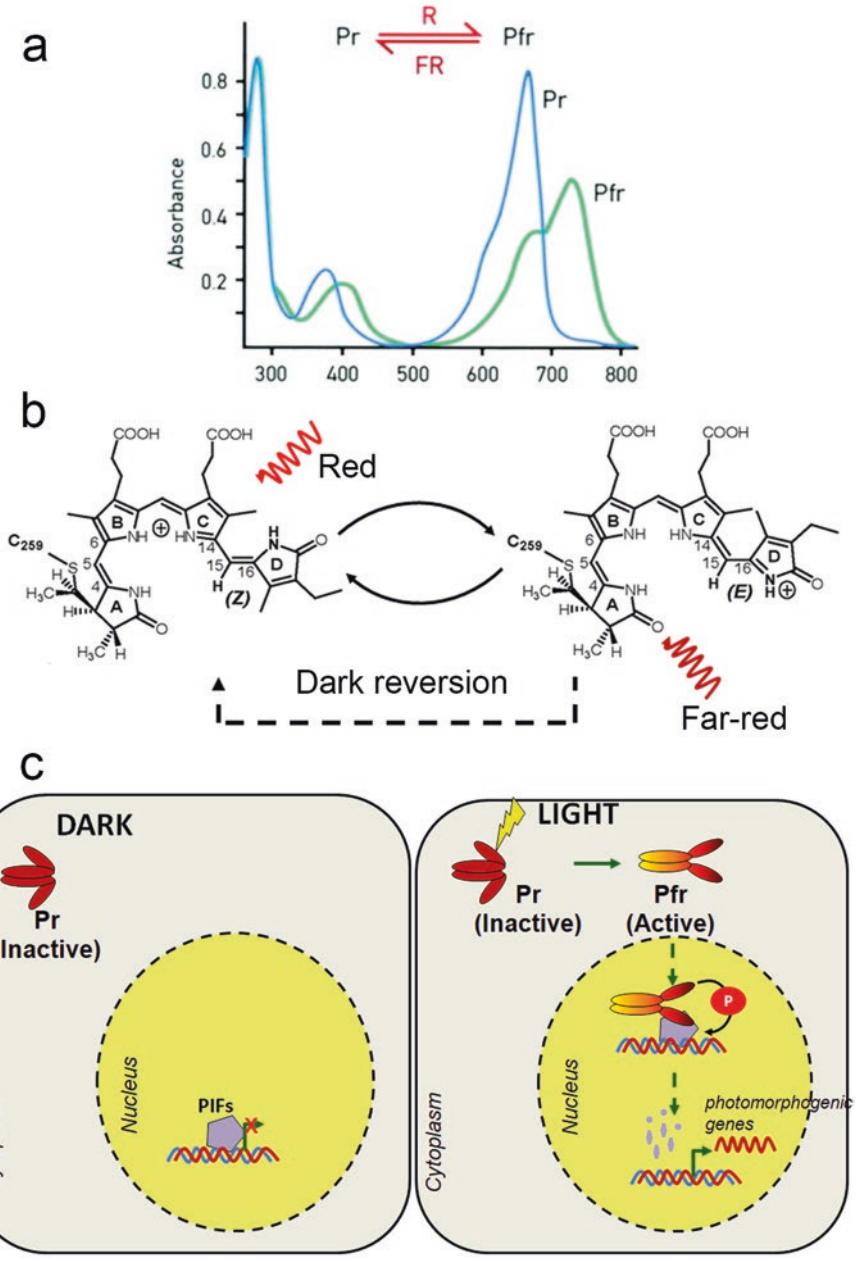

Fig. 2.4 Absorption spectra and mechanism of action of phytochrome (**a**) Activation of dark-state phytochrome (Pr, λ_{max} 660 nm) by red light (R) shifts the peak absorption towards the far-red (Pfr, λ_{max} 730 nm) region. Pfr form is reverted to Pr form on far-red light exposure. (**b**) The red-light-mediated photoconversion of Pr to Pfr form, or its reversion to Pfr is effected by rotation of the linear tetrapyrrole phytochromobilin chromophore (PΦB) covalently attached to a cysteine residue on phytochrome. The exposure to red light induces photoisomerisation of the C15-C16 double

(CONSTANS) – a key regulator of flowering. It, however, remains to be ascertained how these photoreceptors, which sense the different qualities of light, integrate signal information and transmission using common signalling partners to elicit plant response to light.

2.3.3 Phototropins Signalling Is Also Modulated by Other Photoreceptors

Similar to other photoreceptors discussed above, phototropins also work in tandem with other photoreceptors. However, unlike other photoreceptors that can shuttle between the nucleus and cytosol, phototropins are bound to the plasma membrane. The exposure to blue-light triggers the partial internalisation of Phot1 from the plasma membrane to the cytoplasm (Preuten et al. 2015). Contrastingly, on blue-light exposure, Phot2 is targeted to the Golgi apparatus via its C-terminal domain. Though evidence for direct physical interaction of phototropins with any other photoreceptor is lacking, physiological evidence indicates an intersection of signalling pathways, as phytochromes can also modulate phototropin-induced phototropic responses (Srinivas et al. 2004, Sullivan et al. 2016a).

During phototropism, in etiolated seedlings, unilateral light perceived by phototropins localised at the shoot tip (Preuten et al. 2013; Sullivan et al. 2016b) induces autophosphorylation and initiates signalling leading to the differential growth of the shoot towards the light. The autophosphorylated phototropins in turn directly phosphorylate ABCB19 (ATP-BINDING CASSETTE B) and PKS4 (PHYTOCHROME KINASE SUBSTRATE 4) proteins (Christie et al. 2011; Demarsy et al. 2012) followed by activation of less defined signalling mechanisms involving H^+-ATPase, NPH3 (NON PHOTOTROPIC HYPOCOTYL 3) and PIN (PIN FORMED) proteins (Pedmale and Liscum 2007; Hohm et al. 2014; Rakusová et al. 2015). These changes finally culminate in an asymmetric distribution of the phytohormone auxin towards the shaded side and subsequent curvature of the shoot towards light (Figs. 2.3 and 2.5).

In dark-grown seedlings, phytochromes, particularly PhyA, enhance the phototropic response (Srinivas et al. 2004). In contrast, in photoautotrophic adult plants, phototropic responses under non-homogenous light environments involve co-action of Phots and PhyB (Goyal et al. 2016). It seems PhyB plays a dual role in the regulation of phototropism in green adult plants. In conditions where light is not limiting, PhyB strongly inhibits phototropism; on the contrary, it promotes phototropism

Fig. 2.4 (continued) bond between the C and D rings of the chromophore. The associated conformational changes convert inactive Pr form to the active Pfr form, which absorbs far-red light and reverts to the Pr form. The Pfr form can also revert to Pr form in darkness by a slow thermal reversion. (**c**) Red-light-activated Pfr relocates to the nucleus from the cytosol, where it interacts with various partners including PIFs, which function as negative regulators of photoresponses. The binding of active Pfr promotes PIFs phosphorylation leading to proteolytic degradation of PIFs and triggering of expression of genes regulating photomorphogenesis

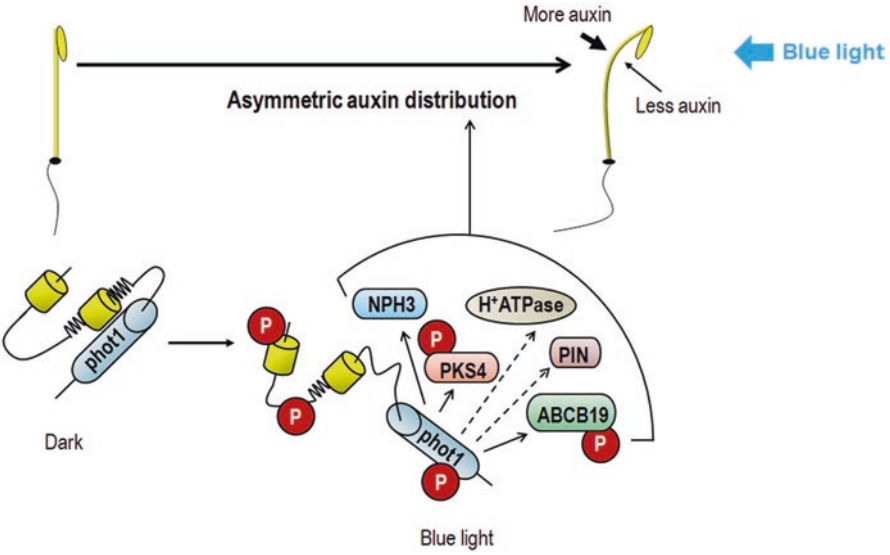

Fig. 2.5 Likely signalling events leading to phototropic curvature in etiolated dicot seedlings. Unidirectional blue-light induces autophosphorylation of phototropins, which in turn directly phosphorylate ABCB19 and PKS4 proteins. Phototropins also activate H^+-ATPase, NPH3 and PIN proteins by yet to be defined signalling mechanisms. These early events activate signalling cascades culminating in an asymmetric distribution of the phytohormone auxin towards the shaded side and subsequent curvature of the shoot towards light

under light-limiting conditions. This dual action of PhyB in modulating phototropic responses is attributed to its ability to relay information of the red/far-red ratio in the immediate surroundings of the plant to the phototropin signalling pathway. To do this, PhyB engages PIFs (such as PIF4, PIF5 and PIF5) whose red/far-red ratio-dependent regulation (discussed in Sect. 2.3.2) in turn modulates phototropism under shade by regulating transcription of the *YUCCA* auxin-biosynthetic genes (Fig. 2.6). The observations that PIFs and YUCCAs promote phototropism only in photoautotrophic, but not in dark-grown, seedlings indicate the operation of different phototropic signalling pathways in green and etiolated seedlings. In natural environments where ambient light tends to be non-uniformly distributed, this integration of phytochrome and phototropin signalling pathways enables plants to re-orient their growth to optimise capture of photosynthetic light.

The exchange of gases through the stomata is critical for plant survival as stomata have to strike a balance between transpirational loss of water and CO_2 fixation by photosynthesis. Stomata are endowed with the ability to optimise stomatal pore size as per the physiological state and the ambient environment of the plant. The regulation of stomatal pore size involves a complex interaction between several endogenous and external signals including light. Among the different wavelengths of light, stomata are most responsive to blue-light. In blue-light-regulated stomatal movement, phosphorylation of phototropins results in subsequent phosphorylation of the guard-cell specific kinase BLUS1 (BLUE LIGHT SIGNALLING 1), which

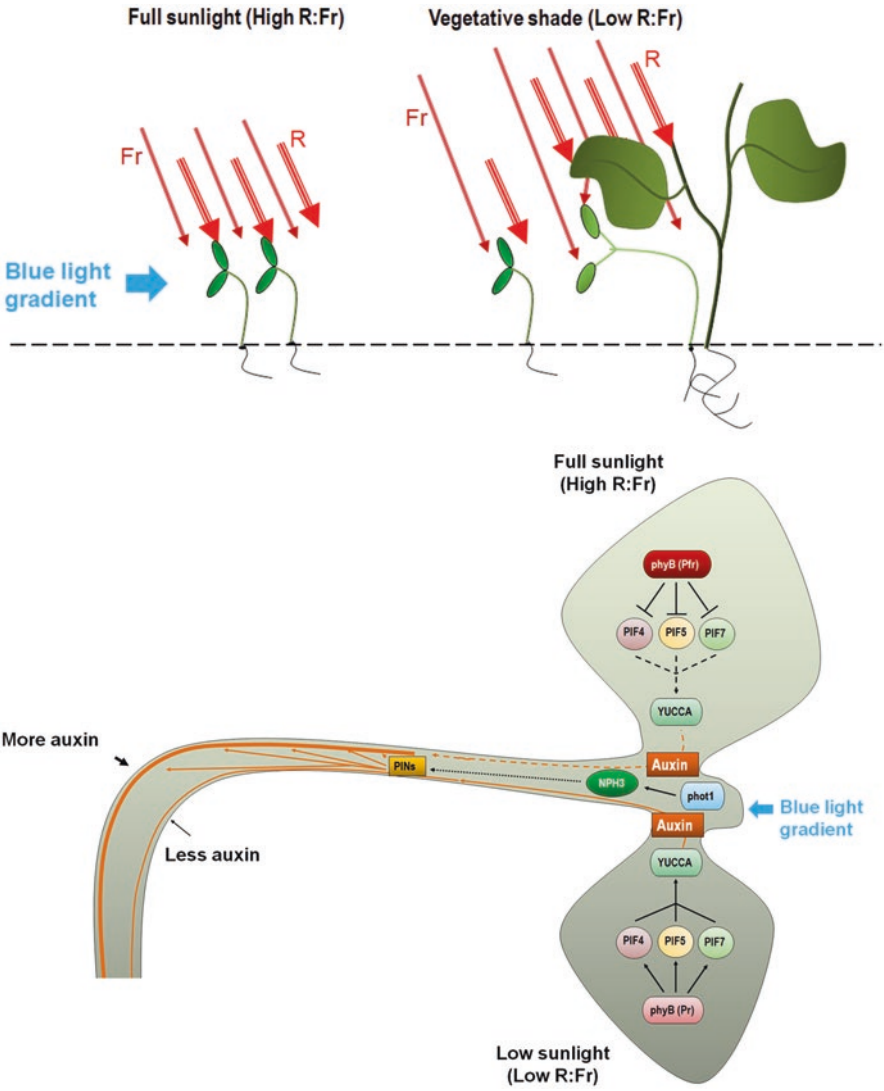

Fig. 2.6 Modulation of phototropism in de-etiolated seedlings by PhyB. Unlike etiolated seedlings where localised auxin gradients induce phototropic curvature, the phototropic curvature of de-etiolated seedlings is mediated by auxin transported from the cotyledons to the hypocotyl. In well-lighted environments (having high R:Fr), PhyB mainly present in the active Pfr form inhibits PIF activity. The inhibition of PIFs leads to reduced auxin biosynthesis in the cotyledons due to decreased expression of *YUCCA* auxin-biosynthetic genes. In turn, a reduced amount of auxin is transported from cotyledon to the hypocotyl. The deficiency of auxin in hypocotyls results in reduced phototropic curvature towards the directional light. In contrast, under low-light environments (such as vegetation-induced shade), PhyB is mainly present in the inactive Pr form (due to low R:Fr). The inactive Pr form relieves inhibition of PIFs leading to high levels of auxin caused by high expression of *YUCCA* genes. The higher amount of auxin transported from the cotyledons to the hypocotyl results in increased phototropic curvature in response to directional blue-light

leads to activation of plasma membrane H^+-ATPase via type 1 protein phosphatase and its regulatory subunit, PRSL1 (Takemiya et al. 2013a, b). The plasma membrane H^+-ATPase together with inward-rectifying K^+ channels facilitate the influx of K^+ ions, which is a key process initiating stomatal opening (Inoue and Kinoshita 2008).

2.3.4 ZTL/FKF1/LKP2 Signalling

The exposure of blue-light induces the covalent binding of FMN to the LOV domain of the FKF1 protein, which plays an important role in the photoperiodic regulation of flowering. Unlike the fast dark-reversion observed in blue-light-activated phototropins, the photoactivated FKF1 signalling state is stable for several days. In flowering plants, expression of the transcription factor CO is critical for photoperiodic regulation of flowering. While the circadian clock regulates *CO* mRNA transcription, the accumulation and the stabilisation of the CO protein involve additional components. The stability of the CO protein is also diurnally regulated, wherein it is stabilised in light and degraded in darkness. In Arabidopsis, a long-day plant, light-activated FKF1 interacts with the plant-specific nuclear protein GI (GIGANTEA) to degrade inhibitors of *CO* transcription such as CDFs (CYCLING DOF FACTOR). The degradation of the CDFs enables the accumulation of CO in light. Activated FKF1 also interacts with CO and stabilises it, resulting in expression of *flowering time* (*FT*) mRNA and induction of flowering. It is important to note here that the regulation of *FT* expression is also mediated by signalling pathways involving phytochromes and Cry2, which regulate the stability of the COP1-SPA1 complex (Sawa et al. 2007; Song et al. 2012; Lee et al. 2017; Andrés and Coupland 2012), thereby inhibiting COP1-SPA1-dependent CO degradation in light (Fig. 2.7).

2.3.5 UVR8 Signalling Is Mechanistically Opposite of Phytochromes and Cryptochromes

The signalling of UVR8 differs from other photoreceptors such as cryptochrome and phytochrome, which function as a dimer. In its inactivated state, UVR8 is present as a homodimer, which then monomerises upon activation by UV-B absorption. Following its monomerisation, activated UVR8 initiates transmission of light signals via interaction with COP1 (Favory et al. 2009; Huang et al. 2014), which then further interacts with SPA1-SPA4 proteins, which are four partially redundant SPA protein family members critical for most of COP1 activities. In this sense, the interaction of UVR8 with COP1 differs from that of Phys and Crys, in which the light-activated photoreceptor proteins constitutively interact with SPA proteins. Therefore,

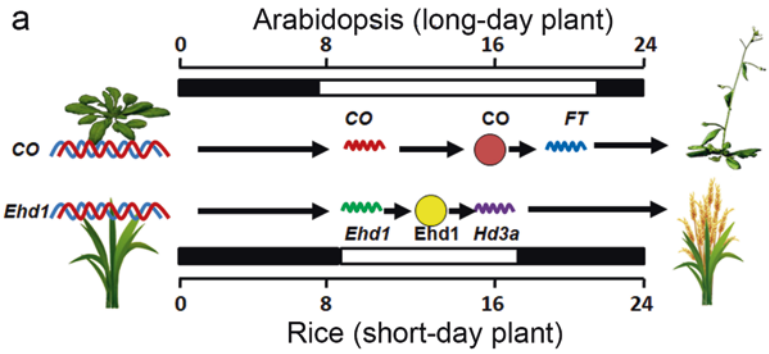

Fig. 2.7 Photoperiodic regulation of flowering. (a) In long-day plants (Arabidopsis), flowering is initiated by the expression of *FT* (*FLOWERING TIME*) that requires CO (CONSTANS) protein for its transcription. Since the CO protein specifically accumulates during the latter half of the long-day period, the flowering is initiated only under long days and not under short days. In short-day plants (rice), accumulation of Ehd1 (EARLY HEADING DATE 1), an ortholog of Arabidopsis CO protein, occurs under short days that promotes expression of *Hd3a* (*HEADING DATE 3a*). The activation of *Hd3a*, which is the rice ortholog of *FT*, results in the initiation of flowering responses under short days. (b) Induction of flowering in Arabidopsis under long days depends on the enhanced transcription of *FT* gene mediated by CO (CONSTANS). *CO* mRNA transcription is diurnally regulated with higher transcription during the dark period. However, in darkness CO protein is degraded by COP1-SPA1 ubiquitin ligase activity. The accumulation of *CO* mRNA is suppressed during the early light period by CDF proteins. Additionally, any CO protein present in the early part of the day is degraded by a COP1-independent pathway activated by PhyB by a yet undefined mechanism. During the latter half of the long-day, light-activated FKF1 interacts with the plant-specific nuclear protein GI (GIGANTEA) to degrade inhibitors of *CO* transcription such as CDFs, leading to the accumulation of *CO* mRNA and protein. Additionally, the activated FKF1 also interacts with CO protein and stabilises it. CO protein expressed during the latter half of the long day is further stabilised by inhibition of COP1-SPA1 activity. This inhibition is partly due to interactions of light-activated Cry2 with SPA1 and COP1. Additionally, PhyA also inhibits the COP1-SPA1 complex by an unknown mechanism. The stabilisation of CO protein during the latter half of the long light period results in the expression of *FT* mRNA and accumulation of FT protein. On transition to darkness, the above-mentioned inhibition of COP1-SPA1 is relieved due to the absence of active photoreceptors. In turn, the COP1-SPA1 complex triggers the degradation of CO protein during the dark period. (c) Under non-inductive photoperiods such as short days, the rhythms of optimal accumulation of GI and FKF1 are not synchronised. Consequently, GI and FKF1 fail to relieve repression of *CO* transcription by CDFs. Additionally, endogenous circadian rhythmicity promotes accumulation of CDFs in morning hours of short days, which further repress *CO* transcription. Though the repression of *CO* transcription is relieved after dusk, the *FT* transcription is blocked due to the unavailability of stable CO protein during the short day. Therefore, flowering is not induced under such non-inductive photoperiods

even though the signalling in Phys, Crys and UVR8 occurs via the COP1-SPA signalling pathway, their signal transduction chains are mechanistically different since activated UVR8 does not interact directly with SPA proteins. Moreover, the

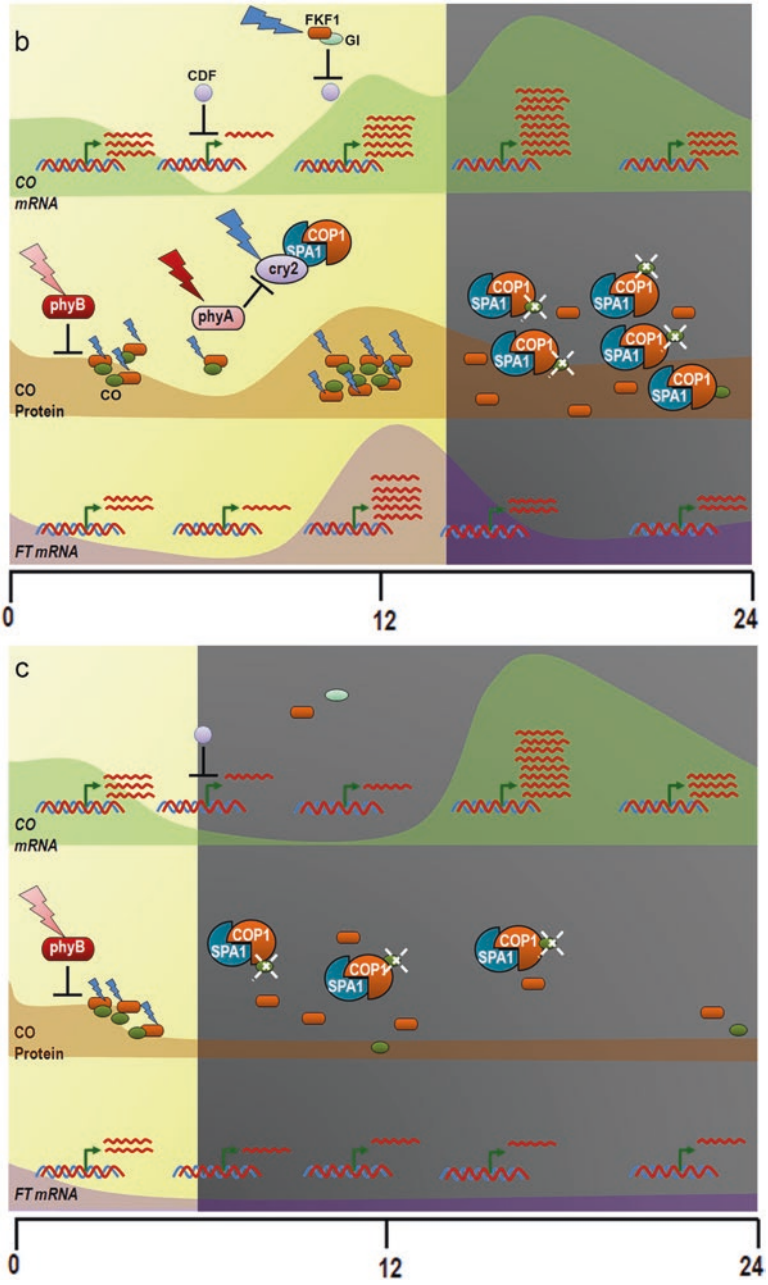

Fig. 2.7 (continued)

UVR8-COP1 interaction does not result in degradation of UVR8, which is also in contrast to the E3 ubiquitin ligase activity of COP1. Once the light signal from activated UVR8 is transmitted to the COP1-SPA complex, the photoreceptor reverts to its inactive homodimeric ground state by re-dimerisation, which restores its UV-B responsiveness. This re-dimerisation of UVR8 is facilitated by the activity of RUP1 and RUP2 (REPRESSOR OF UV-B PHOTOMORPHOGENESIS), both of which are essential for maintaining UVR8 homodimer/monomer photo-equilibrium under natural conditions (Fig. 2.8).

Activation of UVR8 by UV-B partially shifts the activated monomer from the cytosol to the nucleus. In the nucleus, monomeric UVR8 initiates a series of molecular interactions culminating in altered gene expression in response to UV-B. While UV-B signalling shares molecular components with red- and blue-light signalling, there appear to be distinct differences in the roles they accomplish. For instance, unlike in visible light signalling where COP1 plays a repressive role by degrading its target substrates, COP1 activity is essential for promoting UV-B responses (Yin et al. 2016;). UV-B exposure induces nuclear accumulation of activated UVR8 and COP1 along with that of the HY5 transcription factor (Huang et al. 2012; Yin et al. 2016), which otherwise is a target for degradation through COP1 ubiquitination. Contrarily, COP1 seems to be required for inducing expression of HY5 in UVR8-mediated UV-B signalling. In young seedlings, HY5 plays a prominent role in inducing light responses but has much lesser significance in adult plants. However, in UVR8 signalling HY5 retains its functional significance even in advanced stages of plant development.

Further, recent studies revealed that HY5 along with the transcription factor FHY3 contribute to *COP1* transcript abundance under UV-B in a UVR8-dependent fashion (Huang et al. 2012). On the other hand, expression of *FHY3*, whose translated products participate in the nuclear translocation of activated PhyA, is repressed in far-red light but is contrastingly induced by UV-B. In essence, all these observations highlight that UV-B and visible light signalling pathways are mechanistically different.

2.4 Lighting Up Plant Development

2.4.1 Skotomorphogenesis Involves Suppression of Photomorphogenesis

Skotomorphogenic development of plants is an adaptation that is executed by repression of photomorphogenesis. Skotomorphogenesis is an active process, modulated by hormones like brassinosteroids, as BR-deficient mutants show a COP-mutant-like phenotype in the dark (Li et al. 1996). The repression of photomorphogenesis involves suppression of light-responsive genes by nuclear accumulation of the repressor proteins such as COP1 and SPA1. Consequently, mutations in COP1 and SPA1 genes that preclude their action trigger a photomorphogenic phenotype even in darkness. As a result, COP1 mutant seedlings show

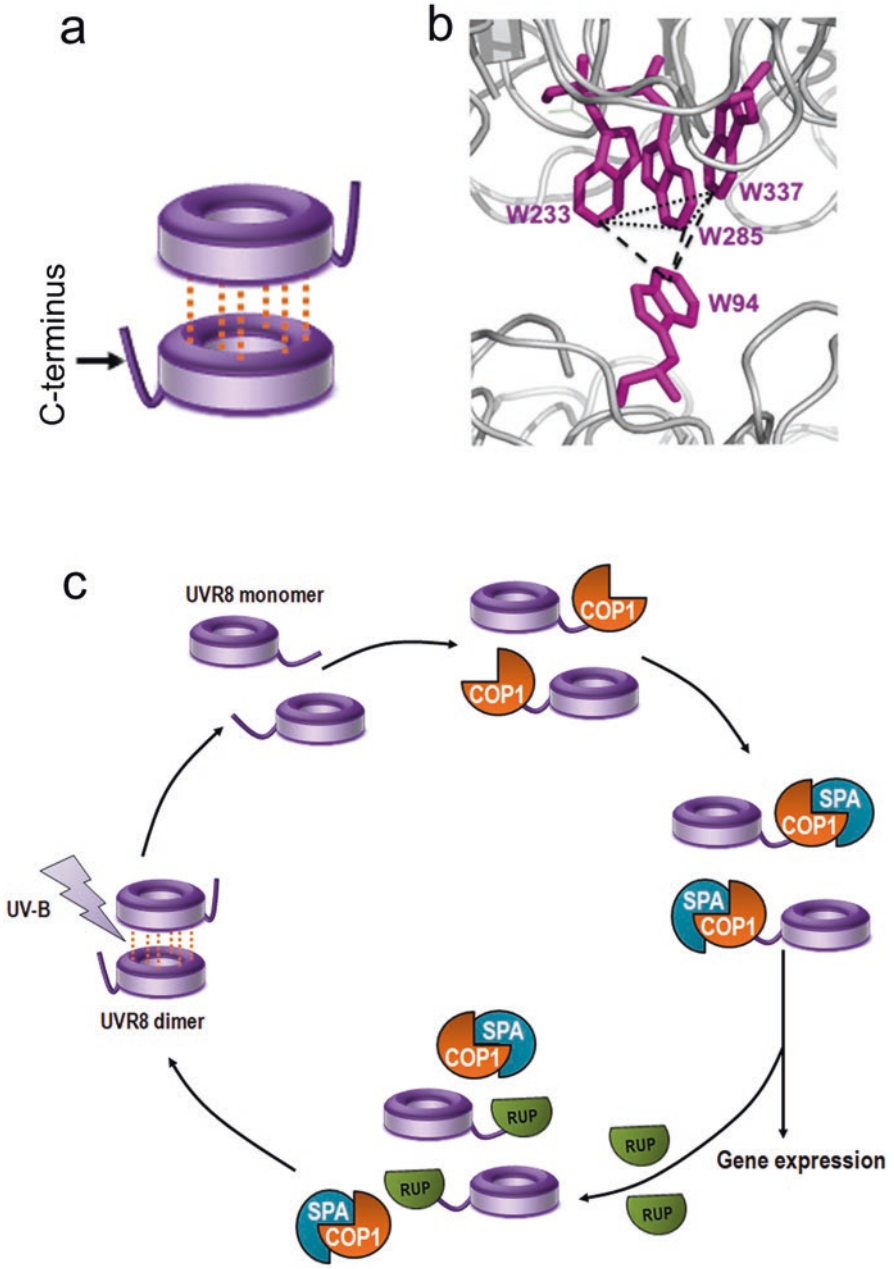

Fig. 2.8 The photo cycle of UVR8 signal transmission. (**a**, **b**) UVR8 in its inactive state is composed of two identical monomers held together by two pyramid clusters each of which is formed by identical tryptophan (W) residues on each monomer. (**c**) Light-activation monomerises UVR8 and induces a conformational change in the C-terminus, which allows for binding of COP1 followed by binding of SPA proteins to the UVR8-COP1 complex. UVR8 bound to COP1/SPA regulates the expression of target genes regulating photomorphogenic UV-B responses, including those encoding RUP (REPRESSOR OF UV-B PHOTOMORPHOGENESIS) proteins. RUP proteins then bind to the C-terminal region of the UVR8 monomer and displace COP1. Additionally, RUP proteins facilitate the re-dimerisation of UVR8 monomers, thereby regenerating the inactive dimer

short hypocotyls, expanded cotyledons and longer roots than the wild-type seedlings in darkness.

Emerging evidence indicated that in addition to COP1 and SPA1, tri- and tetra-galacturonate pectin fragments released from the cell walls of the etiolated seedlings also execute skotomorphogenesis. Sensing of these pectin fragments set up a feed-forward loop stimulating cell elongation (Sinclair et al. 2017) most likely by binding to hitherto unknown receptors. Consistent with the above role of the cell wall in skotomorphogenesis, dark-grown seedlings of several cell wall mutants show photomorphogenic phenotypes. The application of the pectin-derived tri- and tetra-galacturonate to these mutants restores normal dark-specific morphology.

2.4.2 Plants Detect Neighbour Proximity by Monitoring Spectral Quality

One of the biggest challenges of plants is the need to cope with continually changing light conditions. Under natural conditions, plants have to determine whether conditions of low-light availability are transient (e.g. a cloudy day) or more permanent (e.g. light capture by neighbouring plants). In the latter case, the plant must re-program its growth in order to outdo the competition for light by activating a series of developmental changes in response to less available light. Light filtered by neighbouring plants would have distinct spectral properties compared to light due to cloud cover. Under such circumstances, plants need to detect these specific changes in the spectral properties of light they receive and accordingly direct their growth.

Since plants maximally absorb red and blue-light for photosynthesis, plants growing under shade or dense stands receive filtered light having reduced red and blue-light but enriched in far-red and green lights. This results in a drop in the red/far-red light ratio that the plant receives. The photoreversible property of phytochromes to Pr and Pfr forms enables a plant to quantify shade by sensing changes in the red/far-red ratios (R/Fr). The perception of shade triggers a series of developmental responses such as stimulation of elongation growth coupled with reduced leaf development, increased apical dominance and reduction in branching (Franklin 2008). Collectively, these responses provide the plant with a competitive advantage over its neighbours. Such developmental plasticity to diminished light, termed shade-avoidance responses (SAR), enables a plant to increase its survival percentage under limiting light conditions.

Among the phytochromes, PhyB plays the most dominant role in overcoming vegetation shade, with PhyD and PhyE redundantly participating in it. The low red/far-red ratio releases the suppression of the PIFs, which in turn activate genes that stimulate SAR. However, the detection of shade is not restricted to phytochromes; the SAR is also regulated by cryptochromes, which monitor the ratio of blue/green light that plants receive. Long-term exposure to low levels of blue-light in combination with reduced red/far-red ratio triggers SAR responses in plants. Similar to

phytochrome, cryptochrome-mediated regulation of PIF4 and PIF5 is part of signalling mechanisms to stimulate SAR (Pedmale et al. 2016; Xu et al. 2016). Ostensibly, in densely growing populations, blue- and red-light signals are integrated to enable plants to adequately respond to competition from neighbouring plants (Fig. 2.9). Moreover, cross-talk between these two light-signalling pathways is not only restricted to the elicitation of SAR under low light but also promotes phototropic growth regulated by phototropins under foliar shade as discussed earlier. This indicates that plants have evolved very complex sensory mechanisms that can utilise spectral information to mediate plastic growth and development depending on the available light conditions.

The shade avoidance of plants comes with a premium, where the plants lower their immunity as low red/far-red desensitises plants to defence-associated plant hormones, such as jasmonic acid (JA) and salicylic acid (SA). In addition, plants use their metabolic resources to compete with other plants, leading to a reduction in the yield. In agriculture, shade avoidance of crop plants precludes their planting at higher density. Efforts are thus being made to desensitise plants to shade avoidance to allow planting at higher density in the open field.

2.4.3 Plants Can Distinguish Kin from Non-kin

In nature, where natural selection governs growth and survival, the fitness of an individual is a primary determinant of its successful growth and propagation. However, it is observed that lower organisms such as bacteria can form groups among kin, which have resultant co-operative behaviours that surpass the ability of the individual (West et al. 2007; Platt and Bever 2009; Hibbing et al. 2010). Such interactions require a high degree of recognition specificity deemed critical to the inclusion of kin and exclusion of non-kin. While allelopathic interactions among receptor and donor plants are widely accepted, kin and non-kin recognition in plants is still highly contentious. Since the first report of Dudley and File (2007), there have been conflicting observations on whether plants can indeed differentiate their kin from non-kin in their surroundings. Recent emerging evidence, however, seems to indicate that plants can utilise the spectral properties of light reflected from their neighbours to differentiate between kin and non-kin (Crepy and Casal 2015).

Fig. 2.9 (continued) to the nucleus where it phosphorylates PIFs resulting in their degradation and subsequent inhibition of expression of genes involved in SAR. In addition, the activated Cry sequesters PIFs further inhibiting transcription of SAR genes requiring PIFs. *Right Panel* – Under the shade, the combined low R:Fr and low blue-light induces the expression of genes involved in SAR. In low R:Fr, Phy is inactivated and remains in the cytosol, which allows accumulation of PIFs in the nucleus and subsequent expression of genes involved in SAR. In parallel, under low blue-light, inactivation of Cry relieves inhibition of PIF-mediated transcription of SAR genes resulting in promotion of shade-induced responses

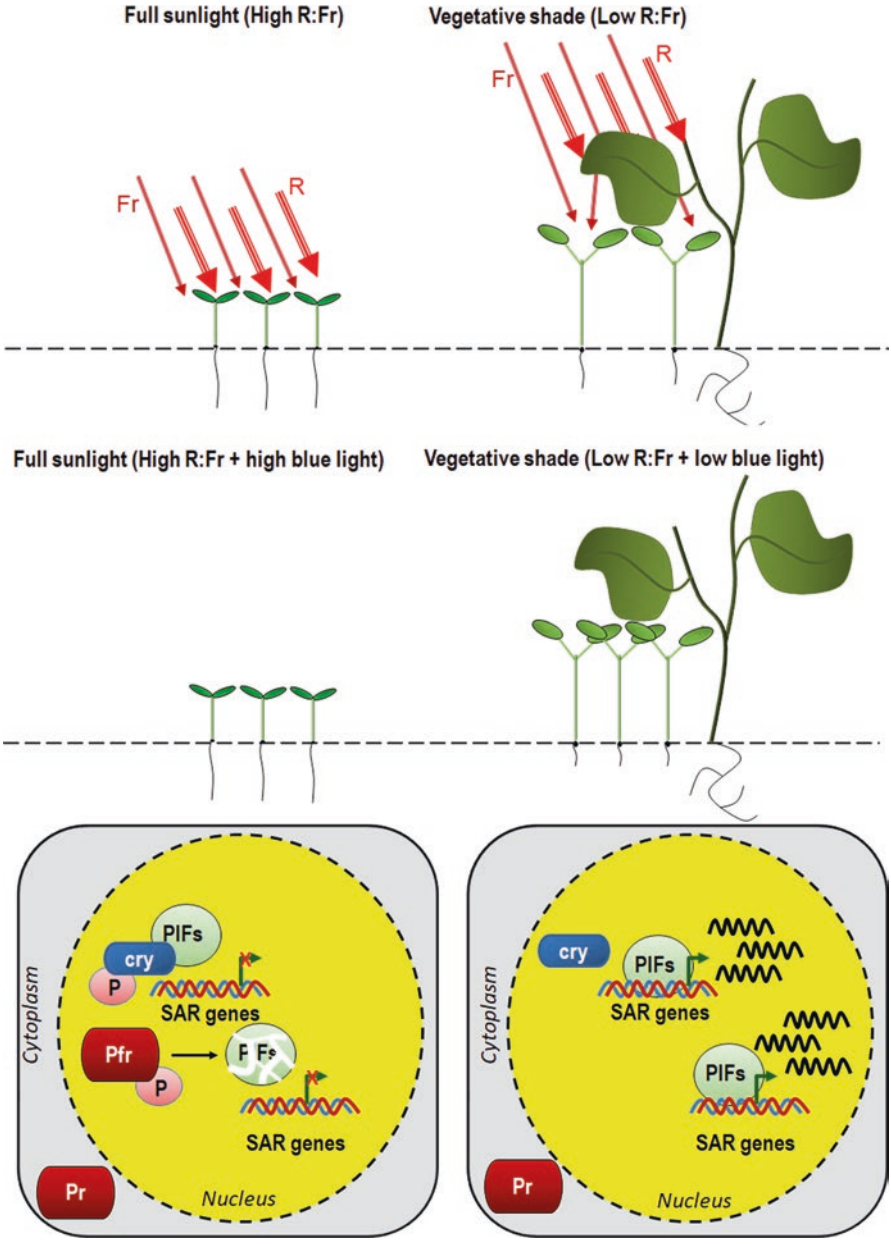

Fig. 2.9 Regulation of shade-avoidance response by phytochrome and cryptochrome. (a) Induction of shade-avoidance responses (SAR) in plants growing under shade. Plants growing in full sunlight have short hypocotyl and longer roots while those grown under canopy shade experience low red/far-red ratios triggering shade-avoidance response (SAR) characterised by longer hypocotyl and shorter roots. (b) Model for integration of phytochrome (Phy) and cryptochrome (Cry) signalling during growth under shade. *Left Panel* – In high light, activated Phy translocates

By using a complex interpretation of reflected light, Arabidopsis engages the light-perception properties of PhyB, Cry1, Cry2, Phot1 and Phot2 to distinguish between kin and non-kin among different ecotypes. In a dense stand where plants are competing for the light, Arabidopsis ecotypes assist their kin to maximise their photosynthetic potential. This is manifested by the redirection of leaf growth away from the neighbours perceived as kin to reduce mutual shading but not away from the non-kin. This leaf positioning response requires similar body shapes and vertical light profile, a parameter not met by non-kin plants. It is therefore believed that kin recognition is a mutually beneficial altruistic response not shared with non-kin, which might serve to increase fitness among neighbouring kin by decreasing competition for the local pool of resources.

2.4.4 Day and Night Sensing

Most parts of the earth experience seasonal variations in temperatures and lengths of the day during the 12 months that make up a calendar year. Plants growing in these regions experience a continually changing day/night cycle of light and temperature. Under such conditions, it is imperative that plants adapt their life cycles and growth to these seasonal variations. Initially reported for the Maryland Mammoth variety of tobacco, photoperiod regulation of flowering is now widely recognised as an important adaptation of plants to available daylight. It is now well established that plants utilise photoreceptors and internal circadian clock to perceive day length variations and adjust the timing of various developmental processes including seed germination, flowering, the setting of buds and others to ensure the highest probability of their survival and propagation. Annual plants, which complete their life cycle in a single year, can be broadly categorised into long-day plants (when the day exceeds a critical length), short-day plants (when the day is shorter than a critical length) and day-neutral plants (independent of a critical length) depending on the day length they require for the onset of flowering.

Long-day plants, such as Arabidopsis, utilise the increased duration of available light during spring to initiate developmental processes resulting in the flowering of plants as the day length increases. On the other hand, the decreasing day lengths occurring at the end of summer enables short-day plants, such as rice, to time their flowering with the onset of autumn. Similarly, growth cessation and bud set in perennial trees growing in temperate regions must be tightly regulated so that the buds can develop hardiness before the onset of frost. For all these plants, utilisation of light signals to facilitate correct day length sensing is critical in ensuring flowering at the most appropriate time of the year and avoidance of frost damage to the developing seed.

To synchronize sensing of day and night, the circadian clock constitutively operating in the plants is entrained by multiple photoreceptors via Phys, Crys and ZTL/FKF1/LKP2. Arabidopsis is a quantitative long-day plant and optimally flowers after receiving a certain number of long days. The induction of flowering under long days critically depends on the availability of three proteins, namely, GI, CO and the

blue-light photoreceptor FKF1. For flowering to occur under inductive photoperiods, these proteins must be available at optimal levels in order to initiate the flowering response. As mentioned earlier (Sect. 2.3.4), under longer photoperiods, light-activated FKF1 interacts with GI to degrade inhibitors of *CO* transcription such as CDFs. The loss of CDF results in accumulation of CO transcripts and subsequent CO proteins, which is in turn stabilised by interactions with activated FKF1. The availability of CO then drives transcription of *FT* and, subsequently, it leads to the induction of flowering by FT protein. However, under non-inductive photoperiods such as short days, the rhythms of optimal accumulation of GI and FKF do not match. Consequently, the GI-FKF protein complex remains below the threshold levels necessary for relieving inhibition of *CO* transcription by CDFs. Additionally, endogenous circadian rhythmicity promotes accumulation of CDFs in the morning hours of short days, which further repress *CO* transcription. Though the repression of *CO* transcription is relieved after dusk, the unavailability of stable CO protein during the short day cannot promote *FT* transcription under such non-inductive photoperiods.

On the other hand, in plants flowering under short days, such as rice, additional players appear to be involved in the regulation of flowering in response to photoperiods. Under short days, accumulation of Ehd1 (EARLY HEADING DATE 1), an ortholog of the Arabidopsis CO protein, occurs which promotes expression of Hd3a (HEADING DATE 3a). The activation of Hd3a, which is the rice ortholog of FT, results in the initiation of flowering responses under short days. Under non-inductive day length, the expression of Ehd1 is however repressed by the action of Ghd7 (GRAIN NUMBER PLANT HEIGHT AND HEADING DATE 7), which is in turn regulated by light input, leading to subsequent prevention of flowering (Greenup et al. 2009; Itoh et al. 2010; Osugi et al. 2011). Therefore, it appears that while the roles of CO and FT in regulating flowering in response to day length is conserved, there seem to be key differences in the molecular mechanisms involved in controlling these responses in long-day and short-day plants (Fig. 2.7).

Interestingly day/night sensing involves the co-operative operation of multiple photoreceptors. While FKF1 complexes with GI, the other two members, ZTL and LKP2, inhibit degradation of the CIB1 protein whose interaction with Cry2 is required for promoting trancription of *FT* (Liu et al. 2013). CO is also stabilised by activated Cry2 under blue-light and by inhibiting the activity of the COP1-SPA1 complex whereas PhyA stabilises CO under far-red light and long days. In contrast, PhyB promotes degradation of CO in red light and early in the photoperiod. The fact that *phyA* and *cry1cry2* mutations delay flowering of plants overexpressing CO whereas the *phyB* mutation accelerates flowering shows the important role of these photoreceptors in modulating CO levels.

The role of photoreceptors in regulating other time keeping processes in plants is emerging. In developing tomato fruits, multiple phytochrome species regulate the duration of on-vine transitions from mature green to the breaker, breaker to the red-ripe stage and red-ripe to the abscission stage. A comparison of time needed to transit from one to another stage revealed that different phytochrome species either singly or in combination regulate the duration of these transitions. Consistent with

the need for time keeping by phytochromes, a *phyAB1B2* mutant showed accelerated ripening with the shortest time to fruit abscission (Gupta et al. 2014).

2.4.5 Light and Inter-organ Communications

Plants being multicellular organisms require tightly regulated co-ordination between different cells to ensure proper growth and development. Such cell-to-cell communication is also pivotal in maintaining optimal growth and development in light. The communication of light signals from one organ to another is most elegantly manifested by the photoperiodic perception of light by leaves and induction of flowering at the apex. It is now established that FT acts as a mobile signal that transmits photoperiod information from the leaf to the apical meristem (Wigge 2011). However, the movement of FT from leaves is not restricted to the shoot apices. In potato, FT also translocates from shoot towards the roots to induce tuberisation (Navarro et al. 2011).

Root growth is also influenced by other light-derived signals emanating from above-ground parts towards the root (Fig. 2.10). Etiolated seedlings tend to have shorter roots as compared to light-grown seedlings due to the absence of a photosynthesis-derived sugar (sucrose), produced in the cotyledons, which functions as a long-distance mobile signal to stimulate root growth. On the other hand, cotyledons of light-grown seedlings produce ample amounts of sucrose that is transmitted through the phloem to stimulate root meristem growth (Kircher and Schopfer 2012).

Root development is also influenced by other key components of above-ground light signalling pathways. HY5, a key integrator of light signalling during photomorphogenesis, also functions as a mobile signal from shoot to root upon stabilisation by light involving PhyA (van Gelderen et al. 2018). HY5 promotes the production and transport of sucrose to the root. In root, the transported sugar enhances binding of shoot-derived HY5 to the promoter of the nitrate transporter NRT2.1. Thus, HY5 acts as a mobile signal synthesised in the above-ground illuminated plant organ and transported to the root, resulting in promotion of nitrate uptake through NRT2.1 (Chen et al. 2016).

Likewise, low red/far-red ratio encountered by plants in shade inhibits the emergence of lateral roots. This response is regulated by the phytochrome-dependent accumulation of HY5 in the lateral root primordia (van Gelderen et al. 2018). In these primordia, HY5 decreases the abundance of PIN3 and LAX3 (AUX1-LIKE PROTEIN 3) auxin transporters, thus reducing auxin level that is needed for the emergence of the lateral roots. Using grafted plants that had inactive PhyB in the shoot or root tissue, it was demonstrated that root-localised PhyB is necessary for the induction of root genes in response to above-ground light (Lee et al. 2016). These experiments indicated that the light signals were transduced from shoot to root most likely via the stem, resulting in light-activation of PhyB in the roots. It is suggested that the fully turgid xylem vessels of plants are somewhat mechanically equivalent to the light-conducting pipes or optic fibres (Lee et al. 2016). Using these vessels, plants may be funnelling ambient light directly into the underground roots through the plant tissues.

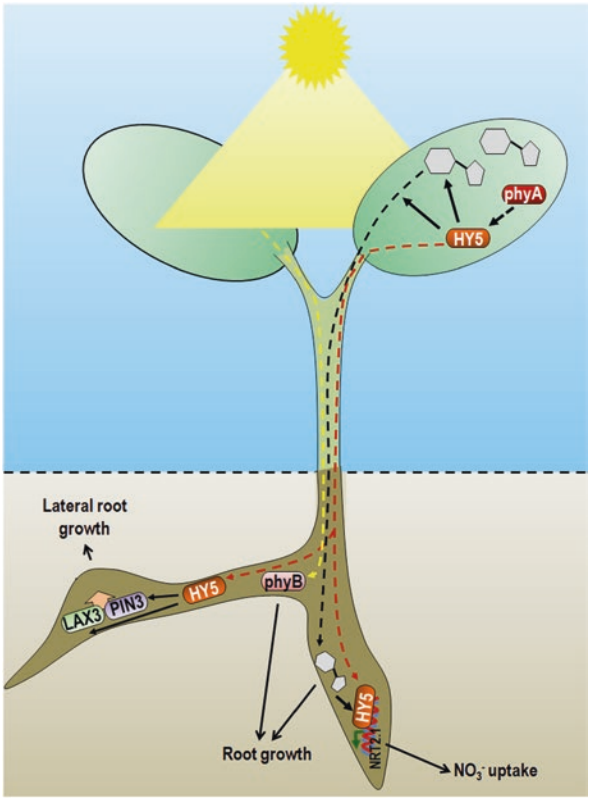

Fig. 2.10 Light and inter-organ communication. Photosynthesis-derived sugar (sucrose, black dashed lines) from leaves functions as a long-distance mobile signal to stimulate root growth in light-grown plants. The synthesis and transport of sucrose, in turn, are promoted by the action of HY5 (LONG HYPOCOTYL 5). HY5 (red dashed lines) is also transported from the aerial parts to the root where it promotes nitrate uptake by enhancing the expression of the nitrate transporter *NRT2.1*. The HY5-induced expression of *NRT2.1* is further enhanced by the presence of leaf-derived sucrose. HY5 from the aerial parts also increases the abundance of PIN3 and LAX3 auxin transporters, thus enhancing auxin levels needed for the emergence of lateral roots. Root growth is further promoted by induction of genes by root-localised PhyB in response to light perceived by the above-ground plant parts (yellow dashed lines) wherein the light signals are transduced from shoot to root via the stem resulting in light-activation of PhyB in the roots

2.5 Can Plant Photoreceptors Have Multiple Functions?

2.5.1 Temperature Sensing by Phototropins and Phytochrome

The overlap of signalling transduction pathways among photoreceptors coupled with functional redundancy among some of the photoreceptors raises the likelihood that these photoreceptors may have evolved additional functional roles in plants

apart from conveying light signals. It is well known that plants can utilise variations in their surrounding temperature as cues to direct their development. Many crop plants grown in temperate regions require prolonged exposure to low temperatures to induce flowering, a response termed as vernalisation. Arabidopsis seeds also germinate faster if they are provided with low temperatures of upto 4°C for 12–24 h. Additionally, plants can also respond to diurnal fluctuations in temperature and adjust their growth to the ambient temperature regimes, a phenomenon termed as thermomorphogenesis. Since most photoreceptors across different plants contain light-activated chromophores that undergo thermal reversion, it would seem likely that such thermal characteristics would allow photoreceptors to function as thermosensors as well.

The analysis of Arabidopsis mutants led to the recognition that changes in temperature regulate expression of several genes. Many of these genes are part of the phytochrome signalling chain such as PIFs (Franklin et al. 2011). Among the phytochromes, PhyB mutants lose sensitivity to temperature perception and thermal tolerance, indicating an additional role of PhyB as a thermosensor (Legris et al. 2017; Song et al. 2017). Detailed analyses indicated that the sensing of temperature by PhyB is accomplished by its temperature-dependent reversion from the Pfr to the Pr form. Since PhyB forms dimer, the reversion to the Pr form happens in two stages. The conversion of the Pfr-Pfr homodimer to the Pfr-Pr heterodimer is slower and more temperature sensitive compared to faster and less temperature sensitive conversation of the Pfr-Pr heterodimer to the Pr-Pr homodimer. The activity of the Pfr form of PhyB at different temperatures is thus directly linked to a temperature-dependent suppression of Pfr activity, which increases with higher temperature. The extent of PhyB activity, therefore, provides plants with a sort of sensory mechanism by which to gauge their surrounding temperature and respond accordingly (Legris et al. 2016).

In addition to PhyB, phototropins also perceive temperature based on the temperature-dependent lifetime of the photoactivated chromophore and regulate chloroplast positioning to maximise photosynthesis. Since the lifetime of phototropins is short ($t_{1/2}$ 30 s) and phytochrome is long ($t_{1/2}$ = > 30 min), phototropins may sense sudden changes in ambient temperature (Fujii et al. 2017). Other than phototropins and phytochromes, other photoreceptors in plants such as cryptochromes having a photosensitive chromophore also exhibit a temperature-dependent lifetime. It remains to be established whether other photoreceptors also function as thermoreceptors using similar lifetime-mediated mechanisms for perceiving temperature.

2.5.2 Geomagnetic Field Sensing by Cryptochromes

Among all plant photoreceptors discovered to date, the cryptochromes are the only photoreceptors that are also present in other organisms including humans. In migratory birds, retinal cryptochromes are proposed to function as magnetoreceptors enabling them to sense the earth's magnetic field and use it for navigation during

migration. Similarly, in Arabidopsis, it is suggested that cryptochromes can also act as a chemical magnetoreceptor (Ahmad et al. 2007; Ritz et al. 2010; Liedvogel and Mouritsen 2010). This suggestion is based on to its ability to form photo-induced radical pairs under weak geomagnetic fields after photo-excitation (Bouly et al. 2007). These transient radical pairs formed by electron transfer reactions in light-activated cryptochrome proteins are considered to have the required properties to respond to earth's geomagnetic field at physiological temperatures.

Similar radical pair formation in Arabidopsis Cry1 has been demonstrated to be sensitive to changes in magnetic fields in vitro (Maeda et al. 2012) as seedlings grown under near-null magnetic field conditions have reduced cryptochrome-mediated blue-light responses (Xu et al. 2012). Emerging reports on the photodynamic properties of isolated cryptochromes under low magnetic fields, coupled with the effects of variable magnetic fields on cryptochrome-mediated responses, seem to suggest that cryptochrome in plants may sense geomagnetic information (Maffei 2014; Occhipinti et al. 2014). However, more experiments are needed to establish whether plants do sense geomagnetic fields and the role of cryptochromes in it.

2.6 Do Plants Have Vision?

Throughout this chapter, we described the various mechanisms by which plant photoreceptors detect the surrounding light information to regulate growth and development. However, recent reports on pattern recognition by plants in their immediate surroundings have triggered a debate on the concept of plant vision. Originally formulated over a century ago by Francis Darwin, the question whether plants have eyes is mostly contentious. The upper epidermal cells of many leaves are shaped like convex or planoconvex lenses that can converge light rays on the light-sensitive subepidermal cells. These cells were considered as plant ocelli, a type of simple eye common to invertebrates (Haberlandt 1905). Baluška and Mancuso (2016) proposed that focusing of light by these cells on plastoglobuli of epidermal amyloplasts and subepidermal chloroplasts can impart some form of vision capability.

The recent reports on the behaviour of higher plants towards their kin or their host plants rekindled the concept of plants having a form of vision. *Boquilla trifoliolata*, a climbing wood vine, can modify its leaves with perfect mimicking of the host plant leaves with respect to colour, shape, sizes, orientation and even petiole length. Moreover, this mimicry is not restricted to one host as the plant can mimic leaves of over a dozen species (Gianoli and Carrasco-Urra 2014). Interestingly, leaf mimicry also occurs even when there is no direct contact between the vine of *B. trifoliolata* and the mimicked tree for which there is no current mechanistic explanation. As described earlier, Arabidopsis can recognise kin from non-kin by perceiving the plant shape of their neighbours presumably by monitoring the reflected light. While it is argued that such kin recognition and leaf mimicry may involve plant-specific

vision using plant ocelli (Baluška and Mancuso 2016), there are concerns about this possibility of plant vision (Gianoli 2017). However, Mancuso and Baluška (2017) are of the opinion that as defined by Nilsson and Daniel (2014) for bacteria, the behaviour or movement based on directional light perception can be regarded as vision.

2.7 Conclusion: Seeing Light At the End of the Tunnel

Molecular genetics analyses of plant responses to light have revealed that a complex regulatory network governs how a plant responds to light and dark cues in its environment. From Darwin's observations of the simple process of a seedling growing towards a light source, research in plant photobiology has advanced by leaps and bounds. We are now aware that beneath the relatively simple exterior of plants lies a labyrinth of complex molecular and cellular processes that determines how a plant regulates its developmental program to incident light. However, much remains to be uncovered. It is still poorly understood how growth is co-ordinated in different plant organs in response to the same light status.

We are yet to decipher why vegetation shade leads to rapid elongation of the hypocotyl and petioles, whereas leaf and root growths are inhibited. It is also not known how photoreceptor activation brings about the differential distribution of auxin in phototropically stimulated hypocotyls. While it is known that the FT protein acts as a 'mobile florigen" to promote flowering, the underlying mechanism of counting number of photoperiods is still largely unknown. There are also contentious opinions regarding magnetic sensing in plants or plants having some form of vision. In order to have a complete understanding of how light regulates important developmental responses of plants, the questions highlighted above and many more still need to be answered. This is particularly relevant for responses such as shade avoidance and flowering time where research can be directly linked to crop performance in the field.

Acknowledgements EK is supported by grant no. SB/EMEQ-152/2014 from the Science and Engineering Research Board, Government of India. RS and YS are supported by the Department of Biotechnology grant no. BT/COE/34/SP15209/2015 and YS is supported by grant no BT/PR6983/PBD/16/1007/2012.

References

Ahmad M, Galland P, Ritz T, Wiltschko R, Wiltschko W (2007) Magnetic intensity affects cryptochrome-dependent responses in *Arabidopsis thaliana*. Planta 225:615–624

Al-Sady B, Ni W, Kircher S, Schäfer E, Quail PH (2006) Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. Mol Cell 23:439–446

Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13:627–639

Baluška F, Mancuso S (2016) Vision in plants via plant-specific ocelli? Trends Plant Sci 21:727–730

Banerjee R, Schleicher E, Meier S, Viana RM, Pokorny R, Ahmad M et al (2007) The signaling state of Arabidopsis cryptochrome 2 contains flavin semiquinone. J Biol Chem 282:14916–14922
Bauer D, Viczián AS, Kircher S, Nobis T, Nitschke R, Kunkel T et al (2004) Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3: a transcription factor required for light signaling in Arabidopsis. Plant Cell 16:1433–1445
Bouly J, Schleicher E, Dionisio-Sese M, Vandenbussche F, Van Der Straeten D, Bakrim N et al (2007) Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. J Biol Chem 282:9383–9391
Briggs WR, Christie JM (2002) Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci 7:204–210
Burgie ES, Bussell AN, Walker JM, Dubiel K, Vierstra RD (2014) Crystal structure of the photosensing module from a red/far-red light-absorbing plant phytochrome. Proc Natl Acad Sci U S A 111:10179–10184
Chen M, Chory J (2011) Phytochrome signaling mechanisms and the control of plant development. Trends Cell Biol 21:664–671
Chen X, Yao Q, Gao X, Jiang C, Harberd Nicholas P, Fu X (2016) Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr Biol 26:640–646
Cho HY, Tseng TS, Kaiserli E, Sullivan S, Christie JM, Briggs WR (2007) Physiological roles of the light, oxygen, or voltage domains of phototropin 1 and phototropin 2 in Arabidopsis. Plant Physiol 143:517–529
Christie JM, Salomon M, Nozue K, Wada M, Briggs WR (1999) LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide. Proc Natl Acad Sci U S A 96:8779–8783
Christie JM, Yang H, Richter GL, Sullivan S, Thomson CE, Lin J et al (2011) phot1 inhibition of ABCB19 primes lateral auxin fluxes in the shoot apex required for phototropism. PLoS Biol 9:e1001076
Christie JM, Arvai AS, Baxter KJ, Heilmann M, Pratt AJ, O'Hara A et al (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. Science 335:1492–1496
Christie JM, Blackwood L, Petersen J, Sullivan S (2015) Plant flavoprotein photoreceptors. Plant Cell Physiol 56:401–413
Crepy MA, Casal JJ (2015) Photoreceptor-mediated kin recognition in plants. New Phytol 205:329–338
Demarsy E, Schepens I, Okajima K, Hersch M, Bergmann S, Christie JM et al (2012) Phytochrome Kinase Substrate 4 is phosphorylated by the phototropin 1 photoreceptor. EMBO J 31:3457–3467
Demkura PV, Ballaré CL (2012) UVR8 mediates UV-B-induced Arabidopsis defense responses against *Botrytis cinerea* by controlling sinapate accumulation. Mol Plant 5:642–652
Devlin PF, Yanovsky MJ, Kay SA (2003) A genomic analysis of the shade avoidance response in Arabidopsis. Plant Physiol 133:1617–1629
Dudley SA, File AL (2007) Kin recognition in an annual plant. Biol Lett 3:435–438
Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M et al (2009) Interaction of COP1 and UVR8 regulates UV-B induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J 28:591–601
Franklin KA (2008) Shade avoidance. New Phytol 179:930–944
Franklin KA, Lee SH, Patel D, Kumar SV, Spartz AK, Gu C et al (2011) Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. Proc Natl Acad Sci U S A 108:20231–20235
Fujii Y, Tanaka H, Konno N, Ogasawara Y, Hamashima N, Tamura S et al (2017) Phototropin perceives temperature based on the lifetime of its photoactivated state. Proc Natl Acad Sci U S A 114:9206–9211
Gianoli E (2017) Eyes in the chameleon vine? Trends Plant Sci 22:4–5

Gianoli E, Carrasco-Urra F (2014) Leaf mimicry in a climbing plant protects against herbivory. Curr Biol 24:984–987
Giovani B, Byrdin M, Ahmad M, Brettel K (2003) Light-induced electron transfer in a cryptochrome blue-light photoreceptor. Nat Struct Mol Biol 10:489–490
Goyal A, Karayekov E, Galvão VC, Ren H, Casal JJ, Fankhauser C (2016) Shade promotes phototropism through phytochrome B-controlled auxin production. Curr Biol 26:3280–3287
Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Ann Bot 103:1165–1172
Gupta SK, Sharma S, Santisree P, Kilambi HV, Appenroth K, Sreelakshmi Y et al (2014) Complex and shifting interactions of phytochromes regulate fruit development in tomato. Plant Cell Environ 37:1688–1702
Haberlandt G (1905) Die Lichtsinnesorgane der Laubblätter. W. Engelmann, Leipzig
Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8:15–25
Hohm T, Demarsy E, Quan CM, Petrolati LA, Preuten T, Vernoux T et al (2014) Plasma membrane H^+-ATPase regulation is required for auxin gradient formation preceding phototropic growth. Mol Syst Biol 10:751
Huang X, Ouyang X, Yang P, Lau OS, Li G, Li J et al (2012) Arabidopsis FHY3 and HY5 positively mediate induction of COP1 transcription in response to photomorphogenic UV-B light. Plant Cell 24:4590–4606
Huang X, Yang P, Ouyang X, Chen L, Deng XW (2014) Photoactivated UVR8-COP1 module determines photomorphogenic UV-B signaling output in Arabidopsis. PLoS Genet 10:e1004218
Inoue S, Kinoshita T (2008) Blue light regulation of stomatal opening and the plasma membrane H+-ATPase. Plant Physiol 174:531–538
Ito S, Song YH, Imaizumi T (2012) LOV domain-containing F-box proteins: light-dependent protein degradation modules in Arabidopsis. Mol Plant 5:573–582
Itoh H, Nonoue Y, Yano M, Izawa T (2010) A pair of floral regulators sets critical day length for Hd3a florigen expression in rice. Nat Genet 42:635
Iwabuchi K, Minamino R, Takagi S (2010) Actin reorganization underlies phototropin-dependent positioning of nuclei in Arabidopsis leaf cells. Plant Physiol 152:1309–1319
Jones MA, Feeney KA, Kelly SM, Christie JM (2007) Mutational analysis of phototropin 1 provides insights into the mechanism underlying LOV2 signal transmission. J Biol Chem 282(9):6405–6414
Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, Kato T et al (2001) Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. Science 291:2138–2141
Kasahara M, Torii M, Fujita A, Tainaka K (2010) FMN binding and photochemical properties of plant putative photoreceptors containing two LOV domains, LOV/LOV proteins. J Biol Chem 285:34765–34772
Kircher S, Schopfer P (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in Arabidopsis. Proc Natl Acad Sci U S A 109:11217–11221
Lee H, Ha J, Kim S, Choi H, Kim Z, Han Y et al (2016) Stem-piped light activates phytochrome B to trigger light responses in Arabidopsis thaliana roots. Sci Signal 9:ra106
Lee B, Kim MR, Kang M, Cha J, Han S, Nawkar GM et al (2017) The F-box protein FKF1 inhibits dimerization of COP1 in the control of photoperiodic flowering. Nat Commun 8:2259
Legris M, Klose C, Burgie ES, Rojas CCR, Neme M, Hiltbrunner A et al (2016) Phytochrome B integrates light and temperature signals in Arabidopsis. Science 354:897–900
Legris M, Nieto C, Sellaro R, Prat S, Casal JJ (2017) Perception and signalling of light and temperature cues in plants. Plant J 90:683–697
Leivar P, Monte E (2014) PIFs: systems integrators in plant development. Plant Cell 26:56–78
Leivar P, Monte E, Al-Sady B, Carle C, Storer A, Alonso JM et al (2008) The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell 20:337–352

Li J, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of Arabidopsis. Science 272:398–401
Li J, Li G, Wang H, Wang Deng X (2011) Phytochrome signaling mechanisms. The Arabidopsis Book, American Society for Plant Biologists, Rockville
Lian H, He S, Zhang Y, Zhu D, Zhang J, Jia K et al (2011) Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. Genes Dev 25:1023–1028
Liedvogel M, Mouritsen H (2010) Cryptochrome-a potential magnetoreceptor: what do we know and what do we want to know? J R Soc Interface 7:S147–S162
Lin C, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, Dutton PL et al (1995) Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269:968–970
Liu H, Wang Q, Liu Y, Zhao X, Imaizumi T, Somers DE et al (2013) Arabidopsis CRY2 and ZTL mediate blue-light regulation of the transcription factor CIB1 by distinct mechanisms. Proc Natl Acad Sci U S A 110:17582–17587
Maeda K, Robinson AJ, Henbest KB, Hogben HJ, Biskup T, Ahmad M et al (2012) Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. Proc Natl Acad Sci U S A 109:4774–4779
Maffei ME (2014) Magnetic field effects on plant growth, development, and evolution. Front Plant Sci 5:445
Mancuso S, Baluška F (2017) Plant ocelli for visually guided plant behavior. Trends Plant Sci 22:5–6
Martínez-García JF, Gallemí M, Molina-Contreras MJ, Llorente B, Bevilaqua MRR, Quail PH (2014) The shade avoidance syndrome in Arabidopsis: the antagonistic role of phytochrome A and B differentiates vegetation proximity and canopy shade. PLoS One 9:e109275
Matsuda S, Kajizuka T, Kadota A, Nishimura T, Koshiba T (2011) NPH3-and PGP-like genes are exclusively expressed in the apical tip region essential for blue-light perception and lateral auxin transport in maize coleoptiles. J Exp Bot 62:3459–3466
Nagatani A (2004) Light-regulated nuclear localization of phytochromes. Curr Opin Plant Biol 7:708–711
Nakasako M, Zikihara K, Matsuoka D, Katsura H, Tokutomi S (2008) Structural basis of the LOV1 dimerization of Arabidopsis phototropins 1 and 2. J Mol Biol 381:718–733
Nakasone Y, Zikihara K, Tokutomi S, Terazima M (2013) Photochemistry of Arabidopsis phototropin 1 LOV1: transient tetramerization. Photochem Photobiol Sci USA 12:1171–1179
Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J et al (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 478:119
Nilsson T, Daniel G (2014) Developments in the study of soft rot and bacterial decay. In: Forest products biotechnology. CRC Press, Boca Raton, pp 47–72
Occhipinti A, De Santis A, Maffei ME (2014) Magnetoreception: an unavoidable step for plant evolution? Trends Plant Sci 19:1–4
Oide M, Okajima K, Nakagami H, Kato T, Sekiguchi Y, Oroguchi T et al (2018) Blue light-excited LOV1 and LOV2 domains cooperatively regulate the kinase activity of full-length phototropin2 from Arabidopsis. J Biol Chem 293:963–972
Osugi A, Itoh H, Ikeda-Kawakatsu K, Takano M, Izawa T (2011) Molecular dissection of the roles of phytochrome in photoperiodic flowering in rice. Plant Physiol 157:1128–1137
Paik I, Yang S, Choi G (2012) Phytochrome regulates translation of mRNA in the cytosol. Proc Natl Acad Sci U S A 109:1335–1340
Park E, Park J, Kim J, Nagatani A, Lagarias JC, Choi G (2012) Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. Plant J 72:537–546
Pedmale UV, Liscum E (2007) Regulation of phototropic signaling in Arabidopsis via phosphorylation state changes in the phototropin 1-interacting protein NPH3. J Biol Chem 282:19992–20001
Pedmale UV, Huang SC, Zander M, Cole BJ, Hetzel J, Ljung K et al (2016) Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. Cell 164:233–245
Pfeifer A, Mathes T, Lu Y, Hegemann P, Kottke T (2010) Blue light induces global and localized conformational changes in the kinase domain of full-length phototropin. Biochemistry 49:1024–1032

Pham VN, Kathare PK, Huq E (2018) Phytochromes and phytochrome interacting factors. Plant Physiol 176:1025–1038

Platt TG, Bever JD (2009) Kin competition and the evolution of cooperation. Trends Ecol Evol 24:370–377

Preuten T, Hohm T, Bergmann S, Fankhauser C (2013) Defining the site of light perception and initiation of phototropism in Arabidopsis. Curr Biol 23:1934–1938

Preuten T, Blackwood L, Christie JM, Fankhauser C (2015) Lipid anchoring of Arabidopsis phototropin 1 to assess the functional significance of receptor internalization: should I stay or should I go? New Phytol 206:1038–1050

Rakusová H, Fendrych MÅ, Friml J (2015) Intracellular trafficking and PIN-mediated cell polarity during tropic responses in plants. Curr Opin Plant Biol 23:116–123

Ritz T, Yoshii T, Foerster C, Ahmad M (2010) Cryptochrome: a photoreceptor with the properties of a magnetoreceptor? Commun Integr Biol 3:24–27

Rizzini L, Favory J, Cloix C, Faggionato D, O'Hara A, Kaiserli E et al (2011) Perception of UV-B by the Arabidopsis UVR8 protein. Science 332:103–106

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR et al (2001) Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. Proc Natl Acad Sci U S A 98:6969–6974

Salomon M, Zacherl M, Rudiger W (1997) Phototropism and protein phosphorylation in higher plants: unilateral blue light irradiation generates a directional gradient of protein phosphorylation across the oat coleoptile. Plant Biol 110:214–216

Sawa M, Nusinow DA, Kay SA, Imaizumi T (2007) FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science 318:261–265

Sharma R, López-Juez E, Nagatani A, Furuya M (1993) Identification of photo-inactive phytochrome A in etiolated seedlings and photo-active phytochrome B in green leaves of the *aurea* mutant of tomato. Plant J 4:1035–1042

Sharma S, Kharshiing E, Srinivas A, Zikihara K, Tokutomi S, Nagatani A et al (2014) A dominant mutation in the light-oxygen and voltage2 domain vicinity impairs phototropin1 signaling in tomato. Plant Physiol 164:2030–2044

Shen Y, Khanna R, Carle CM, Quail PH (2007) Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. Plant Physiol 145:1043–1051

Sinclair SA, Larue C, Bonk L, Khan A, Castillo-Michel H, Stein RJ et al (2017) Etiolated seedling development requires repression of photomorphogenesis by a small cell-wall-derived dark signal. Curr Biol 27:3403–3418

Song YH, Smith R, To BJ, Millar AJ, Imaizumi T (2012) FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science 336:1045–1049

Song J, Liu Q, Hu B, Wu W (2017) Photoreceptor PhyB involved in Arabidopsis temperature perception and heat-tolerance formation. Int J Mol Sci 18:1194

Srinivas A, Behera RK, Kagawa T, Wada M, Sharma R (2004) High pigment1 mutation negatively regulates phototropic signal transduction in tomato seedlings. Plant Physiol 134:790–800

Sullivan S, Hart JE, Rasch P, Walker CH, Christie JM (2016a) Phytochrome A mediates blue-light enhancement of second-positive phototropism in Arabidopsis. Front Plant Sci 7:290

Sullivan S, Takemiya A, Kharshiing E, Cloix C, Shimazaki KI, Christie JM (2016b) Functional characterization of Arabidopsis phototropin 1 in the hypocotyl apex. Plant J 88:907–920

Takemiya A, Sugiyama N, Fujimoto H, Tsutsumi T, Yamauchi S, Hiyama A et al (2013a) Phosphorylation of BLUS1 kinase by phototropins is a primary step in stomatal opening. Nat Commun 4:2094

Takemiya A, Yamauchi S, Yano T, Ariyoshi C, Shimazaki KI (2013b) Identification of a regulatory subunit of protein phosphatase 1 which mediates blue light signaling for stomatal opening. Plant Cell Physiol 54:24–35

van Gelderen K, Kang C, Pierik R (2018) Light signaling, root development, and plasticity. Plant Physiol 176:1049–1060

Wang H, Wang H (2015) Phytochrome signaling: time to tighten up the loose ends. Mol Plant 8:540–551

Wang H, Ma L, Li J, Zhao H, Deng XW (2001) Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. Science 294:154–158
Wang Q, Zuo Z, Wang X, Gu L, Yoshizumi T, Yang Z et al (2016) Photoactivation and inactivation of Arabidopsis cryptochrome 2. Science 354:343–347
Wang X, Wang Q, Han Y, Liu Q, Gu L, Yang Z et al (2017) A CRY-BIC negative-feedback circuitry regulating blue light sensitivity of Arabidopsis. Plant J 92:426–436
West SA, Diggle SP, Buckling A, Gardner A, Griffin AS (2007) The social lives of microbes. Annu Rev Ecol Evol Syst 38:53–77
Wigge PA (2011) FT, a mobile developmental signal in plants. Curr Biol 21:R374–R378
Xu C, Yin X, Lv Y, Wu C, Zhang Y, Song T (2012) A near-null magnetic field affects cryptochrome-related hypocotyl growth and flowering in Arabidopsis. Adv Space Res 49:834–840
Xu P, Lian H, Wang W, Xu F, Yang H (2016) Pivotal roles of the phytochrome-interacting factors in cryptochrome signaling. Mol Plant 9:496–497
Yang H, Tang R, Cashmore AR (2001) The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell 13:2573–2587
Yin R, Skvortsova M, Loubéry S, Ulm R (2016) COP1 is required for UV-B induced nuclear accumulation of the UVR8 photoreceptor. Proc Natl Acad Sci U S A 113:E4415–E4422
Yu X, Sayegh R, Maymon M, Warpeha K, Klejnot J, Yang H et al (2009) Formation of nuclear bodies of Arabidopsis CRY2 in response to blue light is associated with its blue light dependent degradation. Plant Cell 21:118–130
Zeugner A, Byrdin M, Bouly J, Bakrim N, Giovani B, Brettel K et al (2005) Light-induced electron transfer in Arabidopsis cryptochrome-1 correlates with in vivo function. J Biol Chem 280:19437–19440

Eros Kharshiing obtained his Ph.D. from University of Hyderabad (UOH) in the area of blue-light signaling in tomato. After his Ph.D., he joined the laboratory of Giuliano Giovanni at ENEA, Italy, as an ENEA Research Fellow. Currently he holds a teaching and research position at St. Edmund's College, Meghalaya, India. Since joining St. Edmund's, he has visited John Christie's laboratory in Glasgow, under a Biotechnology Associateship. Currently his research interests involve evaluating photoreceptor function, signaling, and application in higher plants.

Yellamaraju Sreelakshmi obtained her Ph.D. from UOH for the work done on physiology, biochemistry, and signal transduction in a light-signaling mutant of tomato. As a Postdoctoral Fellow, she continued to work on mutants and their effect on structure-function relation of proteins in both humans (Mason Eye Institute, USA) and tomato (UOH, India). She is currently working as an Assistant Professor at Repository of Tomato Genomics Resources, Department of Plant Sciences, UOH, and her lab uses functional genomics approaches to understand the influence of light on tomato fruit development and quality.

Rameshwar Sharma obtained his doctoral degree from Jawaharlal Nehru University (JNU), New Delhi (with the Editor), on phytochrome-mediated enzyme regulation. He had his postdoctoral training in photomorphogenesis as an Alexander Von Humboldt Fellow in the lab of Prof. Peter Schopfer at the University of Freiburg, Germany. He was a Visiting Scientist in Prof. Masaki Furuya lab at Frontier Research Program, Institute of Physical and Chemical Research, Tokyo, Japan. He is currently a Professor at Repository of Tomato Genomics Resources, Department of Plant Sciences, UOH, Hyderabad, India. In the past, his research focused on molecular physiology of plant development using mutants. His current research interest is on enhancing micronutrients in tomato fruits using mutagenesis, involving TILLING, NGS, and genome editing by CRISPR/CAS9.

Nutrient Perception and Signaling in Plants

3

Dinesh Kumar Jaiswal and Nandula Raghuram

Abstract

Plants have developed mechanisms to sense the fluctuating availability of nutrients, water, carbon dioxide, oxygen, etc. for their adaptation and survival under constantly changing atmospheric and soil conditions. The biological interventions for crop improvement for nutrient use efficiency have long been limited by the lack of adequate understanding of the sensing and signaling of nutrients and the targets for their improvement. Moreover, nutrient fluctuations could contribute to or accentuate the effects of other abiotic stresses such as drought, flood, salt, extreme light, heat, cold, and wind velocity or biotic stresses due to pests and pathogens. The global warming due to increased atmospheric CO_2 emissions also affects drought, salt stress, and nutrient status in plants. This chapter highlights several developments in the last two decades that have improved our understanding of the molecular physiology of nutrient sensing, signaling pathways, and their crosstalk, revealing the nature of plant responses toward its survival. We deal with sensing at the levels of roots for a few nutrients and sensing at the level of shoots for oxygen and carbon dioxide and how a balance of all these factors ensures growth and development. The sensing of water and stress environment is covered separately in two chapters.

Keywords

Carbon dioxide · Nitrogen · Nutrients · Oxygen · Phosphorus · Potassium · Sensing · Signaling

D. K. Jaiswal · N. Raghuram (✉)
University School of Biotechnology, G.G.S. Indraprastha University, New Delhi, India
e-mail: raghuram@ipu.ac.in

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_3

3.1 Introduction

The sensing of nutrient availability regulates the plants' growth and development. Plants require macronutrients viz., nitrogen (N), phosphorus (P), potassium (K), sulfur (S), magnesium (Mg), and calcium (Ca), and micronutrients such as copper (Cu), iron (Fe), nickel (Ni), zinc (Zn), boron (B), molybdenum (Mo) chlorine (Cl), and manganese (Mn). These nutrients play a significant role in various cellular pathways; however, deficiency of N, P, and K macronutrients significantly affects plant growth. Deficiency of these nutrients in the soil could be due to their non-availability in usable forms, physicochemical properties of soil, soil pH, less solubility/stability in water, and slow diffusion rate among others. Depending upon the developmental stage, plants are in constant need of nutrients. In cultivated plants, this is usually complemented with the exogenous application of various doses and forms of fertilizers to replenish nutrients in the soil or even directly to the plants through foliar sprays, etc. However, even in the best of circumstances such as precision farming with drip irrigation providing just the right amounts of water and nutrients on daily basis, the plants do not uptake and use all the nutrients. The losses can be far worse when farmers in most countries apply large amounts of fertilizers in a few divided doses in the entire cropping season. The unutilized fertilizers pollute the ground water and surface water bodies apart from air pollution by volatilization, posing serious threats to the environment, within and across national boundaries (Sutton et al. 2013). While slow-release fertilizers and crop management practices such as timing, dose, and method of application yield some agronomic benefits in the short term (Li et al. 2018a), the inherent inefficiency of the crop cultivar to use the available nutrients has to be tackled biologically. The problem of biological or genetic improvement of nutrient use efficiency (NutUE) of crops is compounded by the fact that many efficient genotypes may have even been selected out unintentionally during screening under high-nutrient input conditions in yield-centric crop improvement programs of the earlier decades. Finding them and bringing them back for low-nutrient input screening for NutUE are huge challenges because their phenotypes remain largely unknown. Fortunately, there has been some progress in the basic understanding of molecular mechanisms underlying nutrient sensing and signaling, at least with respect to the development of root system architecture (RSA), which is increasingly emerging as an important target for phenotype development and phenomics. *Arabidopsis* has been extensively used as a model system to study nutrient-responsive changes in RSA. Low dose of nitrate induces elongation of lateral root (LR), whereas high doses suppress LR elongation in *Arabidopsis* (Zhang and Forde 1998). A phenotypic screening of *Arabidopsis* natural population under controlled condition using different doses of nitrate showed the pronounced variation in RSA trait at low dose across different accessions (De Pessemier et al. 2013). Nitrate-induced ANR1 gene, which encodes a MADS box transcription factor, has been shown to control lateral root branching in *Arabidopsis* (Zhang and Forde 1998; Gan et al. 2012). The GS3 and DEP1, atypical heterotrimeric G-protein gamma subunits, interact with OsMADS1 in rice (Liu et al. 2018). Rice OsMADS1 transcription factor is encoded by grain yield-associated qLGY3 QTL (Liu et al. 2018)

and its NutUE function yet to be established. Molecular and genetic analyses have led to the identification of key components regulating nutrient uptake, transport, and assimilation inside cell and their biological role in growth and development of plants even under adverse agricultural climatic conditions. As case studies, we will discuss sensing mechanism of a few well-studied nutrients.

3.2 Sensing the Nutrients

3.2.1 Nitrogen Sensing

N is the integral component of biomolecules including nucleic acids and proteins. Plants cannot utilise atmospheric N_2 and depend on compounded forms such as nitrate or ammonium ions or urea. The cellular N level is known to regulate the shoot and root developmental processes, which in turn regulate nitrogen use efficiency (NUE) in plants (Wang et al. 2018a; Gent and Forde 2017). In most of the plants, the intracellular N level could be sensed by glutamine concentration, an amino acid and an end product of N assimilation (Chellamuthu et al. 2014). N-regulated long-distance signaling from shoot to root is possibly mediated via the phloem sap containing high amino acid contents. Therefore, understanding the role of glutamine in long-distance signaling would provide new insight for N-sensing in plants. Recently, the role of elongated hypocotyl 5 (Hy5) was identified as a phloem mobile signal for shoot to root mediated enhancement of nitrate uptake (Chen et al. 2016). It may be possible that the N status may be sensed by C/N ratio as shown for PII protein pathway in lower organism (Arcondeguy et al. 2001). PII proteins are evolutionarily conserved, but their signaling is more complex and diverse in higher organisms. Glutamine is known to bind PII proteins in plants. PII proteins are localized in the plastids of plants, and their interaction with NAGK (N-acetyl-L-glutamate kinase) regulates fatty acid metabolism (Sugiyama et al. 2004). This suggests that the N-sensing role of PII proteins is yet to be established in plants.

Among N, P, and K, only the function of N, especially nitrate, has been proved as a nutrient and signaling molecule affecting cell physiology in plants (Krouk et al. 2010). Genome-wide effect of N starvation and N sources and doses revealed a large-scale transcriptional reprograming in plants (Shin et al. 2018; Sun et al. 2017, Wei et al. 2016; Yang et al. 2015; Misyura et al. 2014; Sharma et al., unpublished data). NO_3^- induces expression of associated transporters; however, no such sensing and signaling role is known for NH_4^+ in the activation of its own transporters (AMTs). Instead, NH_4^+ functions in the opposite way and inhibits the expression of many AMTs in most of the plant species. In case of rice, however, exogenous application of NH_4^+ has been reported to induce OsAMT1;1 and OsAMT1;2 expressions and inhibited by N starvation condition (von Wiren et al. 2000). The signaling role of urea is not well documented; however, urea induces the expression of urea transporter (AtDUR3) similar to nitrate transporter but repressed by NO_3^- and NH_4^+ in *Arabidopsis* (Kojima et al. 2007). This suggests that the transceptor function of urea transporters needs to be investigated.

Fluctuation in cellular N level and its demand enhances the perception of soil N, ammonium, nitrate, and urea, concentrations by root cells, which modulate the cellular acquisition, assimilation, and other processes in plants during optimal and adverse environmental condition (Tsay et al. 2011). NO_3^- concentration is much higher than NH_4^+ in soil, but the acquisition of both the ions is crucial as NH_4^+ significantly affects the activity of nitrate transporters in the root. Transport of urea occurs inside the cell in either a non-metabolized form or a metabolized form, CO_2 and ammonia, produced by bacterial urease activity (Wang et al. 2012). Influx and efflux rates control the acquisition processes in root cells via either high-affinity transport systems (HATS) or low-affinity transport systems (LATS) localized on the plasma membrane (Miller et al. 2007). HATS have been characterized for both NO_3^- and NH_4^+ whereas LATS for NO_3^- (Noguero and Lacombe 2016). Nitrate transporter 1/peptide transporter family (NPF), nitrate transporter 2 (NRT2), slow anion associated channel homologs (SLAC/SLAH), and chloride channel family (CLC) proteins are involved in nitrate acquisition and may play a role in the sensing mechanism as reported for NRT1.1/NPF6.3 transporter (O'Brien et al. 2016). NRT1.1/NPF6.3 acts as a transceptor and is phosphorylated by CIPK23, a calcineurin B-like interacting protein kinase 23, which alters its function for low-affinity or high-affinity state (Ho et al. 2009). NO_3^- induced local gene expression, and long-term feedback repression due to nitrate resupply is regulated by NRT1.1/NPF6.3 in *Arabidopsis*. A diverse set of transcriptional regulators have been implemented to regulate N-sensing/transport in plants (O'Brien et al. 2016). Nodule inception-like protein 7 (NLP7), a NIN family transcription factor, is a positive regulator of nitrate signaling as evidenced from nitrate-induced impaired expression of nitrate transporter (NRT2.1) and nitrate reductase (NR) genes in *nlp7* knockout mutants (Castaings et al. 2009). NLP7 works upstream of NRT1.1-dependent nitrate signaling in the presence of ammonium, whereas in the absence of ammonium, NLP7 functions in NRT1.1-independent signaling in *Arabidopsis* (Zhao et al. 2018). AMT, ammonium transporter/methylammonium permease/rhesus (AMT/MEP/Rh), family proteins are involved in root-mediated NH_4^+ transport along the electrochemical potential gradient in plants (Ludewig et al. 2007). Six AMT family members were reported in *Arabidopsis*, and most of them were expressed in the root. A total of ten AMT transporters were reported in rice, and three OsAMT1 genes, viz., OsAMT1;1, OsAMT1;2, and OsAMT1;3, were characterized for their expression and NH_4^+ transport function in yeast (Sonoda et al. 2003). Increased NH_4^+ influx was observed in the root of transgenic plants overexpressing OsAMT1;1 in rice (Hoque et al. 2006). In plants, two types of urea transporters were reported, namely, DUR3 orthologue and MIPs, i.e., major intrinsic proteins (Wang et al. 2012). MIPs belong to a low-affinity transporter group, whereas DUR3 is a high-affinity urea transporter. DUR3 orthologue has been reported in algae, fungi, mosses, and higher plants; DUR3 urea transporter activity was demonstrated in the root of *Arabidopsis* (Wang et al. 2012); however, its role in urea sensing and signaling, if any, is yet to be discovered.

Target of rapamycin (TOR) is an evolutionarily conserved protein kinase that regulates the nutrient sensing mechanism in yeast and mammals. Plants do not have all the homologs of TORC1 complex present in animals (Dobrenel et al. 2016). Diverse roles of TOR kinase including growth and development functions have been characterized in plants (Dobrenel et al. 2016). *Arabidopsis* mutants defective in TOR signaling components showed induction of genes associated with amino acid recycling and reduced expression of nitrate assimilatory genes (Ahn et al. 2011). Despite the important role of TOR complex, its N-sensing mechanism is unknown in plants. It has been shown that GCN2 or general amino acid control nonderepressible 2 protein kinase maintains the cellular amino acid pool during N deficiency in yeast (Chantranupong et al. 2015). GCN2 protein kinase phosphorylates eukaryotic translation initiation factor (eIF2α) and inhibits protein synthesis during N deprivation (Chantranupong et al. 2015). The GCN2 kinase and eIF2α in plants have been implicated in seed germination and development and multiple stresses (Li et al. 2018b). It has been shown that GCN2 kinase phosphorylates eIF2α in *Arabidopsis* under various conditions including amino acid starvation; however, in-depth analyses are required to enlighten the molecular aspect of GCN2 and eIF2α in sensing N level in plants. Another important candidate for N sensing is glutamate-like receptors (GLRs) in plants. *Arabidopsis* genome codes for 20 GLRs showing homology with mammalian ionotropic glutamate receptor (iGluR) that act as glutamate-gated cation channels (Weiland et al. 2016). GLRs are localized in different membranous systems including plasma membrane (Weiland et al. 2016) and showed broad range specificity to amino acids (Tapken et al. 2013). Experiment with AtGLR1.1 knockdown mutants showed that specifically sucrose has an inhibitory effect on germination in an N-depleted medium, which was restored after exogenous NO_3^- supply to the medium (Kang and Turano 2003). However, the N-sensing role of GLRs is not known and needs more experimentation.

3.2.2 Phosphorus Sensing

Soil is often limited in the concentration of phosphorus (P), an essential macronutrient, and therefore plants have developed efficient mechanisms for phosphate uptake, remobilization, and recycling to maintain growth. P is an essential constituent of biomolecules such as lipids, proteins, and ATP among others, and phosphate deficiency in soil affects the agronomical performance of crops. Plant utilizes inorganic phosphate (orthophosphate, Pi) from soil, and Pi deficiency is due to slow diffusion rate and complex chemical fixation in soil (Raghothama 1999). Arbuscular mycorrhizal fungi (AMF) colonization with root does not involve indirect Pi acquisition; however, AFM enhances the uptake via mineralization of organic P and solubilization of insoluble inorganic P in plants (Smith et al. 2011). AMF-induced P uptake is mediated by the regulation of PSI, Pi starvation-inducible, genes including Pi transporters in plants (Yang et al. 2012; Xu et al. 2007). Pi deficiency also induces remobilization of P, between root and shoot involving phosphate transporters and purple

acid phosphatase among others. Insoluble P compounds are not usable by plants; therefore, secretion of acid phosphatases such as purple acid phosphatase and organic acids by roots solubilizes these compounds to expedite the efficient P acquisition in plants (Robinson et al. 2012). Membrane transporters associated with Pi uptake have been identified and characterized in many plant species (Wang et al. 2018b; Mlodzinska and Zboinska 2016). Phosphate transporter traffic facilitator 1 (PHF1) regulates the targeting of high- and low-affinity Pi transporters from ER to plasma membrane and therefore plays an important role in Pi uptake in plants (Bayle et al. 2011).

Cellular Pi homeostasis is regulated through the combinatorial effects of local and systemic sensing and signaling under Pi-deficient condition in plants. The Pi deficiency in soil is sensed by root cells, which transmit the signal to the shoot for activation of adaptive responses at whole plant level. Root tips perceive the Pi deficiency signal, and root cells activate Pi uptake either by membrane-localized receptors for soil Pi level or by intracellular receptors (Nagarajan and Smith 2012). Local as well as systemic Pi signaling is regulated through sugars, ABA, ethylene, cytokinins, and auxin, among others in plants (Chiou and Lin 2011).

During Pi deficiency, the plant enhances the Pi acquisition from soil and remobilization within plant systems. Phosphate stress responses (PSRs) are Pi deficiency-induced adaptive responses, which include changes in the root system architecture, viz., increased root hair and lateral root density; reduction in primary root length; enhanced PSI (phosphate starvation-induced) gene expression and high-affinity Pi transporter activities; change in root/shoot ratio; starch, sugar, and anthocyanin accumulation; and release of phosphatases and organic acids into the soil (Lynch 2011). Pi acquisition-efficient crops showed better growth response as compared to relatively less efficient genotypes due to shallower root growth angles in Pi-rich soil (Lynch 2011). Pi deficiency-induced PSR genes showed delayed induction in response to media lacking Pi, suggesting that internal Pi levels regulate the PSR expression in *Arabidopsis*. Reduced primary root growth was observed under Pi deficiency in many ecotypes with natural variation in *Arabidopsis* (Chevalier et al. 2003), whereas such responses were lagging in crops like maize and rice, suggesting that different adaptive mechanisms are involved to regulate RSA in Pi-deficient soil (Shimizu et al. 2004).

Transcriptomic and genetic analyses of different mutants to delineate the Pi sensing and signaling mechanism showed that the root tip senses the Pi deficiency in soil (O'Rourke et al. 2013; Lan et al. 2012; Thibaud et al. 2010). Transcriptomic analyses have provided in-depth information on the differential regulation of many genes associated with Pi deficiency-induced signaling cascades governing adaptive responses in plants. The differentially expressed genes were phosphate transporters, SPX domain-containing proteins, and acid phosphatases among others associated with Pi uptake, remobilization, and recycling in plants. The genes induced by Pi deficiency include early signaling event genes such as 14-3-3 proteins, CDPKs, MAPKs, WRKY, bHLH, NAC, MYB TFs, cytochrome P450, and peroxidases including those that belong tohormone- and stress-related pathways (Chiou and Lin 2011); among others were the transcriptional regulators also identified. Genes

associated with late signaling events were associated with the adaptive response pathways, viz., metabolic process, protein synthesis and degradation, and photosynthesis among others (O'Rourke et al. 2013; Thibaud et al. 2010).

Nitrate signaling has provided the evidence that plasma membrane-localized transporter, CHL1, can act as transceptor i.e., transporter and receptor molecules in *Arabidopsis* (Ho et al. 2009). Yeast Pho84 works as a transceptor in Pi sensing and transport mechanism (Popova et al. 2010). By analogy, PHT1 may work as a transceptor to sense and transport Pi in plants. Regulation of Pi-induced signaling by inositol polyphosphates (IPs), ROS, and Ca^{2+} molecules is known in the plants (Chiou and Lin 2011). The IP signaling mutant, atipk1, showed a hypersensitive phenotype to Pi and was less responsive to the changes in Pi level. The *atipk1* mutant showed increased accumulation of internal Pi as compared to wild-type plants, confirming their role in Pi sensing pathways (Stevenson-Paulik et al. 2005). The spatial ROS distribution in the RSA is regulated by Pi deficiency in *Arabidopsis* (Tyburski et al. 2009). ROS accumulation was observed in the elongation zone and other parts of the root under high Pi concentration, whereas ROS accumulation was absent in the elongation zone under low Pi, highlighting the importance of ROS in Pi sensing mechanism (Chiou and Lin 2011). Pi deficiency induces the higher expression of Ca^{2+} transporter, suggesting its possible role in Pi-mediated signaling in plants. Pi deficiency-induced local signal generated in the root cells may transport to the shoot via the xylem to regulate the various responses associated with increased accumulation of sugar and anthocyanin, reduced photosynthesis, and shoot development among others (Bouain et al. 2016). Molecular mechanism involving systemic signaling and shoot-associated responses under Pi deficiency is yet to be discovered. Pi deficiency regulates the expression of auxin-responsive transcription factors, which corroborate the auxin-mediated increase in lateral root density and inhibition of primary root length (O'Rourke et al. 2013). The downregulation of gibberellin-responsive genes was observed in Pi deficiency condition (O'Rourke et al. 2013). The expression of genes associated with ethylene and cytokinin pathways were induced under Pi deficiency (O'Rourke et al. 2013).

Pi deficiency-mediated inhibition of primary root length was due to reduced cell division and cell elongation processes in *Arabidopsis* (Svistoonoff et al. 2007). A PDR2 (phosphate deficiency response 2) gene encodes for P5-type ATPase, and the *pdr2* mutant showed a hypersensitive phenotype to Pi deficiency due to defectiveness in the viability of the meristem in root (Ticconi et al. 2009). Low-phosphate root 1 (LPR1), a protein localized in the endoplasmic reticulum, is a part of the quantitative trait loci (QTL) that affect the primary root growth and genetically interact with PDR2 to regulate meristem activity via SCARECROW (SCR) regulation (Ticconi et al. 2009). Both LPR1 and PDR2 proteins have been documented in sensing of extracellular Pi in soil (Ticconi et al. 2009). The SPX domain proteins (SPX) control the phosphate starvation response 1 (PHR1) activity in response to Pi level in rice and *Arabidopsis* (Zhou et al. 2015). Pi sensing role of SPX is yet to be established.

3.2.3 Potassium Sensing

Potassium (K^+) is the most abundant macronutrient involved in many biological processes including membrane transport, osmoregulation, and enzyme activation among others. Fluctuation in K^+ level affects many physiological processes such as transport and photosynthesis, which ultimately regulate the growth responses in plants (Hafsi et al. 2014). Due to limited concentration of K^+ in soil, plants have developed complex signaling network to sense the K^+ deficiency and activate the adaptive responses under adverse condition. Roots are the main organs to absorb K^+ from the soil; therefore, root cells are likely to play a K^+ sensing role in plants. Plant cells sense the reduction in cellular K^+ level and activate physiological, biochemical, and molecular changes to enhance K^+ uptake and K^+ homeostasis (Schachtman and Shin 2007). The concentration of K^+ regulates the membrane potential and hyperpolarization state of the membrane in root cells, which is the earliest known event during K^+ deficiency sensing (Nieves-Cordones et al. 2008). Plasma membrane-localized AHA proteins, i.e., H^+-ATPases, are responsible for the hyperpolarization of the membrane (Falhof et al. 2016).

Transcriptomic analyses of nutrient deficiencies led to the identification of many genes involved in various biological processes including transcriptional regulators. Transcriptomic analyses under K^+ deficiency identified many genes involved in K^+ acquisition and assimilation, metabolism, and regulatory responses among others (Shen et al. 2017; Zhang et al. 2017; Ma et al. 2012). Transcriptomic studies under N and P deficiency have also identified many genes involved in K^+ sensing and signaling pathways. This overlapping signal transduction may be due to similar physiological changes and adaptive responses for efficient cellular ion homeostasis.

Plant genomes encode a number of K^+ transporters and channels, and among them many of the potential candidates showed differential selectivity and affinity to K^+ (Ward et al. 2009). Shaker family AKT1 subfamily and KUP/HAK/KT transporter HAK5 include most of the K^+ transports in the studied plants (Fuchs et al. 2005; Buschmann et al. 2000; Hartje et al. 2000). Despite the functional redundancy of these AKT1 transporters, there is significant variation in the K^+ acquisition and assimilation across plant species. Root cells sense the K^+ deficiency, and therefore, the plasma membrane-localized proteins could be potential K^+ sensors to sense the changes in the environmental condition. However, there is no report of K^+ sensors in plants till today. The AKT1 involved in the influx of K^+ could function as K^+ sensor similar to NO_3^- transporter, which not only senses the NO_3^- level but is also involved in acquisition in *Arabidopsis* (Ho et al. 2009). The possible reasons for AKT1 as a K^+ sensor are (1) detection of K^+ fluctuation and efficient functioning in high and low affinities, (2) plasma membrane localization in the epidermal cells of root, (3) *akt1* mutant phenotype similar to K^+ deficiency condition, (4) absence of K^+ deficiency-induced hyperpolarization of membrane in *akt1* mutant plants, and (5) CIPK23-mediated AKT1 phosphorylation, which affects K^+ transport (Xu et al. 2006). It has been shown that K^+ binds to H^+-ATPase to regulate membrane polarization (Buch-Pedersen et al. 2006). Sensing of K^+ deficiency, possibly by AKT1, immediately slows down the ATP hydrolysis by inducing the uncoupling of plasma

membrane-localized H^+-ATPase from ATP hydrolysis reaction and initiates the hyperpolarization state of membrane in root tissues.

It has been well documented that Ca^{2+} acts as a second messenger in stress signaling pathways. Stress conditions induce ROS production, which in turn enhances Ca^{2+} accumulation to activate downstream signaling cascades in plants. K^+-deficient soil induces the accumulation of cytosolic Ca^{2+} (Allen et al. 2001), which activates the Ca^{2+} sensor for efficient K^+ accumulation (Li et al. 2006). The cyclic nucleotide-gated channel (CNGC) and glutamate receptor channel (GLR) are Ca^{2+}-permeable channels, localized in the root cells of plants (Michard et al. 2011). This clearly suggests that study of these Ca^{2+} channels during K^+ deficiency would provide new insight into K^+ sensing in plants. The activity of pyruvate kinase, a glycolytic enzyme, was regulated by cytosolic K^+ level (Ramirez-Silva et al. 2001), and K^+ deficiency condition had significantly reduced its substrate pyruvate content in cytosol (Armengaud et al. 2009). Therefore, pyruvate kinase has been proposed as an intracellular potential sensor to perceive the K^+ fluctuation inside plants (Schachtman and Shin 2007; Armengaud et al. 2009). Further investigation is needed to understand the sensing role of pyruvate kinase and related enzymes as K^+ sensors in plants.

3.3 Sensing Gaseous Atmosphere

3.3.1 CO_2 Sensing

Stomatal movement and their development are regulated by CO_2 levels, which directly affect gaseous exchange and stomatal conductance in plants. Low concentration of CO_2 stimulates the opening of stomatal apertures, whereas CO_2 concentration above threshold level promotes the closure of stomatal apertures in plants. The elevated atmospheric CO_2 level enhances the concentration of leaf internal CO_2 (Ci), which represses the stomatal development in plants (Engineer et al. 2016; Santrucek et al. 2014). The guard cells and mesophyll tissues can sense CO_2 level in plants. In most of the plant species, changes in the leaf CO_2 level regulate the aperture of stomatal pores; however, similar phenomena were not observed under increased CO_2 level in a few plant species (Ferris and Taylor 1994). The cellular Ci level depends upon light condition, and a significant increase in leaves Ci level was observed in the night due to respiration, whereas this Ci level rapidly drops in daylight condition (Hanstein et al. 2001). The negative effect of increased CO_2 level is the reduction in total numbers of stomata per unit leaf area and rate of stomatal conductance in plants. The long-term effect of CO_2 is the reduced development of stomata in the leaf epidermis. Decrease in the stomatal conductance protects water loss from leaves (Keenan et al. 2013). Under drought condition, increased CO_2 levels promote heat stress in the leaf due to less evapotranspiration caused by either more closed stomata or less number of stomata present in the leaf (Long and Ort 2010). It has been reported that the higher stomatal conductance can be correlated with better crop performance (Bahar et al. 2009), and therefore reduced stomatal

conductance by elevated CO_2 may be responsible for poor agronomical performance of the crop.

Plant hormone abscisic acid (ABA) is known to regulate stomatal movement and development, and ABA promotes the CO_2 responses in stomata. ABA-insensitive mutants such as *abi1-1* and *abi2-1* showed conditional insensitivity to CO_2 level (Leymarie et al. 1998), whereas partial stomatal response was observed in the case of ABA receptors PYR/RCAR mutants (Merilo et al. 2013). There are three types of plant carbonic anhydrases, alpha, beta and gamma, and among them beta carbonic anhydrases play an important role in CO_2-regulated stomatal movements (Hu et al. 2010). However, the functions of alpha and gamma classes of carbonic anhydrases are needed to be characterized for their CO_2-mediated stomatal regulation in plants. The genetic complementation experiment of carbonic anhydrase double mutants with mammalian carbonic anhydrase restored the wild-type response in *Arabidopsis* (Hu et al. 2010), suggesting the importance of carbonic anhydrase catalytic activity in CO_2 sensing mechanism. Recently, RHC1, a MATE transporter-like protein, has been identified as a bicarbonate sensor (Tian et al. 2015), which may play an important role in CO_2 sensing and signaling. Photosynthesis reduces the Ci level and indirectly controls the CO_2-mediated regulation of stomatal pore in leaves. Though the direct sensing of CO_2 is not known as there are no mutants showing insensitivity to CO_2 level, studies have shown that guard cells (Young et al. 2006) and mesophyll cells (Mott et al. 2008) are involved in direct CO_2 sensing. It is known that Ci affects stomatal conductance than external CO_2 present on the leaf surface. A limited response of CO_2 was observed in the stomata isolated from epidermal tissues whereas increased CO_2 response for mesophyll stomata, suggesting the role of mesophyll tissue CO_2 sensing and signaling (Mott et al. 2008). Further, stomatal response to CO_2 was reversible when mesophyll tissues and leaf epidermis tissues were used together in the experiment (Mott et al. 2008). It was proposed that these responses may involve diffusible small substances like ABA, sugar, or malate (Lawson et al. 2014). Synergistic role of ABA in the CO_2 response is well documented. It has been shown that elevated CO_2 levels inhibit the stomatal development in *Arabidopsis* and this reduced stomatal development was observed in different plant species, suggesting the regulatory role of CO_2 in stomata development. The *hic* mutant, encoding for a putative 3-keto acyl coenzyme A synthase, defective in cell wall wax biosynthesis showed the production of higher number of stomata at elevated CO_2 level (Gray et al. 2000). Further, mutants defective in cell wall wax deposition also showed a defect in stomatal development (Jenks et al. 1995). The signals responsible for stomatal density changes are not known, and it was hypothesized that cuticular waxes may affect the movement of diffusible signals. Carbonic anhydrase mutants also showed increased stomatal development at increased CO_2 levels (Engineer et al. 2014). The *epf2* mutant, encoding for epidermal patterning factor gene EPF2, also showed opposite development of stomata at elevated CO_2 levels (Engineer et al. 2014). EPF2 gene binds to ERECTA receptor kinase to regulate stomatal index, which in turn affects water use efficiency in plants (Masle et al. 2005). It has been shown that the CRSP protease can cleave the EPF2 pro-peptide to produce active EPF2. Mutants of EPF2, CRSP, and carbonic anhydrases (CA1

and CA4) showed similar stomatal development phenotype in response to increased CO2 level (Engineer et al. 2014). The exact mechanism involving ERECTA, EPF2, CRSP and carbonic anhydrases in stomatal development at elevated CO_2 level is hitherto undiscovered.

3.3.2 Oxygen Sensing

Cellular energy status is regulated through the ATP pool generated by oxidative phosphorylation reaction and molecular di-oxygen (O_2) is required for efficient ATP production in all aerobic organisms. Oxygen acts as an electron acceptor in the electron transport chain reaction that operates inside the mitochondria. When cellular oxygen level drops below the threshold level, the cell senses the altered oxygen level and modulates the expression of genes associated with metabolic and energy consumption processes, which ultimately regulate the growth and development of plants. Plant cells encounter oxygen-limited condition during seed germination and fruit development which could be due to the high rate of metabolic processes and/or slow diffusion of oxygen into highly active meristematic cells (van Dongen and Licausi 2015; Bailey-Serres et al. 2012). Depleted oxygen level inside the cell could be directly sensed by receptor/sensor proteins interacting with the oxygen molecule, which are not yet established in plants. An indirect sensing mechanism may be activated by either fluctuations in energy levels or redox homeostasis involving the formation of nitric oxide (NO), hydrogen peroxide (H_2O_2), and other ROS species in cells (van Dongen and Licausi 2015).

Cells present in the different organs of plants respond differently to the depleted oxygen level. For example, low oxygen level induces high expression of ADH1 in the roots as compared to shoots in *Arabidopsis* (Ismond et al. 2003). It has been observed that roots show tolerance to low oxygen levels by regulating the ethanol fermentation process, whereas such a phenomenon for tolerance has not been detected in the aerial parts of *Arabidopsis* (Ellis et al. 1999). These findings clearly suggest that different oxygen sensing mechanisms are operated in the root and shoot, which required further investigations to delineate the exact mechanism. Another survival strategy for oxygen-depleted condition is the long-distance signaling involving oxygen transport from the areal organ to root (Drew 1997). Oxygen deprivation condition is often encountered during flooding conditions, which create oxygen deprivation condition by reducing the diffusion of oxygen. Submergence of plant creates hypoxia conditions, which promote the transportation of ACC from the root to shoot for the production of ethylene in the presence of oxygen (Shiu et al. 1998). During complete submergence, oxygen deficiency depends on (1) photosynthesis-dependent oxygen replenishment, (2) inward movement of water, and (3) higher metabolic activity for oxygen consumption. Effect of submergence/waterlogging-induced hypoxia is less effective in case of plants like rice, due to the presence of aerenchyma that helps in the gaseous transportation from the submerged region to the aerial region. Lack of aerenchyma in many plants rapidly induces cellular oxygen deficit status during submergence (Voesenek et al. 2006). Mitochondrial

respiration is affected by the reduced level of cellular oxygen, which in turn affects the energy-dependent processes by inhibiting the production of cellular ATP pool (Howell et al. 2007). Cell enhances the oxidative phosphorylation reaction via carbohydrate metabolism to meet the consistently increasing demands for ATP to maintain the proper functioning of associated cellular processes (Banti et al. 2013). In mammals, the transcription factor hypoxia-inducible factor (HIF) 1a/b is directly regulated during oxygen sensing (Kaelin and Ratcliffe 2008). Prolyl hydroxylase-mediated hydroxylation of HIF1a controls its nuclear localization and transcriptional activation function during low oxygen condition (Kaelin and Ratcliffe 2008). Despite the presence of prolyl hydroxylases in plants, such evidence for direct oxygen sensing is lacking due to the absence of HIF1a homologs (Mustroph et al. 2010). Sucrose non-fermenting 1 (SNF1)/AMP-activated protein kinases have been implemented to sense the energy status in animals (Carling et al. 2011). In plants, such kinases, viz., KIN10 and KIN11, have been implemented to cellular energy level in low oxygen condition (Baena-Gonzalez et al. 2007). Sucrose signaling is discussed in detail in Chap. 13.

Another important class of sensing proteins includes the APETALA2 (AP2) domain-containing group VII ERF TFs, which have been shown to regulate low-oxygen responses in plants (van Dongen and Licausi 2015). SUB1A, the group VII ERFs, has been shown to fine-tune gene expressions in hypoxia condition generated during submergence. Further, hypoxia-responsive genes (HER1 and HER2) and knockout mutants (*hre1hre2*) have been characterized for their roles in the seedling survival during oxygen-lacking condition in *Arabidopsis* (Hess et al. 2011). Biochemical, molecular, and genetic characterization of group VII ERF TFs and other related important genes would provide more information about direct and indirect sensing in plants.

3.4 Conclusion

In conclusion, this chapter summarizes the recent findings primarily associated with sensing mechanism and physiological consequences in the regulation of nutrients (NPK), CO_2, and O_2. Studies of the past two decades have provided new insights into signaling mechanisms and adaptive responses, which led to the identification of unique and overlapping signaling responses and associated marker genes in plants (Fig. 3.1). The basic understanding of nitrate sensing pathways has been established, but other nutrient sensors are still not clear. The use of genome-wide association study (GWAS) and other functional genomics techniques will help to characterize these unknown sensors and their NutUE. The ROS, Ca^{2+}, metabolic products, and phytohormones constitute the common components in all the studied signaling pathways. Understanding and integration of these overlapping and unique signaling components will help in better understanding of plant responses to changing nutrient levels in the underground environment and oxygen and carbon dioxide in the atmosphere and how plants coordinate and integrate all the information for sustaining energy requirement for their survival.

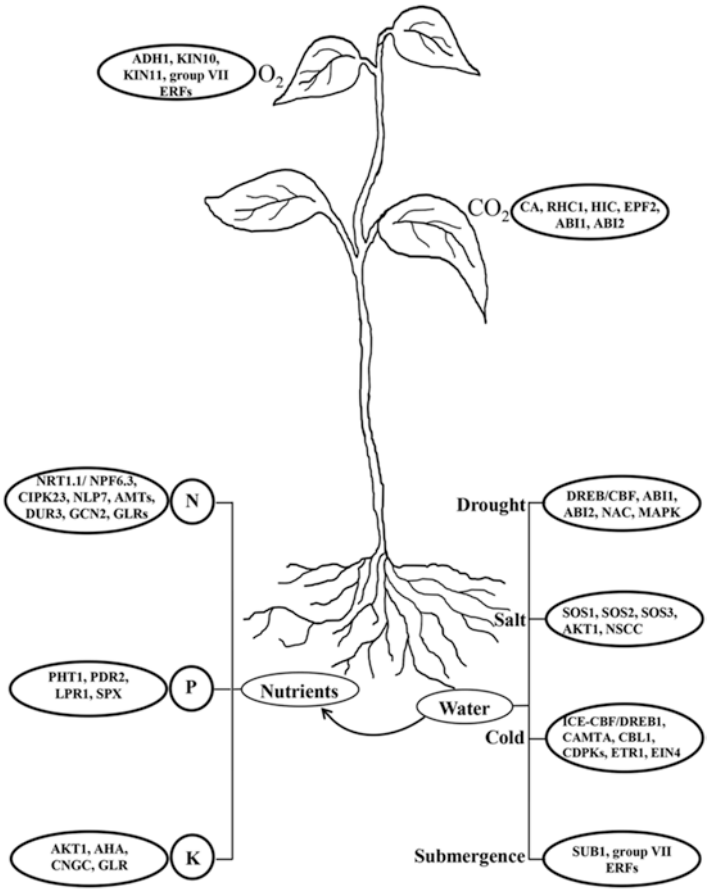

Fig. 3.1 Schematic representation of potential sensing/signaling genes associated with various responses in plants. Environmental factors affecting the corresponding key gene expressions are shown. *ADH1* alcohol dehydrogenase 1, *KIN10* SNF1 kinase homolog 10, *KIN11* SNF1 kinase homolog 11, *group VII ERFs* group VII ethylene response factors, *CA* carbonic anhydrase, *RHC1* resistant to high CO2 1, *HIC* high carbon dioxide, *EPF2* epidermal patterning factor gene 2, *ABI1* abscisic acid-insensitive 1, *ABI2* abscisic acid-insensitive 2, *NRT1.1* nitrate transporter 1.1, *CIPK23* CBL-interacting serine/threonine protein kinase 23, *NLP7* NIN-like protein 7, *AMTs* ammonium transporters, *DUR3* degradation of urea 3, *GCN2* general control non-repressible 2, *GLRs* glutamate receptor channels, *PHT1* phosphate transporter 1, *PDR2* phosphate deficiency response 2, *LPR1* low-phosphate root 1, *SPX* SPX domain proteins, *AKT1 Arabidopsis* K^+ transporter 1, *AHA Arabidopsis* H^+-ATPase, *CNGC* cyclic nucleotide gated channel, *DREB/CBF* dehydration-responsive element-binding protein/C-repeat binding factor, *NAC* NAM/ATAF/CUC transcription factors, *SOS1* salt overly sensitive 1, *SOS2* salt overly sensitive 2, *SOS3* salt overly sensitive 3, *NSCC* non-selective cation channel, *ICE-CBF/DREB1* inducer of cbf expression (ICE)-C-repeat binding factor/DRE binding factor1, *CAMTA* calmodulin-binding transcription activators (CAMTA) factors, *CBL1* calcineurin B-like protein 1, *CDPKs* calcium-dependent protein kinases, *ETR1* ethylene response 1, *EIN4* ethylene-insensitive 4, *SUB1* submergence-tolerant 1

Acknowledgments The work in NR lab was supported by research grants [GGSIPU/DRC/PhD/Adm/2016/1549] and [38(1246)/10/EMRII] from GGS Indraprastha University and Council of Scientific and Industrial Research (CSIR), respectively. DKJ was supported by a fellowship from the Indo-UK Virtual Nitrogen Centre on Nitrogen Efficiency of Whole-cropping Systems (NEWS) BT/IN/UK-VNC/44/NR/2015-16.

References

Ahn CS, Han JA, Lee HS, Lee S, Pai HS (2011) The PP2A regulatory subunit Tap46, a component of the TOR signaling pathway, modulates growth and metabolism in plants. Plant Cell 23:185–209

Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffmann T, Tang YY, Grill E, Schroeder JI (2001) A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature 411:1053–1057

Arcondeguy T, Jack R, Merrick M (2001) P(II) signal transduction proteins, pivotal players in microbial nitrogen control. Microbiol Mol Biol Rev 65:80–105

Armengaud P, Sulpice R, Miller AJ, Stitt M, Amtmann A, Gibon Y (2009) Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in Arabidopsis roots. Plant Physiol 150:772–785

Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signalling. Nature 448:938–942

Bahar B, Yildirim M, Barutcular C (2009) Relationships between stomatal conductance and yield components in spring durum wheat under Mediterranean conditions. Not Bot Horti Agrobot Cluj Napoca 37:45–48

Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LA, van Dongen JT (2012) Making sense of low oxygen sensing. Trends Plant Sci 17:129–138

Banti V, Giuntoli B, Gonzali S, Loreti E, Magneschi L, Novi G, Paparelli E, Parlanti S, Pucciariello C, Santaniello A, Perata P (2013) Low oxygen response mechanisms in green organisms. Int J Mol Sci 14:4734–4761

Bayle V, Arrighi JF, Creff A, Nespoulous C, Vialaret J, Rossignol M, Gonzalez E, Paz-Ares J, Nussaume L (2011) Arabidopsis thaliana high-affinity phosphate transporters exhibit multiple levels of posttranslational regulation. Plant Cell 23:1523–1535

Bouain N, Doumas P, Rouached H (2016) Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in Arabidopsis. Curr Genomics 17:308–304

Buch-Pedersen MJ, Rudashevskaya EL, Berner TS, Venema K, Palmgren MG (2006) Potassium as an intrinsic uncoupler of the plasma membrane H+-ATPase. J Biol Chem 281:38285–38292

Buschmann PH, Vaidyanathan R, Gassmann W, Schroeder JI (2000) Enhancement of Na(+) uptake currents, time-dependent inward-rectifying K(+) channel currents, and K(+) channel transcripts by K(+) starvation in wheat root cells. Plant Physiol 122:1387–1397

Carling D, Mayer FV, Sanders MJ, Gamblin SJ (2011) AMP-activated protein kinase: nature's energy sensor. Nat Chem Biol 7:512–518

Castaings L, Camargo A, Pocholle D, Gaudon V, Texier Y, Boutet-Mercey S, Taconnat L, Renou JP, Daniel-Vedele F, Fernandez E, Meyer C, Krapp A (2009) The nodule inception-like protein 7 modulates nitrate sensing and metabolism in Arabidopsis. Plant J 57:426–435

Chantranupong L, Wolfson RL, Sabatini DM (2015) Nutrient-sensing mechanisms across evolution. Cell 161:67–83

Chellamuthu VR, Ermilova E, Lapina T, Luddecke J, Minaeva E, Herrmann C, Hartmann MD, Forchhammer K (2014) A widespread glutamine-sensing mechanism in the plant kingdom. Cell 159:1188–1199

Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X (2016) Shoot-to-root Mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr Biol 26:640–646

Chevalier F, Pata M, Nacry P, Doumas P, Rossignol M (2003) Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between Arabidopsis accessions. Plant Cell Environ 26:1839–1850

Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol 62:185–206

De Pessemier J, Chardon F, Juraniec M, Delaplace P, Hermans C (2013) Natural variation of the root morphological response to nitrate supply in Arabidopsis thaliana. Mech Dev 130:45–53

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016) TOR signaling and nutrient sensing. Annu Rev Plant Biol 67:261–285

Drew MC (1997) OXYGEN DEFICIENCY AND ROOT METABOLISM: injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223–250

Ellis MH, Dennis ES, Peacock WJ (1999) Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. Plant Physiol 119:57–64

Engineer CB, Ghassemian M, Anderson JC, Peck SC, Hu H, Schroeder JI (2014) Carbonic anhydrases, EPF2 and a novel protease mediate CO2 control of stomatal development. Nature 513:246–250

Engineer CB, Hashimoto-Sugimoto M, Negi J, Israelsson-Nordstrom M, Azoulay-Shemer T, Rappel WJ, Iba K, Schroeder JI (2016) CO2 sensing and CO2 regulation of stomatal conductance: advances and open questions. Trends Plant Sci 21:16–30

Falhof J, Pedersen JT, Fuglsang AT, Palmgren M (2016) Plasma membrane H(+)-ATPase regulation in the center of plant physiology. Mol Plant 9:323–337

Ferris R, Taylor G (1994) Stomatal characteristics of four native herbs following exposure to elevated CO_2. Ann Bot 73:447–453

Fuchs I, Stolzle S, Ivashikina N, Hedrich R (2005) Rice K+ uptake channel OsAKT1 is sensitive to salt stress. Planta 221:212–221

Gan Y, Bernreiter A, Filleur S, Abram B, Forde BG (2012) Overexpressing the ANR1 MADS-box gene in transgenic plants provides new insights into its role in the nitrate regulation of root development. Plant Cell Physiol 53:1003–1016

Gent L, Forde BG (2017) How do plants sense their nitrogen status? J Exp Bot 68:2531–2539

Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, Schuch W, Hetherington AM (2000) The HIC signalling pathway links CO2 perception to stomatal development. Nature 408:713–716

Hafsi C, Debez A, Abdelly C (2014) Potassium deficiency in plants: effects and signaling cascades. Acta Physiol Plant 36:1055–1070

Hanstein S, de Beer D, Felle HH (2001) Miniaturised carbon dioxide sensor designed for measurements within plant leaves. Sensors Actuators B Chem 81:107–114

Hartje S, Zimmermann S, Klonus D, Mueller-Roeber B (2000) Functional characterisation of LKT1, a K+ uptake channel from tomato root hairs, and comparison with the closely related potato inwardly rectifying K+ channel SKT1 after expression in Xenopus oocytes. Planta 210:723–731

Hess N, Klode M, Anders M, Sauter M (2011) The hypoxia responsive transcription factor genes ERF71/HRE2 and ERF73/HRE1 of Arabidopsis are differentially regulated by ethylene. Physiol Plant 143:41–49

Ho CH, Lin SH, Hu HC, Tsay YF (2009) CHL1 functions as a nitrate sensor in plants. Cell 138:1184–1194

Hoque MS, Masle J, Udvardi MK, Ryan PR, Upadhyaya NM (2006) Over-expression of the rice OsAMT1-1 gene increases ammonium uptake and content, but impairs growth and development of plants under high ammonium nutrition. Funct Plant Biol 33:153–163

Howell KA, Cheng K, Murcha MW, Jenkin LE, Millar AH, Whelan J (2007) Oxygen initiation of respiration and mitochondrial biogenesis in rice. J Biol Chem 282:15619–15631

Hu H, Boisson-Dernier A, Israelsson-Nordstrom M, Bohmer M, Xue S, Ries A, Godoski J, Kuhn JM, Schroeder JI (2010) Carbonic anhydrases are upstream regulators of CO2-controlled stomatal movements in guard cells. Nat Cell Biol 12:87–93; sup pp 1–18

Ismond KP, Dolferus R, de Pauw M, Dennis ES, Good AG (2003) Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. Plant Physiol 132:1292–1302

Jenks MA, Tuttle HA, Eigenbrode SD, Feldmann KA (1995) Leaf epicuticular waxes of the eceriferum mutants in Arabidopsis. Plant Physiol 108:369–377

Kaelin WG Jr, Ratcliffe PJ (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 30:393–402

Kang J, Turano FJ (2003) The putative glutamate receptor 1.1 (AtGLR1.1) functions as a regulator of carbon and nitrogen metabolism in Arabidopsis thaliana. Proc Natl Acad Sci U S A 100:6872–6877

Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson AD (2013) Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. Nature 499:324–327

Kojima S, Bohner A, Gassert B, Yuan L, von Wiren N (2007) AtDUR3 represents the major transporter for high-affinity urea transport across the plasma membrane of nitrogen-deficient Arabidopsis roots. Plant J 52:30–40

Krouk G, Crawford NM, Coruzzi GM, Tsay YF (2010) Nitrate signaling: adaptation to fluctuating environments. Curr Opin Plant Biol 13:266–273

Lan P, Li W, Schmidt W (2012) Complementary proteome and transcriptome profiling in phosphate-deficient Arabidopsis roots reveals multiple levels of gene regulation. Mol Cell Proteomics 11:1156–1166

Lawson T, Simkin AJ, Kelly G, Granot D (2014) Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour. New Phytol 203:1064–1081

Leymarie J, Vavasseur A, Lascève G (1998) CO2 sensing in stomata of abi1-1 and abi2-1 mutants of Arabidopsis thaliana. Plant Physiol Biochem 36:539–543

Li L, Kim BG, Cheong YH, Pandey GK, Luan S (2006) A ca(2)+ signaling pathway regulates a K(+) channel for low-K response in Arabidopsis. Proc Natl Acad Sci U S A 103:12625–12630

Li T, Zhang W, Yin J, Chadwick D, Norse D, Lu Y, Liu X, Chen X, Zhang F, Powlson D, Dou Z (2018a) Enhanced-efficiency fertilizers are not a panacea for resolving the nitrogen problem. Glob Chang Biol 24:e511–e521

Li N, Zhang SJ, Zhao Q, Long Y, Guo H, Jia HF, Yang YX, Zhang HY, Ye XF, Zhang ST (2018b) Overexpression of tobacco GCN2 stimulates multiple physiological changes associated with stress tolerance. Front Plant Sci 9:725

Liu Q, Han R, Wu K, Zhang J, Ye Y, Wang S, Chen J, Pan Y, Li Q, Xu X, Zhou J, Tao D, Wu Y, Fu X (2018) G-protein betagamma subunits determine grain size through interaction with MADS-domain transcription factors in rice. Nat Commun 9:852

Long SP, Ort DR (2010) More than taking the heat: crops and global change. Curr Opin Plant Biol 13:241–248

Ludewig U, Neuhauser B, Dynowski M (2007) Molecular mechanisms of ammonium transport and accumulation in plants. FEBS Lett 581:2301–2308

Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol 156:1041–1049

Ma TL, Wu WH, Wang Y (2012) Transcriptome analysis of rice root responses to potassium deficiency. BMC Plant Biol 12:161

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature 436:866–870

Merilo E, Laanemets K, Hu H, Xue S, Jakobson L, Tulva I, Gonzalez-Guzman M, Rodriguez PL, Schroeder JI, Brosche M, Kollist H (2013) PYR/RCAR receptors contribute to ozone-, reduced air humidity-, darkness-, and CO2-induced stomatal regulation. Plant Physiol 162:1652–1668

Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliham M, Liu LH, Obermeyer G, Feijo JA (2011) Glutamate receptor-like genes form Ca2+ channels in pollen tubes and are regulated by pistil D-serine. Science 332:434–437

Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM (2007) Nitrate transport and signalling. J Exp Bot 58:2297–2306

Misyura M, Guevara D, Subedi S, Hudson D, McNicholas PD, Colasanti J, Rothstein SJ (2014) Nitrogen limitation and high density responses in rice suggest a role for ethylene under high density stress. BMC Genomics 15:681

Mlodzinska E, Zboinska M (2016) Phosphate uptake and allocation – a closer look at Arabidopsis thaliana L. and Oryza sativa L. Front Plant Sci 7:1198

Mott KA, Sibbernsen ED, Shope JC (2008) The role of the mesophyll in stomatal responses to light and CO2. Plant Cell Environ 31:1299–1306

Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J (2010) Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. Plant Physiol 152:1484–1500

Nagarajan VK, Smith AP (2012) Ethylene's role in phosphate starvation signaling: more than just a root growth regulator. Plant Cell Physiol 53:277–286

Nieves-Cordones M, Miller AJ, Aleman F, Martinez V, Rubio F (2008) A putative role for the plasma membrane potential in the control of the expression of the gene encoding the tomato high-affinity potassium transporter HAK5. Plant Mol Biol 68:521–532

Noguero M, Lacombe B (2016) Transporters involved in root nitrate uptake and sensing by Arabidopsis. Front Plant Sci 7:1391

O'Brien JA, Vega A, Bouguyon E, Krouk G, Gojon A, Coruzzi G, Gutierrez RA (2016) Nitrate transport, sensing, and responses in plants. Mol Plant 9:837–856

O'Rourke JA, Yang SS, Miller SS, Bucciarelli B, Liu J, Rydeen A, Bozsoki Z, Uhde-Stone C, Tu ZJ, Allan D, Gronwald JW, Vance CP (2013) An RNA-Seq transcriptome analysis of orthophosphate-deficient white lupin reveals novel insights into phosphorus acclimation in plants. Plant Physiol 161:705–724

Popova Y, Thayumanavan P, Lonati E, Agrochao M, Thevelein JM (2010) Transport and signaling through the phosphate-binding site of the yeast Pho84 phosphate transceptor. Proc Natl Acad Sci U S A 107:2890–2895

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol 50:665–693

Ramirez-Silva L, Ferreira ST, Nowak T, Tuena de Gomez-Puyou M, Gomez-Puyou A (2001) Dimethylsulfoxide promotes K+-independent activity of pyruvate kinase and the acquisition of the active catalytic conformation. Eur J Biochem 268:3267–3274

Robinson WD, Park J, Tran HT, Del Vecchio HA, Ying S, Zins JL, Patel K, McKnight TD, Plaxton WC (2012) The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by Arabidopsis thaliana. J Exp Bot 63:6531–6542

Santrucek J, Vrablova M, Simkova M, Hronkova M, Drtinova M, Kveton J, Vrabl D, Kubasek J, Mackova J, Wiesnerova D, Neuwithova J, Schreiber L (2014) Stomatal and pavement cell density linked to leaf internal CO2 concentration. Ann Bot 114:191–202

Schachtman DP, Shin R (2007) Nutrient sensing and signaling: NPKS. Annu Rev Plant Biol 58:47–69

Shen C, Wang J, Shi X, Kang Y, Xie C, Peng L, Dong C, Shen Q, Xu Y (2017) Transcriptome analysis of differentially expressed genes induced by low and high potassium levels provides insight into fruit sugar metabolism of pear. Front Plant Sci 8:938

Shimizu A, Yanagihara S, Kawasaki S, Ikehashi H (2004) Phosphorus deficiency-induced root elongation and its QTL in rice (Oryza sativa L.). Theor Appl Genet 109:1361–1368

Shin SY, Jeong JS, Lim JY, Kim T, Park JH, Kim JK, Shin C (2018) Transcriptomic analyses of rice (Oryza sativa) genes and non-coding RNAs under nitrogen starvation using multiple omics technologies. BMC Genomics 19:532

Shiu OY, Oetiker JH, Yip WK, Yang SF (1998) The promoter of LE-ACS7, an early flooding-induced 1-aminocyclopropane-1-carboxylate synthase gene of the tomato, is tagged by a Sol3 transposon. Proc Natl Acad Sci U S A 95:10334–10339

Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057

Sonoda Y, Ikeda A, Saiki S, von Wiren N, Yamaya T, Yamaguchi J (2003) Distinct expression and function of three ammonium transporter genes (OsAMT1;1-1;3) in rice. Plant Cell Physiol 44:726–734

Stevenson-Paulik J, Bastidas RJ, Chiou ST, Frye RA, York JD (2005) Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. Proc Natl Acad Sci U S A 102:12612–12617

Sugiyama K, Hayakawa T, Kudo T, Ito T, Yamaya T (2004) Interaction of N-acetylglutamate kinase with a PII-like protein in rice. Plant Cell Physiol 45:1768–1778

Sun L, Di D, Li G, Kronzucker HJ, Shi W (2017) Spatio-temporal dynamics in global rice gene expression (Oryza sativa L.) in response to high ammonium stress. J Plant Physiol 212:94–104

Sutton MA, Bleeker A, Howard C, Erisman J, Abrol Y, Bekunda M, Datta A, Davidson E, de Vries W, Oenema O (2013) Our nutrient world. The challenge to produce more food & energy with less pollution. Centre for Ecology & Hydrology, Edinburgh

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T (2007) Root tip contact with low-phosphate media reprograms plant root architecture. Nat Genet 39:792–796

Tapken D, Anschutz U, Liu LH, Huelsken T, Seebohm G, Becker D, Hollmann M (2013) A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids. Sci Signal 6:ra47

Thibaud MC, Arrighi JF, Bayle V, Chiarenza S, Creff A, Bustos R, Paz-Ares J, Poirier Y, Nussaume L (2010) Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. Plant J 64:775–789

Tian W, Hou C, Ren Z, Pan Y, Jia J, Zhang H, Bai F, Zhang P, Zhu H, He Y, Luo S, Li L, Luan S (2015) A molecular pathway for CO(2) response in Arabidopsis guard cells. Nat Commun 6:6057

Ticconi CA, Lucero RD, Sakhonwasee S, Adamson AW, Creff A, Nussaume L, Desnos T, Abel S (2009) ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. Proc Natl Acad Sci U S A 106:14174–14179

Tsay YF, Ho CH, Chen HY, Lin SH (2011) Integration of nitrogen and potassium signaling. Annu Rev Plant Biol 62:207–226

Tyburski J, Dunajska K, Tretyn A (2009) Reactive oxygen species localization in roots of Arabidopsis thaliana seedlings grown under phosphate deficiency. Plant Growth Regul 59:27–36

van Dongen JT, Licausi F (2015) Oxygen sensing and signaling. Annu Rev Plant Biol 66:345–367

Voesenek LA, Colmer TD, Pierik R, Millenaar FF, Peeters AJ (2006) How plants cope with complete submergence. New Phytol 170:213–226

von Wiren N, Gazzarrini S, Gojon A, Frommer WB (2000) The molecular physiology of ammonium uptake and retrieval. Curr Opin Plant Biol 3:254–261

Wang WH, Kohler B, Cao FQ, Liu GW, Gong YY, Sheng S, Song QC, Cheng XY, Garnett T, Okamoto M, Qin R, Mueller-Roeber B, Tester M, Liu LH (2012) Rice DUR3 mediates high-affinity urea transport and plays an effective role in improvement of urea acquisition and utilization when expressed in Arabidopsis. New Phytol 193:432–444

Wang YY, Cheng YH, Chen KE, Tsay YF (2018a) Nitrate transport, signaling, and use efficiency. Annu Rev Plant Biol 69:85–122

Wang F, Deng M, Xu J, Zhu X, Mao C (2018b) Molecular mechanisms of phosphate transport and signaling in higher plants. Semin Cell Dev Biol 74:114–122

Ward JM, Maser P, Schroeder JI (2009) Plant ion channels: gene families, physiology, and functional genomics analyses. Annu Rev Physiol 71:59–82

Wei Z, Zeng X, Qin C, Wang Y, Bai L, Xu Q, Yuan H, Tang Y, Nyima T (2016) Comparative transcriptome analysis revealed genes commonly responsive to varied nitrate stress in leaves of Tibetan hulless barley. Front Plant Sci 7:1067

Weiland M, Mancuso S, Baluska F (2016) Signalling via glutamate and GLRs in Arabidopsis thaliana. Funct Plant Biol 43:1–25

Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, Wu WH (2006) A protein kinase, interacting with two calcineurin B-like proteins, regulates K+ transporter AKT1 in Arabidopsis. Cell 125:1347–1360

Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghothama KG, Levy AA, Silber A (2007) Functional characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced expression. J Exp Bot 58:2491–2501

Yang SY, Gronlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika H, Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski U (2012) Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the phosphate transporter1 gene family. Plant Cell 24:4236–4251

Yang SY, Hao DL, Song ZZ, Yang GZ, Wang L, Su YH (2015) RNA-Seq analysis of differentially expressed genes in rice under varied nitrogen supplies. Gene 555:305–317

Young JJ, Mehta S, Israelsson M, Godoski J, Grill E, Schroeder JI (2006) CO(2) signaling in guard cells: calcium sensitivity response modulation, a Ca(2+)-independent phase, and CO(2) insensitivity of the gca2 mutant. Proc Natl Acad Sci U S A 103:7506–7511

Zhang H, Forde BG (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 279:407–409

Zhang X, Jiang H, Wang H, Cui J, Wang J, Hu J, Guo L, Qian Q, Xue D (2017) Transcriptome analysis of rice seedling roots in response to potassium deficiency. Sci Rep 7:5523

Zhao L, Zhang W, Yang Y, Li Z, Li N, Qi S, Crawford NM, Wang Y (2018) The Arabidopsis NLP7 gene regulates nitrate signaling via NRT1.1-dependent pathway in the presence of ammonium. Sci Rep 8:1487

Zhou Z, Wang Z, Lv Q, Shi J, Zhong Y, Wu P, Mao C (2015) SPX proteins regulate Pi homeostasis and signaling in different subcellular level. Plant Signal Behav 10:e1061163

Dinesh Kumar Jaiswal received his Ph.D. from the National Institute of Plant Genome Research (NIPGR), New Delhi, India. During doctoral training, he studied dehydration-responsive proteomes in crop plants such as chickpea and rice. He was a Postdoctoral Research Associate in the laboratory of Prof. Alan M. Jones at the University of North Carolina, Chapel Hill, USA, where he worked on activation mechanism of G-protein signaling in *Arabidopsis*. He is working as a project Research Scientist with the author. Currently, he is interested to understand the role of heterotrimeric G-protein complexes in nutrient regulation.

Nandula Raghuram obtained his doctoral degree from JNU, New Delhi (with the Editor), on light-mediated signal transduction and nitrate reductase gene expression in maize. He did his postdoctoral training in photomorphogenesis from JNU. He joined as a Senior Lecturer (Assistant Professor) in the Department of Life Sciences, University of Mumbai, and later joined Guru Gobind Singh Indraprastha University, New Delhi. He was INSA-Royal Society Visiting Fellow at IACR-LARS, Long Ashton, UK, and Visiting Research Scientist at the Institute of Arable Crops Research, Long Ashton, UK. Currently, he is a Professor in the University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi. As a Co-founder of the Indian Nitrogen Group, Director of the South Asian Nitrogen Centre, and Steering Committee member of the UNEP Global Partnership on Nutrient Management, he facilitated interdisciplinary international consultations on the research and policy aspects of reactive nitrogen and other nutrients in agriculture, industry, and environment. He is Editor-in-Chief of the Springer journal *Physiology and Molecular Biology of Plants* and Elected Chair of the International Nitrogen Initiative. The main research focus of his lab has been in the functional biology of nitrogen metabolism in rice and spirulina (*Arthrospira*), using a combination of biochemical, molecular, genetic, genomic, and bioinformatic approaches. Another area of interest has been in the functional genomics of G-protein and GPCR signaling in rice and *Arabidopsis*.

Water Sensing in Plants

4

Hillel Fromm and Yosef Fichman

This water was indeed a different thing from ordinary nourishment. Its sweetness was born of the walk under the stars, the song of the pulley, the effort of my arms. It was good for the heart, like a present. The Little Prince (Antoine de Saint-Exupéry).

Abstract

Water is a key factor in plant life. Therefore, reaching and holding water is a crucial part in plant survival. Plants sense water through a set of sensors which includes sensors for water activity (potential), for specific components of water potential, or for specific solutes contributing to water potential and for hydraulic signals. While these sensors are common to different plants and other organisms, their functions and modes of action are yet far from being understood. It is also unknown how these sensing mechanisms are linked to cellular and whole-plant responses to changes in water status in the soil or in the atmosphere. Advanced technologies that would provide means for single-cell physiological manipulations together with high-throughput noninvasive real-time monitoring systems of shoots and roots and advanced biochemistry and structural studies at atomic resolution of sensor proteins and protein complexes are imperative for understanding water sensing by plants.

Keywords

Cell wall integral (CWI) signaling · Extracellular matrix (ECM) · Hydraulic pressure · Hydrotropism · Mechanosensors · Osmosensing · Receptor-like wall-associated kinases (WAKs)

H. Fromm (✉) · Y. Fichman
School of Plant Sciences and Food Security, Faculty of Life Sciences,
Tel Aviv University, Tel Aviv, Israel
e-mail: hillelf@tauex.tau.ac.il

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_4

4.1 Introduction: The Roles of Water in Plant Biology

The life of plants like that of all other organisms relies on biochemistry in an aqueous medium with nearly 80% of the body composed of water under optimal conditions. However, water has many more functions in plants than just being the milieu where biochemical reactions occur. Water is the source of electrons in light-driven photosynthesis, from which energy is funneled to fix carbon. Water pressure is responsible for plant stature; it drives cell expansion, stomata opening, and burst of the pollen tube tip to release male gametes. Water pressure is also responsible to drive the distribution of solutes and signaling molecules throughout the plant via the phloem as suggested by the "'Pressure-Flow" model (reviewed by De Schepper et al. 2013). Water is driven from the soil through the roots and then throughout the plant's shoot to the atmosphere via the xylem by the driving force generated from the differences in water potentials between plant tissues and the outer environment, as suggested by the Cohesion-Tension model (reviewed by Steudle 2001), although the model has been challenged over the years (Steudle 2001; Bentrup 2017). This transport of water is also crucial for plant cooling (Cook et al. 1964). Water transport in plants is also being used to remove hazardous chemicals such as heavy metals (Lasat et al. 2000) and salt (Wilson et al. 2017) either to subcellular compartments or by secretion out of the plant.

To obtain and maintain the necessary amounts of water, plants evolved complex mechanisms to find water in the soil, to reduce evaporation from the plant bodies by depositing layers with low water permeability (e.g., the waxy cover of leaves) and tight regulation of water release through regulated pores (stomata). Moreover, plants require to communicate their water status between the different parts, for example, from roots to shoots (Takahashi et al. 2018), from root to root (Falik et al. 2012) and between different root tissues (Choi et al. 2017; Shkolnik et al. 2018), and for that they use a variety of signals, including chemicals (e.g., ions as Ca^{2+}; Dodd et al. 2010; Choi et al. 2017; Shkolnik et al. 2018), peptides (Takahashi et al. 2018), electric signals (Choi et al. 2017), and hydraulic signals (Christmann et al. 2013). Furthermore, plants have complex systems that deal with situations of water deficiency in the soil or in response to environmental conditions that may cause rapid depletion of water (e.g., heat and wind). Plants have also evolved memory for drought episodes to be more ready for subsequent situations of water deficiency (Auler et al. 2017). These defense mechanisms against water loss and water deficiency operate at the cellular, organ, and the whole-plant levels and involve diverse regulatory processes from modifications of cytoskeleton, membranes, and cell walls to changes in enzyme activities and modulation of gene expression. Some of these responses are rapid, like closure of stomata within minutes to hours (Buckley 2005); some are slower and regulated by developmental processes like reduction in stomata density in response to water deficiency (Yoo et al. 2010), which is a matter of days, and other developmental changes may be even slower. In addition to the different time scales of defense responses to water deficiency, the defense mechanism may be classified as mechanisms of (i) "escape" which consists of developmental reprograming to protect from stress, such as seasonal-dependent germination (regulation by day length), stimulus-dependent germination (regulation by water availability or temperature),

flowering time (terminal drought); (ii) "avoidance" that includes morphological and physiological adaptations to minimize stress, such as osmotic adjustments (Blum 2017), stomata aperture control to maintain leaf water potential (isohydric versus un-isohydric; Sade et al. 2012), reducing stomata density (GTL1 – SDD1; Yoo et al. 2010); and (iii) "tolerance," namely, the ability to survive a stressful situation while maintaining basic plant processes (e.g., the ABA-controlled pathways in resurrection plants; Giarola et al. 2017).

Several reviews have been published over the years on drought responses in plants, on adaptation to water deficiency, and on biotechnological approaches to achieve drought tolerance for improved crop production (Zhu 2002; Seki et al. 2007; Hussain et al. 2011; Shanker et al. 2014; Feller 2016; Joshi et al. 2016; Basu et al. 2016; Ghatak et al. 2017; Blum 2017; Buckley et al. 2017). This review focuses on water sensing. It aims at explaining the biochemical and molecular aspects of water sensing (depicted in Fig. 4.1). For an introduction of water–plant relationships, the readers are advised to consider Williams et al. (2014) and Taiz et al. (2015a). In short, water activity (potential), which is typically measured in megapascal (MP = ~ 10 Atmospheres), is defined according to the following equation, $\Psi w = \Psi_{\Pi} + \Psi_{\gamma +} \Psi_{p}$, where Ψ_{Π} refers to the osmotic potential (also referred to as osmotic pressure), which is defined as zero for pure water at atmospheric pressure but otherwise always negative and is proportional to the molar concentration (but not type) of the molecules in the solution; Ψ_{g} is the gravitational potential, which is only relevant when height differences of several meters are considered; and Ψ_{p} which is the hydrostatic potential (pressure) which could be positive (e.g., when turgor pressure occurs) or negative in case of water adhesion to soil particles or to cell-wall microfibrils in evaporating leaves (Taiz et al. 2015b).

4.2 The Molecular–Biochemical Basis of Water Sensing

4.2.1 Direct Osmosensing: Sensing the Solvent or the Solutes?

Within cells or at their immediate extracellular milieu, in the absence of hydrostatic pressure (potential), the osmotic pressure of an aqueous solution is proportional to its water activity (potential) and is determined by the activities (but not the identities) of all its solutes. To operate like the ligand-specific receptors (chemosensors) that initiate other signal transduction cascades, a direct osmosensor would detect water activity. However, osmotic shifts alter many cellular properties, which could be detected by an indirect osmosensor. These include cell volume, turgor pressure, membrane strain as well as the concentration of individual solutes, the ionic strength and the crowding of macromolecules in the cytoplasm (Wood 2006). In bacteria, various osmosensors have been described. For example, three glycine betaine transporters are activated by different mechanisms of osmosensing. OpuA is suggested to be an ionic-strength sensor (Mahmood et al. 2006). BetP is activated when internal K^+ is concentrated, thus altering the conformation and interactions of the C-terminus (Schiller et al. 2004), and ProP senses its own hydration state and is activated when it is partially dehydrated, retaining water molecules that contribute

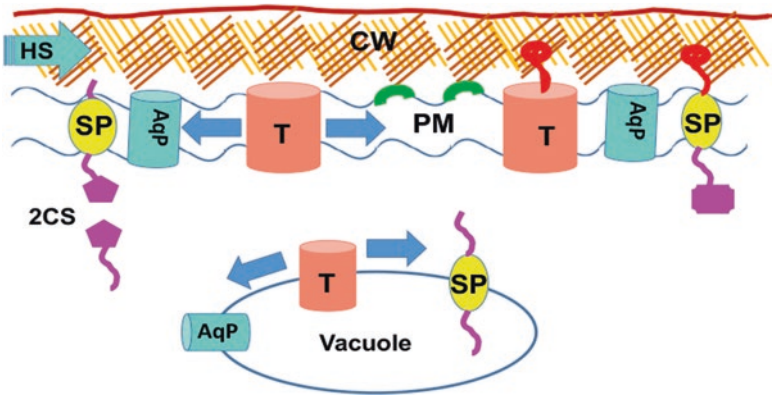

Fig. 4.1 Water signals, sensors, and balance in plants. Water status is perceived by plant cells in various direct and indirect ways. Water balance in the plant requires the dynamic coordination of osmoregulation of organelles (e.g., the vacuole) and cytosol. Water transport across membranes between cellular compartments and to/from the apoplast is driven by water potential differences and is facilitated by aquaporin water channels (AqP), which are subject to regulation at different levels (e.g., transcriptional and posttranscriptional). Such regulations modulate the hydraulic conductivity of membranes. Aquaporins may function as true sensors of water potential difference across membranes (Hill and Shachar-Hill 2015). Specialized sensory membrane proteins (SP) may directly sense either osmotic pressure (regardless of the identity of the solutes) or specific ions or the dehydration state of SPs. The perception of water status by SPs is transduced to the cytosol either through a two-component kinase cascade (2CS; e.g., AtHK1) or other mechanisms. Direct water sensors may function as transporters that are permeable to ions or osmolytes (like the bacterial OpuA, BetP, and ProP). SPs may be linked to cell wall structures (red) and convey water status changes to the cytoplasm, initiating a signaling cascade (e.g., WAK2 kinase, PERKs and CWI signaling; Voxeur and Höfte 2016). Stretch-activated transporters (T; in the PM and organelles) sense changes in membrane tension resulting from differences in the osmotic pressure such as OSCA1, a plant Ca^{2+}-permeable plasma membrane (PM) channel (belongs to a family of 15 members) and perhaps MCA1 and MCA2. Arabidopsis and perhaps other plants possess a single homolog of the large mechanosensor piezo nonselective cation channel (Peyronnet et al. 2014; Demidchik 2014). In addition, transporters may be tethered to the cell wall (CW) structures (brown; e.g., lectins or pectin) and respond to mechanical perturbations of the CW resulting from changes in osmotic pressure or by hydraulic signals (HS). Other types of mechanosensors (i.e., other than transporters) may also be involved in the perception of HS. Physical connections between CW, cytoskeleton, and PM (green crescents) may also be relevant to water sensing. Suggestions for the effects of electric fields (EF) on water sensing and the perception of water sound vibrations (SV) need to be more thoroughly examined experimentally and therefore are not included here

to the pathway for H^+ transport. Dehydration occurs because the water activity decreases (Wood 2006). These examples from bacteria describe different modes of direct osmosensing, but they are not necessarily mutually exclusive. Under different physiological conditions, osmosensors may respond to the hydration and/or to specific solutes.

While the mechanisms described above truly represent mechanisms of sensing water activity, osmotic pressure or specific solvents on one side of the membrane, theoretically, there should be transmembrane osmosensors which directly detect a

water potential difference spanning a membrane, or, in other words, a sensor that detects water potential differences between the outside and inside of the cell. In biological systems water is usually divided into three distinctly different classes (Nalepa et al. 2017): (i) internal water molecules (also referred to as structural water) that are hydrogen-bonded to specific amino acid residues in a water pocket or are mobile along inner-protein channels, and they are often of key importance for protein function, (ii) surface water molecules in the hydration shell of the protein at the solute–solvent interface, and (iii) bulk water molecules randomly distributed in the protein matrix. Water molecules in the protein hydration layer have restricted dynamics with respect to water molecules in the bulk (Nalepa et al. 2017). Hill and Shachar-Hill (2015) suggested that aquaporins (AqPs) may be functioning as direct transmembrane osmosensors. The hypothesis states that a water pore spanning a membrane that is impermeable to solutes, which exert significant osmotic pressure in the two bounding aqueous phases, will have a gradient of hydrostatic pressure along the pore, according to basic thermodynamics (Hill and Shachar-Hill 2015). While this suggestion is still controversial, no doubt AqPs play a major role in water homeostasis and osmoregulation in plants. They are encoded by large gene families in all plant species, and their protein products are distributed in membranes of different cellular compartments in both roots and shoots. As the major pathways for water across the membranes, it would be logical to assume that they are in fact transmembrane water potential sensors, which is supported by structural considerations (Hill and Shachar-Hill 2015). Nalepa et al. (2017), using electron paramagnetic resonance (EPR) to investigate hydration of bacterial photosynthetic reaction centers, suggested that hydration water plays a crucial role in protein dynamics and structural relaxation on different time scales. Changes in the amount of hydration water affect not only the protein's energy landscape but also significantly influence structural fluctuations between conformational sub-states, thereby controlling biological function. These authors conclude that changes in water-mediated hydrogen-bonding patterns usually have a crucial impact on the global function of a biological system. Therefore, differences in hydration of channel proteins (e.g., AqPs) on both sides of the membrane may be transmitted as a proxy to differences in water potential.

Not all osmosensors are transporters. In yeast, the high-osmolarity glycerol response (HOG) mitogen-activated (MAP) kinase pathway is activated in response to hyperosmotic stress via two independent osmosensing branches: Sln1 branch and Sho1 branch. The Sln1 branch is most sensitive to hyperosmolarity and functions as a two-component histidine kinase phosphorelay that consists of an autophosphorylating protein histidine kinase sensor (Sln1), a histidine-containing phosphotransfer protein (Ypd1) and a downstream MAP kinase cascade. The mechanism of osmosensing is not clear, but recent studies (Tanigawa et al. 2012) suggest that both Sln1 and Sho1 are distributed in raft-enriched detergent-resistant membranes (DRMs, also referred to as nanoclusters or microdomains) and that sphingolipid depletion and osmotic stress similarly lead to dissociation of an Sln1-containg protein complex and elevated association of Sho1with DRMs. Sln1 has similar organization to the bacterial osmosensor EnvZ (Ota and Varshavsky 1993).

Both proteins possess two N-terminal transmembrane domains connected by an extracellular loop, which consists of approximately 300 amino acid residues, which are responsible for sensing turgor pressure changes. The bacterial protein has an autophosphorylated histidine residue that is relayed to an aspartic acid residue in the OmpR-associated protein. The two-component signaling systems are prevalent in both bacteria and eukaryotes, some of which compose osmosensing and osmoregulation modules.

Plants also possess two-component histidine kinase signaling modules (Schaller et al. 2011). The *Arabidopsis thaliana* AHK1 histidine kinase (At2g17820), which is a member of two-component signaling systems in plants, is able to complement the yeast *sln1* mutant and was thus proposed to act as a plant osmosensor (Urao et al. 1999). Other studies suggest that AHK1 plays a role in stomatal density and transcription of stress-responsive genes, but its role as an osmosensor was questioned (Kumar et al. 2013; Sussmilch et al. 2017). It is possible that AHK1 is not an osmosensor on its own but is activated by an associated protein that is an actual osmosensor (Wohlbach et al. 2008). However, this possibility has not been tested experimentally. Nevertheless, similar proteins are found in other plants, for example, in rice (Kushwaha et al. 2014) and Populus (Chefdor et al. 2006; Hericourt et al. 2013), and the roles of some members of the histidine kinase family as osmosensors in plants cannot be ruled out.

4.2.2 Indirect Osmosensing Through Membrane Tension

A large group of osmosensors may be referred to as indirect osmosensors because they respond to changes in membrane tension or cytoskeletal changes either due to changes in water potential and hydration status or specifically respond to changes in osmotic pressure or specific solutes that affect membrane topology. Such proteins may constitute transporters or signaling proteins. Among the prototypic mechanosensitive channels are MscS and MscL from bacteria. When turgor increases under hyperosmotic conditions, these perceive stretch forces acting on the plasma membrane to allow rapid release of solutes and water from the cell. MscS-like proteins but not MscL homologs have been found in all plant genomes examined to date. The Arabidopsis genome encodes ten MscS-related proteins, two of which, MSL1 (AT4G00290) and MSL3 (AT1G58200), may play a role in osmoprotection similar to the role of MscS (Haswell et al. 2011). The MSL proteins have different subcellular locations, including the plasma membrane, endoplasmic reticulum, and plastid. Recent studies revealed that MSL1 is localized in the inner membrane of the mitochondria (Lee et al. 2016) although its osmoprotective function there is still unclear. A hypo-osmotic protection role for MSLs within plastids and during pollen germination has been demonstrated for specific MSL family members (Veley et al. 2012; Hamilton et al. 2015). Nevertheless, MSL proteins also appear to have other functions in plants, including plastid division (Wilson et al. 2011) and activation of programmed cell death by the plasma membrane-localized MSL10 (Veley et al. 2014).

In plants, a forward genetics approach based on screening for mutants that do not evoke cytosolic Ca^{2+} signals in response to osmotic stress, revealed the first bona fide Ca^{2+}-permeable osmosensor transporter in plants designated OSCA1 (Yuan et al. 2014). OSCA1 in Arabidopsis is a member of a family of 15 genes, and their homologues are found in other plant species and other eukaryotes. In its activity, OSCA1 resembles TRPV4 from vertebrates (Arnadóttir and Chalfie 2010). Many other mechanosensitive channels are present in prokaryotes and in eukaryotes (Haswell et al. 2011; Arnadóttir and Chalfie 2010), but only some of them are associated with osmoregulation (e.g., TRPY1; Arnadóttir and Chalfie 2010). Other plant proteins that share homology with the yeast stretch-activated channel MID1 and mediate hypo-osmolarity-induced Ca^{2+} increases and mechanical responses are MCA1(AT4G35920) and MCA2 (AT2G17780) in Arabidopsis and Ca^{2+} influx in response to hypo-osmotic shock in rice (Kurusu et al. 2012a, b) and tobacco (Kurusu et al. 2012c). Both proteins share 74% amino acid sequence identity, form homotertramers, have no homology to any known ion channels or transporters, and mediate Ca^{2+} influx upon mechanical stimulation, such as hypo-osmotic shock. Genes of this family are found exclusively in the plant kingdom (Kamano et al. 2015). While these proteins are not typical pore-forming subunits (Yamanaka et al. 2010), recent studies suggest that MCA1 and MCA2 are structurally unique mechanosensory channels responsive to osmotic changes and are permeable to Ca^{2+} (Kamano et al. 2015). The Arabidopsis genome possesses also a single homolog of Piezo, a large mechanosensitive nonselective cation channel (gene number AT2G48060; Peyronnet et al. 2014). Interestingly, recent findings (Tran et al. 2017) suggest that the activity of at least some mechanosensitive Ca^{2+} channels in the plant plasma membrane is dependent on the developmental regulator DEK1.

The need for coordinating water status in different cellular compartments suggests that osmosensors function in organelle membranes. An interesting vacuolar two-pore K^+ channel (TPK) appears to act as an osmosensor as it responds to osmotic changes and to membrane stretch. This was shown with TPKs from Arabidopsis, rice, and barley (Maathuis 2011). This report is consistent with previous electrophysiological studies that indicated the occurrence of a pressure-sensitive osmosensitive vacuolar ion channel, where high turgor increases vacuolar ion efflux, reducing vacuolar volume and hence turgor, whereas at low turgor the vacuolar ion efflux is reduced, helping to restore vacuolar volume and turgor (MacRobbie 2006). Consistent with the suggested role of TPKs in osmoregulation, overexpression of the potassium channel TPKb in small vacuoles confers osmotic and drought tolerance to rice (Ahmad et al. 2016). These studies emphasize the importance of coordinating osmoregulation across different cellular compartments.

4.2.3 Extracellular Matrix Proteins in Mechanosensing Osmoregulators

In addition to integral membrane proteins that may function as stretch-activated mechano-osmosensors, some proteins may be linked to extracellular matrix (ECM) components and respond to changes in osmotic pressure (water potential).

External perturbations are likely to act on the plant's cell wall and be conveyed to the plasma membrane directly or through proteins that link the cell wall with the plasma membrane, often with a cytosolic extension that links cell wall perturbation to cytosolic signaling cascades. The notion of cell wall integral (CWI) signaling has been developed in the past decade to reflect the plasticity and complexity of the cell wall and its role in signaling both in biotic and abiotic stresses (Voxeur and Hofte 2016). A potential family of such proteins is the receptor-like kinases (RLKs) that bind lectin and participate in protein-protein interactions to mediate plasma membrane-cell wall adhesion (Gouget et al. 2006). Similarly, receptor-like wall-associated (pectin binding) kinases (WAKs) and WAK-like kinases (WAKLs) are positioned to communicate cell wall perturbation to the cytoplasm (Anderson et al. 2001). However, evidence that WAKs and RLKs are involved in osmoregulation is lacking. Nevertheless, activation of WAK2 (AT1G21270) initiates a turgor increase via induction of vacuolar invertase (Kohorn and Kohorn 2012), which links WAK activity to water status homeostasis. Interestingly, both protein families are associated with plant immunity against pathogens (Balagué et al. 2017; Harkenrider et al. 2016). In other organisms, transporters linked to cytoskeletal tethers may operate by a mechanism referred to as "gating spring" (Kung 2005; Haswell et al. 2011). It is very likely that protein linking plasma membrane transporters and other proteins with cell wall molecules (e.g., pectin and lectin) or cytoskeletal proteins function as osmosensors in plants as well. Other types of potential cell wall-plasma membrane linkers are the proline-rich extensin-like receptor kinases (PERKs). PERKs are involved in Ca^{2+} signaling in response to mechanical stimuli (Nakagawa et al. 2007) and thus might translate osmotic changes at the cell wall into cytosolic Ca^{2+} signal.

4.2.4 Sensing of Water Pressure (Hydraulic Sensing)

Changes in hydrostatic pressure can be rapidly propagated in plant tissues and can function as hydraulic signals in response to various external and internal stimuli. For example, a hydrostatic signal may result from mechanical perturbations in shoots (Louf et al. 2017). This hydromechanical coupling may be responsible for hydraulic pulses of signals between distant parts of the plant (Louf et al. 2017). In addition, changes in water status in the soil may also be rapidly transmitted by hydraulic signals (Christmann et al. 2013). Other functions in plants are known to be driven by hydrostatic pressure differences (rather than by water potential differences), such as phloem transport driven by the Pressure-Flow model (Sevanto 2014; Ham and Lucas 2014; De Schepper et al. 2013), cell expansion necessary for cell growth and development (Mathur 2006), and cell burst of the apical region of the pollen tube, which is necessary for the release of male sperm cells (Amien et al. 2010). Thus, decoding of hydraulic signals is an important component of the plant's ability to sense water. The hydraulic signal generated by water deficit causes first a reduction in turgor and second a moderate increase in solute concentration because of water withdrawal from cells and, third, mechanical forces exerted at the cell wall and the cell wall-plasma membrane interface. Therefore, both direct and indirect osmosensors may be active in sensing hydraulic signals.

4.3 How Do Plants Actively Search for Water?

Plants like all other organisms actively search for water. Since the time of Darwin (Darwin and Darwin 1880) and even earlier, it was known that plants use moisture gradients to direct their roots through the soil once a water source is detected. In heterogeneous natural habitats, plant roots have the capacity to grow spontaneously toward places with adequate moisture and nutrients, exhibiting hydrotropism and chemotropism by which plants adapt to arid soil environments via root growth (Feng et al. 2016). However, how plants sense water in the natural environment is still an open question, and it may also differ in species occupying a variety of habitats (Cole and Mahall 2006). In particular, the plant sensors that detect water in this context are unknown. Nevertheless, these elusive water sensors transmit a signal to the elongation zone where differential cell elongation across the root occurs and confers root bending toward the water source. In spite of this important process in the life of the plant, the molecular components and cell signals involved are largely unknown. The positive mediation of hydrotropism by ABA signaling (Dietrich et al. 2017) and negative mediation by ROS (Krieger et al. 2016; Shkolnik and Fromm 2016) and auxin (Shkolnik et al. 2016) have been described. Recently, Shkolnik et al. (2018) reported on the role of long-distance Ca^{2+} signaling in hydrotropism, which is mediated by the inhibition of ECA1, an endoplasmic reticulum Ca^{2+} pump, by the direct binding of MIZ1. However much of this process is enigmatic especially regarding the water sensors (osmosensors) and how these are linked to intercellular signaling and concomitant root bending. The fact that ABA signaling is required for hydrotropism may suggest that the water sensors underlying hydrotropism are the same or similar to those mediating other water and osmotic responses, such as the direct and indirect osmosensors described above.

A recent study of hydrotropism of pea (*Pisum sativum*) roots (Gagliano et al. 2017) suggested that roots are able to locate a water source by sensing the vibrations generated by water movement inside pipes. When both moisture and acoustic cues were available, roots preferentially used moisture in the soil over acoustic vibrations, suggesting that acoustic gradients enable roots to broadly detect a water source at a distance, while moisture gradients help them to reach their target more accurately. These studies are consistent with other sound vibration-tracking responses in plants (Mishra et al. 2016). These sound vibrations are likely perceived by membranes, possibly by transporters or cell wall-associated proteins linked to plasma membrane proteins. Upon perception of a water signal (i.e., change in water potential or hydrostatic signal), a cytosolic signal is evoked (possibly Ca^{2+}) that initiates a secondary signaling cascade which modulates the activity of downstream effectors underlying responses at the intra- and intercellular levels and the whole organism. Alternatively, vibrations may be transduced as systemic hydraulic signals that are perceived by mechanisms discussed earlier.

Interestingly, there are several studies suggesting that electric fields around the root affect tropic responses (Marcum and Moore 1990), and the term electrotropism has been used (Gorgolewski and Rozej 2001). According to Ramthun (2017), humidity water droplets have a net charge of zero. However, when water droplets

are exposed to an electric field, they will be polarized and become a dipole. The cloud of polarized droplets will therefore be electrostatically connected in three dimensions. The electrostatic connections are able to transfer small push-pull forces within the humidity cloud. According to Ramthun (2017), it is possible that the earth's electric field is polarizing the humidity. The roots may then electrostatically be attracted to the polarized water droplet field and, hence, to the moist soil patches (Ramthun 2017). Although these suggestions require further experimental validation, the possible effects of electric fields on water sensing should not be ruled out.

4.4 Open Questions

- How are specific water sensory mechanisms linked to whole-plant responses to water availability/deficiency? We do not know if the water sensory mechanism that underlies hydrotropism is the same as that controlling other osmotic stress responses. In other words, does hydrotropism have a specialized set of water sensors?
- How do plant water sensory mechanisms interpret the root's environment in three dimensions?
- How are water sensors spatially distributed and how their responses are coordinated to evoke proper responses at the organ and whole-plant levels?
- How does the plant quantify the water status in the root's environment?

4.5 Future Perspectives

4.5.1 3D Structure at Single-Cell Resolution

While research at the cellular and molecular levels continue to flourish with the advent of novel 'omic' technologies, spatial and architectural organization of the sensory system must be addressed because it is likely to be a crucial factor in the plant's ability to map its environment with regard to water status, particularly considering the dynamics of heterogeneity in the roots' microenvironment. Therefore, characterizing the topology of such sensors at single-cell resolution is required. Such organization would not be surprising considering that other processes require asymmetric distribution of the underlying molecular machinery. The well-known asymmetric organization of the auxin influx carrier AUX1 and PIN transporters (Gälweiler et al. 1998) required for polar auxin transport is just one example.

4.5.2 High-Resolution Physiological Manipulations of Roots

Roots and their microenvironment (i.e., rhizosphere) constitute a diverse ecosystem with great complexity and dynamics; therefore, current methods for analyzing root biology always represent a compromise between physiological relevance and

accuracy and imaging capabilities regarding resolution, dynamics, and dimension. A recent technological development of a dual-flow-root chip, based on a microfluidic platform, was able to demonstrate cell-autonomous adaptation of root hair development under asymmetric phosphate perfusion (Stanley et al. 2018). Interestingly, the asymmetric root environment resulted in asymmetric gene expression of a key gene involved in root hair growth. Similarly, using the same microfluidic platform, these authors also demonstrated asymmetric Ca^{2+} signaling in roots undergoing asymmetric osmotic stimulus. Another interesting platform that enables multidimensional characterization of soil-grown roots is the "growth and luminescence observatory for roots" (GLO-Roots) (Rellan-Alvarez et al. 2015). It utilizes image analysis algorithms that allow spatial integration of soil properties, gene expression and root system architecture traits. The method provides biological and physical characterization of roots and their growth environment, yet in an artificial lab-based system, not in the field.

4.5.3 Atomic Resolution Protein Structure Dynamics Under Dehydration: Hydration Shifts

To understand how a protein may function as a sensor for water activity will require protein structural analysis at single-atom resolution to resolve the exact status of water interactions with the protein and their effects on protein dynamics. An example of atomic resolution of water-protein interaction is the X-ray crystallography analysis of oxygen-evolving photosystem II at a resolution of 1.9 A (Umena et al. 2011). These authors identified more than 1300 water molecules in each photosystem II monomer. Some of them formed extensive hydrogen-bonding networks that may serve as channels for protons, water, or oxygen molecules.

4.5.4 Roots in Their Natural Environment: Throughput Versus Resolution Compromise

In addition to the necessary tightly controlled high-resolution platforms for research of roots in the lab, there is a need to advance our ability to study root growth and development in their natural environment. Unfortunately, there is yet no ideal system that covers high-throughput analysis in 3D of roots in their natural environment (namely, soil). However, the possibility to obtain 3D images of individual plant root systems combined with the ability to monitor real-time in situ water status is improving. Examples are the combined MRI-PET (Jahnke et al. 2009) or X-ray CT-phenotyping platforms (Rogers et al. 2016; Tardieu et al. 2017). To date, high-throughput root phenotyping systems are suitable only for plants that are grown in artificial systems (Clark et al. 2011), although some field systems are becoming available, albeit with serious limitations of resolution. A portable fluorescence spectroscopy imaging system for automated root phenotyping in soil in the field was

recently reported (Wasson et al. 2016). Quantitative 3D analysis of roots in soil is possible by magnetic resonance imaging (van Dusschoten et al. 2016); however, this is not suitable for field tests and is not a high-throughput technology.

References

Ahmad I, Devonshire J, Mohamed R, Schultze M, Maathuis FJ (2016) Overexpression of the potassium channel TPKb in small vacuoles confers osmotic and drought tolerance to rice. New Phytol 209:1040–1048

Amien S, Kliwer I, Márton ML, Debener T, Geiger D, Becker D, Dresselhaus T (2010) Defensin-like ZmES4 mediates pollen tube burst in maize via opening of the potassium channel KZM1. PLoS Biol 8:e1000388

Anderson CM, Wagner TA, Perret M, He ZH, He D, Kohorn BD (2001) WAKs: cell wall-associated kinases linking the cytoplasm to the extracellular matrix. Plant Mol Biol 47:197–206

Arnadóttir J, Chalfie M (2010) Eukaryotic mechanosensitive channels. Annu Rev Biophys 39:111–137

Auler PA, do Amaral MN, Rodrigues GDS, Benitez LC, da Maia LC, Souza GM, Braga EJB (2017) Molecular responses to recurrent drought in two contrasting rice genotypes. Planta 246:899–914

Balagué C, Gouget A, Bouchez O, Souriac C, Haget N, Boutet-Mercey S, Govers F, Roby D, Canut H (2017) The Arabidopsis thaliana lectin receptor kinase LecRK-I.9 is required for full resistance to Pseudomonas syringae and affects jasmonate signalling. Mol Plant Pathol 18:937–948

Basu S, Ramegowda V, Kumar A, Pereira A (2016) Plant adaptation to drought stress. F1000 Res 5:1554

Bentrup FW (2017) Water ascent in trees and lianas: the cohesion-tension theory revisited in the wake of Otto Renner. Protoplasma 254:627–633

Blum A (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ 40:4–10

Buckley TN (2005) The control of stomata by water balance. New Phytol 168(2):275–292

Buckley TN, Sack L, Farquhar GD (2017) Optimal plant water economy. Plant Cell Environ 40:881–896

Chefdor F, Bénédetti H, Depierreux C, Delmotte F, Morabito D, Carpin S (2006) Osmotic stress sensing in Populus: components identification of a phosphorelay system. FEBS Lett 580:77–81

Choi WG, Miller G, Wallace I, Harper J, Mittler R, Gilroy S (2017) Orchestrating rapid long-distance signaling in plants with Ca2+, ROS and electrical signals. Plant J 90:698–707

Christmann A, Grill E, Huang J (2013) Hydraulic signals in long-distance signaling. Curr Opin Plant Biol 16:293–300

Clark RT, MacCurdy RB, Jung JK, Shaff JE, McCouch SR, Aneshansley DJ, Kochian LV (2011) Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiol 156:455–465

Cole ES, Mahall BE (2006) A test for hydrotropic behavior by roots of two coastal dune shrubs. New Phytol 172:358–368

Cook GD, Dixon JR, Leopold AC (1964) Transpiration: its effects on plant leaf temperature. Science 144:546–547

Darwin C, Darwin F (1880) The power of movement in plants. John Murray, London

De Schepper V, De Swaef T, Bauweraerts I, Steppe K (2013) Phloem transport: a review of mechanisms and controls. J Exp Bot 64:4839–4850

Demidchik V (2014) Mechanisms and physiological roles of K+ efflux from root cells. J Plant Physiol 171:696–707

Dietrich D, Pang L, Kobayashi A, Fozard JA, Boudolf V, Bhosale R, Antoni R, Nguyen T, Hiratsuka S, Fujii N, Miyazawa Y, Bae TW, Wells DM, Owen MR, Band LR, Dyson RJ, Jensen OE, King

JR, Tracy SR, Sturrock CJ, Mooney SJ, Roberts JA, Bhalerao RP, Dinneny JR, Rodriguez PL, Nagatani A, Hosokawa Y, Baskin TI, Pridmore TP, De Veylder L, Takahashi H, Bennett MJ (2017) Root hydrotropism is controlled via a cortex-specific growth mechanism. Nat Plants 8:17057

Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant Biol 61:593–620

Falik O, Mordoch Y, Ben-Natan D, Vanunu M, Goldstein O, Novoplansky A (2012) Plant responsiveness to root-root communication of stress cues. Ann Bot 110:271–280

Feller U (2016) Drought stress and carbon assimilation in a warming climate: reversible and irreversible impacts. J Plant Physiol 203:84–94

Feng D, Huang X, Liu Y, Willison JH (2016) Growth and changes of endogenous hormones of mulberry roots in a simulated rocky desertification area. Environ Sci Pollut Res Int 23:11171–11180

Gagliano M, Grimonprez M, Depczynski M, Renton M (2017) Tuned in: plant roots use sound to locate water. Oecologia 184:151–160

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science 282:2226–22230

Ghatak A, Chaturvedi P, Weckwerth W (2017) Cereal crop proteomics: systemic analysis of crop drought stress responses towards marker-assisted selection breeding. Front Plant Sci 8:757

Giarola V, Hou Q, Bartels D (2017) angiosperm plant desiccation tolerance: hints from transcriptomics and genome sequencing. Trends Plant Sci 22:705–717

Gorgolewski S, Rozej B (2001) Evidence for electrotropism in some plant species. Adv Space Res 28:633–638

Gouget A, Senchou V, Govers F, Sanson A, Barre A, Rougé P, Pont-Lezica R, Canut H (2006) Lectin receptor kinases participate in protein-protein interactions to mediate plasma membrane-cell wall adhesions in Arabidopsis. Plant Physiol 140:81–90

Ham BK, Lucas WJ (2014) The angiosperm phloem sieve tube system: a role in mediating traits important to modern agriculture. J Exp Bot 65:1799–1816

Hamilton ES, Jensen GS, Maksaev G, Katims A, Sherp AM, Haswell ES (2015) Mechanosensitive channel MSL8 regulates osmotic forces during pollen hydration and germination. Science 350:438–441

Harkenrider M, Sharma R, De Vleesschauwer D, Tsao L, Zhang X, Chern M, Canlas P, Zuo S, Ronald PC (2016) Overexpression of rice wall-associated kinase 25 (OsWAK25) alters resistance to bacterial and fungal pathogens. PLoS One 11:e0147310

Haswell ES, Phillips R, Rees DC (2011) Mechanosensitive channels: what can they do and how do they do it? Structure 19:1356–1369

Héricourt F, Chefdor F, Bertheau L, Tanigawa M, Maeda T, Guirimand G, Courdavault V, Larcher M, Depierreux C, Bénédetti H, Morabito D, Brignolas F, Carpin S (2013) Characterization of histidine-aspartate kinase HK1 and identification of histidine phosphotransfer proteins as potential partners in a populus multistep phosphorelay. Physiol Plant 149:188–199

Hill AE, Shachar-Hill Y (2015) Are aquaporins the missing transmembrane osmosensors? J Membr Biol 248:753–765

Hussain SS, Kayani MA, Amjad M (2011) Transcription factors as tools to engineer enhanced drought stress tolerance in plants. Biotechnol Prog 27:297–306

Jahnke S, Menzel MI, van Dusschoten D, Roeb GW, Bühler J, Minwuyelet S, Blümler P, Temperton VM, Hombach T, Streun M, Beer S, Khodaverdi M, Ziemons K, Coenen HH, Schurr U (2009) Combined MRI-PET dissects dynamic changes in plant structures and functions. Plant J 59:634–644

Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL (2016) Transcription factors and plants response to drought stress: current understanding and future directions. Front Plant Sci 7:1029

Kamano S, Kume S, Iida K, Lei KJ, Nakano M, Nakayama Y, Iida H (2015) Transmembrane topologies of Ca2+-permeable mechanosensitive channels MCA1 and MCA2 in Arabidopsis thaliana. J Biol Chem 290:30901–30909

Kohorn BD, Kohorn SL (2012) The cell wall-associated kinases, WAKs, as pectin receptors. Front Plant Sci 3:88

Krieger G, Shkolnik D, Miller G, Fromm H (2016) reactive oxygen species tune root tropic responses. Plant Physiol 172:1209–1220

Kumar MN, Jane WN, Verslues PE (2013) Role of the putative osmosensor Arabidopsis histidine kinase1 in dehydration avoidance and low-water-potential response. Plant Physiol 161:942–953

Kung C (2005) A possible unifying principle for mechanosensation. Nature 436:647–654

Kurusu T, Iida H, Kuchitsu K (2012a) Roles of a putative mechanosensitive plasma membrane Ca2+-permeable channel OsMCA1 in generation of reactive oxygen species and hypo-osmotic signaling in rice. Plant Signal Behav 7:796–798

Kurusu T, Nishikawa D, Yamazaki Y, Gotoh M, Nakano M, Hamada H, Yamanaka T, Iida K, Nakagawa Y, Saji H, Shinozaki K, Iida H, Kuchitsu K (2012b) Plasma membrane protein OsMCA1 is involved in regulation of hypo-osmotic shock-induced Ca2+ influx and modulates generation of reactive oxygen species in cultured rice cells. BMC Plant Biol 12:11

Kurusu T, Yamanaka T, Nakano M, Takiguchi A, Ogasawara Y, Hayashi T, Iida K, Hanamata S, Shinozaki K, Iida H, Kuchitsu K (2012c) Involvement of the putative Ca2+-permeable mechanosensitive channels, NtMCA1 and NtMCA2, in Ca2+ uptake, Ca2+-dependent cell proliferation and mechanical stress-induced gene expression in tobacco (Nicotiana tabacum) BY-2 cells. J Plant Res 125:555–568

Kushwaha HR, Singla-Pareek SL, Pareek A (2014) Putative osmosensor–OsHK3b–a histidine kinase protein from rice shows high structural conservation with its ortholog AtHK1 from Arabidopsis. J Biomol Struct Dyn 32:1318–1332

Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator Thlaspi caerulescens. J Exp Bot 51:71–79

Lee CP, Maksaev G, Jensen GS, Murcha MW, Wilson ME, Fricker M, Hell R, Haswell ES, Millar AH, Sweetlove LJ (2016) MSL1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress. Plant J 88:809–825.

Louf JF, Guéna G, Badel E, Forterre Y (2017) Universal poroelastic mechanism for hydraulic signals in biomimetic and natural branches. Proc Natl Acad Sci USA 114:11034–11039

Maathuis FJM (2011) Vacuolar two-pore K+ channels act as vacuolar osmosensors. New Phytol 191:81–91

MacRobbie EA (2006) Osmotic effects on vacuolar ion release in guard cells. Proc Natl Acad Sci USA 103:1135–1140

Mahmood NA, Biemans-Oldehinkel E, Patzlaff JS, Schuurman-Wolters GK, Poolman B (2006) Ion specificity and ionic strength dependence of the osmoregulatory ABC transporter OpuA. J Biol Chem 281:29830–29839

Marcum H, Moore R (1990) Influence of electrical fields and asymmetric application of mucilage on curvature of primary roots of zea mays. Am J Bot 77:446–452

Mathur J (2006) Local interactions shape plant cells. Curr Opin Cell Biol 18:40–46

Mishra RC, Ghosh R, Bae H (2016) Plant acoustics: in the search of a sound mechanism for sound signaling in plants. J Exp Bot 67:4483–4494

Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, Kishigami A, Sokabe M, Kojima I, Sato S, Kato T, Tabata S, Iida K, Terashima A, Nakano M, Ikeda M, Yamanaka T, Iida H (2007) Arabidopsis plasma membrane protein crucial for Ca2+ influx and touch sensing in roots. Proc Natl Acad Sci USA 104:3639–3644

Nalepa A, Malferrari M, Lubitz W, Venturoli G, Möbius K, Savitsky A (2017) Local water sensing: water exchange in bacterial photosynthetic reaction centers embedded in a trehalose glass studied using multiresonance EPR. Phys Chem Chem Phys 19:28388–28400

Ota IM, Varshavsky A (1993) A yeast protein similar to bacterial two-component regulators. Science 262:566–569

Peyronnet R, Tran D, Girault T, Frachisse JM (2014) Mechanosensitive channels: feeling tension in a world under pressure. Front Plant Sci 5:558

Ramthun AD (2017) Plant electro-tropism. Water J 8:47–106

Rellán-Álvarez R, Lobet G, Lindner H, Pradier PL, Sebastian J, Yee MC, Geng Y, Trontin C, LaRue T, Schrager-Lavelle A, Haney CH, Nieu R, Maloof J, Vogel JP, Dinneny JR (2015)

GLO-Roots: an imaging platform enabling multidimensional characterization of soil-grown root systems. eLife 4:–e07597
Rogers ED, Monaenkova D, Mijar M, Nori A, Goldman DI, Benfey PN (2016) X-Ray computed tomography reveals the response of root system architecture to soil texture. Plant Physiol 171:2028–2040
Sade N, Gebremedhin A, Moshelion M (2012) Risk-taking plants: anisohydric behavior as a stress-resistance trait. Plant Signal Behav 7:767–770
Schaller GE, Shiu SH, Armitage JP (2011) Two-component systems and their co-option for eukaryotic signal transduction. Curr Biol 21:R320–R330
Schiller D, Krämer R, Morbach S (2004) Cation specificity of osmosensing by the betaine carrier BetP of Corynebacterium glutamicum. FEBS Lett 563:108–112
Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10:296–302
Sevanto S (2014) Phloem transport and drought. J Exp Bot 65:1751–1759
Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B (2014) Drought stress responses in crops. Funct Integr Genomics 14:11–22
Shkolnik D, Fromm H (2016) The Cholodny-Went theory does not explain hydrotropism. Plant Sci 252:400–403
Shkolnik D, Krieger G, Nuriel R, Fromm H (2016) hydrotropism: root bending does not require auxin redistribution. Mol Plant 9:757–759
Shkolnik D, Nuriel R, Bonza MC, Costa A, Fromm H (2018) MIZ1 regulates ECA1 to generate a slow, long-distance phloem-transmitted ca signal essential for root water tracking in Arabidopsis. Proc Natl Acad Sci U S A 115:8031–8036
Stanley CE, Shrivastava J, Brugman R, Heinzelmann E, van Swaay D, Grossmann G (2018) Dual-flow-root chip reveals local adaptations of roots towards environmental asymmetry at the physiological and genetic levels. New Phytol 217:1357–1369
Steudle E (2001) The cohesion-tension mechanism and the acquisition of water by plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:847–875
Sussmilch FC, Brodribb TJ, McAdam SAM (2017) Up-regulation of NCED3 and ABA biosynthesis occur within minutes of a decrease in leaf turgor but AHK1 is not required. J Exp Bot 68:2913–2918
Taiz L, Zeiger E, Moller IM and Murphy A (2015a) Plant physiology and development, 6th edn (ed. Sinauer AD), pp 83–118. Sinauer Associates Sunderland, MA
Taiz L, Zeiger E, Moller IM and Murphy A (2015b) Plant physiology and development, 6th edn (ed. Sinauer AD), pp 104—110. Sinauer Associates, Sunderland MA
Takahashi F, Suzuki T, Osakabe Y, Betsuyaku S, Kondo Y, Dohmae N, Fukuda H, Yamaguchi-Shinozaki K, Shinozaki K (2018) A small peptide modulates stomatal control via abscisic acid in long-distance signalling. Nature 556:235–238.
Tanigawa M, Kihara A, Terashima M, Takahara T, Maeda T (2012) Sphingolipids regulate the yeast high-osmolarity glycerol response pathway. Mol Cell Biol 32:2861–2870
Tardieu F, Cabrera-Bosquet L, Pridmore T, Bennett M (2017) Plant phenomics, from sensors to knowledge. Curr Biol 27:R770–R783
Tran D, Galletti R, Neumann ED, Dubois A, Sharif-Naeini R, Geitmann A, Frachisse JM, Hamant O, Ingram GC (2017) A mechanosensitive Ca2+ channel activity is dependent on the developmental regulator DEK1. Nat Commun 8:1009
Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473:55–60
Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell 11:1743–1754.
van Dusschoten D, Metzner R, Kochs J, Postma JA, Pflugfelder D, Bühler J, Schurr U, Jahnke S (2016) Quantitative 3d analysis of plant roots growing in soil using magnetic resonance imaging. Plant Physiol 170:1176–1188

Veley KM, Marshburn S, Clure CE, Haswell ES (2012) Mechanosensitive channels protect plastids from hypoosmotic stress during normal plant growth. Curr Biol 22:408–413
Veley KM, Maksaev G, Frick EM, January E, Kloepper SC, Haswell ES (2014) Arabidopsis MSL10 has a regulated cell death signaling activity that is separable from its mechanosensitive ion channel activity. Plant Cell 26:3115–3131
Voxeur A, Höfte H (2016) Cell wall integrity signaling in plants: "To grow or not to grow that's the question". Glycobiology 26:950–960
Wasson A, Bischof L, Zwart A, Watt M (2016) A portable fluorescence pectroscopy imaging system for automated root phenotyping in soil cores in the field. J Exp Bot 67:1033–1043
Williams M, Oliver M, Pallardy S (2014) Teaching tools in plant biology™: lecture notes. water relations 1: uptake and transport. The Plant Cell. www.plantcell.org. American Society of Plant Biologists
Wilson ME, Jensen GS, Haswell ES (2011) Two mechanosensitive channel homologs influence division ring placement in Arabidopsis chloroplasts. Plant Cell 23:2939–2949
Wilson H, Mycock D, Weiersbye IM (2017) The salt glands of Tamarix usneoides E. Mey. ex Bunge (South African Salt Cedar). Int J Phytoremediation 19:587–595
Wohlbach DJ, Quirino BF, Sussman MR (2008) Analysis of the Arabidopsis histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20:1101–1117
Wood JM (2006) Osmosensing in bacteria. Sciences STKE 357:pe48
Yamanaka T, Nakagawa Y, Mori K, Nakano M, Imamura T, Kataoka H, Terashima A, Iida K, Kojima I, Katagiri T, Shinozaki K, Iida H (2010) MCA1 and MCA2 that mediate Ca2+ uptake have distinct and overlapping roles in Arabidopsis. Plant Physiol 152:1284–1296
Yoo CY, Pence HE, Jin JB, Miura K, Gosney MJ, Hasegawa PM, Mickelbart MV (2010) The Arabidopsis GTL1 transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via transrepression of SDD1. Plant Cell 22:4128–4141
Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B, Johnson DM, Swift GB, He Y, Siedow JN, Pei ZM (2014) OSCA1 mediates osmotic-stress-evoked Ca2+ increases vital for osmosensing in Arabidopsis. Nature 514:367–371.
Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Hillel Fromm comes from an agricultural background and did his undergraduate studies at the Faculty of Agriculture of the Hebrew University of Jerusalem (Israel). He further did his graduate studies at the Weizmann Institute of Science (Israel) on chloroplast molecular biology. His acquaintance with the Editor started then and developed over the years. Fromm subsequently spent 3 years at the Rockefeller University, New York (USA), as a Postdoctoral Fellow in the lab of Plant Molecular Biology. Fromm is currently a Professor at Tel Aviv University, Israel, and as an Independent Researcher and Group Leader, he has been studying Ca^{2+} signaling in plant responses to abiotic stresses at the Weizmann Institute of Science, the University of Leeds (UK), and at Tel Aviv University. His focus in recent years has been on the study of root-water relations and hydrotropism. In Tel Aviv University, Prof. Fromm served as Head of the Department of Plant Sciences, the first Head of the School of Plant Sciences and Food Security, and the Director of the Israeli Center for Research Excellence on Plant Adaptation to Changing Environment.

Yosef Fichman was a Ph.D. student at Tel Aviv University, co-mentored by Prof. Fromm, and is currently a Postdoctoral Fellow in the University of Missouri, USA.

Gravitropism of Plant Organs Undergoing Primary Growth

5

Shih-Heng Su and Patrick H. Masson

Abstract

As sessile organisms anchored to their substrate, plants have to develop in such a way that their organs can fulfill essential primary functions, which include photosynthesis, gas exchange and reproduction for shoots, and anchoring as well as water and nutrients uptake for roots. To do so, these organs have to use directional information within their environments as growth guides. Gravity, a constant parameter on Earth, is one of the cues used by most organs to direct growth, a process named gravitropism. Typically, shoots will grow against the gravity vector whereas roots will follow it. Furthermore, lateral organs will grow along shallower vectors relative to gravity, whose obliqueness is dictated by endogenous/hormonal and environmental cues. In this chapter, we review the molecular mechanisms that allow angiosperm organs to use gravity as a growth guide. Gravity-sensing cells named statocytes contain dense starch-filled plastids (amyloplasts) that sediment within their cytoplasm. These cells are located in the columella region of the root cap and in the endodermis that surrounds the vasculature in shoots. Amyloplast sedimentation in these cells promotes a polarization of auxin efflux facilitators to the bottom membrane, creating a downward flow of auxin that results in a lateral gradient across the stimulated organ. Differential auxin accumulation on opposite flanks of the organ results in differential cellular elongation upon transmission to the site of response, a process that is responsible for upward curvature in shoots and downward growth in roots. Lateral organs, on the other hand, respond to similar stimuli by developing weaker lateral auxin gradients, leading to shallower growth angles from gravity. An abundance of research carried out by multiple laboratories around the world has recently led to important new insights into the mechanisms that govern these complex processes and the machinery that fine-tunes them to ultimately yield highly controlled and

S.-H. Su · P. H. Masson (✉)
Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI, USA
e-mail: phmasson@wisc.edu

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_5

amazingly complex responses. This chapter attempts to discuss these mechanisms and identify some of the areas in need of further investigation in this important area of plant biology.

Keywords
Amyloplasts · Columella cells · Cyclic nucleotide-gated ion channel · Gravistimulation · Gravity set point angle · Root cap · Small auxin up RNA (SAUR) · Statocytes

5.1 Introduction

As sessile organisms, plants have to direct the growth of their organs to reach out for light, oxygen, carbon dioxide, water and nutrients. Hence, cues required for guidance should be associated, either directly or indirectly, with these environmental gradients in such a way that each organ can better fulfill its primary functions, including gas exchange, photosynthesis and reproduction for shoots, and plant anchoring as well as water and nutrient uptake for roots. One key parameter in the environment that broadly favors organs growth in directions that are compatible with their primary functions is gravity. Indeed, plant organs are equipped with machinery that allows them to detect the direction of gravity and guide their growth relative to it. Named gravitropism, this directional growth response to gravity has received much attention since its recognition two centuries ago (Knight 1806).

Upon germination, seedling primary roots tend to grow vertically downward into the soil, whereas shoots tend to expand in the opposite direction, reaching out for light. Both organs can do this even when the seedlings are exposed to complete darkness, using gravity as a guide. This directional growth response to gravity remains critical throughout the life cycle of a plant. In fact, different plant organs will follow distinct growth vectors relative to gravity. For many species, the primary organs will grow along the gravity vector, as discussed above, whereas lateral organs will emerge from a lower-order organ at a stereotypical angle from it, before curving toward a target vector at a defined angle from the vertical, named gravity set point angle (GSA) (Firn and Digby 1997). Named plagiogravitropism, the latter process may facilitate exploration of the three-dimensional space around a plant, improving its access to essential resources and, consequently, influencing its performance.

In natural environments, resources are often distributed unevenly in the immediate vicinity of a plant. For instance, light will be differently oriented depending on the time of the day, and its intensity and spectrum will vary depending on the density of shading plants in the canopy, cloud cover and period of day and year. Similarly, neighboring soil particles may have varied biophysical properties that confer distinct abilities to retain water, ions, or specific nutrients, thereby creating local gradients in humidity and/or nutrients that may directly influence root growth rate, direction, and/or branching. Hence, the gravity set point for a defined plant

organ will be established in coordination with responses to a variety of developmental, environmental, and/or hormonal cues, leading to drastically distinct morphologies under diverse conditions, which may help improve the plant's ability to cope with a rapidly changing environment (Firn and Digby 1997).

In this chapter, we will introduce the reader to the mechanisms that govern plant responses to gravity, with emphasis on organs that undergo primary growth from apical meristems. Many of the experiments described in this chapter were carried out on *Arabidopsis thaliana* roots and shoots. This is because most of the recent work uncovering key mechanisms involved in gravity sensing and signal transduction has been carried out using this model dicot organism.

We will first describe the mechanisms that allow shoots and roots to grow and then discuss the mechanisms that govern gravitropism in these organs.

5.1.1 Cell Division and Elongation Contribute to Plant Organs' Growth

To grow, plant organs use a combination of cell divisions in apical meristems and cell elongation in subapical regions named the elongation zones, where cells also differentiate into defined cell types (Fig. 5.1). In roots, the apical meristem is covered by a cap, which contributes to environmental sensing as well as meristem protection against mechanical damage during root growth in soil. The root cap is made of large central columella cells as well as lateral cap cells that wrap around the root meristem, and tip cells. Both columella and lateral cap cells are being constantly replaced by new cells originating from the asymmetrical division of lateral and distal initial cells that surround the quiescent center within the promeristem. As we will see later on in this chapter, the central columella cells of the root cap are critical for gravity sensing whereas the lateral cap cells contribute to signal transmission from the gravisensing cap to the graviresponding elongation zone.

Shootward to the tip-organizing quiescent center, additional initials also divide asymmetrically to generate concentric files of cells that will ultimately form the different tissue types that constitute a root, including the lateral root cap, epidermis, cortex, endodermis, pericycle and the rest of the vasculature. Rapid anticlinal cell divisions occur along a defined region of the root tip named meristem. As more divisions occur in that region, those cells that are located more proximally (closer to the shoot) eventually cease division after reaching a defined distance from the root tip. Those interphase cells start expanding along a vector that is parallel to the longitudinal axis of the root. They continue to elongate and differentiate as their distance from the tip increases due to continued division at the meristem. Ultimately, those cells will stop expanding and differentiate into the different cell types listed above. The region where cells elongate is named the elongation zone (EZ). This region is, in fact, complex, being composed of two distinct sub-regions: (1) a distal elongation zone (DEZ) and (2) a central elongation zone (CEZ). The DEZ is made of cells that transit from a state of division to a state of elongation. The region flanking the DEZ on its shootward side is the CEZ. It is made of cells that elongate at rates higher than

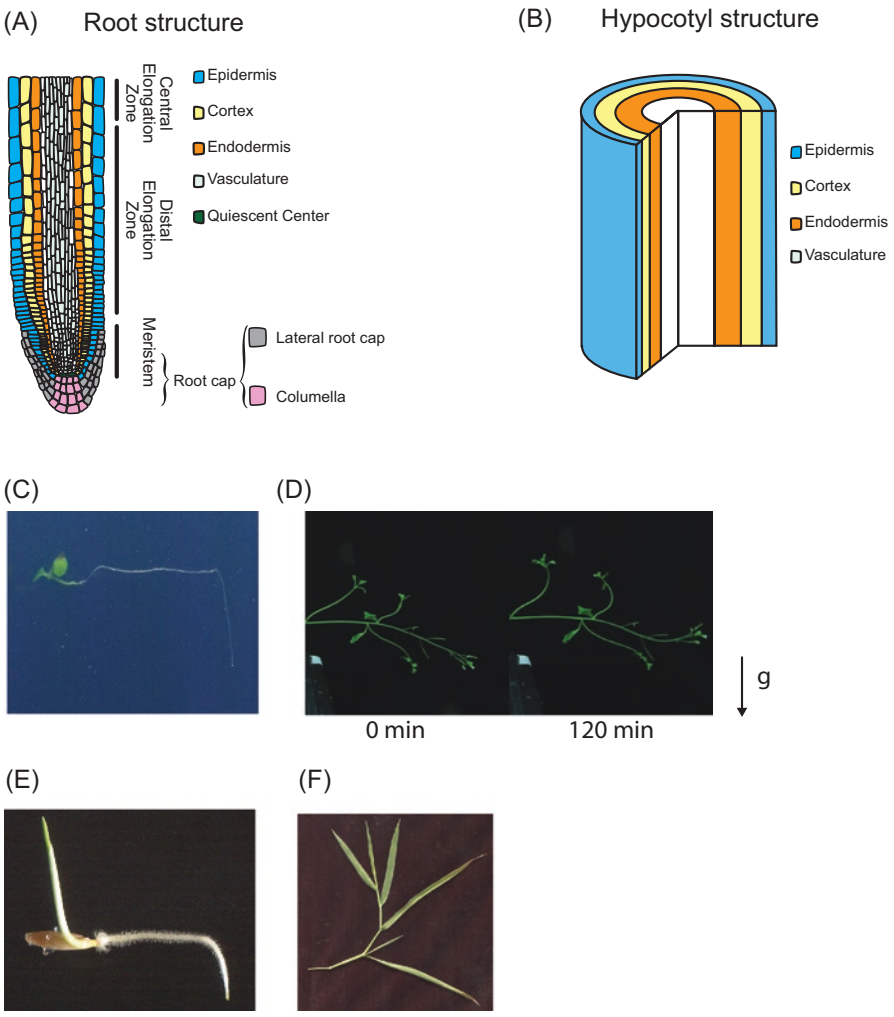

Fig. 5.1 Upon reorientation within the gravity field, plant organs respond by developing tip curvatures that involve differential cell elongation between upper and lower flanks of elongation zones. Panels (**a**) and (**b**) show the cellular organization and longitudinal patterning of the responding growth zone of dicot *Arabidopsis thaliana* root (**a**) and hypocotyl (**b**). Panels (**c**–**f**) show the gravitropic responses of (**c**) a gravistimulated *Arabidopsis thaliana* seedling, (**d**) an *Arabidopsis* inflorescence stem 0 and 120 min after reorientation, and (**e**, **f**) monocot *Brachypodium distachyon* seedling (**e**) and stem (**f**)

30% of their maximal value (Fig. 5.1a) (Ishikawa and Evans 1995). The DEZ is the site where the curvature response to gravistimulation (GS) initiates in roots, as discussed below (Ishikawa and Evans 1995).

Arabidopsis stems have also been used quite extensively in investigations of gravitropism. In young *Arabidopsis* seedlings, an embryonic stem named the

hypocotyl separates the root from the cotyledons and shoot apical meristem. This hypocotyl is made of several concentric layers of cells, including the epidermis, the cortex (2 layers), the endodermis and the stele containing vasculature tissues (Fig. 5.1b). The hypocotyl grows only by cell elongation, at least when etiolated (Gendreau et al. 1997). In fact, the pattern of cell elongation differs significantly between light-grown and dark-exposed hypocotyls. In light-grown seedlings, all epidermal cells elongate continuously during the entire growth period. However, in etiolated seedlings, elongation occurs along a steep acropetal spatial and temporal gradient (Gendreau et al. 1997). In both cases, the gravitropic curvature (Fig. 5.1c) occurs in the region of maximum cellular expansion.

In adult plants, the inflorescence stems are also made of several tissue types arranged in concentric circles, including the epidermis (one layer of cells), the cortex (three layers of cells), the endodermis (one cell layer), and the stele containing the vasculature. Cell divisions occur in the apical meristems, whereas elongation occurs along most of the stem length in young *Arabidopsis* plants. In older plants cellular elongation is restricted to more distal regions of the stem. The rootward side of older inflorescence stems is formed of mature cells that are surrounded by inextensible walls that contain lignin (Weise et al. 2000). The gravitropic curvature occurs only in the distal region of older mature stems, within their elongation zone (Fig. 5.1d).

In monocots, seedling coleoptiles and shoot pulvini develop strong gravitropic curvatures (Fig. 5.1e). The coleoptiles are seedling leaf sheaths that enclose the primary leaves and protect them as they grow up through the soil. Coleoptiles grow mostly by cell elongation, although some evidence of cell division has also been noted in wheat (Lu et al. 2006). The tip of a coleoptile is important for its growth and gravitropism, as a main source of auxin. The growing coleoptile remains capable of strong gravitropism as long as the developing leaves remain enclosed within it. As soon as the first growing leaf emerges from the tip, the coleoptile loses its ability to develop a gravicurvature (Iino 1995).

In adult monocot plants, shoot gravitropism typically involves the contribution of pulvini, which are short segments of tissue that are apical to the nodes and collectively contribute to bringing a shoot tip that was previously prostrated by wind or rain, back to a more vertical position. In *Panicoid* species like maize, the pulvini constitute disc-shaped segments of the stem, whereas the pulvini of *Festucoid* grasses, such as wheat, oat, and barley, are made of a tissue that encircles the leaf sheath immediately apical to the point where it attaches to the node. The cells making up the pulvini of an adult plant typically do not grow any more in the absence of a gravistimulus. However, when monocot stems are being prostrated by wind or rain, cells at the bottom side of several pulvini (two or four in maize) along the stem resume elongation, resulting in local segmental upward curvature (Fig. 5.1f). In maize, each pulvinus can provide a maximum of 30-degree curvature in response to gravistimulation. This process plays an important role in agriculture because it keeps seed away from soil moisture and pathogens, accessible to mechanical harvesting, even after the plants have been prostrated by heavy storms.

In addition to this important economical impact of the gravitropic response in cereals, pulvini have been the target of many investigations on plant gravitropism because their responses are very slow, making it possible to independently investigate the molecular mechanisms that contribute to gravity sensing and/or signal transduction, relative to those involved in the curvature response.

After this brief description of root and shoot elements that display gravitropism in monocot and dicot plants, we will now discuss some of the cellular and molecular mechanisms that drive gravitropism in plants, a process that includes several important steps: (1) gravity sensing and signal transduction, (2) signal transmission, and (3) curvature response.

5.2 Gravity Sensing and Signal Transduction

5.2.1 Amyloplast Settling Within Statocytes Contributes to Gravity Sensing

It has long been recognized that cells located at the center of the root cap (the columella cells) and the endodermis tissue surrounding the vasculature in shoots, coleoptiles and pulvini, are well suited for gravity sensing in plants because they contain dense starch-filled plastids that sediment to the bottom upon plant reorientation within the gravity field (Darwin 1880; Haberlandt 1900; Nemec 1900). Those plastids function as *statoliths* (solid structures/organelles that settle to the bottom of a cell). So, how do we know these cells actually perform a gravity-sensing function in plants?

To illustrate some of the experiments that allowed scientists to answer this question, we will use the root as an example. Indeed, roots have been extensively used to investigate the cellular and molecular mechanisms that govern gravitropism because they physically separate the primary site for gravity sensing (the cap) from the locale of curvature response (the EZ; Fig. 5.1a), facilitating assignment of key molecular mechanisms to distinct phases of a graviresponse.

Several experiments have demonstrated a key role for the root cap in gravity sensing. For example, removing the cap from primary roots by surgical ablation (Barlow 1995), killing specific cap cells with heavy-ion microbeam irradiation (Tanaka et al. 2002), genetically obliterating cap cells by targeted expression of the diphtheria toxin (Tsugeki and Fedoroff 1999) or mutating transcription factor genes that are needed for proper root cap specification (Wang et al. 2005), all resulted in roots that continued to grow, but were unable to develop a gravitropic response. Hence, the root cap is important for root gravitropism. However, does it function in the gravity-sensing phase of the process?

Before explaining some of the experiments that addressed the role of the root cap in gravisensing, we will discuss the concept of gravisensitivity. We will start this discussion by recognizing that plant organs appear to respond to transient gravistimulation by developing curvatures that vary linearly with the logarithm of the dose of stimulation, defined as the product of gravity level multiplied by the duration of

stimulation. Known as *the reciprocity law*, this relationship between curvature angle and dose of stimulation implies that plant organs might be able to sense the force of gravity and use this information to guide their growth. This concept of *force sensing* has been widely accepted in the field for many years, and researchers have used it to develop methods aimed at evaluating the gravisensitivity of plant organs (although we now know that this is an oversimplification of the process, as discussed later on in this chapter). One of these methods involves the quantification of organ curvature responses to small doses of gravistimulation. On Earth, such experiments involve reorienting the plant within the gravity field (1xg) for short periods of time and quantifying the resulting curvatures. A linear function associating the angles of curvature to the logarithm of stimulation times is then retrofitted to the data and extended to the time axis, intersecting it at a value that can be defined as the minimal gravistimulation time needed to induce a detectable curvature response. This minimal time is named the *presentation time*, and it is often viewed as a measure of gravisensitivity (Fig. 5.2).

While this strategy of presentation time determination seems simple at first glance, it is, in reality, complicated by the fact that most plant organs have presentation times that are much shorter than the time needed for the curvature responses to develop. Yet, the plants cannot be returned to the vertical after having been gravistimulated, to allow for curvature development, because this reorientation would constitute a second gravistimulus that would confound the data. To resolve this problem, researchers have used a rotating device, named the *clinostat*, to randomize the plant orientation within the gravity field while it responds to an initial transient gravistimulus.

Therefore, a typical experiment aimed at evaluating the presentation time of a plant organ involves the following sequence of events (Fig. 5.2). First, short gravistimuli are provided by reorienting the plants within the gravity field. After a defined period of gravistimulation, the plants are positioned on a clinostat, which continuously rotates them along a horizontal axis at a speed of approximately 1–4 revolutions per minute (rpm), over a period of 3–5 h. During this period, the plant organs are not exposed to directional gravistimulation for enough time to reset the gravity signal transduction pathway. Consequently, they develop a curvature that is a direct consequence of the initial short gravistimulus that preceded the clinorotation.

Many researchers have used the presentation time to represent gravisensitivity in plants. However, other investigators have warned that this concept might be misleading. First, earlier experiments had indicated that successive exposures to very short pulses of gravistimulation (much shorter than the presentation time) still allow plant organs to develop curvature responses, implying that such short stimuli are still perceived by the plant. Second, Dr. Perbal and his collaborators (Perbal et al. 2002) noted that the logarithmic model correlating observed angles of curvature to the logarithm of the dose/time of gravistimulation is actually not the best fit to the observed experimental data. In fact, a hyperbolic model better represents those data (Fig. 5.2b). It is quite significant that such a hyperbolic model intersects the X-axis (dose of stimulation) at the origin, invalidating the presentation time/dose concept. Therefore, these authors proposed to use the slope of the hyperbolic curve at the origin to estimate gravisensitivity.

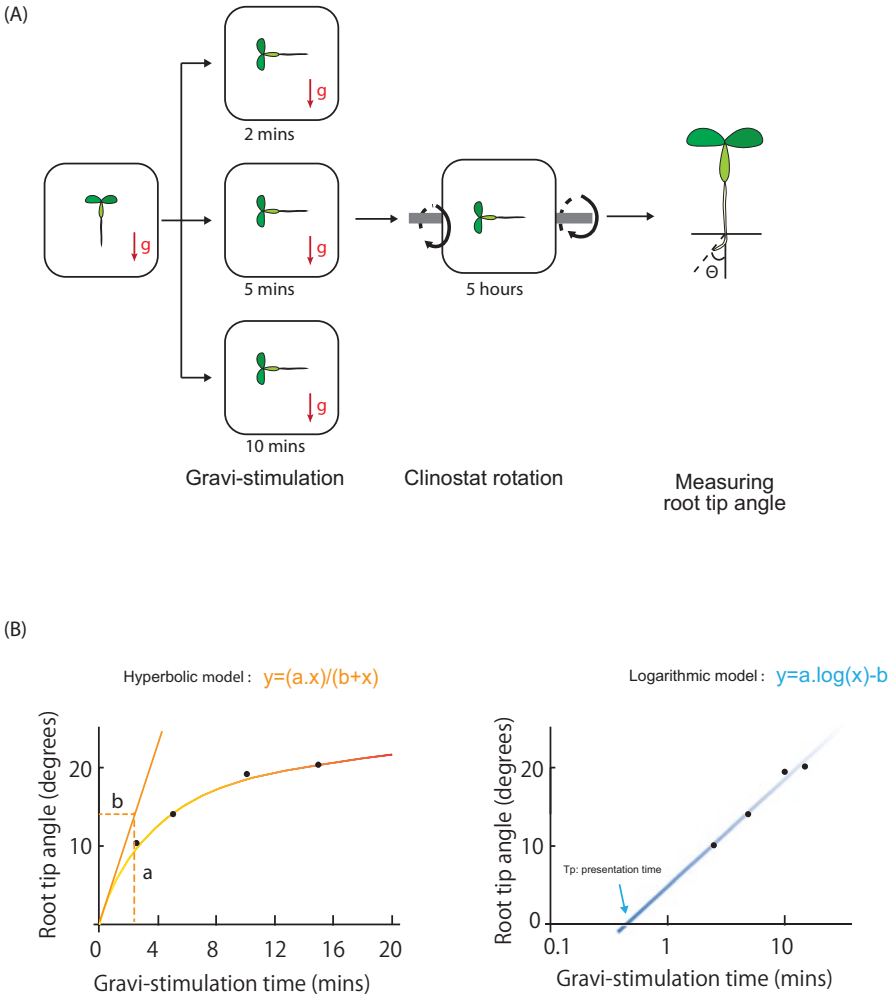

Fig. 5.2 Methods used to evaluate plant organ sensitivity to gravistimulation. Panel (**a**). Seedlings growing on agar-based media in petri dishes are reoriented to the horizontal for short periods of time (2, 5, and 10 mins in this case). After stimulation, the plates are transferred into a clinostat and rotated at a speed of 1–4 rpm for a period of 5 h, thereby constantly modifying the organs' orientation relative to gravity and avoiding additional gravistimulation (middle section of the drawing). During this period of clinorotation, plant organs will develop a curvature response to the initial stimulus. At the end of this process, the orientations of organ tips are measured (right side of panel **a**), providing data that relate the resulting tip angle to the dose (period) of gravistimulation. Panel (**b**). Two mathematical models have been used to represent the relationship between angle of curvature and dose/period of gravistimulation: the hyperbolic model (left) and the logarithmic model (right). The logarithmic model intersects the time axis at a point named the *presentation time*, which represents the minimal gravistimulation time needed to activate a curvature response. The hyperbolic model, on the other hand, intersects the time axis at the origin. It has been proposed that the slope of the curve at the origin (S = a/b) is another measure of gravisensitivity. The data points shown in these two graphs are identical, deriving from *Arabidopsis* roots subjected to the protocol described in panel A by Caspar and Pickard (1989), as cited in Perbal et al. (2002)

Choosing between these two alternative methods of gravisensitivity determination would require a better understanding of the molecular mechanisms that govern gravity sensing and signal transduction in plants. Short of such information, researchers have adopted both models to evaluate root gravisensitivity in mutants or pharmacologically treated seedlings. In general, these models resulted in similar ranking of gravisensitivities between mutants/treated seedlings and wild-type/control samples (for instance, see (Blancaflor 2013)).

The two methods described above allow estimation of organ gravisensitivity on Earth, exposed to 1xg. However, access to the microgravity environment of the International Space Station allows the design of more direct experiments aimed at measuring plant organ sensitivity to gravity. Under microgravity, fractional g conditions can be created by centrifugation. The minimal centrifugation force needed to trigger an organ tip curvature can be determined. Although very expensive, these experiments have demonstrated that the plant gravisensing machinery is overbuilt, reacting to forces that are well below those encountered under 1 g conditions on Earth (Kiss et al. 2012).

Despite the heavy reliance on presentation time to evaluate gravisensitivity of plant organs, it is important to note that recent studies have suggested that the force sensor model of gravity sensing described above, which is key to the concept of presentation time as a measure of gravisensitivity, may actually not be an adequate or complete representation of the actual mechanism used by plant statocytes to sense gravity. In fact, a mechanism that would sense the *inclination* of the plant relative to gravity rather than the force of gravity may actually be at play (Chauvet et al. 2016). This *inclination sensor model* of gravity sensing will be described in the next section.

5.2.2 The Columella Cells Serve as Statocytes in Roots

The experiments described in the previous section suggest that the root cap contributes to gravity sensing in roots. Which cap cells contribute to this process? As noted above, a simple morphological and cytological analysis of the root cap provides a possible answer to this question. In fact, at the center of the root cap, a group of large cells (the columella cells) appear well suited to serve as statocytes. Devoid of large vacuoles, these cells display a unique organization that suggests a role in gravity sensing. Their nucleus is located in their upper (shootward) half, and their central cytoplasm is depleted of organelles. The endoplasmic reticulum (ER) lines the periphery at the distal side of these cells, as do most other organelles. Importantly, these cells contain large and dense starch-filled plastids (amyloplasts). In most other cell types within the plant, plastids are tightly associated with the actin cytoskeleton network. However, in the columella cells, these organelles are only loosely associated with the cytoskeleton. As a consequence, they do sediment to the bottom while also bouncing around in a saltatory movement that probably derives from transient interactions with a highly dynamic actin cytoskeleton present in these cells as well as with subtending ER membranes (Bérut et al. 2018; Leitz et al. 2009). When a

plant is reoriented within the gravity field, columella amyloplasts (also called *statoliths*) quickly settle to the new bottom side of the cells, following a liquid-like behavior that is dependent upon cellular activity (Bérut et al. 2018). This amyloplast system repositioning within the statocytes triggers a gravity signal transduction pathway that is largely uncharacterized.

How do we know that the columella cells of the root cap contribute to gravity sensing in plants? To answer this question, Alison Blancaflor and his collaborators evaluated the presentation time (interpreted as a measure of gravisensitivity) of *Arabidopsis* roots after distinct cells of the root cap had been killed by laser ablation (Blancaflor et al. 1998). In these experiments, wild-type *Arabidopsis thaliana* seedling roots (Columbia accession) displayed a presentation time of approximately 1.16 min. When cells from layers S1 and S2 of the columella region of their root caps were ablated, the presentation time was increased to 7.13 min, suggesting a decrease in root gravisensitivity relative to control (Blancaflor et al. 1998). A similar alteration was observed when all central columella cells were ablated, suggesting that layers S1 and S2 of columella cells contribute most to gravisensing in roots (Blancaflor et al. 1998). On the other hand, ablating lateral cap or distal tip cells did not affect much the presentation time of treated roots, confirming that gravisensing is mostly performed by a few specialized cells at the center of the root cap – precisely those that contain amyloplasts with the highest sedimentation capability.

Does amyloplast repositioning within the statocytes contribute to gravity sensing? Investigations of gravitropism in starchless and starch-deficient mutants seem to support a role for amyloplast sedimentation in gravity sensing. Indeed, root cap amyloplasts of starchless mutants do not sediment under 1 g because of their lower density in the absence of starch. This phenotype is associated with altered gravitropism, suggesting a role for amyloplast sedimentation in gravity signal transduction (Band et al. 2012; Kim et al. 2011; Kiss et al. 1989, 1996; MacCleery and Kiss 1999). Second, starch-deficient mutants that carry amyloplasts with limited amount of starch do not show evidence of amyloplast sedimentation upon gravistimulation under normal conditions. They also show an altered gravitropic phenotype. However, increased g forces provided by centrifugation can promote a lateral displacement of these starch-deficient amyloplasts, allowing resumption of gravitropism (Fitzelle and Kiss 2001). On the other hand, mutations that affect starch-degrading enzymes, such as *starch excess 1 (sex1)* in *Arabidopsis thaliana*, or conditions that result in larger amyloplasts, are associated with greater sensitivity to gravity (Vitha et al. 2007).

Another key experiment that addressed a role for amyloplast settling in gravity signaling relied on the use of high-gradient magnetic fields to laterally displace amyloplasts within the statocytes of vertically oriented seedlings. Being diamagnetic, starch grains can be displaced laterally by application of a local high-gradient magnetic field. The corresponding ponderomotive force is sufficient to displace the statoliths in a direction that is dictated by the geometry of the gradient. Placing paramagnetic particles in proximity of vertical plant organs within a magnetic field creates local high-gradient magnetic fields that are sufficiently large to mobilize amyloplasts within the root cap columella cells, moving them laterally. This lateral displacement of amyloplasts within the statocytes was associated with the

development of a tip curvature in the direction dictated by statolith movement (Kuznetsov and Hasenstein 1996). The curvature was not an indirect consequence of exposure to magnetic fields because it did not occur when starchless mutants (whose plastids cannot be displaced by the magnetic fields) were used.

While amyloplast movement within the statocytes is sufficient to trigger a tip curvature, it should still be cautioned that these statoliths are not completely free to sediment. As mentioned above, a dynamic actin-filament network is also present, which transiently interacts with the statoliths, promoting saltatory movements that may fine-tune the gravitropic response (Leitz et al. 2009; Zheng et al. 2014). Interestingly, mutations that affect actin dynamics, such as *distorted1*, lead to slower kinetics of gravitropism (Zheng et al. 2014). On the other hand, treatments with agents that affect actin-filament dynamics (such as latrunculin B or D) resulted in enhanced kinetics of gravitropism, increased gravisensitivity, and gravitropic signal persistence leading to overshooting the vertical at the end of a response (Hou et al. 2003; Yamamoto et al. 2002).

The experiments described above document a key role for root cap amyloplast sedimentation in gravisensing. However, several experiments have also suggested the existence of a secondary site for gravisensing in roots, localized at the DEZ (Kiss et al. 1999). Indeed, to better characterize the spatiotemporal distribution of gravisensing in responding plant organs, investigators developed a novel device named the *rotato*, which maintains a specific region of the root tip at a predefined angle from the vertical over time. The *rotato* is a microscope equipped with a rotating vertical platform that carries petri dishes with growing seedlings in front of the objective (Fig. 5.3a). This platform is equipped with a motor that automatically rotates it to maintain a defined angle between a small, predefined segment of the root tip and the vertical. If the system is set up to maintain the root cap at a defined angle from the vertical over time, the platform will continue to rotate as the root curves, attempting to return the tip to the vertical (Fig. 5.3b). The speed of rotation defines the kinetics of tip curvature.

If the *rotato* is set up to maintain a subapical region of the root tip at a defined angle from the vertical, the tip is expected to return to the vertical. As soon as the vertical is reached, the root should stop curving, and the tip should resume vertical downward growth without platform rotation (as the gravity set point angle is reached). However, when Wolverton and his collaborators carried out the latter experiment by attempting to keep the DEZ at a constant angle from the vertical, the platform continued to rotate even after the cap had reached the vertical and gone beyond it (Wolverton et al. 2002). This result was surprising and important because it suggested that cells within the root DEZ may also be able to sense gravity. In fact, this conclusion could be corroborated by other observations. For instance, in maize seedlings, decapped roots remain somewhat gravitropic, a response that can be enhanced by disrupting actin filaments or manipulating myosin activity (Mancuso et al. 2006).

These observations are puzzling because the cells in the DEZ of roots do not contain starch-filled plastids, suggesting that a different mechanism of gravity sensing might be at play in these cells. In fact, researchers have postulated that DEZ

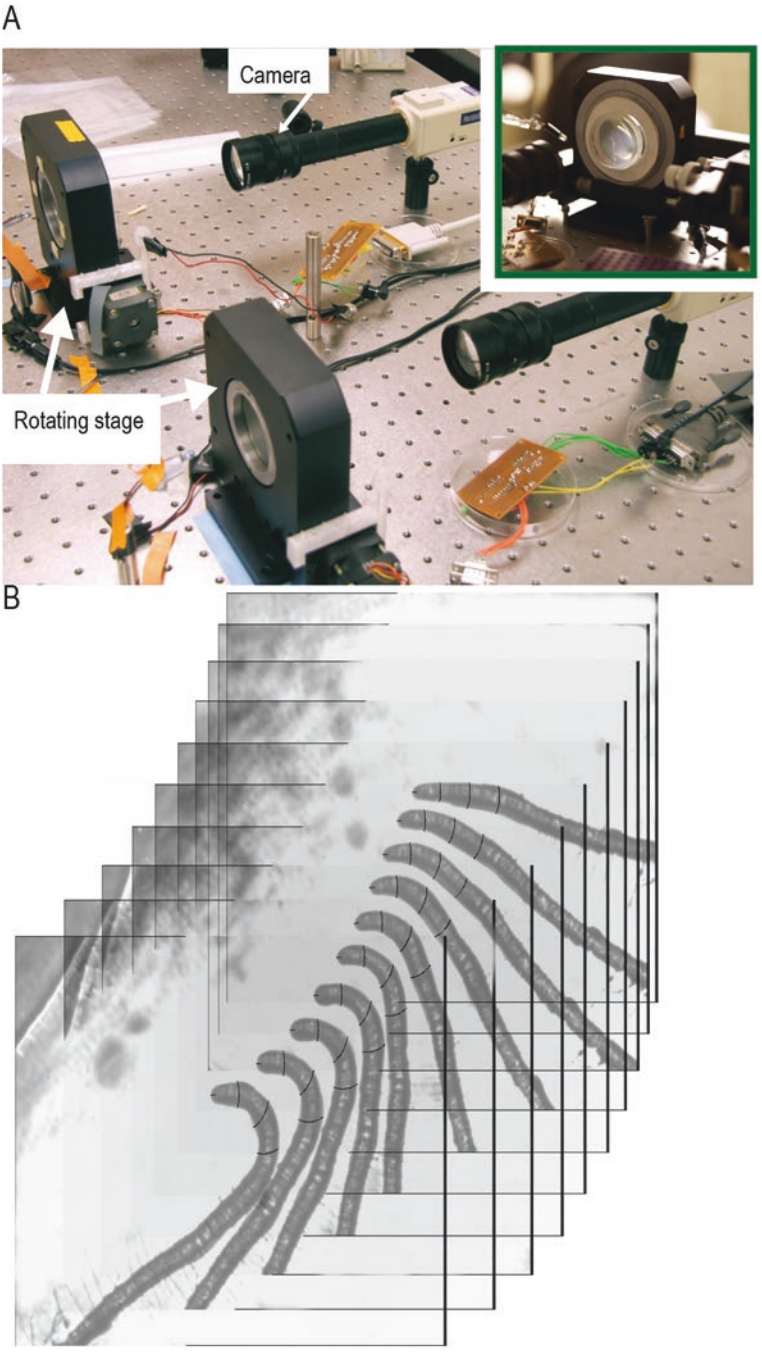

Fig. 5.3 *Rotato* is a useful device to study gravitropism. Panel (**a**) shows two *rotato* devices working side-by-side in the Wolverton laboratory at Ohio Wesleyan University. Each device is made of

cells might be able to sense gravity by detecting the overall pressure exerted by the protoplast on its wall. A similar mechanism was previously suggested to explain gravity sensing by the large internodal cells of *Chara* and rice root tips (Staves et al. 1992, 1997).

5.2.3 Transduction of the Information Conferred by Amyloplast Movement and/or Position Within the Statocytes

The conclusion of the experiments described in the previous sections of this chapter is that the settling of amyloplasts within the statocytes constitutes a first step in gravity sensing by plants. We will now describe the molecular mechanisms that govern gravity signal transduction in root statocytes.

In roots, the physical separation that exists between sites of gravity sensing and the location of curvature response implies that there is a need for communication between these two regions in order for an adequate response to occur. In other words, the physical information provided by amyloplast settling in the columella cells of the cap has to be converted into a biochemical signal that needs to be transmitted to the DEZ to trigger the resulting curvature. As discussed below, this signal takes the form of a lateral auxin gradient generated across the root cap upon gravistimulation. A first question that comes to mind is: How is amyloplast settling within the statocytes transduced into a repolarization of auxin transport, leading to the formation of a lateral auxin gradient across the cap?

Two models have been suggested to explain the transduction of information provided by amyloplast settling in the statocytes. The first model suggests that sedimenting statoliths press upon sensitive membranes on the inside of the statocytes, triggering the opening of mechanosensitive ion channels, with consequent Ca^{2+} spikes in the cytoplasm (Sievers et al. 1991). As a second messenger, Ca^{2+} would trigger a local transduction pathway leading to statocyte repolarization. The second model (named "ligand-receptor hypothesis") postulates the existence of ligands on the surface of sedimenting statoliths. These ligands would interact with receptors located within sensitive membranes on the side of the statocytes to activate the gravity signaling pathway (Limbach et al. 2005). This model emerged from investigations of gravitropism in single-cell rhizoids from the green algae *Chara*. Whether it also applies to the statocytes of flowering plant organs remains unknown.

As pointed out above, the first model postulates a contribution of mechanosensitive ion channels to gravity signaling within the statocytes. Unfortunately, the

Fig. 5.3 (continued) a camera located in front of a rotating platform that holds a Petri dish with growing seedlings. An automatic software controls the rotation of the platform to maintain a defined region of the plant organ (root in this case) at a pre-specified angle from the vertical. The software records the speed of stage rotation needed to fulfill this condition. Panel (**b**) (copied from Wolverton et al. 2002) shows images from a 4-h time-lapse analysis of an *Arabidopsis* root growing on the *rotato* system, which was set up to maintain the root tip region at 90° from the vertical. The stage rotates clockwise as the root curves, to maintain the tip at 90°. These two panels were kindly provided by Dr. Chris Wolverton, Ohio Wesleyan University

channels responsible for this process have not been identified. However, pharmacological studies using drugs that inhibit the opening of ion channels, chelate Ca^{2+}, or inhibit Ca^{2+} sensors, such as calmodulins, calmodulin-like proteins, and/or Ca^{2+}/calmodulin-dependent protein kinases, strongly affected plant gravitropism, suggesting a role for Ca^{2+} in gravitropic signaling (Lu and Feldman 1997; Sinclair and Trewavas 1997). Unfortunately, gravity-induced changes in cytosolic Ca^{2+} levels within the statocytes have not been documented. For instance, investigators have used a transgenic AEQUORIN Ca^{2+}-reporter system to analyze possible changes in cytosolic Ca^{2+} levels early in response to gravistimulation. This system involves expressing a protein named AEQUORIN in transgenic plants. When present in the cytoplasm, this protein can be altered to emit photons in a Ca^{2+}-dependent manner by simply adding a luminophore named coelenterazine to the medium. This compound is taken up by the plant and accumulates in the cytoplasm of exposed cells. Expressed AEQUORIN binds to available coelenterazine within the cell, forming a complex that emits light in a Ca^{2+}-dependent manner. Using this system, investigators demonstrated the existence of biphasic spikes in cytosolic Ca^{2+} within seconds of a gravistimulus (Plieth and Trewavas 2002; Toyota et al. 2008). Yet, these Ca^{2+} spikes derived only from hypocotyls and petioles, not roots, and they could not be assigned to specific cell types because the signal was too small to allow cell-specific mapping (Toyota et al. 2008). In fact, we now know that Ca^{2+} contributes to plant cell responses to auxin ((Monshausen et al. 2011); see below). Whether it also contributes to gravity signal transduction in the statocytes remains unclear.

A possible involvement of Ca^{2+} in gravity signal transduction has also been suggested based on observations of changes in inositol 1,4,5-trisphosphate ($InsP_3$) levels in oat coleoptiles and *Arabidopsis* inflorescence stems upon gravistimulation (Perera et al. 2006). As a component of the phosphoinositide-signaling pathway, $InsP_3$ is a signaling molecule that has been implicated in the regulation of cytosolic Ca^{2+} levels and vesicle trafficking in both animal and plant systems (Munnik and Nielsen 2011; Perera et al. 2006). Interestingly, overexpression of human inositol trisphosphatase, which hydrolyzes $InsP_3$, in *Arabidopsis* roots, stems and hypocotyls, caused altered gravitropism (Perera et al. 2006). Similarly, inhibiting the synthesis of $InsP_3$ with a phospholipase C inhibitor (U73122) led to altered root gravitropism (Andreeva et al. 2010), whereas mutations affecting the *Arabidopsis INOSITOL POLYPHOSPHATE 5-PHOSPHATASE 13* gene enhanced gravitropism while reducing endomembrane trafficking (Wang et al. 2009). Hence, $InsP_3$ and/or other molecules of the phosphoinositide-signaling pathway may play a role in gravity signaling (Perera et al. 2006). Whether this $InsP_3$ contribution to gravity signaling implies a role for Ca^{2+} in this process remains unclear, though, as the ability of $InsP_3$ to gate the opening of Ca^{2+} channels at intracellular locations is not as obvious in plants as it is in animals (Munnik and Nielsen 2011).

The first models described above assume that the plant gravisensing machinery is a force sensor. This assumption was initially based on the fact that the gravitropic response of plant organs appears to follow *the reciprocity law* (the curvatures resulting from transient gravistimulation vary linearly with the logarithm of the dose of gravistimulation, as discussed above). However, recent, clever experiments carried

out by Hugo Chauvet and collaborators cast doubts on this assumption. Growing wheat seedlings in growth chambers carried by a rotating table, these authors were able to show that the developing coleoptiles respond to *continuous gravistimulation* within a large range of effective gravity forces ($0.5xg_{earth}$ to $2g_{earth}$) with *similar angles of curvature*. In other words, under their experimental conditions, the coleoptiles did *not* follow the reciprocity law. On the other hand, the curvature response developed by these coleoptiles followed the so-called sine rule, which postulates that the curvature response to gravistimulation is proportional to the sine of the angle of stimulation (the inclination of the plant). The authors interpreted their results by suggesting that the gravity sensing machinery in plants functions as an *inclination/position sensor* rather than a force sensor (Fig. 5.4). The inclination sensor would be sensitive to the position of the *bulk of amyloplasts* within the statocytes, rather than responding to the force exerted by sedimenting amyloplasts (or the entire protoplast) on sensitive membranes. Under their model, plant organs subjected to transient gravistimulation (such as those subjected to a presentation-time assay) would follow the reciprocity law simply because these experiments involve transient stimuli that are sufficiently short to only allow incomplete repositioning of the amyloplasts within the statocytes under regular $1xg_{earth}$ conditions. Consequently, under such conditions, higher doses of g would promote a faster sedimentation of the plastids to the bottom of the cells, allowing a stronger graviresponse. In experiments that involve continuous gravistimulation, amyloplasts are allowed to fully settle at the bottom of the statocytes, allowing for a full response to develop. Therefore, the presentation time experiment is a better setup to evaluate effectiveness of amyloplast sedimentation than it is to estimate organ gravisensitivity. Similar results and conclusions were obtained when these experiments were repeated with seedlings of a wide range of plant species, including representatives of *Asterids*, *Rosids*, and *Commelids*. Although roots were not tested in these experiments, the shoots of these diverse plant groups developed gravitropic responses that obeyed the sine law and were independent of gravity intensity. Therefore, the shoots of these diverse species may also use a mechanism of gravisensing that involves an inclination sensor system (Bérut et al. 2018; Chauvet et al. 2016).

The ligand-receptor model discussed earlier in this section would function as an inclination sensor mechanism, as would other models that postulate functional interactions between the group of sedimented amyloplasts and the vesicle trafficking machinery that is critical for proper location of auxin transporters (Pouliquen et al. 2017).

It is interesting to note here that the inclination sensor hypothesis is, in fact, compatible with the ability of plant organs to respond to very low inclinations from the vertical while not overreacting to the vibrations created by wind, rain, or other temporary environmental perturbations (Pouliquen et al. 2017).

In summary, two main models have been suggested to explain gravity sensing by amyloplast sedimentation in the statocytes: (1) the force sensor model suggests that amyloplasts settling on side membranes of a statocyte, or the entire weight of the protoplast on its cell wall, may trigger a transduction pathway within these cells, possibly by promoting the opening of mechanosensitive ion channels that remain to

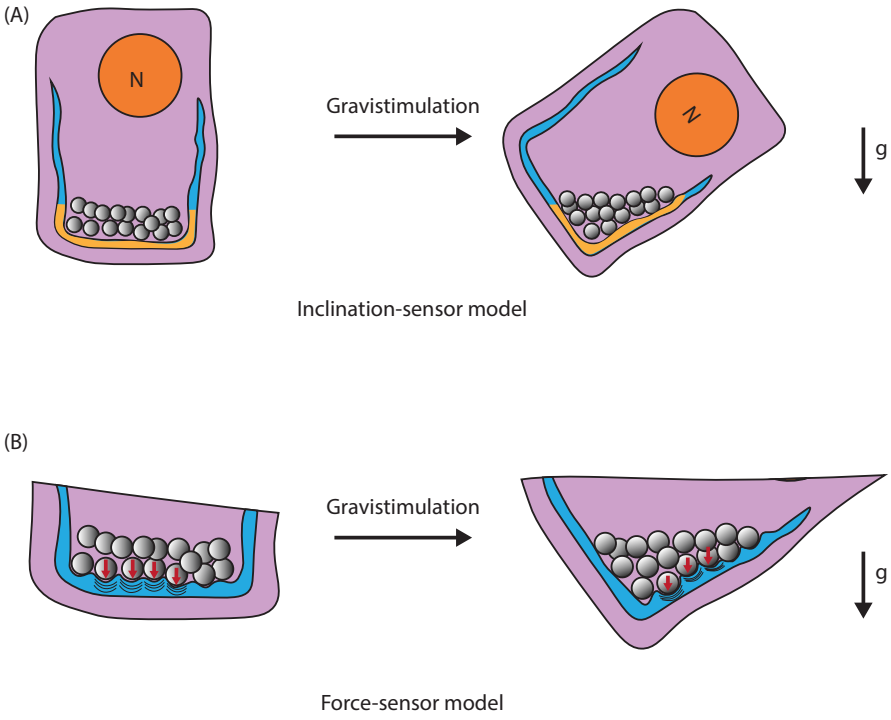

Fig. 5.4 Two models have been proposed to explain gravisensing by plant statocytes. In the *inclination sensor* model (panel **a**), the position of sedimenting amyloplasts within the statocytes determines the polarity of auxin transport. In the statocyte illustrated here, amyloplasts occupy a larger surface on the right side of the cell after reorientation (right drawing) relative to the vertically oriented cell (left). The area of peripheral ER covered by sedimenting amyloplasts is represented in yellow. This model predicts that the curvature response to gravistimulation will not depend upon the pressure level. It also predicts that very small levels of inclination can trigger a curvature response. In the *pressure sensor* model (panel **b**), the force exerted by sedimenting amyloplasts on sensitive membranes (red arrows) triggers the opening of mechanosensitive ion channels, activating a transduction pathway that leads to statocyte polarization. This model predicts that a curvature response to gravistimulation will depend upon the pressure level. In these drawings, the peripheral ER is represented in blue and yellow, whereas amyloplasts are represented by gray circles. *N* Nucleus

be characterized, and (2) the recently proposed inclination sensor model, which postulates that the location of the amyloplast system within the statocytes, rather than the pressure exerted by individual amyloplasts or the overall protoplast, is the main response trigger. While most investigators have focused many years of research on attempting to resolve the molecular machinery that makes up a force sensor in plant statocytes, the clever experiments carried out by Moulia and collaborators suggest a distinct mechanism responding to organ inclination rather than force. Recognition of this possibility is reshaping our view of the process, and it is likely

to catalyze new exciting research to identify the molecular mechanisms that contribute to gravisensing in plants.

In conclusion, we do not know the identity of the gravitropic receptors that function to activate the gravitropic signal transduction pathway in the statocytes in response to gravistimulation. However, we do know that this pathway leads to an asymmetric redistribution of auxin across the root tip, ultimately responsible for differential cell elongation between upper and lower flanks at a distal side of the elongation zone. We will now review some of the molecular mechanisms that lead to gravity-induced lateral polarization of the statocytes. However, before we do so, we will provide a brief description of the molecular mechanisms that govern auxin synthesis, transport and response in plants.

5.2.4 How Is Auxin Synthesized and Transported Within the Plant, and How Do Plant Cells Respond to It?

Auxin is a hormone that contributes to many facets of plant growth and development regulation as well as responses to the environment. Before discussing its contribution to gravitropism, we will first describe some of the molecular mechanisms that specifically contribute to auxin transport and response.

Auxin is mostly synthesized in young shoot tissues, using a combination of tryptophan-dependent and tryptophan-independent pathways (Zhang and Peer 2017). From there, it is transported to other regions of the plant where it regulates a variety of cellular processes including cell division, elongation, differentiation and death. It is also transported through the vasculature into the root tip, where it adds to a pool of locally synthesized auxin and accumulates in the quiescent center and upper layers of the columella cells. From this maximum center at the root tip, auxin is redistributed to more peripheral tissues and then transported back toward the root meristem and elongation zone, where it regulates cell division, inhibits elongation, and modulates cell differentiation (Brumos et al. 2018; Ding and Friml 2010; Mironova et al. 2010). Auxin transport is a highly regulated process that follows cell files. In each transporting cell within a file, auxin import facilitators of the *AUX1/LAX* family contribute to auxin uptake from the apoplast, helped along by free auxin diffusion across the plasma membrane. The latter process is possible because indole-3-acetic acid (IAA, the most common natural auxin in plants) is a weak acid, and the acidic condition of the apoplast (pH~5.6) facilitates its protonation, a process that is needed for free diffusion across the plasma membrane. On the other hand, plant cell cytoplasm has a neutral pH, resulting in the ionization of almost all auxin molecules within the cell. Ionized auxin cannot cross membranes. Therefore, auxin efflux facilitators of the PIN family, along with P-glycoprotein-type transporters (such as AtPGP1 and AtPGP19 in *Arabidopsis*; (Geisler et al. 2005; Noh et al. 2001)), are needed to export it away from the cell interior. Interestingly, the PIN proteins are often distributed asymmetrically within the plasma membrane, accumulating at one side of the transporting cells. Therefore, the polarity of auxin

transport through cell files is dictated by the polar localization of the PIN auxin efflux facilitators in transporting cells (Fig. 5.5).

Auxin influx and efflux carriers are encoded by rather large gene families in plants, with each gene within a family displaying specific expression patterns and protein localizations. In *Arabidopsis* roots, the PIN1, PIN3, PIN4, and PIN7 proteins contribute to auxin transport toward the tip ("rootward" transport) through provasculature cell files. By contrast, the PIN2 protein contributes to auxin transport from cap to elongation zone ("shootward" transport) within peripheral tissues. The *PIN2* protein localizes on the shootward-facing side of the lateral cap and epidermal cells of the elongation zone, moving auxin in a shootward direction (away from the root tip toward the shoot). Additionally, PIN2 is also expressed in the cortical cells of the elongation zone, where it localizes on the inner and rootward-facing side of the cells, thereby refluxing auxin from the shootward peripheral stream back to the central rootward flow (directed toward the root tip; Fig. 5.5) (Adamowski and Friml 2015).

The molecular mechanisms that contribute to cellular responses to auxin have been well investigated, involving a combination of gene expression-dependent and gene expression-independent processes. A fast response to auxin involves the direct activation of ion channels (Fig. 5.6a). In roots, auxin promotes a depolarization of the responding cell, associated with an alkalinization of the apoplast, which leads to increased cell wall rigidity, decreased cell elongation and slower root growth (Cosgrove 2000; Monshausen et al. 2011). This process is dependent upon auxin uptake by the cell, and its use by a cytoplasmic pool of an SCF-based ubiquitylation complex that contains the TIR1/AFB auxin receptor. This process leads to the activation of a cyclic nucleotide-gated ion channel (CNGC14). Opening of this channel results in increased levels of cytoplasmic Ca^{2+}, leading to the activation of a plasma membrane H^+/OH^- conductance, with concomitant alkalinization of the apoplast (Monshausen et al. 2011; Mullen et al. 1998). This alkalinization leads to increased cell wall rigidity, decreased cell elongation, and lower root growth rate (Cosgrove 2000; Dindas et al. 2018; Monshausen et al. 2011).

In gene expression-dependent responses, intracellular auxin interacts with the same $SCF^{TIR1/AFB}$ complex, allowing its interaction with and ubiquitylation of a group of AUX/IAA proteins, targeting them to degradation by the proteasome (Fig. 5.6a). Because these AUX/IAA proteins serve as inhibitors of AUXIN RESPONSE FACTORS (transcription factors that regulate the expression of specific subgroups of target genes), auxin allows ARF-dependent regulation of target gene expression (Dharmasiri et al. 2005; Kepinski and Leyser 2005; Lau et al. 2011). Participation of this system in root gravitropism is evidenced by the altered gravitropism phenotype displayed by *Arabidopsis tir1* mutants (Dharmasiri et al. 2005; Kepinski and Leyser 2005; Lau et al. 2011).

Plants contain many genes that encode AUX/IAA (29 in *Arabidopsis*, for instance) and ARF proteins (23 in *Arabidopsis*). Each cell type expresses specific subsets of *AUX/IAA* and *ARF* genes. Furthermore, different AUX/IAA proteins display distinct binding preferences for different groups of ARF proteins. This combinatorial system leads to cell-specific regulation of gene expression in response to

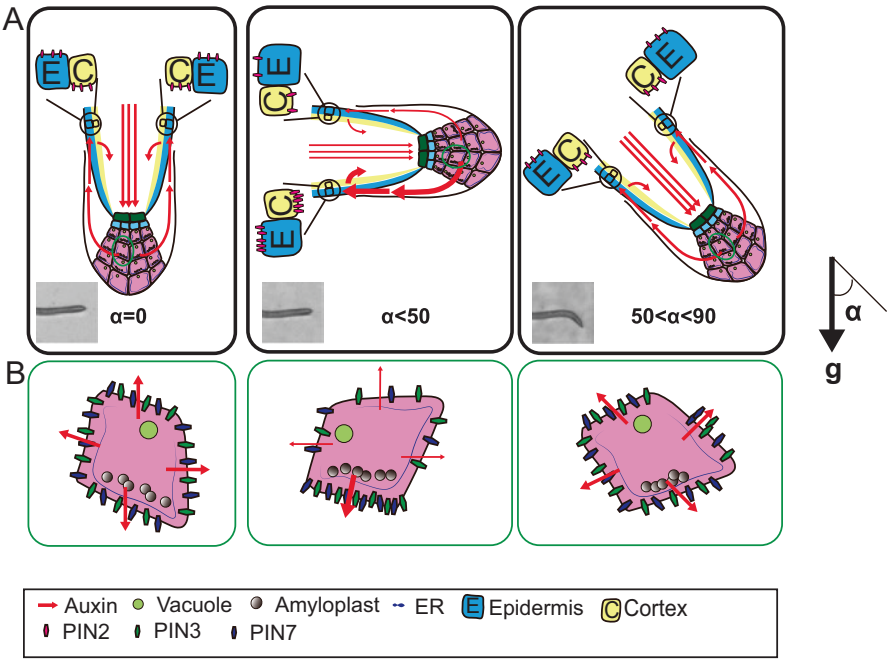

Fig. 5.5 In roots, gravistimulation promotes the formation of a lateral auxin gradient across the cap, which is responsible for a tip curvature upon transmission to the elongation zone. Panel (**a**) shows the flow of auxin in vertical (left) and gravistimulated seedlings (middle and right drawings), whereas panel (**b**) shows amyloplast sedimentation and PIN protein relocalization within the statocytes at different phases of a graviresponse. The reverse fountain model of auxin transport is shown in the left drawing of panel A. Mainly synthesized in young shoot tissues, auxin is transported through the vasculature into the root tip where it accumulates at the quiescent center and upper tiers of the columella region of the cap. It is then redistributed laterally to more peripheral tissues of the lateral cap, using the PIN3 and PIN7 auxin efflux carriers. From the lateral cap, auxin is transported back toward the elongation zone through lateral cap and epidermal cells. Auxin transport through cell files involves AUX1 influx carriers and polarized PIN2 efflux transporters. PIN2 is also expressed in the cortical cells of the root distal elongation zone, where it localizes to the inner and rootward membranes, contributing to a reflux of peripheral auxin toward the provasculature. Upon gravistimulation (middle panel), amyloplast sedimentation within the statocytes triggers a gravity signal transduction pathway that leads to a polarization of the PIN3 and PIN7 proteins to the lower side of the cells. Consequently, auxin is transported preferentially to the lower flank of the cap, and the resulting gradient is transferred toward the elongation zone where it triggers a downward curvature. Increased levels of auxin on the lower side of the root also result in increased retention of the PIN2 auxin efflux carrier within the plasma membrane relative to cells on the topside, contributing to auxin gradient maintenance. When the graviresponding root tip reaches an angle of 50° from the vertical (right panel), amyloplasts have settled back to their original position within the statocytes, and PIN3 and PIN7 redistribute more or less symmetrically on all sides of the statocytes. Consequently, the lateral auxin gradient dissipates, and the remaining curvature toward the vertical proceeds in the absence of an auxin gradient. In these drawings, the direction of auxin transport is represented by red arrows, whose widths represent auxin flow intensities. C represents a cortical cell whereas E represents the epidermis. Other symbols are defined in the legend provided underneath the figure. This figure is a modification from Figures 2 and 3 in Su et al. (2017)

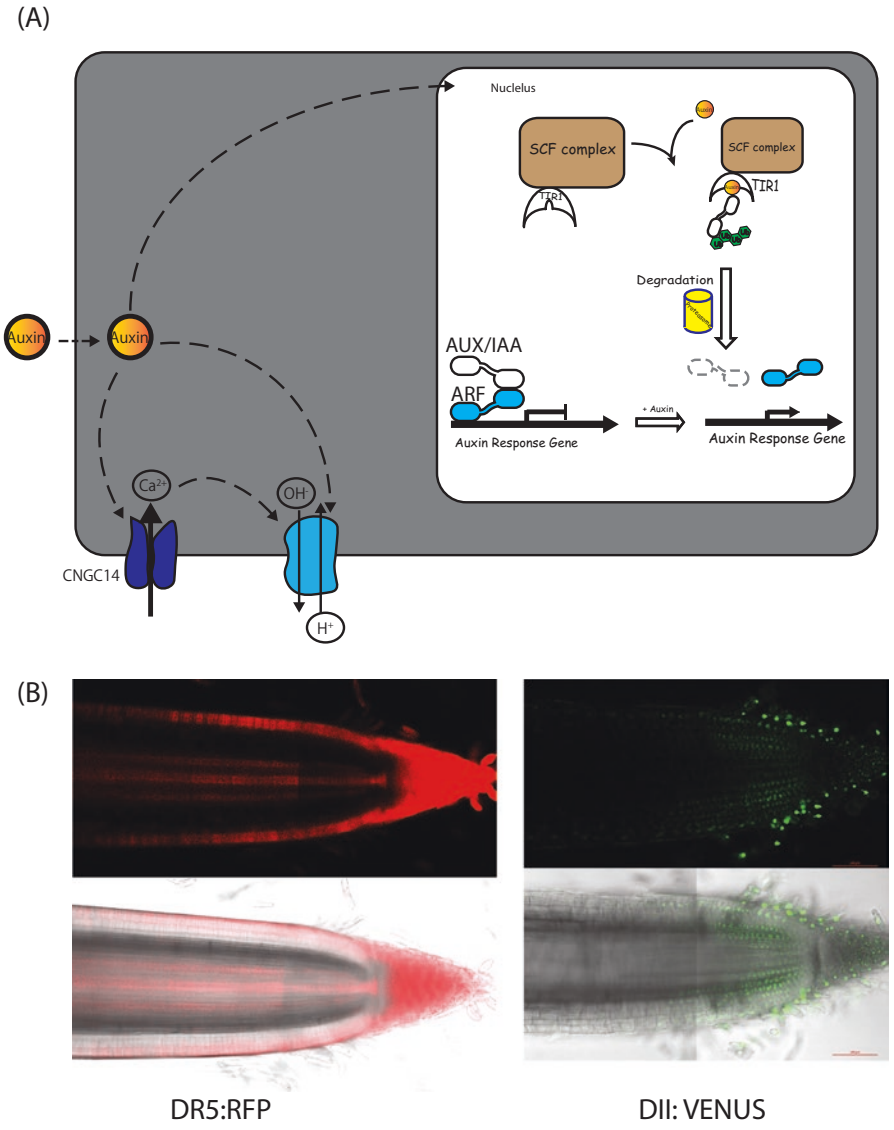

Fig. 5.6 Plant cellular responses to auxin involve expression-dependent and expression-independent processes. Panel (**a**) represents an *Arabidopsis* root cell responding to auxin (orange circle). Auxin penetrates the cell by free diffusion through the plasma membrane or through AUX1-like transporters. Once in the cytoplasm, auxin activates the opening of a cyclic nucleotide-gated ion channel (CNGC14), allowing a pulse in cytoplasmic Ca^{2+}. This triggers a pathway that leads to activation of a H^+/OH^- antiporter, responsible for alkalinization of the apoplast and inhibition of cell expansion. Some auxin molecules can also enter the nucleus, where they favor the formation of a complex between the SCF^{TIR1} ubiquitylation enzyme and Aux/IAA target proteins. Without auxin, Aux/IAA interacts with auxin response transcription factors (ARFs), inhibiting their function. In the presence of auxin, the Aux/IAA proteins are ubiquitylated by SCF^{TIR1} and

auxin. Some of the auxin-responsive genes expressed in the epidermis were shown to encode proteins that contribute to cell wall remodeling (Swarup et al. 2005). Others were shown to encode proteins that modulate auxin conjugation, thereby contributing to feedback regulation of the pathway (Zhang and Peer 2017). The *SMALL AUXIN UP RNA* (*SAUR*) genes, which are among the fastest auxin responders, were shown to encode small proteins that inhibit PP2C.D phosphatases, thereby activating plasma membrane proton ATPases and modulating cell expansion (Ren and Gray 2015).

Interestingly, the discovery of those auxin response pathways in plants led to the development of two complementary transgenic auxin reporter systems that allowed detection of auxin gradients generated across the root tip upon gravistimulation (Fig. 5.6b). The first reporter is a fusion between a synthetic promoter (*DR5*) that carries several copies of an auxin response element (enhancer elements recognized by ARF transcription factors) and the open reading frame of a gene that encodes a reporter protein (fluorescent protein such as GFP or VENUS or β-glucuronidase, a bacterial enzyme that converts a colorless soluble compound named *X-glu* into a blue insoluble precipitate that stains the cells expressing it). When plants are transformed with such a reporter construct, the levels of reporter transcripts (hence, the level of reporter protein) increase when auxin levels increase or when expressing cells become more sensitive to auxin.

The second type of auxin reporter involves a sensor protein (typically the fluorescent protein VENUS) engineered to carry a motif (named *dII*) that is recognized by the $SCF^{TIR1/AFB}$ ubiquitylation complex in the presence of auxin. This transgenic reporter is expressed in plants under the control of a ubiquitous promoter. When expressing cells are exposed to increased levels of auxin, the dII-VENUS protein is targeted by the $SCF^{TIR1/AFB}$ complex, which ubiquitylates it and targets it to degradation by the proteasome. Therefore, with the DR5 reporter system, increased auxin levels (or auxin sensitivity) lead to increased reporter expression, whereas the

Fig. 5.6 (continued) targeted to the proteasome where they get degraded. As a consequence, the ARF transcription factors are free to modulate the expression of multiple auxin response genes. Panel (**b**) shows transgenic *Brachypodium distachyon* roots expressing a DR5p:RFP auxin-activity reporter (left) or a dII-Venus auxin-level reporter (right). In both cases, the top picture shows the fluorescent signals displayed by the root, whereas the bottom picture is an overlay of the fluorescent signals with a bright-field image of the root. The red-fluorescent protein (RFP) reporter shown on the left is expressed under the control of the synthetic DR5 promoter, which carries several copies of an auxin-responsive transcriptional enhancer targeted by auxin response factors. Its transcription is modulated by the expression-dependent pathway described under panel A. The *dII-Venus* reporter (right), on the other hand, is expressed ubiquitously in the plant. It produces a dII-Venus fluorescent protein carrying a dII motif that allows its recognition by SCF^{TIR1} in the presence of auxin. Consequently, increased levels of auxin result in increased polyubiquitylation of dII-Venus, a modification that targets it to degradation by the proteasome. Therefore, increased levels of auxin within the cell lead to lower reporter signals. Please note that DR5:RFP expression is highest around the quiescent center and columella region of the cap, whereas the dII-Venus reporter is mostly visible in peripheral tissues. These two transgenic lines were provided by Devin O'Connor, University of Cambridge, UK

dII-VENUS reporter is degraded in the presence of auxin. The latter construct is a more direct sensor of auxin levels than the former.

When transgenic plants expressing a *DR5-GFP* reporter are subjected to gravistimulation (reorientation within the gravity field), their roots quickly develop a lateral gradient of reporter expression across the root cap, with increased expression on the lower flank. This gradient then progresses along the root tip toward the elongation zone. When such gravistimulated plants express dII-VENUS instead of DR5-GFP, the fluorescence signal decreases in cells at the bottom flank of the cap. The corresponding fluorescent-signal gradient also propagates toward the elongation zone over time, again reflecting formation of a lateral auxin gradient across the root cap upon gravistimulation (Fig. 5.6). In the next few sections, we will summarize the molecular mechanisms that contribute to gravity-induced formation of lateral auxin gradients across gravistimulated root tips.

5.2.5 Gravistimulation Promotes a Relocalization of Auxin Efflux Facilitators in the Statocytes

Critical to gravitropic regulation is a root cap-specific lateral auxin redistribution stream that connects the auxin maximum at the center of the root tip (quiescent center and upper columella cells) to its peripheral shootward stream. The PIN3 and PIN7 proteins, which are expressed in overlapping domains within the columella region of the root cap, play key roles in this lateral redistribution. In fact, *AtPIN3* is expressed in the upper two tiers of columella cells, whereas *AtPIN7* is expressed in tiers 2 and 3 (Fig. 5.1) (Friml et al. 2002; Kleine-Vehn et al. 2010; Wang et al. 2015). The PIN3 and PIN7 proteins are distributed uniformly within the plasma membrane on all sides of the statocytes in vertically oriented roots, allowing symmetrical auxin redistribution to the lateral cap cells.

Upon plant reorientation within the gravity field, the PIN3 and PIN7 proteins quickly relocalize to the lower side of the statocytes, thereby generating a downward stream of auxin across the cap, with accumulation in its lower side (Fig. 5.5) (Friml et al. 2002; Kleine-Vehn et al. 2010). This process appears to be mediated by a transcytotic mechanism that involves endocytosis of PIN3/7-carrying vesicles from the plasma membrane and their recycling toward the lower membrane of the cells. It is regulated by PIN protein phosphorylation and is dependent upon several factors that are known to contribute to vesicle trafficking within plant cells such as small GTPases of the ADP-ribosylation factor (ADP-RF) type, associated with GDP/GTP exchange factors (GEFs) of the GNOM type (Ganguly et al. 2012; Kleine-Vehn et al. 2010). Brefeldin A, a pharmacological agent of fungal origin that inhibits the GNOM-dependent step of vesicular trafficking, also affects gravitropism, supporting a role for vesicular trafficking in this response.

The regulatory molecules that contribute to the modulation of PIN3/7 trafficking toward the lower membrane upon gravistimulation remain poorly characterized. In fact, genetic approaches have been carried out to identify some of the contributing factors. The corresponding screens involved seeking mutations that specifically

affect gravitropism while having no effects on phototropism and/or organs growth responses to exogenous auxin or auxin transport inhibitors. The rationale for such screening criteria is as follows. Because both gravitropism and phototropism involve the formation of an auxin gradient across stimulated organs, mutations that affect both gravi- and phototropism are more likely to affect auxin transport and/or response. Mutations that specifically affect gravitropism, on the other hand, are more likely to affect early (and specific) steps of gravity sensing and/or signal transduction. On the other hand, mutations that affect both gravitropism and organs' growth responses to exogenous auxin and/or polar auxin transport inhibitors are more likely to affect the later phases of auxin transport and/or curvature response.

When mutations fulfilling the criteria defined above are found, contribution of the corresponding genes to early steps of gravity sensing and/or signal transduction in the statocytes can be verified by demonstrating a lack of PIN3/7 relocalization in the statocytes upon gravistimulation and an absence of lateral auxin gradient across gravistimulated root tips in mutant seedlings.

Using this approach, researchers were able to isolate several mutations that affect gravity sensing and/or early steps of gravity signal transduction in the root statocytes. The first *Arabidopsis* mutations found to alter at the same time root gravitropism, PIN3 relocalization and lateral auxin gradient formation upon gravistimulation, affected two genes that encode paralogous proteins named ALTERED RESPONSE TO GRAVITY 1 (ARG1) and ARG1-LIKE2 (ARL2). These mutations affected both root and hypocotyl gravitropism without altering phototropism. *arg1* and *arl2* mutant seedlings displayed wild-type root growth responses to auxin and auxin transport inhibitors, and their statocytes contained starch-filled amyloplasts that sedimented like wild type (Boonsirichai et al. 2003; Harrison and Masson 2008). The *ARG1* and *ARL2* genes were found to encode peripheral membrane proteins that associate with the plasma membrane, ER, Golgi and endosome, thereby probably regulating vesicular trafficking, a process needed for PIN3/7 protein relocalization in the statocytes upon gravistimulation (Boonsirichai et al. 2003; Harrison and Masson 2008).

One interesting feature of the *arg1* and *arl2* mutant seedlings is that they display only partial defects in root and hypocotyl gravitropism. Therefore, *arg1* (or *arl2*) plants can be used as sensitized lines to isolate novel mutations that either enhance or suppress their gravitropic responses. Such genetic modifiers of *arg1* (or *arl2*) would likely also contribute to early steps of gravity sensing and/or signal transduction. A secondary screen for genetic enhancers of *arg1* was carried out, identifying plants with enhanced gravitropism defects relative to *arg1*. *modifier of arg1-1* (*mar1-1*) carried a missense mutation in *TOC75*, a gene that encodes the channel component of plastidic TRANSLOCON ON THE OUTER CHLOROPLAST MEMBRANE (TOC) complex, which mediates the import of cytoplasmic proteins through the outer membrane of plastids. A second modifier of *arg1*, named *mar2-1*, was also isolated, carrying a missense mutation in *TOC132*, which encodes another component of the same TOC complex (Stanga et al. 2009). These two *mar* mutations did not obliterate TOC's function as protein importer. In fact, mutant root cap amyloplasts accumulated starch like wild type, and they sedimented at wild-type

rates upon gravistimulation. These data suggested a role for amyloplasts in gravity signal transduction that goes beyond their ability to sediment as statoliths (Stanga et al. 2009; Strohm et al. 2014). A differential proteomic analysis comparing wild-type and *toc132* mutant roots identified candidate gravity signal transducers, whose functions remain to be characterized (Strohm et al. 2014).

Genetic investigations of gravity sensing and signal transduction have not been limited to *Arabidopsis thaliana*. In fact, work done in the legume model *Medicago truncatula* uncovered a mutation that leads to upward-oriented roots that grow out of the soil. Named *negative gravitropic response of roots* (*ngr*), this mutation was found to affect a gene that encodes a plant-specific protein of unknown function (Ge and Chen 2016). Interestingly, this protein shares similarity with LAZY1, a protein known to contribute to gravitropism in rice, maize, and *Arabidopsis* (Dong et al. 2013; Li et al. 2007; Yoshihara et al. 2013). In *Arabidopsis*, six genes with spatially distinct expression patterns encode LAZY1-like proteins. Phenotypic analysis of higher-order mutants revealed key contributions played by distinct members of this gene family to root and shoot branch angles as well as seedling primary organs gravitropism (Yoshihara and Spalding 2017; Taniguchi et al. 2017). Importantly, the reversed gravitropic response displayed by some higher-order *Atlazy* mutants relative to wild type was associated with reversed asymmetric distribution of PIN3 in gravistimulated statocytes and a reverse lateral gradient of auxin (Taniguchi et al. 2017; Yoshihara and Spalding 2017). Starch content and amyloplast sedimentation were not affected in analyzed mutants, indicating that the *NGR*/*LAZY* genes contribute to a step of gravity sensing and/or signal transduction that follows amyloplast sedimentation. In one of the triple mutants, the reversed gravitropism phenotype could be rescued by expression of a wild-type *LAZY1* transgene specifically in the statocytes, demonstrating a statocyte-specific contribution of the gene to gravitropism. Taken together, these exciting results position the LAZY/NGR proteins at an important step of the transduction pathway that is needed for proper interpretation of the gravity vector by the statocytes (Ge and Chen 2016).

In conclusion, genetic investigations of root gravitropism have identified a number of loci that contribute to the transduction of information provided by amyloplast settling into a transcytotic process that results in a relocalization of auxin efflux facilitators PIN3 and PIN7 to the lower membranes of the statocytes, thereby leading to the formation of a lateral auxin gradient across the cap, and ultimately a curvature response at the DEZ. It should however be cautioned that the pathway may be a little more complicated. Indeed, the *pin3* and *pin7* knockout mutants, as well as the *pin3 pin7* double mutants, still display significant root curvature responses to gravistimulation. This implies that other unknown auxin transporters may also contribute to gradient formation across the root tip and to gravitropism.

Another important point that should be raised here is that auxin transport across the root cap may be facilitated by other physiological changes that occur in the root cap statocytes in response to gravistimulation. For instance, gravistimulated statocytes have been shown to undergo a rapid alkalinization of their cytoplasm, accompanied by an acidification of the apoplast (Fasano et al. 2001). This process is ARG1-dependent, and it may result from an activation of plasma membrane proton

pumps and/or vacuolar ATPases. It is critical for full responsiveness to gravity, possibly by decreasing the fraction of ionized auxin in the apoplast and facilitating its mobility through membranes and/or transporters (Fasano et al. 2001).

5.3 Auxin Gradient Propagation from the Root Cap to the DEZ

The mechanisms discussed above allow establishment of a lateral auxin gradient across the root cap upon gravistimulation. Yet, the initial phases of curvature response occur at the distal side of the EZ. Therefore, auxin transport machinery has to move this auxin gradient from root tip to EZ, maintaining it along the road. Which mechanisms contribute to this complex process?

The lateral root cap, epidermis and cortex all contribute to the shootward flow of auxin in roots. In *Arabidopsis*, these cells take up auxin using mostly the AUX1 influx carrier, whereas PIN2, along with members of the p-glycoprotein family, contribute to its export from the transporting cells. The polar localization of PIN2 within these transporting cells dictates shootward transport in lateral cap and epidermal cells and rootward reflux in the cortical cells at the distal side of the elongation zone (Blilou et al. 2005) (Fig. 5.5). Both flows of auxin (shootward in epidermal and lateral cap cells and rootward in cortical cells) are critical for an efficient gravitropic response (Blilou et al. 2005). Interestingly, reversible phosphorylation of PIN2 plays a key role in its localization within transporting cells, with the serine/threonine protein kinases PINOID (PID) and PID-like WAG1 and WAG2 kinases contributing to its phosphorylation, whereas type-IIA protein phosphatase complexes (PP2A) contribute to its dephosphorylation. When phosphorylated, PIN2 localizes at the rootward side of the transporting cells, whereas it associates with the shootward side of the cell when dephosphorylated (Barbosa et al. 2014; Dhonukshe et al. 2010).

The shootward transport of auxin from root cap to elongation zone is also subject to feedback regulatory mechanisms that assure lateral gradient maintenance during its transfer toward the EZ. Indeed, as emphasized earlier in this chapter, auxin has been shown to promote the maintenance of PIN proteins within the plasma membrane of transporting cells (Abas et al. 2006). As a consequence, auxin accumulation on the bottom side of a gravistimulated root tip leads to increased auxin transport potential on that side and lower transport potential on the upper side. Therefore, the gravity-induced lateral auxin gradient that was generated across the root cap becomes increasingly pronounced as it progresses toward the elongation zone (Abas et al. 2006; Li and Xue 2007; Lin et al. 2012). This process is exacerbated by increased auxin-dependent production of small signaling peptides *GOLVEN1* (*GLV1)* and *GLV2* by cells on the lower side of the stimulated roots, triggering a response pathway that also favors PIN2 association with the plasma membrane (Whitford et al. 2012).

On the other hand, the increased apoplast alkalinization that occurs on the lower side of a graviresponding root as a consequence of increased auxin levels is

responsible for decreasing the fraction of protonated IAA molecules in the apoplast, thereby decreasing the rate of free diffusion through the plasma membrane of transporting cells. Consequently, the auxin influx carrier AUX1 is needed for adequate shootward auxin transport and root gravitropism (Dharmasiri et al. 2006; Monshausen et al. 2011). In fact, the *AUX1* gene is expressed broadly in the root tip, including the provasculature, root cap, and epidermal cells. However, restricting its expression to the lateral cap and epidermal cells of the root meristem and EZ is sufficient to rescue the altered root gravitropism phenotype displayed by *aux1* mutant seedlings (Dharmasiri et al. 2006; Swarup et al. 2005). The latter observation is important because it demonstrates that the contribution of AUX1 to root gravitropism requires its expression only within the peripheral tissues of the root tip, where shootward auxin transport occurs (Dharmasiri et al. 2006; Swarup et al. 2005).

From the preceding discussion, it appears that *Arabidopsis* PIN2 plays a key role in root gravitropism, transporting the gravity-induced auxin gradient from the root cap toward the elongation zone, where it regulates differential cellular elongation and curvature. It is therefore quite surprising that *Arabidopsis pin2* mutant roots retain some gravitropic capability (Baldwin et al. 2013). This implies that PIN2 function may be redundant with other transporters. In agreement with this contention, P-glycoprotein-type transporters, which use ATP hydrolysis to carry specific molecules through membranes (auxin in this case), may fulfill this redundant auxin transport function. Indeed, *Arabidopsis AtPGP1* and *AtPGP19* genes are also expressed in the root EZ, and functional studies in heterologous systems (plant protoplasts, yeast and mammalian cells) have demonstrated their ability to transport auxin (Geisler et al. 2005; Yang and Murphy 2009). Furthermore, *pgp19* single mutants and *pgp1 pgp19* double mutants exhibited reduced basipetal auxin transport (Lewis et al. 2007). Surprisingly, these mutants displayed an enhancement of gravitropism and phototropism. This phenotype is, in fact, a consequence of these genes being expressed more proximally (shootward) than *PIN2* in the root tip. Hence, *pgp1 pgp19* mutant roots develop a stronger auxin gradient across the DEZ relative to wild type, allowing for enhancement of the curvature response (Noh et al. 2001; Rojas-Pierce et al. 2007).

5.4 Root Curvature Response to Gravistimulation

5.4.1 The Gravitropic Curvature Involves Differential Cell Elongation Between Opposite Root Tip Flanks

Upon transmission to the EZ, the gravity-induced lateral auxin gradient promotes differential cell elongation between upper and lower flanks of the gravistimulated root, leading to initiation of a curvature within 10–15 min of reorientation. Which mechanisms contribute to gravitropic curvature development?

As mentioned above, gravistimulation promotes a lateral movement of auxin across the root cap, with accumulation at the bottom half. This gradient is then transmitted to the EZ. There, higher auxin level on the lower side of the

gravistimulated root leads to an inhibition of cell elongation, whereas lower auxin level on the upper side leads to increased elongation. As a consequence, a downward curvature develops.

Increased auxin levels on the upper half of gravistimulated roots leads to the activation of a plasma membrane H^+/OH^- conductance, which results in alkalinization of the apoplast, as described in Sect. 5.2 (D part) of this chapter (Monshausen et al. 2011; Mullen et al. 1998). The resulting increase in cell wall rigidity leads to decreased rate of cellular elongation on the lower side of the root. The upper flank, on the other hand, is exposed to lower auxin levels, resulting in increased cell wall acidity. Lower wall pH is known to promote the breakage of intermolecular cross-links between wall polymers by expansins and xyloglucan endotransglucosylases/hydrolases (XTHs), favoring cellular elongation. Consequently, increased cell elongation on the topside and decreased expansion at the bottom results in a downward curvature (Cosgrove 2000; Monshausen et al. 2011).

In addition to these direct effects of auxin via its $SCF^{TIR1/AFB}$ receptor on cellular expansion, other signaling molecules have also been implicated in the curvature response to gravistimulation. For instance, nitric oxide (NO) was found to accumulate on the lower side of gravistimulated roots in response to auxin accumulation, where it inhibits auxin transport and modulates auxin signaling through S-nitrosylation of TIR1 (Terrile et al. 2012). Similarly, reactive oxygen species (ROS) have been shown to accumulate at the bottom side of roots in an auxin-dependent manner, where they contribute to the regulation of gravicurvature (Krieger et al. 2016).

The epidermis is believed to be the main driver of root tip curvature. Indeed, expressing an auxin response repressor (*axr3-1*) in epidermal cells of the elongation zone is sufficient to obliterate the gravitropic response, whereas expressing it in different cell types within the elongation zone has little impact (Swarup et al. 2005).

5.4.2 How Does a Root Know It Has Curved Enough?

As discussed earlier in this chapter, the availability of in situ auxin sensors (*DR5-GFP*; *dII-VENUS*) and fluorescent protein-PIN fusion reporters in *Arabidopsis* has allowed careful spatiotemporal investigations of PIN3/7 relocalization to the bottom membrane of statocytes and auxin gradient formation across the root tip upon gravistimulation, this in relation with the timing of amyloplast sedimentation in the statocytes and root curvature response. Such experiments have demonstrated that the auxin gradient generated across the root disappears when the tip reaches an approximate angle of 50° from vertical. At this point, the statoliths have returned to the distal side of the statocytes as a consequence of cellular morphology, and the PIN3/7 proteins have returned to a symmetrical distribution on all sides of the statocytes (Band et al. 2012). This suggests that auxin asymmetry during a gravitropic response is susceptible to a tipping-point mechanism that is triggered when the tip reaches a 50° angle from vertical. At that point, the curvature has to proceed in the absence of a lateral auxin gradient, until the tip reaches the vertical. Unfortunately, the

molecular mechanisms that contribute to this second auxin gradient-independent phase of gravicurvature and its termination when the tip reaches the vertical remain unexplained (Band et al. 2012).

5.5 The Gravity Set Point Angle of Lateral Roots Differs from That of Primary Roots

While the primary roots of most plants tend to grow *orthogravitropically* (parallel to gravity), the lateral roots that develop from pericycle initials within the primary root will tend to grow either *diagravitropically* (horizontally) or *plagiogravitropically* (obliquely), allowing for better soil exploration for water and nutrients. The environmental conditions can alter the angle of lateral root growth from the vertical, favoring either steeper angles (allow better adaptation to drought, for instance) or shallower growth (when there is a need for better exploitation of surface resources such as phosphate) (Bai et al. 2013). What do we know of the mechanisms that allow lateral roots to grow at a different GSA from the vertical?

In *Arabidopsis*, lateral roots emerge perpendicularly from the primary root and then progressively acquire plagiogravitropism as starch accumulates in the statocytes, and the EZ becomes established (Guyomarch et al. 2012; Kiss et al. 2002; Rosquete et al. 2013). These roots curve toward an initial GSA, which is rather shallow, and then straighten up and grow along this vector for some time. Subsequently, these laterals may start curving again, leaning toward positive orthogravitropism (vertically downward). The initial plagiogravitropic phase of lateral root growth may result from an auxin-dependent antigravitropic offset mechanism that opposes gravitropism to regulate the distribution of auxin levels and response between opposing sides (Roychoudhry et al. 2013).

Regulation of auxin transport in lateral root statocytes seems responsible for the regulation of positive orthogravitropism (Fig. 5.7). Early after emergence, only *PIN3* is expressed in the columella cells of the *Arabidopsis* lateral root cap. This PIN3 protein is quickly redistributed asymmetrically toward the bottom side of the statocytes, yielding a lateral gradient of auxin that triggers downward curvature. When the young lateral root reaches its first GSA plateau, *PIN3* expression decreases, and *PIN4* and *PIN7* are activated to very low expression levels. At this stage, the overall level of PIN expression in the statocytes is low, and the PIN proteins are symmetrically distributed in the statocytes, allowing the laterals to continue growing straight along the GSA. Subsequently, the *PIN4* and *PIN7* genes increase their expression, and the corresponding proteins redistribute to the bottom side of the statocytes, again creating a lateral auxin gradient that is responsible for a new phase of downward curvature (Roychoudhry et al. 2013). It is tempting to speculate that the regulatory system discussed above may constitute a target for developmental and environmental signals to dictate whether a root system will be radially expanded or organized axially (Rosquete et al. 2013). Interestingly, the LAZY proteins described in Sect. 3.5. of this chapter may play an important role in this process (Taniguchi et al. 2017).

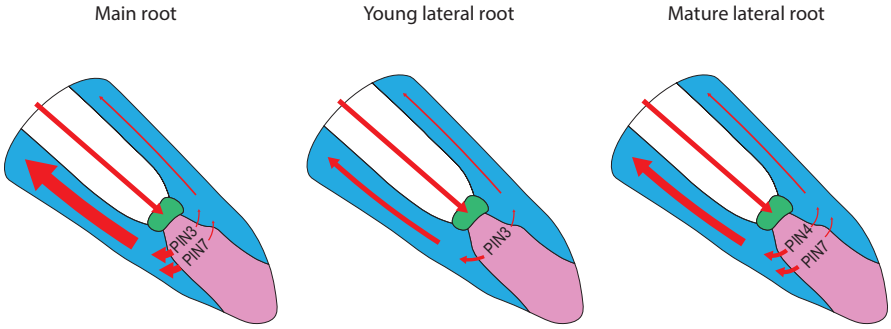

Fig. 5.7 Lateral root gravity set point angle (GSA) correlates with decreased auxin flow at the tip. In gravistimulated primary *Arabidopsis thaliana* roots, polar localization of PIN3 and PIN7 auxin efflux facilitators in the statocytes leads to the formation of a strong lateral auxin gradient across the cap, which is responsible for root tip curvature upon transmission toward the distal side of the elongation zone. These primary roots will tend to grow vertically downward (orthogravitropism). In young lateral roots (center drawing), *PIN3* is expressed at lower levels in the root cap, decreasing over time. Consequently, the lateral gradient of auxin that develops across the cap remains mild. This leads to shallower GSA relative to primary roots (plagiogravitropism). In older lateral roots, *PIN3* expression ceases and is replaced by stronger expression of the *PIN4* and *PIN7* genes in the statocytes, leading to a stronger lateral auxin gradient across the tip relative to your laterals. As a consequence, the older lateral root will curve back to a steeper angle. (Roychoudhry et al. 2013)

5.6 How Do Shoots Respond to a Reorientation Relative to Gravity?

In dicots, shoot and hypocotyl gravitropism also involves a differential elongation between top and bottom sides, typically leading to upward curvatures. Unlike roots, stems do not show evidence of a physical separation between sites of gravity sensing and curvature response. Instead, the curvature occurs along the entire length of the EZ, and gravity-sensing statocytes occupy the entire region, forming the endodermal layer or starch sheath parenchyma that surrounds the vasculature. This implies that the curvature response to gravistimulation will typically follow more complex kinetics in shoots. As an example, *Arabidopsis* inflorescence stems are characterized by different rates of elongation along their length. Upon gravistimulation, the apical region will initially curve faster than the basal (rootward) region. When this apical region reaches the vertical, the basal region is still curving, implying that the tip will soon overshoot the vertical. As a consequence, the apical segment will sense an opposite gravistimulus and will start curving in the opposite direction. This back-and-forth oscillation may occur several times before the stem eventually reaches its final posture. This complex behavior is, in fact, compatible with the existence of gravity-sensing cells along the entire length of the EZ in shoots, along with differential rates of cellular elongation between upper and lower segments, and an added mechanism of autostraightening (Bastien et al. 2013; Fukaki et al. 1996; Morita 2010).

Gravity sensing by shoot endodermal statocytes also involves sedimenting amyloplasts. However, the endodermal cells of shoots differ from the columella cells of the root cap by the existence of a large central vacuole that pushes all organelles to the cell periphery. Consequently, amyloplasts have to traverse transvacuolar strands during their sedimentation. This implies that vacuolar integrity and biogenesis are critical for normal gravitropism. We know this because many of the mutations that have been identified in *Arabidopsis* for their impact on shoot gravitropism were shown to affect either endodermal cell fate specification (*sgr1*/*scr* and *sgr7*/*shr*, for instance) or vacuolar biogenesis and function (*sgr2*, *sgr3*, *sgr4*, *sgr8*, for instance).

The SHOOT GRAVITROPIC RESPONSE 7/SHORTROOT (SGR7/SHR) and SHOOT GRAVITROPISM 1/SCARECR*OW* (SGR1/SCR) transcription factors are critical for endodermis specification. Indeed, *sgr1*/*scr* and *sgr7*/*shr* mutant plants lack a fully differentiated endodermal layer with sedimenting amyloplasts. These developmental phenotypes are accompanied by an inability for mutant shoots and hypocotyls (but not roots) to respond to gravistimulation (Fukaki et al. 1998). The *sgr2*, *sgr3*, *sgr4* and *sgr8* mutations, on the other hand, display altered vacuolar phenotypes. In fact, the proteins encoded by the *SGR3*, *SGR4* and *SGR8* genes appear to contribute to vesicular trafficking between Golgi and vacuole, providing an environment that is favorable to amyloplast sedimentation upon gravistimulation (Silady et al. 2007; Yano et al. 2003; Zheng et al. 1999). *SGR2*, on the other hand, encodes a putative phospholipase that also localizes to vacuolar membranes, possibly modifying their composition and their biophysical properties, thereby interfering with amyloplast sedimentation (Kato et al. 2002; Morita et al. 2002).

That the gravitropic defect associated with these mutations is a consequence of their negative impact on amyloplast sedimentation in endodermal statocytes was supported by centrifugation experiments, which demonstrated a concomitant rescue by higher g forces (provided by centrifugation) of amyloplast sedimentation and gravitropism for *sgr2*, *sgr9* and *pgm* (Toyota et al. 2013). The authors summarized the results of their experiments by indicting that "*Arabidopsis* shoots have a gravisensing mechanism that linearly converts the number of amyloplasts that settle to the 'bottom' of the cell into gravitropic signals" (Toyota et al. 2013).

So, amyloplast sedimentation within the endodermal statocytes of shoots leads to the activation of a gravity signal transduction pathway that promotes an upward curvature. What are the mechanisms that contribute to this gravity transduction pathway? In fact, as already discussed for roots, the mechanisms that transduce the information derived from amyloplast sedimentation into a biochemical signal that is responsible for the curvature remain poorly understood. However, we do know that this pathway leads to the development of a lateral auxin gradient across the stem, with auxin accumulation on the lower flank. As for roots, we know this because auxin-level or auxin-activity reporters (such as DR5-GFP) demonstrated asymmetric activation on opposite flanks of the shoot upon gravistimulation, consistent with increased auxin accumulation on the lower side. Because auxin promotes cell elongation in shoots, this gravity-induced auxin gradient leads to an upward curvature.

Research involving *Arabidopsis thaliana* hypocotyls and inflorescence stems led to a better characterization of the molecular mechanisms that control establishment

of this auxin gradient across gravistimulated shoots (Fig. 5.8). Indeed, seedling reorientation within the gravity field was shown to promote a relocalization of the PIN3 auxin efflux facilitator to the lower membrane of the statocytes, with accumulation in the inner membrane of upper-half endodermal cells and outer membrane of lower half cells. This repolarization of PIN3 is consistent with a lateral downward transport of auxin from the upper to lower flanks of the stem, leading to an upward curvature (Fig. 5.8). As for root statocytes, gravity-induced PIN3 polarization in the endodermal cells requires a GNOM-dependent endocytotic recycling pathway which is modulated by PINOID-dependent protein phosphorylation (Rakusová et al. 2011).

In experiments carried out with *Arabidopsis* hypocotyls, the gravitropic curvature initiated within 2 h, and it proceeded quickly during the initial phases of the

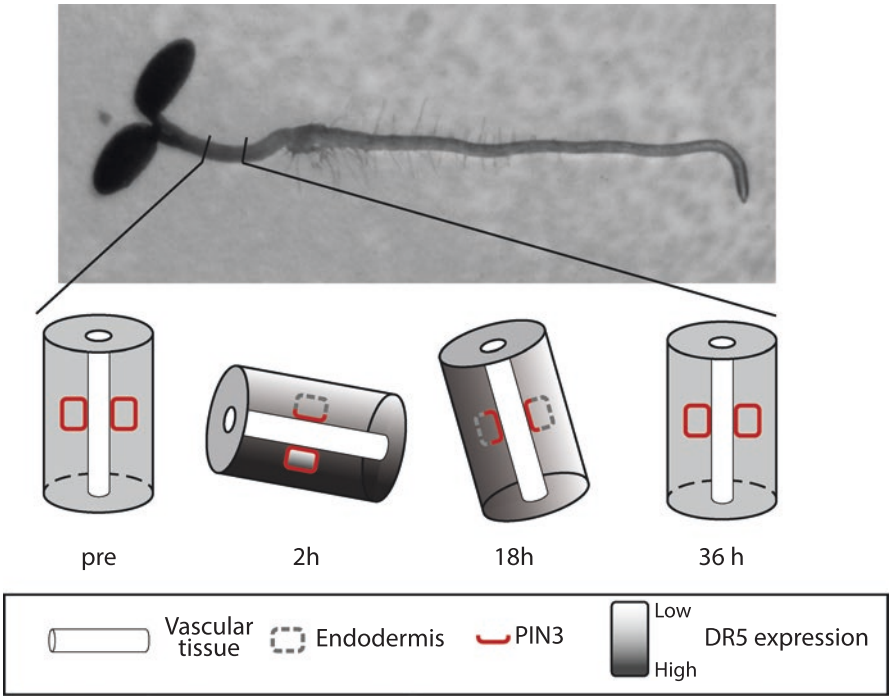

Fig. 5.8 PIN3 polarization in endodermal cells surrounding the vasculature contributes to auxin gradient formation and gravitropism in hypocotyls. In vertically oriented hypocotyls, the PIN3 protein is localized symmetrically at the plasma membrane of endodermal cells surrounding the vasculature. As a consequence, auxin is distributed equally on all sides of the hypocotyl, and the organ can grow straight up (left). Upon seedling reorientation within the gravity field, the PIN3 protein relocalizes to the lower membrane of endodermal statocytes, leading to lateral auxin gradient formation and upward curvature (2 h). Over time (18 h), auxin accumulation on the lower half of the hypocotyl leads to an inner lateralization of PIN3 in the lower endodermal cells, resulting in a reflux of excessive auxin from the lower half into the vasculature. As a consequence, the gradient dissipates and the differential growth responsible for increasing curvature ends. (Rakusová et al. 2016)

response. However, after approximately 18 h, the rate of curvature diminished to eventually taper off. Interestingly, the termination of this curvature followed a second change in PIN3 protein distribution within endodermal cells at the lower side of the responding hypocotyl, with relocalization from outer to inner membrane (Fig. 5.8). Amazingly, this inner lateralization of the PIN3 protein was shown to be a consequence of auxin accumulation in the endodermal cells (Rakusová et al. 2016). This relocalization results in a back flow of auxin from the lower flank of the responding hypocotyl into the vasculature. As a consequence, the auxin gradient initially created across the stimulated hypocotyl progressively dissipates, eventually disappearing. With the auxin gradient gone, the curvature ceases, and the hypocotyl tip resumes straight upward growth (Rakusová et al. 2016).

The amazing conclusion from the studies described above is that the auxin gradient generated across the hypocotyl upon gravistimulation by PIN3 polarization triggers both an upward curvature and a subsequent condition that is favorable to PIN3 inner lateralization on the lower, auxin exposed, flank of the hypocotyl, leading to gradient dissipation and termination of the response. This elegant analysis provided a simple explanation to the mechanism involved in the termination of shoot gravitropism. Interestingly, mutations or pharmacological treatments leading to alterations in this auxin-dependent inner lateralization of the PIN3 protein in shoot endodermal cells lead to overshooting the gravitropic response, as anticipated by the model.

In monocots, seedling coleoptiles and shoot pulvini develop strong gravitropic responses. Coleoptile gravitropism has received much attention over the years. Immediately after germination, a monocot coleoptile grows mostly by cell expansion for a few hours, enclosing the first developing leaves. It develops strong gravitropic responses, accompanied by auxin accumulation along the lower flank, as long as the leaves remain enclosed. As soon as the first growing leaves emerge from the tip, the coleoptile loses its ability to develop a gravicurvature. As discussed in Sect. 3.3 of this chapter, experiments using wheat coleoptiles allowed Chauvet and collaborators to suggest an inclination/position sensor mechanism of gravity sensing by plant statocytes (Chauvet et al. 2016).

In adult monocot plants, shoot gravitropism typically involves the contribution of pulvini, which are short segments of tissue that are apical to the nodes and collectively contribute to bringing a shoot tip back up after previous prostration by wind or rain. In *Panicoid* species like maize, the pulvini constitute disc-shaped segments of the stem, whereas the pulvini of *Festucoid* grass species, such as wheat, oat, and barley, are made of a tissue that encircles the leaf sheath immediately apical to the point where it attaches to the node. When monocot stems are gravistimulated, such as upon prostration by heavy wind or rain, cells at the bottom side of 3–4 pulvini along the stem resume cell elongation on the lower flank, resulting in local segmental upward curvature (Fig. 5.9). In maize, each pulvinus can provide a maximum of 30° curvature in response to gravistimulation. This process plays an important role in agriculture because it keeps seed away from soil moisture and pathogens after prostration in heavy storms. Another reason for which pulvinus gravitropism has been heavily investigated in monocot plants is that it takes a long time for a

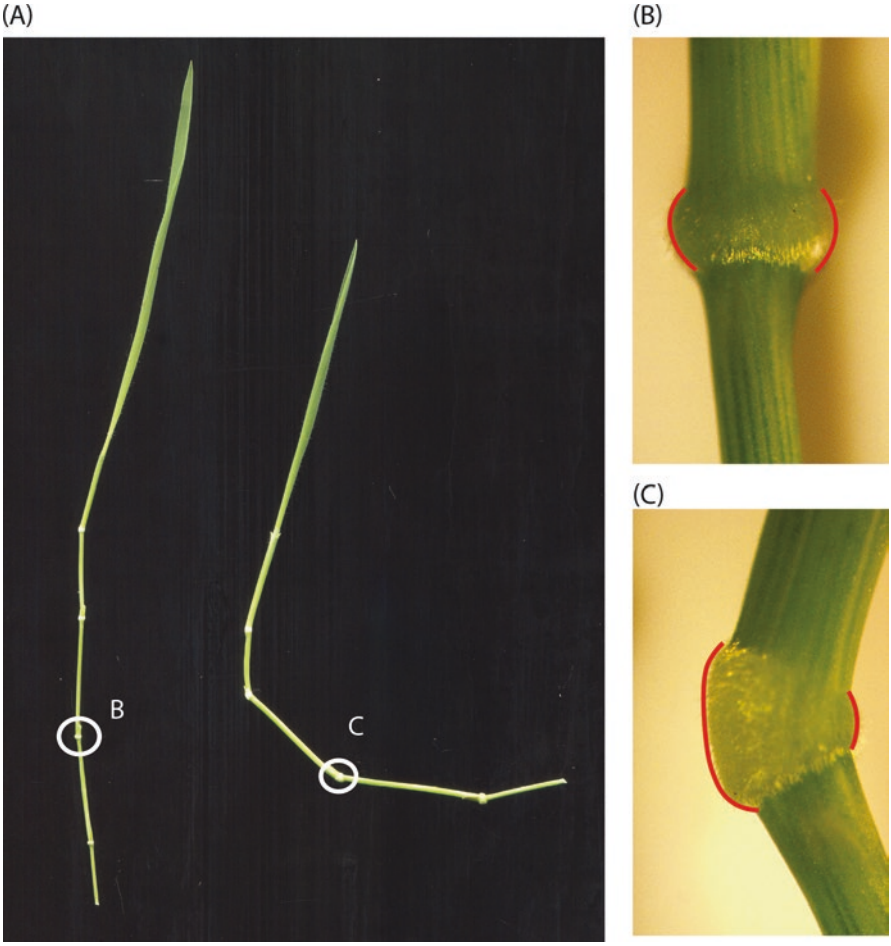

Fig. 5.9 The gravitropic response of a *Brachypodium distachyon* stem involves localized curvatures at several successive leaf pulvini. Panel (**a**) shows vertically oriented (left) and gravistimulated (right) stems (with leaves cut off at their bases to allow better observation of the pulvini). Panels (**b** and **c**) show individual pulvini from vertical control (**b**) and gravistimulated stems (**c**). Please note that gravistimulation promotes cell elongation on the bottom side of the pulvini (left side in panel **c**) relative to control or the upper side

pulvinus to develop a curvature response to gravistimulation. Therefore, this system can be used quite efficiently to independently investigate the molecular mechanisms that contribute to gravity sensing and/or signal transduction, relative to those involved in the curvature response.

Similar to the other shoot systems described above, gravity sensing in cereal pulvini seems to involve the sedimentation of starch-filled plastids within the starch sheath cells that surround the vasculature. Upon gravistimulation, an auxin gradient also forms across stimulated pulvini, with accumulation at the bottom flank. This

gradient leads to increased cellular expansion on the lower side, hence upward curvature. However, before this auxin gradient can form, a number of very fast physiological changes also occur, which may contribute to gravity signal transduction. First, an increase in the levels of $InsP_3$ was documented on the bottom flank of gravistimulated pulvini (Perera et al. 1999, 2001). In maize pulvini, this change occurred already within 10 s of gravistimulation, and it was followed by fluctuations between upper and lower sides over a period of 30 min. Subsequently, a stable increase in the levels of $InsP_3$ was observed in the lower flank of the stimulated pulvini over a period of 3–7 h. The first signs of gravicurvature appeared about 8 h after the onset of gravistimulation (Perera et al. 2001). As previously discussed in this chapter, these changes in $InsP_3$ levels upon gravistimulation may contribute to Ca^{2+} signaling, although gravity-induced Ca^{2+} changes have not been observed in pulvinus statocytes.

Investigators have also demonstrated the existence of fast changes in the levels of reactive oxygen species, including H_2O_2, in pulvinus statocytes upon gravistimulation, with initial changes occurring in proximity of the sedimenting amyloplasts within 1 min and expanding throughout the cytoplasm within 30 min of a gravistimulus (Clore 2013). Subsequently, more H_2O_2 was found on the lower half than on the upper side, possibly contributing directional information for upward bending. This is consistent with a parallel accumulation of a cytoplasmic aconitase/iron regulatory protein 1 (IRP1), which may function as a redox sensor (Clore 2013). The role of reactive oxygen species in gravity signaling remains uncertain. However, reactive oxygen species have been suggested to function both before and after auxin redistribution in gravistimulated maize pulvini (Clore et al. 2008).

In addition to the changes discussed above, pulvinus statocytes also display fast changes in cytosolic pH in response to gravistimulation. A significant alkalinization of the cytoplasm was observed at the bottom side, near the sedimenting amyloplasts, after 30 min of gravistimulation, and it was accompanied by a slight acidification at the sides of the same cells (Johannes et al. 2001). These cytosolic pH changes were suggested to contribute to gravity signaling.

Protein kinases were also implicated in pulvinus responses to gravistimulation. Indeed, MAP kinase activities were shown to first fluctuate in gravistimulated maize pulvini starting 75 min into continuous gravistimulation and followed by a stabilization of the response, with increased activity on the topside after 2 h. Inhibition of MAP kinase activity using the U0106 inhibitor led to alterations in the gravitropic response, suggesting a contribution in gravity signal transduction (Clore et al. 2003). It has been suggested that this sustained increase in MAP kinase activity on the upper section of pulvini may contribute to sustained inhibition of growth on that side.

5.7 Conclusion

In this chapter, we tried to summarize the current state of our understanding of the molecular mechanisms that govern gravitropism in plants undergoing primary growth. We described seminal experiments that allowed mapping regions within

plant organs that contribute to gravity sensing and/or curvature response and discussed several models that attempt to explain gravity sensing by statocytes. We also pointed out recent experiments that suggest a mechanism allowing detection of organ inclination rather than gravity force. We discussed how activation of a signal transduction pathway within the statocytes triggers a change in polar distribution of auxin transporters, resulting in a lateral transport of auxin toward the bottom side of the stimulated organ. A variety of regulatory mechanisms that contribute to the propagation of the resulting auxin gradient from the site of sensing to the site of response, and its maintenance during transport, were also reported, as were the molecular mechanisms that contribute to cellular responses and organ curvature. We described some of the mechanisms that lead to termination of curvature at the end of a response.

Lateral plant organs were reported to grow at distinct angles from the vertical relative to the primary organs they originated from and to be able to modify that angle in response to a variety of environmental and endogenous parameters. Overall, these responses allow a plant to develop a general architecture that allows efficient exploration for acquisition of the resources it needs to sustain growth, development and reproduction.

Yet, despite the tremendous progress recently made toward a better understanding of plant gravitropism, many questions remain unanswered. For instance, the receptors involved in converting information derived from amyloplast sedimentation and/or position within the statocytes into a biochemical signal have not been identified, and the secondary messengers that contribute to this response have not been characterized. The secondary mechanism of gravity sensing known to function in the DEZ of roots remains ill-defined, and functionally redundant auxin transporters contributing to gravity signal transduction and auxin gradient transmission toward the DEZ have not been characterized. Similarly, the mechanisms that modulate root curvature termination at the end of a gravity response remain unknown. Finally, the molecular mechanisms that govern differential cell elongation in response to gravistimulation remain poorly understood, in part because the key regulators of cell wall loosening and/or other aspects of anisotropic cell expansion are functionally redundant.

Yet, we anticipate major progress in our understanding of the mechanisms that govern plant gravitropism in the next few years. Indeed, novel tools are available to answer the remaining questions. Available growth resources in the microgravity environment provided by the International Space Station should allow a better characterization of the mechanisms involved in gravity sensing. Furthermore, the revolution in genome editing driven by the development of CRISPR/Cas9 technologies will be instrumental at identifying and functionally characterizing key genes involved in the different phases of gravitropism (Jiang et al. 2014). Novel biological sensors allow detection of more signaling molecules previously implicated in gravity signaling (Ca^{2+}, H^{+}, $InsP_3$, NO and ROS) (Costa et al. 2013; Hou et al. 2011). Coupled with the development of better real-time imaging approaches and computerized image analysis routines, these sensors should allow the development of better spatiotemporal maps of signal evolution along plant organs during gravitropic

responses. Furthermore, system biology approaches relying on forward and reverse genetics, genome-wide association studies, and transcriptomic, proteomic, and metabolomic approaches should allow the identification of novel gravity signal transducers. Finally, the development of mathematical models that attempt to explain quantitative aspects of the gravitropic response by integrating some of its contributing factors should provide a holistic view of the process (Band et al. 2012).

While our description of gravitropism has focused on plant organs that undergo primary growth (driven by apical meristems), it is important to understand that plant organs that undergo secondary growth, such as the woody stems of trees, can also change their orientation relative to gravity by developing reaction wood that provides a force to reorient upward. This process plays important roles in tree architecture, tree posture, and stem reorientation after prostration by heavy storms. Graviperception by angiosperm woody stems leads to the formation of *tension wood* on their upper side, which creates a tensile force that pulls it upward. On the other hand, a prostrated gymnosperm stem will develop *compression wood* on its bottom side, which generates a compressive force that pushes it upward. These reactions of prostrated woody stems are important because they modulate the architecture of trees and also contribute to plant survival. Furthermore, the reaction wood they generate alters the market value of lumber, thereby leading to important economical impact in forestry. The molecular mechanisms that govern these tree responses have also received some attention from researchers in the last few years. Unfortunately, space constraints do not allow us to further explore these fascinating processes. For further information, we would like to refer interested readers to a recently published review of the process (Groover 2016).

Acknowledgments This chapter was made possible by grants from the *National Aeronautics and Space Administration* (#NNX14AT23G and #80NSSC18K1481), the *University of Wisconsin-Madison College of Agriculture and Life Sciences HATCH program* (#WIS10338), and a *Fall Competition Award from the Office of Vice Chancellor for Research and Graduate Education from the University of Wisconsin-Madison*, to PHM. We thank Dr. Chris Wolverton (Ohio Wesleyan University) for providing information on and images of the *rotato* device.

References

Abas L, Benjamins R, Malenica N, Paciorek T, Wirniewska J, Moulinier-Anzola J, Sieberer T, Friml J, Luschnig C (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. Nat Cell Biol 8:249–256

Adamowski M, Friml J (2015) PIN-dependent auxin transport: action, regulation, and evolution. Plant Cell 27:20–32

Andreeva Z, Barton D, Armour W, Li M, Liao L, McKellar H, Pethybridge K, Marc J (2010) Inhibition of phospholipase C disrupts cytoskeletal organization and gravitropic growth in Arabidopsis roots. Planta 232:1263–1279

Bai H, Murali B, Barker K, Wolverton C (2013) Low phosphate alters lateral root setpoint angle and gravitropism. Am J Bot 100:175–182

Baldwin K, Strohm A, Masson P (2013) Gravity sensing and signal transduction in vascular plant primary roots. Am J Bot 100:126–142

Band L, Wells D, Larrieu A, Sun J, Middleton A, French A, Brunoud G, Sato E, Wilson M, Péret B et al (2012) Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. Proc Natl Acad Sci U S A 109:4668–4673

Barbosa I, Zourelidou M, Willige B, Weller B, Schwechheimer C (2014) D6 PROTEIN KINASE activates auxin transport-dependent growth and PIN-FORMED phosphorylation at the plasma membrane. Dev Cell 29:674–685

Barlow P (1995) Gravity perception in plants – a multiplicity of systems derived by evolution. Plant Cell Environ 18:951–962

Bastien R, Bohr T, Moulia B, Douady S (2013) Unifying model of shoot gravitropism reveals proprioception as a central feature of posture control in plants. Proc Natl Acad Sci USA 110:755–760

Bérut A, Chauvet H, Legué V, Moulia B, Pouliquen O, Forterre Y (2018) Gravisensors in plant cells behave like an active granular liquid. Proc Natl Acad Sci USA 115:5123–5128

Blancaflor E (2013) Regulation of plant gravity sensing and signaling by the actin cytoskeleton. Am J Bot:1–10

Blancaflor E, Fasano J, Gilroy S (1998) Mapping the functional roles of cap cells in the response of *Arabidopsis* primary roots to gravity. Plant Physiol 116:213–222

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433:39–44

Boonsirichai K, Sedbrook J, Chen R, Gilroy S, Masson P (2003) ARG1 is a peripheral membrane protein that modulates gravity-induced cytoplasmic alkalinization and lateral auxin transport in plant statocytes. Plant Cell 15:2612–2625

Brumos J, Robles L, Yun J, Vu T, Jackson S, Alonso J, Stepanova A (2018) Local auxin biosynthesis is a key regulator of plant development. Dev Cell 47:306–318

Caspar T, Pickard B (1989) Gravitropism in a starchless mutant of Arabidopsis: implications for the starch-statolith theory of gravity sensing. Planta 177:185–197

Chauvet H, Pouliquen O, Forterre Y, Legué V, Moulia B (2016) Inclination, not force, is sensed by plants during shoot gravitropism. Sci Rep 6:35431

Clore A (2013) Cereal grass pulvini: Agronomically significant models for studying gravitropism signaling and tissue polarity. Am J Bot 100:101–110

Clore A, Turner W, Morse A, Whetten R (2003) Changes in mitogen-activated protein kinase activity occur in the maize pulvinus in response to gravistimulation and are important for the bending response. Plant Cell Environ 26:991–1001

Clore A, Doore S, Tinnirello S (2008) Increased levels of reactive oxygen species and expression of a cytoplasmic aconitase/iron regulatory protein 1 homolog during the early response of maize pulvini to gravistimulation. Plant Cell Environ 31:144–158

Cosgrove D (2000) Loosening of plant cell walls by expansins. Nature 407:321–326

Costa A, Candeo A, Fieramonti L, Valentini G, Bassi A (2013) Calcium dynamics in root cells of *Arabidopsis thaliana* visualized with selective plane illumination. PLoS One 8:e75646

Darwin C (1880) The power of movement in plants. John Murray, London

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Dharmasiri S, Swarup R, Mockaitis K, Dharmasiri N, Singh S, Kowalchyk M, Marchant A, Mills S, Sandberg G, Bennett M et al (2006) AXR4 is required for localization of the auxin influx facilitator AUX1. Science 312:1218–1220

Dhonukshe P, Huang F, Galvan-Ampudia C, Mähönen A, Kleine-Vehn J, Xu J, Quint A, Prasad K, Friml J, Scheres B et al (2010) Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. Development 137:3245–3255

Dindas J, Scherzer S, Roelfsema M, von Meyer K, Müller H, Al-Rasheid K, Palme K, Dietrich P, Becker D, Bennett M et al (2018) AUX1-mediated root hair auxin influx governs SCFTIR1/AFB-type Ca2+ signaling. Nat Commun 9:1174

Ding Z, Friml J (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc Natl Acad Sci USA 107:12046–12051

Dong Z, Jiang C, Chen X, Zhang T, Ding L, Song W, Luo H, Lai J, Liu R, Chen H et al (2013) Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling and light response. Plant Physiol 163:1306–1322

Fasano J, Swanson S, Blancaflor E, Dowd P, Kao T, Gilroy S (2001) Changes in root cap pH are required for the gravity response of the *Arabidopsis* root. Plant Cell 13:907–921

Firn R, Digby J (1997) Solving the puzzle of gravitropism – has a lost piece been found? Planta 203:S159–S163

Fitzelle K, Kiss J (2001) Restoration of gravitropic sensitivity in starch-deficient mutants of Arabidopsis by hypergravity. J Exp Bot 52:265–275

Friml J, Wisniewska J, Benkova E, Mendgen K, Palme K (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415:806–809

Fukaki H, Fujisawa H, Tasaka M (1996) Gravitropic response of inflorescence stems in *Arabidopsis thaliana*. Plant Physiol 110:933–943

Fukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey P, Tasaka M (1998) Genetic evidence that the endodermis is essential for shoot gravitropism in *Arabidopsis thaliana*. Plant J 14:425–430

Ganguly A, Lee S-H, Cho H-T (2012) Functional Identification of the phosphorylation sites of Arabidopsis PIN-FORMED3 for Its subcellular localization and biological role. Plant J 71:810–823

Ge L, Chen R (2016) Negative gravitropism in plant roots. Nat Plants 2:16155

Geisler M, Blakeslee J, Bouchard R, Lee O, Vincenzetti V, Bandyopadhyay A, Titapiwatanakun B, Peer W, Bailly A, Richards E et al (2005) Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J 44:179–194

Gendreau E, Traas J, Desnos T, Grandjean O, Caboche M et al (1997) Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. Plant Physiol 114:295–305

Groover A (2016) Gravitropisms and reaction woods of forest trees – evolution, functions and mechanisms. New Phytol 211:790–802

Guyomarch S, Léran S, Auzon-Cape M, Perrine-Walker F, Lucas M, Laplaze L (2012) Early development and gravitropic response of lateral roots in *Arabidopsis thaliana*. Philos Trans R Soc Lond B Biol Sci 367:1509–1516

Haberlandt G (1900) Ueber die perzeption des geotropischen reizes. Ber Dtsch Bot Ges 18:261–272

Harrison B, Masson P (2008) ARL2, ARG1 and PIN3 define a gravity signal transduction pathway in root statocytes. Plant J 53:380–392

Hou G, Mohamalawari D, Blancaflor E (2003) Enhanced gravitropism of roots with a disrupted cap actin cytoskeleton. Plant Physiol 131:1360–1373

Hou B-H, Takanaga H, Grossmann G, Chen L-Q, Qu X-Q, Jones A, Lalonde S, Schweissgut O, Wiechert W, Frommer W (2011) Optical sensors for monitoring dynamic changes of intracellular metabolite levels in mammalian cells. Nat Protoc 6:1818–1833

Iino M (1995) Gravitropism and phototropism of maize coleoptiles: evaluation of the Cholodny-Went theory through effects of auxin application and decapitation. Plant Cell Physiol 36:361–367

Ishikawa H, Evans M (1995) Specialized zones of development in roots. Plant Physiol 109:725–727

Jiang W, Yang B, Weeks D (2014) Efficient CRISPR/Cas9-mediated gene editing in Arabidopsis thaliana and inheritance of modified genes in the T2 and T3 generations. PLoS One 9:e99225

Johannes E, Collings D, Rink J, Allen N (2001) Cytoplasmic pH dynamics in maize pulvinal cells induced by gravity vector changes. Plant Physiol 127:119–130

Kato T, Morita M, Fukaki H, Yamauchi Y, Uehara M, Niihama M, Tasaka M (2002) SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE, are involved in the shoot gravitropism of *Arabidopsis*. Plant Cell 14:33–46

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435:446–451

Kim K, Shin J, Lee S, Kweon H, Maloof J, Choi G (2011) Phytochromes inhibit hypocotyl negative gravitropism by regulating the development of endodermal amyloplasts through phytochrome-interacting factors. Proc Natl Acad Sci USA 108:1729–1744

Kiss J, Hertel R, Sack F (1989) Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. Planta 177:198–206

Kiss J, Wright J, Caspar T (1996) Gravitropism in roots of intermediate-starch mutants of *Arabidopsis*. Physiol Plant 97:237–244

Kiss J, Edelmann R, Wood P (1999) Gravitropism of hypocotyls of wild-type and starch-deficient *Arabidopsis* seedlings in spaceflight studies. Planta 209:96–103

Kiss J, Miller K, Ogden L, Roth K (2002) Phototropism and gravitropism in lateral roots of *Arabidopsis*. Plant Cell Physiol 43:35–43

Kiss J, Millar K, Edelmann R (2012) Phototropism of *Arabidopsis thaliana* in microgravity and fractional gravity on the International Space Station. Planta 236:635–645

Kleine-Vehn J, Ding Z, Jones A, Tasaka M, Morita M, Friml J (2010) Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. Proc Natl Acad Sci USA 107:22344–22349

Knight T (1806) On the direction of the radicle and germen during the vegetation of seeds. Philos Trans R Soc 99:108–120

Krieger G, Shkolnik D, Miller G, Fromm H (2016) Reactive oxygen species tune root tropic responses. Plant Physiol 172:1209–1220

Kuznetsov O, Hasenstein K (1996) Intracellular magnetophoresis of amyloplasts and induction of root curvature. Planta 198:87–94

Lau S, Smet I, Kolb M, Meinhardt H, Jürgens G (2011) Auxin triggers a genetic switch. Nat Cell Biol 13:611–615

Leitz G, Kang B-H, Schoenwaelder M, Staehelin L (2009) Statolith sedimentation kinetics and force transduction to the cortical endoplasmic reticulum in gravity-sensing Arabidopsis columella cells. Plant Cell 21:843–860

Lewis D, Miller N, Splitt B, Wu G, Spalding E (2007) Separating the roles of acropetal and basipetal auxin transport on gravitropism with mutations in two Arabidopsis multidrug resistance-like ABC transporter genes. Plant Cell 19:1838–1850

Li G, Xue H (2007) Arabidopsis PLD{zeta}2 regulates vesicle trafficking and is required for auxin response. Plant Cell 19:281–295

Li P, Wang Y, Qian Q, Fu Z, Wang M, Zeng D, Li B, Wang X, Li JJ (2007) LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. Cell Res 17:402–410

Limbach C, Hauslage J, Schäfer C, Braun M (2005) How to activate a plant gravireceptor. Early mechanisms of gravity sensing studied in Characean rhizoids during parabolic flights. Plant Physiol 139:1030–1040

Lin D, Nagawa S, Chen J, Cao L, Chen X, Xu T, Li H, Dhonukshe P, Yamamuro C, Friml J et al (2012) A ROP GTPase-dependent auxin signaling pathway regulates the subcellular distribution of PIN2 in Arabidopsis roots. Curr Biol 22:1319–1325

Lu Y-T, Feldman L (1997) Light-regulated root gravitropism: a role for, and characterization of, a calcium/calmodulin-dependent protein kinase homolog. Planta 203:S91–S97

Lu C, Yuan S, Li J (2006) Contribution of both cell elongation and cell division to wheat coleoptile elongation. Belg J Bot 139:167–172

MacCleery S, Kiss J (1999) Plastid sedimentation kinetics in roots of wild-type and starch-deficient mutants of *Arabidopsis*. Plant Physiol 120:183–192

Mancuso S, Barlow P, Volkmann D, Baluska F (2006) Actin turnover-mediated gravity response in maize root apices. Gravitropism of decapped roots implicates gravisensing outside of the root cap. Plant Signal Behav 1:52–58

Mironova V, Omelyanchuk N, Yosiphon G, Fadeev S, Kolchanov N, Mjolsness E, Likhoshvai V (2010) A plausible mechanism for auxin patterning along the developing root. BMC Syst Biol 4:98

Monshausen G, Miller N, Murphy A, Gilroy S (2011) Dynamics of auxin-dependent Ca2+ and pH signaling in root growth revealed by integrating high-resolution imaging with automated computer vision-based analysis. Plant J 65:309–318

Morita M (2010) Directional gravity sensing in gravitropism. Annu Rev Plant Biol 61:705–720

Morita M, Kato T, Nagafusa K, Saito C, Ueda T, Nakano A, Tasaka M (2002) Involvement of the vacuoles of the endodermis in the early process of shoot gravitropism in *Arabidopsis*. Plant Cell 14:47–56

Mullen J, Ishikawa H, Evans M (1998) Analysis of changes in relative elemental growth rate patterns in the elongation zone of *Arabidopsis* roots upon gravistimulation. Planta 206:598–603

Munnik T, Nielsen E (2011) Green light for polyphosphoinositide signals in plants. Curr Opin Plant Biol 14:489–497

Nemec B (1900) Ueber die art der wahrnehmung des schwekraftreizes bei den pflanzen. Ber Dtsch Bot Ges 18:241–245

Noh B, Murphy A, Spalding E (2001) Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. Plant Cell 13:2441–2454

Perbal G, Jeune B, Lefranc A, Carnero-Diaz E, Driss-Ecole D (2002) The dose-response curve of the gravitropic reaction: a re-analysis. Physiol Plant 114:336–342

Perera I, Heilmann I, Boss W (1999) Transient and sustained increases in inositol-1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. Proc Natl Acad Sci USA 96:5838–5843

Perera I, Heilmann I, Chang S, Boss W, Kaufman P (2001) A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. Plant Physiol 125:1499–1507

Perera I, Hung C-Y, Brady S, Muday G, Boss W (2006) A universal role for inositol 1,4,5-trisphosphate-mediated signaling in plant gravitropism. Plant Physiol 140:746–760

Plieth C, Trewavas A (2002) Reorientation of seedlings in the Earth's gravitational field induces cytosolic calcium transients. Plant Physiol 129:786–796

Pouliquen O, Forterre Y, Bérut A, Chauvet H, Bizet F, Legué V, Moulia B (2017) A new scenario for gravity detection in plants: the position sensor hypothesis. Phys Biol 14:035005

Rakusová H, Gallego-Bartolomé J, Vanstraelen M, Robert H, Alabadí D, Blázquez M, Benková E, Friml J (2011) Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in *Arabidopsis thaliana*. Plant J 67:817–826

Rakusová H, Abbas M, Han H, Song S, Robert H, Friml J (2016) Termination of shoot gravitropic responses by auxin feedback on PIN3 polarity. Curr Biol 26:3026–3032

Ren H, Gray W (2015) SAUR proteins as effectors of hormonal and environmental signals in plant growth. Mol Plant 8:1153–1164

Rojas-Pierce M, Titapiwatanakun B, Sohn E, Fang F, Larive C, Blakeslee J, Cheng Y, Cutler S, Peer W, Murphy A et al (2007) Arabidopsis P-glycoprotein19 participates in the inhibition of gravitropism by gravacin. Chem Biol 14:1366–1376

Rosquete M, von Wangenheim D, Marhavý P, Barbez E, Stelzer E, Benková E, Maizel A, Kleine-Vehn J (2013) An auxin transport mechanism restricts positive orthogravitropism in lateral roots. Curr Biol 23:817–822

Roychoudhry S, Del Bianco M, Kieffer M, Kepinski S (2013) Auxin controls gravitropic setpoint angle in higher plant lateral branches. Curr Biol 23:1497–1504

Sievers A, Buchen B, Volkmann D, Hejnowicz Z (1991) Role of the cytoskeleton in gravity perception. In: Lloyd C (ed) The cytoskeletal basis of plant growth and form. Academic Press, London, pp 169–182

Silady R, Ehrhardt D, Jackson K, Faulkner C, Oparka K, Somerville C (2007) The GRV2/RME-8 protein of Arabidopsis functions in the late endocytic pathway and is required for vacuolar membrane flow. Plant J 53:29–41

Sinclair W, Trewavas A (1997) Calcium in gravitropism: a re-examination. Planta 203:S85–S90

Stanga J, Boonsirichai K, Sedbrook J, Otegui M, Masson P (2009) A role for the TOC complex in Arabidopsis root gravitropism. Plant Physiol 149:1896–1905

Staves M, Wayne R, Leopold A (1992) Hydrostatic pressure mimics gravitational pressure in characean cells. Protoplasma 168:141–152
Staves M, Wayne R, Leopold A (1997) The effect of external medium on the gravitropic curvature of rice (*Oryza sativa*, Poaceae) roots. Am J Bot 84:1522–1529
Strohm A, Barrett-Wilt G, Masson P (2014) A functional TOC complex contributes to gravity signal transduction in Arabidopsis. Front Plant Sci 5:148
Su S-H, Gibbs N, Jancewicz A, Masson P (2017) Review: molecular mechanisms of root gravitropism. Curr Biol 27:R964–R972
Swarup R, Kramer E, Perry P, Knox K, Leyser H, Haseloff J, Beemster G, Bhalerao R, Bennett M (2005) Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. Nat Cell Biol 7:1057–1065
Tanaka A, Kobayashi Y, Hase Y, Watanabe H (2002) Positional effect of cell inactivation on root gravitropism using heavy-ion microbeams. J Exp Bot 53:683–687
Taniguchi M, Furutani M, Nishimura T, Nakamura M, Fushita T, Iijima K, Baba K, Tanaka H, Toyota M, Tasaka M et al (2017) The Arabidopsis LAZY1 family plays a key role in gravity signaling within statocytes and in branch angle control of roots and shoots. Plant Cell 29:1984–1999
Terrile M, París R, Calderón-Villalobos L, Iglesias M, Lamattina L, Estelle M, Casalongué C (2012) Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. Plant J 70:492–500
Toyota M, Furuichi T, Tatsumi H, Sokabe M (2008) Cytoplasmic calcium increases in response to changes in the gravity vector in hypocotyls and petioles of Arabidopsis seedlings. Plant Physiol 146:505–514
Toyota M, Ikeda N, Sawai-Toyota S, Kato T, Gilroy S, Tasaka M, Morita M (2013) Amyloplast displacement is necessary for gravisensing in Arabidopsis shoots as revealed by a centrifuge microscope. Plant J 76:648–660
Tsugeki R, Fedoroff N (1999) Genetic ablation of root cap cells in *Arabidopsis*. Proc Natl Acad Sci USA 96:12941–12946
Vitha S, Yang M, Sack F, Kiss J (2007) Gravitropism in starch-excess mutant of *Arabidopsis thaliana*. Am J Bot 94:590–598
Wang J-W, Wang L-J, Mao Y-B, Cai W-J, Xue H-W, Chen X-Y (2005) Control of root cap formation by microRNA-targeted auxin response factors in Arabidopsis. Plant Cell 17:2204–2216
Wang Y, Lin W, Chen X, Xue H (2009) The role of Arabidopsis 5PTase13 in root gravitropism through modulation of vesicle trafficking. Cell Res 19:1191–1204
Wang H-Z, Yang K-Z, Zou J-J, Zhu L-L, Xie Z, Morita M, Tasaka M, Friml J, Grotewold E, Beeckman T et al (2015) Transcriptional regulation of PIN genes by FOUR LIPS and MYB88 during Arabidopsis root gravitropism. Nat Commun 6:8822
Weise S, Kuznetsov O, Hasenstein K, Kiss J (2000) Curvature in *Arabidopsis* inflorescence stems is limited to the region of amyloplast displacement. Plant Cell Physiol 41:702–709
Whitford R, Fernandez A, Tejos R, Pérez A, Kleine-Vehn J, Vanneste S, Drozdzecki A, Leitner J, Abas L, Aerts M et al (2012) GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. Dev Cell 22:678–685
Wolverton C, Mullen J, Ishikawa H, Evans M (2002) Root gravitropism in response to a signal originating outside of the cap. Planta 215:153–157
Yamamoto K, Pyke K, Kiss J (2002) Reduced gravitropism in inflorescence stems and hypocotyls, but not in roots, of *Arabidopsis* mutants with large plastids. Physiol Plant 114:627–636
Yang H, Murphy A (2009) Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in Schizosaccharomyces pombe. Plant J 59:179–191
Yano D, Sato M, Saito C, Sato M, Morita M, Tasaka M (2003) A SNARE complex containing SGR3/AtVAM3 and ZIG/VTI11 in gravity-sensing cells is important for Arabidopsis shoot gravitropism. Proc Natl Acad Sci USA 100:8589–8594
Yoshihara T, Spalding E (2017) *LAZY* genes mediate the effects of gravity on auxin gradients and plant architecture. Plant Physiol 175:959–969

Yoshihara T, Spalding E, Iino M (2013) AtLAZY1 is a signaling component required for gravitropism of the *Arabidopsis thaliana* inflorescence. Plant J 74:267–279
Zhang J, Peer W (2017) Auxin homeostasis: the DAO of catabolism. J Exp Bot 68:3145–3154
Zheng H, van Mollard G, Kovaleva V, Stevens T, Raikhel N (1999) The plant vesicle-associated SNARE AtVTI1a likely mediates vesicle transport from the trans-Golgi network to the prevacuolar compartment. Mol Biol Cell 10:2251–2264
Zheng Z, Zou J, Li H, Xue S, Wang Y, Le J (2014) Microrheological insights into the dynamics of amyloplasts in root gravity-sensing cells. Mol Plant 8:660–663

Shih-Heng Su is an Assistant Scientist in the Masson laboratory at the University of Wisconsin-Madison. She developed her Ph.D. research in the Krysan laboratory, also at the University of Wisconsin-Madison, and completed a postdoctoral program in the Masson laboratory in 2017. She is currently using the natural variation that exists between *Brachypodium* accessions to investigate the molecular mechanisms that modulate complex root growth behaviors in monocots. Additionally, she is running a research project aimed at investigating *Brachypodium* seedling responses to the microgravity environment encountered during spaceflight.

Patrick H. Masson is a Professor of Genetics at the University of Wisconsin-Madison. He obtained a Ph.D. in Agronomy from the Faculty of Agronomical Sciences in Gembloux, Belgium, in 1986, and completed a postdoctoral program at the Carnegie Institution of Washington in Baltimore, MD, in 1991. Research in his laboratory is aimed at characterizing the molecular mechanisms that control root growth behaviors in response to mechanical information within the environment, including gravity and touch stimulation. His laboratory also investigates the role of polyamines in the control of root growth and system architecture in model plants such as *Arabidopsis thaliana* in the dicots and *Brachypodium distachyon* in the monocots.

Plant Cognition: Ability to Perceive 'Touch' and 'Sound'

6

Ratnesh Chandra Mishra and Hanhong Bae

Abstract

Plants' sessile life-style has enabled them to develop enormous sensitivity towards their dynamic, tactile and clamorous surroundings. Consequently, besides a range of different stimuli, plants can even perceive subtle stimuli, like 'touch' and unanticipatedly 'sound'. Importantly, touch sensitivity in plants is not just limited to sensitive plant and carnivorous species, which respond through eye-catchy movements; instead every plant and living plant cell senses and responds to mechanostimulation, whether intrinsic or extrinsic in nature. For instance, plant roots are extremely touch-sensitive, and upon encountering a barrier in soil, they are able to effectively redirect their growth to transcend it. Similarly, tendrils in climbing plants exhibit extreme sensitivity towards touch, which enable them to sense and grab a support in close vicinity. Unlike touch sensitivity, which was recognized long ago by Robert Hooke and Darwin, plants' sensitivity towards sound has started gaining attention only recently. The past decade has seen major advances in this area of plant biology; many breakthrough discoveries were made that revealed the, otherwise debatable, ecological significance of sound perception in plants' life. It has come to light that plants not just sense but also distinguish relevant sound among a mixture of irrelevant sound frequencies; plants distinguish buzz produced by a true pollinator among pollen thieves in the sophisticated process of buzz pollination. Similarly, plants distinguish sound typical of a herbivore for elicitation of defence response. Interestingly, plant roots can sense sound of flowing water in order to direct their growth towards the water source. Given the similarity in the physical properties of touch and sound stimuli, many recently discovered signaling events and molecular players in touch and sound perception are noted to be common. However, in view

R. C. Mishra · H. Bae (✉)
Department of Biotechnology, Yeungnam University,
Gyeongsan, Gyeongbuk, Republic of Korea
e-mail: hanhongbae@ynu.ac.kr

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_6

of the contrasting responses tailored according to the stimuli, plants appear to distinguish well among the two in an ecologically meaningful manner.

Keywords
Cognition · Development · Growth · Mechanoperception · Plant acoustics · Sound · Thigmonasty · Thigmotropism · Touch · Volatile organic compounds

6.1 Introduction

On evolutionary time scale, plants have consistently preceded animals in successfully inhabiting a niche through ample adjustments and/or modification in their forms. Still, humans have always had a bias that animals are more evolved than plants, in terms of sensing and responding towards a change in their surroundings. One of the underlying reasons behind this procrastinating thought is the quiescent life-style of plants that was argued to leave plants less privileged and sensible towards their environment in comparison with animals. It was proposed that animals are capable of analyzing an undesirable situation and can choose to move away from it, whereas plants do not have this advantage. As a matter of fact, however, inability to move rendered plants to develop mechanisms for scrutinizing their surroundings and utilize every possible cue that fine-tunes their growth and development favoring sustenance. Thus, the sedentary life-style has actually proven to be a boon to plants in exposing them to copious environmental cues, which enabled them to perceive stimuli that are even beyond human's imagination. 'Touch' and more particularly 'sound' are the two such environmental stimuli.

As a rationale for plants to have developed sensitivity to touch, the first need is to envisage the niches they thrive in. It is necessary to be highlighted here that plants live in an extremely tactile environment; mechanically, while winds agitate them furiously, they are also disturbed through animals passing by. It is thus reasonably valid that plants developed sensitivity towards mechanical stimulation or touch for modulation of their growth and development so as to endure such situations. Not just this, plants have also been able to smartly deploy the developed touch sensitivity to maintain their race and fulfill their nutritional requirements. Evidently, there are several plant species relying on animals for their successful pollination, where the pollinator is identified through touch stimulation. The other classical example is the excellent touch sensitivity of carnivorous plants, where even a minute stimulation by an insect at the evolved sensory structure is sufficient to evoke a response in fraction of seconds. Being visually captivating, the rapid movements of carnivorous plants to capture their preys had gathered attention since Darwin's era (Darwin 1875). Clearly, plants' elaborate responsiveness towards touch was discovered long back and the physiological mechanisms behind many such responses were later discovered. However, reports on the mechanism of touch perception and signal transduction with regard to the molecular players involved are very recent and many aspects are still obscure.

Though touch perception in plants succeeded in gathering the requisite attention of biologist long ago, the idea whether plants utilize sound as a modality to interact with their environment remained debatable until recently. The past decade has seen major advancements in this area, ending the procrastinating debate and shifting the focus from 'whether' plants perceive sound to 'how' and 'why' they do it. The first argument was the extreme alertness that plants had evolved towards their surroundings. Importantly, there is no niche colonized by plants on this planet that is quiet. There are several sound frequencies both within the audible or non-audible ranges which plants are exposed to. Sound can either be produced physically by blowing winds or flowing water in the streams or of biological origin in the form of bee buzz, chirping birds, stridulating crickets, etc. Thus, it makes much sense that plants have also developed sensitivity towards sounds of various ecologically relevant frequencies to interact with their environment in a more fruitful manner for their better growth and development. Additionally, considering that plants have attained sensitivity to a level that they can even sense a subtle stimulus like touch, the question was why they cannot perceive sound, especially when both touch and sound have more or less similar physical influence on plants' structure. Sound waves originate from a vibrating body and proceed by vibrating the particles in the medium it travels through. Upon interface with an object, it tends to mechanically vibrate the object as well. In fact, the principle behind hearing in human is the same: sound vibrations travel through ear canal and vibrate the eardrum, which then eventually are sensed by the nerves aiding in the very process of hearing. The similarity in the physical nature of these two stimuli is also the reason why touch and sound fall within the domain of this chapter. Nevertheless, it is necessary and important to make it clear to the readers here itself that plants can very well distinguish within the two stimuli and respond accordingly in a tailored manner. Refuting the doubts whatsoever, recent discoveries have established that plants do perceive sound and respond suitably in an ecologically significant manner. Evidently, plants perceive different sound stimuli for different purposes. The phenomenon of buzz pollination, where plants dehisce anthers and release pollen upon exposure to a particular bee buzz frequency, is one of the bona fide examples of plant-sound interactions in nature (De Luca and Vallejo-Marin 2013). As a well-known fact, plants' exposure to different sound frequencies enhances the growth/yields of several crop species and strengthens plant immunity. Owing to which, application of sound has extensively been exploited in agriculture and biotechnology (Hassanien et al. 2014). Plants can also sense sound typical of an herbivore and respond by producing defence-related toxic molecules (Appel and Cocroft 2014). There are many such instances where plant-animal mutualism is a result of perception of sound by plants. The past 5 years have been rewarding in this area of plant biology, in terms of identification of genes, proteins and hormones affected by sound perception and elucidation of initial signaling events triggered by sound (Jung et al. 2018). This thus forms a perfect platform inviting researchers all over the world to explore more in this emerging field of plant biology – 'plant acoustics'.

Conforming the hierarchy in their discoveries, plants' interaction with mechanical stimuli and/or touch is taken up first in this chapter, and subsequent to which, the recent and fascinating discoveries in plant acoustics are discussed. In so doing, some of the most fascinating and previously hidden facets in plant's sensing abilities are uncovered. Certainly, this will cause a perspective change towards these otherwise quiescent creatures and affirm that plants are much sensitive and smarter than were assumed previously.

6.2 Plants Respond to Mechanostimulation: Lessons from Nature

Diving deep into the nature reveals many instances where there is clear evidence of touch perception by plants. Talking in terms of what biologists see, speculate, understand and believe, it is always the responses that capture first attention, following which the cause and mechanisms are explored. Touch-mediated plant responses were reported long ago during the time of Darwin (1875). For instance, the rapid movements of carnivorous plants, visually being obvious, gathered much attention and reserved elaborate mention in Darwin's famous book *The Power of Movement in Plants*. Alongside, the navigation of roots through barriers in the soil, which is exhibited by almost all plant species, was also appreciated and emphasized in his book. Nevertheless, in nature we merely see the tip of an iceberg; while there are several plant responses that are rapid and highly cognizable, many others are often slow, gradual and underappreciated. Rapid responses can either be thigmotropic or thigmonastic, where the word 'thigma' means touch in Greek. In tropic movements the direction of the responses is influenced by the direction of the touch stimulus, for example, an obstacle in the soil, once encountered, results in the root growth away from it. Contrastingly, thigmonastic responses are not influenced by the direction of the stimulus and thus can occur in any direction, for example, the rapid folding of leaflets in *Mimosa pudica* is independent of the direction of touch stimulus. In contrast to the above two rapid responses against touch stimulation, there are other responses in plants that are more gradual and can be realized as slow morphogenetic alterations. Such responses are called as 'thigmomorphogenesis'. It has been seen that plants with specialized touch-sensitive structures or plant organs, like roots, that are able to sense touch, exhibit rapid thigmotropic and/or thigmonastic types of responses. However, most of the higher plants that do not possess a specialized structure/mechanism and are yet exposed to mechanical stimulation, like touch, wind, etc., come up with gradual thigmomorphogenetic alteration in their growth and development suitable to adapt the mechanical force. Thus, compared to the thigmotropic/thigmonastic responses, thigmomorphogenesis is more common. Advances in the research in the area of thigmomorphogenesis have resulted in the elucidation of the cellular signaling involved in touch perception. The upcoming account first gives a quick overview of some thigmonastic and thigmotropic plant responses and mechanisms, following which, a comprehensive discussion on the thigmomorphogenetic responses and the cellular signaling involved is provided.

6.2.1 Thigmonasty and Thigmotropism: Swift and Captivating

As elaborated earlier, the responses more obvious to human eyes are the ones that were discovered earlier. Comparatively, thigmonastic and thigmotropism are quicker than thigmomorphogenetic responses with thigmonastic movements being fastest of all. The first thigmonastic plant response that came to light was the touch-triggered folding of leaves of *M. pudica* – the 'sensitive plant'. Robert Hooke first identified this response in 1665, which later captured the due attention of the successive biologist for elucidating the involved mechanism. *M. pudica* comprises doubly compound leaves, which folds their leaflets upon touch stimulation (Fig. 6.1). The response depends on the magnitude of the force exerted, as it is not just restricted to the touched leaflet but rather it spreads out to the other leaflets of the doubly compound leaf as well; the higher the pressure, the more number of leaflets are folded. In extreme cases, drooping of the leaf occurs, passing the stimulus even to the other leaves in vicinity, resulting in folding of their leaflets as well. Ecologically, this behavior is explained as a mechanism to limit herbivory. While on one hand, sudden folding of leaves frightens away the predator, on the other hand, it demotivates the foraging predator through visual reduction of foliage volume. Also, folding of leaves conspicuously exposes the protective thorns as a further defence. The broad mechanism operational behind this response is the change in the turgidity of the extensors and flexors cells of the pulvini. Pulvini are the specialized

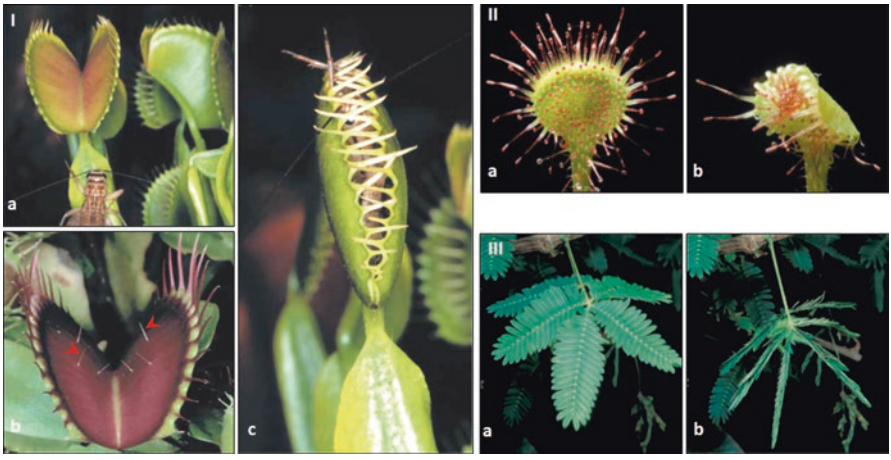

Fig. 6.1 Touch responses in carnivorous species and sensitive plant. (*I*) *Dionaea muscipula* (Venus flytrap). (*I*, *a*) Insect prey approaching an open trap. (*I*, *b*) Close-up of a specialized bilobed leaf (trap) showing three touch-sensitive trigger hairs on the ventral side of each lobe (indicated by arrow head) and needle-shaped spikes at the margin. (*I*, *c*) Prey sandwiched within the bilobed trap with interlaced spikes. (II) *Drosera* (sundew). (*II*, *a*) Open tentacle-laden leaf with shiny, sticky mucilage at the end of each tentacle. (*II*, *b*) Touch-stimulated leaf with a cup-shaped indentation. (*III*) *Mimosa pudica* (sensitive plant). (*III*, *a*) Doubly compound leaves with wide open leaflets before touch perturbation. (*III*, *b*) Touch-stimulated leaves with closed leaflets. (These pictures are adapted from Braam (2005). Originally, these were captured by Barry Rice, Ph.D.; http://www.sarracenia.com/galleria/galleria.html)

motor organs located at the bases of both the petioles and leaflets. While the touch stimulation results in the loss of turgor from the extensor cells, the oppositely oriented flexor cells tend to stretch. These changes in the cell turgidity and thus the volume are reversible and the definitive cause behind the sophisticated leaflet and petiole movements (Braam 2005). There are several lines of explanation for the reason behind loss in extensor cell turgor. However, the precise mechanism behind the propagation of the stimuli to the far located pulvini is still a matter of research. Following the above discovery, the elaborate thigmonastic responses exhibited by carnivorous species were the next to be recognized. While Darwin took much interest in studying the different carnivorous plant species, the one he found to exhibit the most impressive thigmonastic movement was Venus flytrap (*Dionaea muscipula*). He narrated this plant 'as one of the most wonderful in the world'. Morphologically, it possesses a specialized bilobed leaf with needle-shaped spikes on the margins and three trigger hairs on the ventral side of each lobe (Fig. 6.1). This bilobed leaf generally remained spread open to allow insects to crawl over. Once an insect collides with the trigger hair, the bilobe sandwiches the insect through the closure of the trap (Fig. 6.1). Interestingly, the plant knows when to shut the bilobe; multiple stimulations of more than one trigger hair within a matter of few seconds are required for the response to occur (Braam 2005). This ensures that the prey is sufficiently voluminous and will not be able to wiggle out of the trap, justifying the energy spent in capturing and digesting it. Through this the plant ensures fulfillment of its nitrogen demand in the nitrogen-poor environment. Another carnivorous species where both thigmonastic and thigmotropic movements together are involved in capturing the prey is the *Drosera rotundifolia*. In this case, the modified leaf is covered with over 100 tentacles with sticky mucilage at the end of each, which glistens in sunlight justifying the common name of the plant as 'sundew' (Fig. 6.1). The glistening leaf attracts the insect, which gets entangled in the gluey mucilaginous trap. As the insect struggles to escape, the touch-sensitive neighboring tentacles sense the movement and bend towards the prey, thereby forming a cup-shaped indentation that encloses the prey from all possible escape points (Braam 2005). Importantly, it is not just the general mechanical stimulation that results in this response; where the plant remarkably responds to subtle insect touch, it doesn't mount any response when agitated by water drops from heavy rains. The mechanism with which plant distinguished between these stimuli, however, is still obscure. Nevertheless, it highlights that the developed sensitivity is highly sophisticated and precise.

Unlike thigmonasty, where a swift response within a matter of few seconds is warranted either to bluff a predator/herbivore or to resist possible escape of a prey, thigmotropic movements are relatively gradual. Further, while some specialized plants exhibit thigmonasty, thigmotropism is relatively more common. In fact, root growth in most of the land plants exhibits thigmotropic growth while navigating through barriers in soil. To be more specific, however, there are few plants, like the climbing species (e.g., *Bryonia dioica*) that exhibit thigmotropic growth which increases their height to reach sunlight. Instead of expending unnecessary energy in developing supporting trunk, climbing species modified their leaves or stem in touch-sensitive tendrils that encircles a firm object in proximity facilitating the vertical growth of the plant. Touch sensitivity of some tendrils is proposed to be even greater than humans',

where just a 0.25 mg thread in the vicinity of the tendril is sufficient to evoke the coiling response. Once stimulated, the tip of the tendril tends to coil rapidly, sometimes within seconds, securing firm support of the object. Again, the sensitivity is very specific, as touch of a raindrop is never considered a stimulus to elicit a response. Additionally, coiling response to any transient stimulation is often reversed by uncoiling. After identifying and grabbing a firm support, lignification stiffens the coil to avoid unwinding. Coiling is a result of initial turgor-based changes in cell volumes followed by sustained differential cell growth; while ventral cell in a coil tends to contract, the dorsal cell expands more. A continued pattern of such growth results in sustained coiling (Braam 2005). Besides climbing species, there are several flowering plants that bear flowers with evolved touch-sensitive organs. Usually, thigmonastic or thigmotropic petals, stamen filaments and carpels are evolved either to limit self-pollination or to deposit pollens on pollinators, like humming birds, insects, etc. In order to circumvent self-pollination, stigma of the touch-sensitive flowers bends towards the petal in response to an insect approaching anthers of the same flower, so as to avoid contact with the insect while it departs carrying pollen. Similarly, to facilitate pollination, touch-sensitive stamens bend over the insects to dab pollens on it. One of the fascinating examples here is the dimorphic flower of *Catasetum* (Braam 2005). Here, the stamens are rigidly held by petals and thus experience great tension. Once a bee visits and contacts the sensory antennae of the flower, it responds by releasing the held filaments along with a sticky disc of pollen sac, which hits the bee with an extreme force. The force is so strong that besides knocking the traumatized bee with a burden of heavy pollen sac away from the flower, it ensures that the bee preferably selects a female flower over a male for its next visit. This results in effective pollination of the female flower.

One of the best and ubiquitous examples of the thigmotropism is the growth of root through soil barriers. Charles Darwin was the first to observe that roots reorient their downward directional growth upon interface with a flat obstacle; upon such a situation, root tips tend to turn angularly, almost 90°, taking a new direction of growth transcending the barrier. He was the first to hypothesize that root apices are touch-sensitive and upon contact with an obstacle, a transmissible signal is generated changing the root growth. More recently, similar root growth behavior was observed in the model plant *Arabidopsis*, where encounter with a barrier compromises the gravitropic root growth favoring thigmotropism instead. One of the earliest gravitropic responses of root growth at the subcellular level is the settling of starch in the columella cells. Touch has been proposed to delay this response (Braam 2005).

Almost every response discussed above can be explained either through touch-mediated transient and differential alterations in cells/cell layer volumes, as a result of change in cell turgor, or through sustained differential growth in terms of differential cell expansion rates, leading into differently sized cells/cell layers. Broadly, an action potential is triggered by touch stimulation, which propagates through symplastically associated cells eventually evoking turgor changes in the responsive cell (Monshausen and Haswell 2013). This is functional mostly in plants bearing specialized structures responsive to touch and respond through nastic movements. However, in plants exhibiting tropic responses, it goes even beyond entailing other players as

well, like hormonal modulation, leading to sustained differential growth. Regardless of the kind of responses and involved mechanisms, the sensing and transduction of the touch stimulus involve certain mechanisms, most of which have been derived from studies entailing thigmomorphogenesis. In the next account, a comprehensive overview of the thigmomorphogenetic responses is provided, followed by the molecular events involved in the perception and signal transduction of touch, known so far.

6.2.2 Thigmomorphogenesis: Slow Yet Fascinating

Almost all plants, even the ones without the specialized sensory organs/cells, respond to mechanical stimulation through gradual morphogenetic changes. True, they do it slowly over time and the responses are not readily apparent, but the overall responses are quite dramatic. For such touch-modulated gradual morphogenetic and/or developmental responses, the term 'thigmomorphogenesis' was coined by Mark Jaffe (1973), who has been studying touch-induced responses of nonspecialized plants for over the past 40 years. In general, touch results in inhibition of plant growth and acceleration in senescence. As summarized, the hallmark of thigmomorphogenesis in shoot over a range of species is increased radial growth associated with a decreased elongation (Chehab et al. 2009). In fact, the model plant *Arabidopsis*, as examined under in vitro conditions, displays short stature when touched on a regular basis over a period of time. It is believed that such kind of growth behaviour is an adaptive adjustment to withstand continued exposure of mechanical perturbations, for example, trees growing along the coastlines are often short in height with widened trunk to sustain mechanical forces imposed by strong winds. This is seldom associated with increased production of strengthening tissue through secondary growth. In contrast to it, however, some species respond through increase in their tissue flexibility to cope with mechanical stress-induced breakage. It is important to note here that mechanical perturbations are not always imposed externally through environment; in fact, plants experience mechanical stresses also intrinsically throughout their development. As a woody plant grows against gravity and gains mass, it experiences progressively increasing mechanical self-load, which is often counteracted through increased production of supporting tissues and stem thickening through thigmomorphogenetic modifications. Not just in woody plants, perception of longitudinal strain is intrinsic and critical to all land plants as they grow and attain mass. In fact, the extent of longitudinal strain experienced by a plant is strongly correlated with the thigmomorphogenetic adjustment it brings. So much so, that a mutant plant with xylem of reduced tensile stiffness tends to accumulate more xylem tissues to achieve requisite stiffness supporting its longitudinal growth (Braam 2005). Further, studies carried with model plant *Arabidopsis thaliana* suggest that with increasing height and associated weight, there is an increased xylem production (Braam 2005). Furthermore, addition of weight artificially to immature inflorescence results in enhanced cambium development. Applying direct compressive forces to undifferentiated mass of callus cells in vitro also induces cambium-like development. From the foregoing it is amply clear that sensing of mechanical forces is systemic and fundamental to all plant cells. Corroboratively, different kinds of plant cells, like the ones from fully differentiated

shoot and roots, suspension culture cells and even the isolated plant cell protoplast, respond to mechanical stimulation both physiologically and developmentally (Monshausen and Haswell 2013). Moreover, touch-induced changes have also been reported subcellularly. Touching a cell with a glass capillary triggers chloroplast movements away from the site of contact. Contrastingly, nucleus migrates towards the site of cell wall distortion induced by microneedle contact (Braam 2005). Speaking in cellular terms, the principal intrinsic mechanical stress that is endured by all living plant cell is turgor pressure. It is the turgor that contributes towards the structural integrity, at least in the case of all herbaceous plant species. Considering more fundamentally, turgor is the decisive force behind cell expansion and a major determinant of cell size and shape, in concert with tightly modulated cell wall extensibility. Being a regulator of cell expansion, turgor is also critical for proper cell division. Certainly, it is the turgor, which is more fundamental towards the gradual overall thigmomorphogenetic response exhibited by a plant. Further, as highlighted previously, it is the change in the cell turgor that is fundamental also to all thigmonastic and thigmotropic responses. Thus, in large, change in turgor appears to be central in all touch/mechanostimulation response, which then is backed up by more specific changes tailored according to the stimulus entailing other players. This also warrants the initial mechanosensing to be common among all mechanostimulation-induced responses. In accordance to this, Jaffe noticed that even in the slow thigmomorphogenetic responses the primary physiological response in terms of changes in electrical resistance is mounted within seconds of stimulus perception (Chehab et al. 2009), a common feature of thigmonastic and thigmotropic responses. With this enticing background this chapter will delve a bit deeper into the molecular aspect of mechanosensing known so far.

6.3 Mechanoperception: A Molecular Aspect

To be able to elicit a response, a stimulus should first be sensed and identified at the cell surface. Subsequently, a series of biochemical changes are triggered facilitating signal transduction, which couples the stimulus reception to appropriate responses. As a matter of fact, a molecular player facilitates each and every step within a stimulus-response model. For stimulus reception, there should be a molecular receptor and/or an alternative mechanism based on changes in the membrane potential. The message is then passed on to an appropriate second messenger, which in turn excites a series of biochemical modifications of different molecular players. Eventually, this leads to the customization of an appropriate response in terms of gene/protein expression, physiological and/or morphological adjustments. As mentioned earlier, plant responses to mechanical stimulation are often systemic; one can see the thigmomorphogenetic modifications occurring at a region distal to the region directly perturbed by mechanical stimulation. Moreover, responses to mechanostimulation can easily be emulated or antagonized by different pharmacological treatments (Chehab et al. 2009). These observations convincingly advocate the involvement of signaling molecules in plants' responses to mechanical stimuli. The subsequent text discusses the progress made in this direction, with an elaboration on the involved molecular players identified so far.

6.3.1 Mechanoreception and Signal Transduction: A Suite of Early Events

Although it was a century ago when the plants' ability to respond to touch was recognized, the efforts to decipher the involved molecular mechanisms are only recent. Therefore, the knowledge we have gained so far is only preliminary and a lot more is yet to be understood. Interestingly, the earlier proposals that the mechanism of mechanoperception at the plants' cell membrane is possibly similar to what is functional in animals and bacterial cells appear to hold true. Animal and bacterial cells possess certain stretch-activated ion channels that trigger ion flux in response to mechanical disturbances in cell membrane. As discussed in the previous text, the generation of an action potential, electrical resistance and/or associated turgor change as the first physiological response upon mechanical stimulation hints towards an ion channel-based mechanism to be operational. Importantly, in the late 1980s, existence of stretch-activated ion channels and their activities in osmoregulation and signaling was reported in plants (Basu and Haswell 2017). Thereafter, efforts were concentrated towards identifying and characterizing more such channels in plants and to reveal their function in mechanoperception. The past 30 years have seen major advancement in this area with a number of mechanosensitive ion channels been discovered and characterized in plants. One of the scientists actively working on this area is Elizabeth S. Haswell from Washington University, Saint Louis, USA. 'Mechano-sensitive channels of small conductance (MscS)' and 'MscS-like (MSL)' are the ion channels that sense and alleviate mechanical stress and osmotic imbalances in bacteria. Later, MSLs were found to be widely distributed and also present in plants, with 10 MSL proteins in *Arabidopsis* (Hamilton et al. 2015a). Many of these MSLs were found to have similar channel characteristics as bacterial MscS. In fact, *Arabidopsis* MSL3 even complemented the MscS defect in mutant bacteria (Haswell and Meyerowitz 2006). From the foregoing, the involvement of MSLs in maintaining optimum turgor and/or relieving osmotic stress is almost certain. Corroboratively, MSL2 and MSL3, two plastid localized MSLs, are already noted to have direct role in osmoregulation in plastids; *msl2 msl3* mutants exhibit altered plastid shape, size and fission (Haswell and Meyerowitz 2006). Adding to this is the recent work on plasma membrane-localized and pollen-specific MSL8, whose optimal activity is decisive in maintaining turgor balance requisite for proper pollen germination, tube elongation and fertility. While MSL8 mutation leads to pollen tube bursting, its overexpression inhibits pollen germination (Hamilton et al. 2015b). This suggests its prime role as an osmotic mechanosensor and puts it forth as the first identified plant mechanoreceptor. MSLs thus certainly play a role in sensing and regulating mechanical perturbation sensed in terms of turgor imbalances, which may originate intrinsically or be caused due to external factors, like touch. However, whether these are the sole and prime mechanoreceptors that besides maintaining turgor also trigger events typical of a cellular signaling, evoking other molecular responses, like in the case of thigmomorphogenesis, is still under debate. Also, as MSLs are non-selective channels with anionic preference, their activity in conjunction with some other mechanosensitive ion

channels, with plausible preference to a second messenger, say, for example, Ca^{2+}, is more likely. It is worth highlighting here that Ca^{2+} has long been implicated in plant mechanosensing (Chehab et al. 2009). Strengthening the likelihood of existence of a mechanosensitive channel facilitating this Ca^{2+} increase, the plant Mid1-Complementing Activity (MCA) protein was identified. The name MCA was derived based on its ability to complement the yeast Mid1 channel mutant. Mechanosensitive nature of MCA was identified through heterologous expression of *Arabidopsis* MCA1 in *Xenopus laevis* oocyte plasma membrane, which led to the overall enhancement of mechanosensitive channel activity upon stretch (Furuichi et al. 2012). Further, an association of *MCA* expression with enhanced Ca^{2+} influx, noted upon mechanostimulation in several plant species, confirmed it to be a Ca^{2+}-specific channel (Monshausen and Haswell 2013). Although the available information strongly supports MCAs to be the prospective and more general mechanoreceptor, sensing mechanical stimuli and eliciting a signal transduction pathway through Ca^{2+}, affirmative evidence is still needed. Nevertheless, the inability of *Arabidopsis mca1*-null mutant roots to penetrate and grow through hard agar, as the wild-type root does, implies that this stretch-activated channel indeed leads to mechanosensing at least in *Arabidopsis* roots (Monshausen and Haswell 2013). The above discoveries regarding the mechanoreceptors are based on the touch-induced or mechanically induced changes/stretch experienced by the membrane and inbound mechanosensitive channels. The other possible indirect mechanism is the identification of mechanically induced cell wall damage via different receptor like kinases (RLKs). Mutation of one such RLK in *Arabidopsis* root has already been found to have mechanosensing defects, which involves inability to penetrate hard agar media and altered touch-induced Ca^{2+} influx (Monshausen and Haswell 2013).

As hinted above, rapid flux in cellular Ca^{2+} is a trademark of all mechanically perturbed plant cells. Whether it is a point contact achieved through touching a single cell with a glass micropipette or a more general touch affecting an entire tissue, Ca^{2+} influx has been noted as a prime response irrespective of the mode of mechanical perturbation. Ecologically, point contact has been proposed to mimic fungal penetration or herbivore manifestation, whereas a general touch simulates blowing wind. It is interesting to note the occurrence of Ca^{2+} fluxes as a first response both in specialized plants with fast thigmonastic/tropic responses as well as in nonspecialized plants exhibiting thigmomorphogenesis (Monshausen and Haswell 2013). This implies that Ca^{+2} fluxes are functional not only in generating action potentials (propagating electrical cues) for quick responses, but also in facilitating downstream signal transduction to evoke a whole suite of adjustments typical to thigmomorphogenetic response. Indeed, Ca^{2+} is a ubiquitous secondary messenger and while its involvement in mechanosensing is fascinating, it is not surprising. Interesting is the involvement of Ca^{2+} also in animal mechanosensing, where again Ca^{2+} flux is proposed to be facilitated by stretch-activated channels. This highlights that although evolutionarily animals and plants are much diverse, they share steps in their mechanosensing pathway, which are inherited from the primitive unicellular life forms. Furthermore, it is also clear that while thigmonastic/tropic and thigmomorphogenetic responses differ in their pace, they initiate through common mechanisms, where on one hand the action potential,

once generated, swiftly triggers and terminates in a response and, on the other hand, it goes beyond involving other players, bringing a long-lasting morphological response, as seen in thigmomorphogenesis. Interestingly, varying Ca^{2+} signatures are produced depending on the mechanical stimulus and perturbed tissue (Monshausen and Haswell 2013). This corroborates the distinct response plants exhibit to different mechanical stimuli. Further, like it happens in case of few other stresses/cues, Ca^{2+} signaling appears to be closely associated with regulation of extra- and intracellular pH in mechanoperception as well (Monshausen and Haswell 2013). Mechanical stimulation triggers apoplastic alkalinization in roots and this response was noted to be dependent on cytoplasmic Ca^{2+} increase (Monshausen et al. 2009). Although the precise mechanism behind the aforementioned observation is still obscure, pharmacological studies suggest connection with H^+ and/or OH^- transport processes across cell membranes. Corroboratively, mechanical stimulation leads to a transient inhibition of PM-localized H^+-ATPase in *B. dioica* internodes (Monshausen and Haswell 2013). Interestingly, in a recent study on *Arabidopsis*, trichomes have been suggested as the prime mechanosensing site, as pressing and brushing them lead to Ca^{2+} fluxes and shifts in the apoplastic pH, both in the trichome and adjoining cells (Zhou et al. 2017).

Besides Ca^{2+}, the other molecular hallmark of mechanically stimulated plant cell is generation of reactive oxygen species (ROS). While increased accumulation of ROS has been linked with cellular death under acute stress, at optimal levels, it also plays an important role as a signaling molecule in plant morphogenesis and responses to several stimuli. Importantly, like mechanically induced pH changes rely on Ca^{2+} transients, ROS production too is dependent on Ca^{2+} fluxes under mechanical stimulation (Monshausen et al. 2009). Additionally, as ROS has been evidenced to regulate Ca^{2+} channel gating, it is proposed to further facilitate Ca^{2+} fluxes from internal stores/subcellular compartments. Thus Ca^{2+} and ROS are the two cellular signals that are interdependently generated and functionally linked as transducers of mechanical stimulus (Braam 2005).

6.3.2 Touch-Related Transcriptome: Evidences Filling the Gaps

Both earlier and recent discoveries regarding touch-related transcriptomes provide ample evidences supporting the involvement of the above discussed molecular players as transducers of mechanical stimulus. Janet Braam, from Rice University, Texas, USA, is one of the pioneering scientists in this area of research. The touch-inducible genes (also called the *TCH* genes) were originally identified serendipitously. Their m-RNAs were first found to be induced dramatically by spraying plants with gibberellins. Further analysis revealed that they were induced also by spraying other hormones, like abscisic acid, auxin and cytokinin, and surprisingly just by spraying water. Eventually, the actual cause of their induction was found to be the mechanical agitation caused by spray action, as the similar set of genes were induced also by gently touching and bending the plant leaves back and forth (Braam and Davis 1990). Initially, only a few *TCH* genes were identified; however, with the advent of modern technologies over the past

years, around 2.5% of the *Arabidopsis* genome was noted to be touch-inducible with at least twofold expression (Chehab et al. 2009). Interestingly, most of the TCH genes identified so far are either Ca^{2+}-related genes or the ones encoding enzymes involved in cell wall modification. For instance, among the first 4 *TCH* genes identified by Braam, *TCH1* encodes for calmodulins, CAM2, *TCH2* and *TCH3* encode CAM-like (CML) proteins, CML24 and CML12, respectively, and *TCH4* encodes a cell wall modifying enzyme, xyloglucan endotransglucosylase/hydrolase (XTH22) (Chehab et al. 2009). Genome-wide analysis later revealed that besides *CAM2* (*TCH1*), which is the only *CAM* gene induced by touch, around 19 *CMLs* and 12 *XTHs* show up-regulation more than twofold in touched plants (Chehab et al. 2009). The expression of *CAM2* and *CMLs* substantiates the function of Ca^{2+} as the unequivocal secondary messenger and evidenced their function downstream in plant mechanosensing. Likewise, while expression of *XTHs* suggests on the one hand the alteration of cell wall being operational upon touch stimulation, on the other hand it substantiates the indirect sensing of mechanical stimulus by RLKs. Surprisingly, perhaps ecologically more relevant, the third most represented class of touch-induced genes are the ones involved in disease resistance. As highlighted before in the text, touch stimulus at the cellular level may mimic fungal penetration and/or herbivore attack. This is possibly one of the most relevant explanations to this observation. However, further research is underway to find the potential connection between mechanical perturbation and disease resistance responses. In addition to this, the other touch-induced genes are either kinases or transcription factors. Again, kinases are implicated in signal transduction pathways and their touch-induced expression is much expected. Similarly, transcription factors are the eventual target of the touch trigger signal transduction, which then impact additional gene transcriptional activities. Intriguingly, besides mechanical induction, expression of TCH genes is also induced by other cues, like darkness, sub−/supra-optimal temperatures and growth hormones (Braam 2005). This suggests that these environmental cues are also capable of imposing mechanical perturbation (perhaps in terms of ionic imbalances/turgor changes and/or cell wall modification) at the cellular level. Similarly, TCH genes are regulated developmentally as well, which is consistent with changes in the mechanical strains plants experience during development and/or morphogenesis. Overall, the expression of TCH genes under different environmental cues and during developmental course goes hand in hand with the general scheme of transient turgor changes being sufficient for their regulation. This further highlights that turgor is the central player in modulating plant responses to mechanical stimulation.

Although touch-induced transcriptome has been studied in detail, least is known regarding the touch-mediated changes in plant proteome. In a very recent study, employing high-throughput SILIA (stable isotope labeling in *Arabidopsis*)-based quantitative phosphoproteomics analysis, 24 touch-responsive phosphopeptides were identified (Wang et al. 2018). Many of these were noted to be cytoskeleton proteins, membrane proteins, ion transporters, kinases and phosphatases.

6.4 Mechanoperception and Plant Growth Regulators: Emerging Roles of Jasmonates

Mechanostimulation results in a suite of morphological responses that are modulated by plant growth regulators. Phytohormones, like ethylene, abscisic acid (ABA), auxin, brassinosteroids (BR), nitric oxide (NO) and jasmonates (JA), are implicated in one or the other responses against mechanostimulation (Chehab et al. 2009). Retardation of growth upon mechanical stimulation is also one of the functional attributes of ABA accumulation. Moreover, ABA accumulation also couples thigmomorphogenetic responses in many species upon mechanical perturbations. Although it indicates involvement of ABA in mechanoperception, any definitive evidence entailing strong genetic studies is yet lacking. Similarly, BR induced up-regulation of one of the TCH genes, TCH4, links BR to thigmomorphogenesis. However, given a lack of direct evidence, BR function in plant thigmomorphogenesis is still debatable. Likewise, NO has also been proposed to play a role in thigmomorphogenetic responses, as it is highly produced in mechanically stressed *Arabidopsis* plants. Interestingly, like in animals, where Ca^{2+}/CaM modulates NO production, genetic and/or pharmacological alteration in CaM/CML proteins lead to alterations in NO biosynthesis in plants as well. Thus, mechanically induced NO production comes downstream to Ca^{2+}signaling (Chehab et al. 2009), which is one of the initial events triggered upon mechanical perturbation. Auxin has also been implicated in mechanoperception in plants. Precisely, mechanically induced morphological changes involves auxin turnover at the affected tissue. Mechanical induction leads to disappearance of auxin in the lower internodes of *B. dioica*, where it is otherwise normally present. Furthermore, as peroxidase-mediated oxidative decarboxylation is one of the major mechanisms of auxin turnover, mechanically induced peroxidase activity has been proposed to play a role in it (Chehab et al. 2009). Ethylene is the first phytohormone to be identified as a regulator of thigmomorphogenesis and has been studied for the longest. Treating plant exogenously with ethylene results in morphological/physiological changes typical of thigmomorphogenesis. Further, mechanical perturbation results in production of ethylene in plants. Corroboratively, transcripts of the key ethylene biosynthetic enzyme *1-aminocyclopropane-1-carboxylate synthase* (ACS) up-regulate rapidly upon mechanical stimulation. Initially, ethylene was thought to be the prime modulator of thigmomorphogenesis. However, later studies in several species revealed that ethylene production as such peaks around hours post-stimulation. Further, genetic studies entailing ethylene mutants did not exhibit any defect in mechanoresponses. Ethylene thus might modulate aspects of thigmomorphogenetic responses; it is unlikely to be the primary regulator of mechanoresponse (Chehab et al. 2009).

Recently, JA has emerged as a prime phytohormone functioning as a transducer of mechanical signal, coupling the mechanostimulation to thigmomorphogenetic responses. One of the JAs, 12-oxo-10,15-phytodienoic acid (12-OPDA), has already been implicated in touch-induced tendril coiling response of *B. dioica* (Braam 2005). More recently, *Arabidopsis* mutant accumulating higher levels of JA and 12-OPDA was found to exhibit thigmomorphogenetic phenotype (Monshausen and

Haswell 2013). Not only this, exogenous application of JA also triggers physiological responses typical of thigmomorphogenesis. Roots are believed to be touch-sensitive; impeding root growth mechanically also leads to accumulation of JA along with temporary inhibition of root elongation. The prime role of JA in mechanical signal transduction is also consistent with its several-fold level increase within 60 s of mechanical stress. In fact, *D. muscipula* leaves exhibiting thigmonastic response also accumulate JA precursor significantly within minutes of insect capture (Monshausen and Haswell 2013). A single touch treatment to *Arabidopsis* is sufficient to induce JA synthesis within minutes. A link between mechanically induced Ca^{2+} and JA production has been proposed. However, it still needs further research to be documented as evidence. In the light of the foregoing, it is clear that although research in this field has recently gained significant acceleration, there is still a long way to go and many more discoveries are still awaited.

6.5 Plant Acoustics: Evolution of the Concept

Mother Nature unbiasedly provided equal opportunities to every creature on this planet to compete, grow, reproduce and evolve in the very process. Therefore, like animals, plants developed sensitivity towards ecologically significant sound frequencies to better adapt with their environment. The aforementioned phenomenon of 'buzz pollination' is exhibited by over 20,000 plant species and is one of the marvellous examples of plants' interaction with sound. However, since it is visually not as apparent as rapid movements exhibited by *M. pudica* and carnivorous species upon touch, plants' sensitivity for sound failed to gather due attention for a long time. Although studies addressing effect of sound on plants commenced seven decades ago, in the 1950s, most of those were non-scientific works, addressing effect of musical sound on plants. Dr. T. C. Singh from India was the first whose work on the effect of music on plants (conducted during the 1950s) was documented in the famous book *The Secret Life of Plants* (Tompkins and Birds 1973). Another book published by Dorothy Retallack in the same year with the title *The Sound of Music and Plants* was dedicated fully to such debatable studies (Retallack 1973). Nevertheless, despite being controversial, these studies succeeded in attracting increasingly widespread scientific focus. This marked the beginning of scientific research in the field of plant acoustics. Subsequent efforts then addressed the effect of different natural sounds, like bird's chirping, bee's buzzing and cricket's stridulating on plants' growth and development, and surprisingly interesting results were obtained. In some cases, such sound enhanced overall plant growth, while in others the seed germination rate was accelerated, like in okra and zucchini. Playback of natural sounds recorded in nature as such exposes plants with a mixture of different sound frequencies (in terms of Hz). Therefore, to add further precision to such studies, successive researchers started using varying single frequencies. While exposure of plants to different single sound frequencies led to an increase in the oxygen uptake/polyamine content in one species, in the other, the overall growth was enhanced. Most interestingly, the morphological/physiological effects were frequency specific. Here, it is relevant to quickly refer to what the famous

plant biologist Daniel Chamowitz stated: 'music is not ecologically relevant for plants, but there are sounds that could be advantageous for them to hear' (Mishra et al. 2016). Absolutely, treatment of plants with music and chosen single sound frequencies is not a true representation of what is happening in nature. The above studies were relevant, but only to advocate that sound is perceived by plants and it happens to cause molecular/physiological and morphological adjustments in plants. The important point that comes to light, however, was the plants' preference for a particular frequency to exhibit a response. This hinted that plant-sound interaction possibly bears ecological significance, as in nature they do experience different sounds, but are possibly able to choose one as more relevant over the other. Thus, besides establishing that plants do perceive sound, this encouraged researchers to explore more in this fascinating and promising area of plant biology. In spite of this, however, advancement in the field of plant acoustics has yet suffered a delay, as the focus was diverted towards implication of sound in biotechnology and agriculture, owing to the positive effects of sound on various plants' growth parameters. Nonetheless, researchers with the ideology that plants do perceive ecological sounds in nature continued their exploration with full enthusiasm and came up with some important discoveries. The first and foremost scientific report came in 2012 from a young and most dedicated researcher who pioneered the establishment of this field – Monica Gagliano from The University of Queensland, Australia. She noted that plants are able to communicate among themselves even after blocking all the known sources of communication and surprisingly proposed the modality to be acoustic signals (Gagliano et al. 2012b). Her claim was not baseless; right after the above report, her next paper in the same year highlighted that plant roots respond to sound by producing sound themselves, besides showing positive phonotropic growth (Gagliano et al. 2012a). These studies were sufficient to raise awareness among both critics and believers of this ideology and led to the commencement of heated discussions for almost the successive 2 years. Many decent commentaries, reviews and opinion articles on different aspects of plant-sound interaction were out (Gagliano et al. 2012a; Gagliano 2013a, b; Bailey et al. 2013), which readers are strongly recommended to refer. Importantly, these discussions successfully diverted the focus on the earlier question of 'whether' plant perceive sound towards the more relevant questions of 'how' and 'why' they are doing it. Additionally, two broad areas of research in plant acoustics were forwarded – first, tackling plants' communication among alike through sound and, second, addressing the mechanism of perception and transduction of ecologically relevant sound signals in plants. Right after this remarkable advancement, a fascinating discovery was made in 2014; *Arabidopsis* plant was found to mount defence response just upon exposure of the leaf-chewing sound of an herbivore (Appel and Cocroft 2014). By forwarding one of the answers to 'why' perception of sound is important in plant, this study proved to be the turning point in the field of plant acoustics. Subsequent to this discovery, efforts were concentrated on elucidating 'how' sound is sensed and transduced in plants, and the past 3 years have seen significant advancement in this direction. The upcoming account elaborates to the readers some enchanting discoveries made in this emerging field of plant biology and exposes a new facet in plants' sensing ability.

6.6 Sound in Biotechnology and Agriculture: Plant Responses with Ecological Messages

As has been highlighted previously, positive effect of sound treatment on plants has been exploited significantly in agriculture and biotechnology. This impeded the progress of the research towards addressing the ecological relevance of sound in plants' life. Nonetheless, the physiological/morphological benefits exploited in terms of plants' response to sound treatment carry many ecological messages. Under in vitro conditions or agricultural settings, one of the most common responses of plants/plant tissues to sound treatment is enhanced growth. Stimulation of in vitro growth and development by sound treatment has been exploited in case of many species, like *Daucus carota*, *Aloe arborescens*, *Gerbera jamesonii*, *Oryza sativa*, *Corylus avellana*, etc. (Hassanien et al. 2014). Similarly, under agricultural setup, sound treatment has been implicated in acceleration of seed germination, increased growth of shoot and root system and enhanced fresh weight in case of many crop plants. So much so that Qingdao Physical Agricultural Engineering Research Center in China customized a plant acoustic frequency technology (PAFT), an equipment that generates eight different sound frequencies well optimized to accelerate agricultural outputs (Hassanien et al. 2014). Hitherto, PAFT has successfully been used to enhance growth and yield in many crop plants, like cotton, strawberry, rice, etc. Interestingly, the recent transcriptomic studies discussed later in this chapter support many of these growth responses in terms of the genes expressed upon sound treatment. Importantly, plants exposed with sound display, in large, enhancement in growth, which is opposite to stunted growth response that plants exhibit when perturbed mechanically by touch. It thus highlights that although the physical nature of the two stimuli is more or less similar, plants recognize and respond to them in a contrasting manner. Therefore, plants perceive sound and touch as distinct ecological stimuli and come up with responses tailored accordingly.

Apart from growth enhancement, the other major benefit obtained by sound treatment is the increased plant immunity against plant diseases and insect pests. Treatment of rice plant with sound reduced the severity and spread of sheath blight by 50% (Hassanien et al. 2014). Recently, *Arabidopsis* plant pre-exposed with sound was found to be more tolerant to *Botrytis cinerea* infection. Strikingly, this enhancement in immunity was noted to be independent of phytohormone JA (Choi et al. 2017), which is one of the key hormones modulating mechanoresponses. This again brings forth the differences in the mechanoperception of sound and touch stimuli. Apart from this, sound-induced immunity indicates that certain sound frequencies can potentially mimic either the disturbances caused by a plant pathogen mechanically at the cellular level or the acoustic frequencies produced during the herbivore infestation. While the above-stated example corroborates the former, the latter goes hand in hand with the key discovery of defence elicitation in *Arabidopsis* as a response to treatment with sound produced by caterpillar/herbivore chewing (Appel and Cocroft 2014).

6.7 Ecological Relevance of Sound in Plants' Life: A Broader View and Key Discoveries

Although the exploitation of sound treatment for agricultural/biotechnological benefits has been the prime focus in the past two decades, there also exist few recent studies where ecological relevance of sound in plants' life has been addressed. In fact, nature has ample examples, where plants' communication with the environment uses sound as a modality. However, due to the lack of understanding, such phenomenon is still awaiting due scientific recognition. Plants have been actively utilizing the mobility of animals for either maintenance of their race by ensuring successful pollination or fulfilling their nutritional requirement. This usually is achieved through a mutualistic relationship among the species. Interestingly, many of these interactions rely on the acoustic communication between the two partners. The widely spread phenomenon of buzz pollination is one such interaction, where the dehiscence of the anthers rely totally on the buzz sound produced by bees. It is such a precise and sophisticated phenomenon that anthers are dehisced only upon exposure to a particular buzz frequency produced by a specific bee, but not by the buzz produced by other bees and insects, who act as pollen thieves (Mishra et al. 2016). In ecological terms, this highlights the extreme competence of plants to distinguish the relevant sound frequencies over the non-relevant ones. There are few other plant-animal mutualisms where sound is the underlying means of communication. The Cuban vine, *Marcgravia evenia*, has evolved a dish-shaped foliage leaf located right above the inflorescence that functions as an echo beacon and facilitates its detection and pollination by bats (Simon et al. 2011) (Fig. 6.2). Similarly, the pitcher of *Nepenthes hemsleyana* is modified in a way that it reflects ultrasound produced by bats (Fig. 6.2). This again facilitates detection of the plants by the bats; later roost in the pitcher and in turn plant obtains nitrogen from the bat droppings (Schoner et al. 2016). Ecologically, plants evolved these modifications to ensure their pollination and satiate their nutritional requirements. However, the critical question of what plants have taken advantage of in order to attract bat has hardly been studied in physiological and molecular terms. Considering the close and tight alliance between these species of plants and bats, it has recently been proposed that plants sense the ultrasound produced by bats, which enables them to evolve and retain these adaptive structures (Mishra et al. 2016). Certainly, future studies are expected to fill in the knowledge gap we have with regard to the molecular physiological basis behind such responses. Nevertheless, sound has influenced plants' life way more than we imagined. The subsequent account discusses two important discoveries in plant acoustics that very well explain why sensing sound is so important in plants' life.

6.7.1 Sound: One of the Arsenals of Plants' Defence Against Herbivory

The discovery that sound acts as a signal in plant defence mechanism is one of the major breakthroughs in the field of plant acoustics. It has also provided a more straightforward answer to why perceiving sound is ecologically relevant for plants.

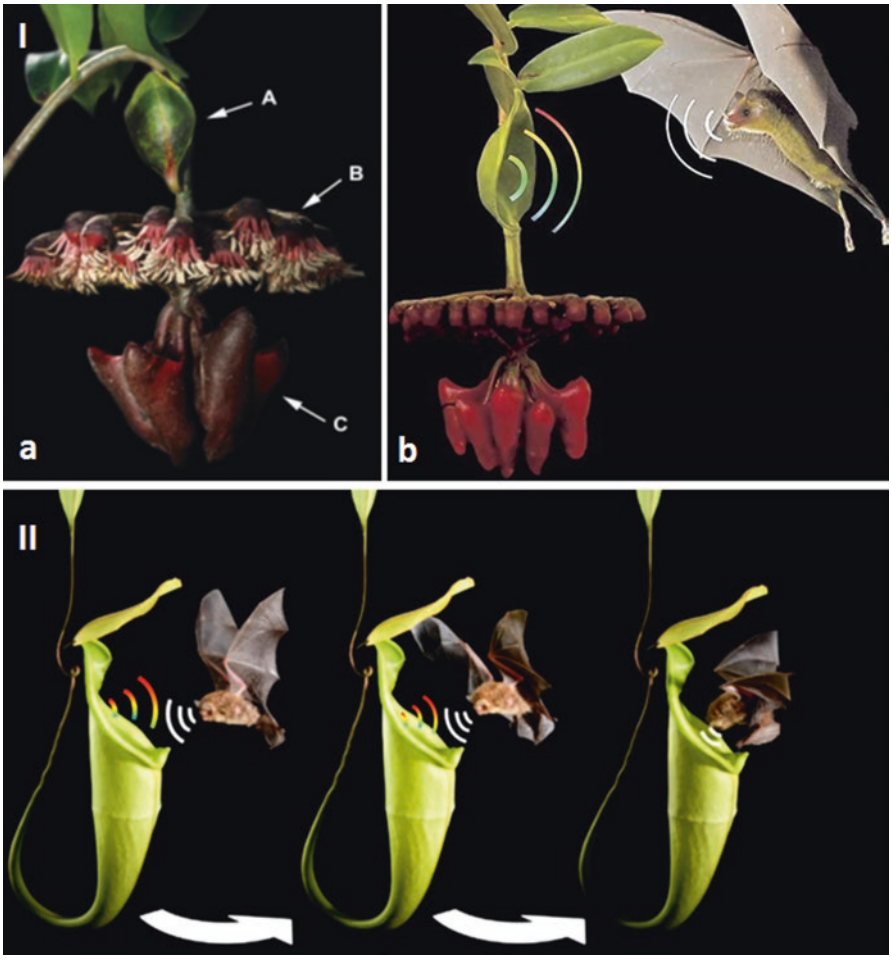

Fig. 6.2 Example of acoustic communication in plant-animal mutualism. (*I*, *a*) A typical inflorescence of *Marcgravia evenia*; (*A*) dish-shaped leaf that serves as an echo beacon, (*B*) ring of flowers with exposed anthers, (*C*) cup-shaped nectaries. (*I*, *b*) A representation of the bat acoustically attracted toward the inflorescence. (These pictures are acquired from Ralph Simon et al. 2011.) (*II*) Detection of *Nepenthes hemsleyana* by bat through echolocation (acoustic communication). (Picture is acquired from Michael G. Schöner et al. 2015)

Appel and Cocroft (2014) came up with the study where they showed that plant identifies sound typical of an herbivore and responds by triggering defence. This well-timed discovery marked the end of the heated discussion on the relevance of sound in plants' life. *Arabidopsis* plant pre-exposed with the sound produced during the act of leaf chewing by the caterpillar *Pieris rapae* was found to be more tolerant to subsequent attack by this herbivore. Interestingly, plants that were exposed with the chewing sound accumulated higher quantities of defence molecules such as anthocyanins and glucosinolate in comparison with unexposed plants. Most importantly, when the

plants were exposed to the recorded sound of a grasshopper or wind, no such defence response was triggered. Thus, plants are not just able to sense, but they can also identify and distinguish the sound typical of an herbivore among other sound frequencies. This convincingly demonstrated that sensing sound is very much relevant in plant life and they do it sophistically in a much precise manner. As a matter of coincidence, the discovery of another player in plant defence system – the volatile organic compounds (VOC) – was also initially met with friction. However, it is now a well-established fact that plants communicate through VOC production. Discovery of sound as a signal in plant defence machinery is thus another entry in the list of signals plant utilizes to synchronize with their surroundings. This further strengthens the view that plants not just utilize cues, but they are smart enough to use signals for their better communication with the environment. There are several advantages of sound signals over VOC, like (1) herbivore-induced sound is costless for plants, whereas VOC generation requires high metabolic cost, (2) sound travels faster than VOC and (3) sound signals are least affected by wind direction unlike the case of VOC. In the light of these added values, sound has recently been proposed to act as first line of defence against herbivory (Mishra et al. 2016). This is indeed backed up by VOC-mediated signaling, owing to the fact that VOC signals are more durable. Nonetheless, future studies will provide definite evidences to substantiate this proposal.

6.7.2 Sound of Flowing Water: Roots Can Trace It Well

As expected, the field of plant acoustics is unveiling many surprises. It is not because plants adopted a particular strategy to literally surprise us, but because we are not yet ready to assimilate the new discoveries being made in this field. Monica Gagliano has recently come up with another enchanting discovery regarding plants' competence to identify and utilize ecologically relevant acoustic signals. It has now come to light that plant roots can sense sound of the flowing water and respond by directing their growth towards the water source (Gagliano et al. 2017). Hitherto, the only known mechanism through which the root detects and reaches water source is via sensing water gradient. However, what when water gradient itself is far and not approachable? The acoustic mechanism of locating water source fits well in an ecological niche, where plants growing far from water flowing in a stream are unable to detect water gradient, like the ones growing relatively closer to the water source. An alternative mechanism is thus absolutely needed at least to approach the water gradient itself. Sound, as it travels long and faster in compact medium, like soil, forms the best signal for the roots to broadly detect the sound source and direct growth towards it. Once a suitable water gradient is reached, it then facilitates further growth in a more precise way to locate the exact water source. Different sets of sophisticated experiments form the basis of the above discovery. Readers are strongly recommended to refer the original paper (Gagliano et al. 2017) to have a comprehensive understanding of the work. To sum up, the following important observations were made: (1) plant prefers water gradient over sound of flowing water (either water actually flowing in a pipe or recorded sound of flowing water) to show the directional

root growth response; (2) in the absence of water gradient, plant directed root growth towards the water sound, equally good as it does towards water gradient; and (3) playback of irrelevant sound (noise) disturbs the root growth response to flowing water sound. Based on the latter observation, the concern of increasing noise pollution and its impact on the critical ecological processes have also been raised.

The field of plant acoustics now has many examples to prove ecological relevance of sound perception by plants, with many more yet to be discovered. The need of the hour now is to decipher 'how' plant perceives and respond sound in molecular terms. Although, the past two decades have produced some scattered pieces of evidence regarding sound-induced molecular/physiological responses, significant discoveries have been made only recently. Upcoming text elaborates the knowledge gathered so far on the molecular/physiological front of plant acoustics.

6.8 Sound Affects Plants' Cellular Activities: Sound Perception and Signal Transduction

To start with, a quick recapitulation of the physical property of sound is important; sound travels as pressure waves, which mechanically impact an object upon interface. Hence, many molecular players and cellular events functional in mechanosensory transduction and the process of hearing are common in animals. It is therefore much likely and expected that perception and signal transduction of sound in plants share similarities with that of the mechanical stimuli. Corroboratively, Liu et al. (2017) analyzed and found that *Arabidopsis* trichomes have vibrational modes in the frequency range of the sounds of caterpillar chewing, leaving the possibility of trichomes to be functioning also as acoustic antennae open. Further, the molecular evidences so far also reveal many similarities. The scattered pieces of preliminary evidences regarding sound-associated cellular episodes are gathered together in a plausible signaling model and discussed at length in one of our recent articles (Mishra et al. 2016). Importantly, results from the subsequent studies on sound-mediated cellular events are going hand in hand with the proposed signaling model. Readers are strongly recommended to refer the article for a comprehensive account.

6.8.1 Early Events Associated with Sound Signaling

Sound exposure triggers changes associated both with cell wall and plasma membranes, just like what happens when cell experiences mechanical stimuli; (a) increased tension in cell membrane, (b) modification in the secondary structure of cell membrane-associated protein and (c) induction of *TCH4* (*XTH*, the cell wall-modifying enzyme) are few of the many adjustments sound triggers (Mishra et al. 2016). Further, as suggested in the signaling model we proposed, previous indications complemented with a very recent study by Rodrigo-Moreno et al. (2017) suggest the influx of the secondary messenger Ca^{2+} as one of the initial events upon sound perception in plants. As discussed previously, Ca^{2+} functions in transduction of mechanical stimuli as well.

Further, as stretch-activated channels have been shown to facilitate touch-mediated Ca^{2+} influx, Rodrigo-Moreno et al. (2017) made use of mechanosensitive channel blocker in a pharmacological assay and showed that sound-mediated Ca^{2+} influx is also facilitated by stretch-activated Ca^{2+} channel. ROS has also been implicated as one of the initial players in sound signaling, as it is induced upon sound exposure (Rodrigo-Moreno et al. 2017). Importantly, ROS is also induced upon mechanical perturbation. Authors have also shown that ROS comes downstream to Ca^{2+} signaling as blocking the Ca^{2+} channels inhibits the sound-induced ROS induction as well. Further, sound-induced Ca^{2+} regulates the ROS formation through activation of NADPH oxidase, as inhibiting its activity blocks ROS induction (Rodrigo-Moreno et al. 2017). Interestingly, similar observations have been made regarding modulation of touch-induced ROS. The signaling model we proposed also implicates K^+ channel and ion in sound signaling. Interestingly, Rodrigo-Moreno et al. (2017) noted that K^+ is indeed involved in sound-mediated responses and its efflux facilitated by K^+ channels is operational in sound-induced responses. It is important to be noted here that changes in Ca^{2+} and K^+ ion fluxes and increased ROS production are also one of the initial signaling events of sound perception in animals.

The preliminary studies published two decades ago suggested that sound induces enhancement in protein kinase activity, which progressively leads to activation of H^+ATPase. Importantly, blocking the Ca^{2+} inhibited H^+ATPase activity directly implicating calcium-dependent protein kinases in this response (Mishra et al. 2016). Future studies involving present day technologies are expected to substantiate many previous claims made with regard to sound-induced cellular changes. Nevertheless, activation of kinases is very likely given the fact that Ca^{2+}-mediated signaling often implicates calcium-dependent kinases. Further, these are kinases, which then affect phosphorylation events modulating the activation of various other signaling protein and transcriptional regulators. Eventually, the signaling culminates in differential regulation of responsive genes.

6.8.2 Sound-Mediated Regulation of Gene Expression, Enzyme Activity and Phytohormones

Previous studies have suggested differential regulation of few genes in plants upon exposure to sound, for example, genes encoding small subunit of rubisco (*RBCS*), aldolase (*ALD*), catalase (*CAT*), phenylalanine ammonialyase (*PAL*), etc. (Mishra et al. 2016). Moreover, the promoter of *ALD* gene drove the sound-induced expression of the reporter gene β-glucouronidase (*GUS*) in a transgenic context, strongly suggesting its sound responsiveness (Mishra et al. 2016). These studies, however, involved primitive techniques, and thus an extensive study entailing present-day gene profiling technologies was highly desired to shed more light on sound-induced transcriptome. In one of our very recent works, global transcriptomic response of *Arabidopsis* plants treated with five different sound frequencies is revealed (Ghosh et al. 2016). Importantly, based on their attributes, the following categories of genes were up-regulated: (1) signaling-related genes (CML and various kinases), (2) transcription

factors (TFs), (3) genes involved in redox homeostasis, (4) biosynthetic genes and (5) defence-related genes. Most interestingly, many of the touch-inducible genes are also up-regulated on sound exposure, like TCH4, CML and few TFs, again highlighting the commonality in the two stimuli. In addition, up-regulation of defence-related genes is also a hallmark of plants' response to mechanical stimuli. This highlights the robustness of plants' defence mechanism, where recognition of pathogen/pest either physically or through sound produced during infestation is treated similarly at least in eliciting the defence response. Up-regulation of CML gene further strengthens the idea of Ca^{2+} to be functioning as a second messenger in sound signaling. Induced expression of several biosynthetic genes corroborates the response of growth enhancement that plant generally exhibits upon sound stimulation. The above study also targeted the proteomic responses and brings forth differential regulation of several proteins involved in photosynthesis, respiration, ROS scavenging, energy metabolism, cellular transport, etc. Furthermore, phytohormonal analysis depicts the interplay of salicylic acid (SA) as the primary hormone modulating sound-induced adjustments (Ghosh et al. 2016). Overall, it has come to light that, like any other stimuli, perception of sound also leads to modulation of signature cellular episodes, like ROS scavenging, primary metabolism, hormone signaling, etc. The above study has indeed pioneered the molecular progress in plant acoustics, but as the sound frequencies used were randomly chosen, the transcriptomic/proteomic response appears to be more general providing an overall glimpse of plant responses to anonymous sound. Therefore, molecular studies involving frequencies that are ecologically more relevant and/or molecular extension of the bona fide work conducted by Apple and Cocroft are expected to reveal more customized molecular responses of plants.

6.9 Sound as a Mode of Communication Among Plants: An Emerging Concept

Raising the curiosity of the readers through the above account, the chapter closes by bringing forth an interesting and evolving concept in this field – communication among plants using sound as a modality. The foundation of this idea was laid in the pioneering paper of Gagliano (Gagliano et al. 2012b), where she revealed that plants are able to communicate even after occluding all known sources of communication and suggested the possible involvement of sound signals. Precisely, she showed that the presence of a neighboring plant did influence the seed germination of other species even after blocking all known sources of communication, like underground and airborne chemicals and light. It is now already established that plants also perceive sound in an ecological manner. In order to strengthen the sound-based communication theory, the only prerequisite is the evidence that plants can produce sound signals themselves. To this end, studies suggest that trees experiencing drought produce sound through the process of cavitation; drought results in popping up of air bubbles in plants' xylem and when these bubbles burst they produce acoustic emissions (Mishra et al. 2016). Moreover there are views that plants employ the cavitation sound to alarm other plants in vicinity of an impending drought condition (Mishra et al. 2016).

Although direct evidence to this is awaited, in experimental setup exposure to frequencies matching the cavitation sound elicited drought response in rice plant (Jeong et al. 2008, 2014). In fact, researchers have come up with the idea to use sound treatment for priming plant against drought. Recently, pre-treatment with sound was shown to enhance drought tolerance in the model plant *Arabidopsis* (Lopez-Ribera and Vicient 2017). Whereas the foregoing raises a possibility of sound-based communication under drought, another mechanism of sound production different from cavitation should exist for the communication to be more general. Strengthening this proposition, Gagliano has shown that root of maize seedlings grown hydroponically can also produce sound (Gagliano et al. 2012a). Further, she has also proposed a model by which a plant cell itself can produce ecologically meaningful acoustics emission that may facilitate their communication (Gagliano 2013b). Thus, cavitation is just one of the several mechanisms by which plant produces sound.

Certainly, the idea is still in its infancy, but with the current state of the art in plant acoustics, nothing seems to be impossible.

Acknowledgement This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03030357).

References

Appel HM, Cocroft RB (2014) Plants respond to leaf vibrations caused by insect herbivore chewing. Oecologia 175:1257–1266

Bailey NW, Fowler-Finn KD, Rebar D, Rodriguez RL (2013) Green symphonies or wind in the willows? Testing acoustic communication in plants. Behav Ecol 24:797–798

Basu D, Haswell ES (2017) Plant mechanosensitive ion channels: an ocean of possibilities. Curr Opin Plant Biol 40:43–48

Braam J (2005) In touch: plant responses to mechanical stimuli. New Phytol 165:373–389

Braam J, Davis RW (1990) Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell 60:357–364

Chehab EW, Eich E, Braam J (2009) Thigmomorphogenesis: a complex plant response to mechano-stimulation. J Exp Bot 60:43–56

Choi B, Ghosh R, Gururani MA, Shanmugam G, Jeon J, Kim J, Park SC, Jeong MJ, Han KH, Bae DW, Bae H (2017) Positive regulatory role of sound vibration treatment in Arabidopsis thaliana against Botrytis cinerea infection. Sci Rep 7:2527

Darwin CR (1875) Insectivorous plants. John Murray, London

De Luca PA, Vallejo-Marin M (2013) What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. Curr Opin Plant Biol 16:429–435

Furuichi T, Iida H, Sokabe M, Tatsumi H (2012) Expression of Arabidopsis MCA1 enhanced mechanosensitive channel activity in the Xenopus laevis oocyte plasma membrane. Plant Signal Behav 7:1022–1026

Gagliano M (2013a) The flowering of plant bioacoustics: how and why. Behav Ecol 24:800–801

Gagliano M (2013b) Green symphonies: a call for studies on acoustic communication in plants. Behav Ecol 24:789–796

Gagliano M, Mancuso S, Robert D (2012a) Towards understanding plant bioacoustics. Trends Plant Sci 17:323–325

Gagliano M, Renton M, Duvdevani N, Timmins M, Mancuso S (2012b) Out of sight but not out of mind: alternative means of communication in plants. PLoS One 7:e37382
Gagliano M, Grimonprez M, Depczynski M, Renton M (2017) Tuned in: plant roots use sound to locate water. Oecologia 184:151–160
Ghosh R, Mishra RC, Choi B, Kwon YS, Bae DW, Park SC, Jeong MJ, Bae H (2016) Exposure to sound vibrations lead to transcriptomic, proteomic and hormonal changes in Arabidopsis. Sci Rep 6:33370
Hamilton ES, Schlegel AM, Haswell ES (2015a) United in diversity: mechanosensitive ion channels in plants. Annu Rev Plant Biol 66:113–137
Hamilton ES, Jensen GS, Maksaev G, Katims A, Sherp AM, Haswell ES (2015b) Mechanosensitive channel MSL8 regulates osmotic forces during pollen hydration and germination. Science 350:438–441
Hassanien RHE, Hou TZ, Li YF, Li BM (2014) Advances in effects of sound waves on plants. J Integr Agric 13:335–348
Haswell ES, Meyerowitz EM (2006) MscS-like proteins control plastid size and shape in Arabidopsis thaliana. Curr Biol 16:1–11
Jaffe MJ (1973) Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation: with special reference to Bryonia dioica. Planta 114:143–157
Jeong MJ, Shim CK, Lee JO, Kwon HB, Kim YH, Lee SK, Byun MO, Park SC (2008) Plant gene responses to frequency-specific sound signals. Mol Breed 21:217–226
Jeong MJ, Cho JI, Park SH, Kim KH, Lee SK, Kwon TR, Park SC, Siddiqui ZS (2014) Sound frequencies induce drought tolerance in rice plant. Pak J Bot 46:2015–2020
Jung J, Kim SK, Kim JY, Jeong MJ, Ryu CM (2018) Beyond chemical triggers: evidence for sound-evokedphysiological reactions in plants. Front Plant Sci 9:25
Liu S, Jiao J, Lu TJ, Xu F, Pickard BG, Genin GM (2017) Arabidopsis leaf trichomes as acoustic antennae. Biophys J 113:2068–2076
Lopez-Ribera I, Vicient CM (2017) Drought tolerance induced by sound in Arabidopsis plants. Plant Signal Behav 12:e1368938
Mishra RC, Ghosh R, Bae H (2016) Plant acoustics: in the search of a sound mechanism for sound signaling in plants. J Exp Bot 67:4483–4494
Monshausen GB, Haswell ES (2013) A force of nature: molecular mechanisms of mechanoperception in plants. J Exp Bot 64:4663–4680
Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S (2009) Ca2+ regulates reactive oxygen species production and pH during mechanosensing in Arabidopsis roots. Plant Cell 21:2341–2356
Retallack DL (1973) The sound of music and plants. DeVorss, Santa Monica
Rodrigo-Moreno A, Bazihizina N, Azzarello E, Masi E, Tran D, Bouteau F, Baluska F, Mancuso S (2017) Root phonotropism: early signalling events following sound perception in Arabidopsis roots. Plant Sci 264:9–15
Schöner MG, Caroline RS, Ralph ST, Ulmar G, Sébastien JP, Liaw LJ, Gerald K (2015) Bats are acoustically attracted to mutualistic carnivorous plants. Curr Biol 25(14):1911–1916
Schoner MG, Simon R, Schoner CR (2016) Acoustic communication in plant-animal interactions. Curr Opin Plant Biol 32:88–95
Simon R, Holderied MW, Koch CU, von Helversen O (2011) Floral acoustics: conspicuous echoes of a dish-shaped leaf attract bat pollinators. Science 333:631–633
Tompkins P, Birds C (1973) The secret life of plants. Harper & Row, New York
Wang K, Yang Z, Qing D, Ren F, Liu S, Zheng Q, Liu J, Zhang W, Dai C, Wu M, Chehab EW, Braam J, Li N (2018) Quantitative and functional posttranslational modification proteomics reveals that TREPH1 plays a role in plant touch-delayed bolting. Proc Natl Acad Sci U S A 115:E10265–E10274
Zhou LH, Liu SB, Wang PF, Lu TJ, Xu F, Genin GM, Pickard BG (2017) The Arabidopsis trichome is an active mechanosensory switch. Plant Cell Environ 40:611–621

Ratnesh Chandra Mishra earned his doctorate from the Department of Plant Molecular Biology, University of Delhi, South Campus (UDSC), under Prof. Anil Grover. His Ph.D. added to the understanding of heat-shock response mechanism with regard to the role of HSP100 in plants and fetched him the prestigious Young Scientist award (2018) from the Indian National Science Academy (INSA). During his Ph.D. term, he came in contact with the Editor. He obtained Short Stay Fellowship from Utrecht University, Netherlands, and worked in the lab of Prof. Rens Voesenek for 3 months on plants' response to flooding. Later, he worked at the Yeungnam University, South Korea, for his first postdoctoral study on the emerging field of "plant acoustic" under Prof. Hanhong Bae. Presently, he is working as a FWO Postdoctoral Fellow in the lab of Prof. Dominique Van Der Straeten at the University of Ghent, Belgium. His current research entails understanding the role of folates in plant growth and development to design fruitful folate biofortification strategy in crops with least repercussions.

Hanhong Bae obtained his doctoral degree under Professors R. Hall/S. Rodermel from Iowa State University on light control of plant organelle development. He had his postdoctoral training in Molecular Plant-Microbial Interaction in the lab of Dr. R. Sicher and B. Bailey at Plant Science Institute (USDA-ARS), Maryland, USA. He is currently a Professor in the Department of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea. His research interests are on characterization of plant response to sound vibration and enhancing the production of metabolites (e.g., ginsenosides in ginseng plants), involving NGS and metabolic profiling.

Perception of Stress Environment in Plants

7

Charanpreet Kaur, Ashwani Pareek, and Sneh Lata Singla-Pareek

Abstract

Any unfavourable condition or constituent that upsets or blocks a plant's metabolism, growth, or development can be termed as stress. As plants lack the ability to escape from these adverse situations, they have evolved elaborate mechanisms to perceive and respond to them. Stress signaling has, therefore, taken a central role in growth and development of plants as they have to endure such situations more frequently during their life cycle. Perception of stress is a critical component of stress signaling which governs the ultimate fate of plant survival. Plasma membrane serves as the primary site for sensing various environmental stimuli through membrane receptors and transduces them via second messengers to downstream intra- and intercellular signaling networks. Further, phytohormones which are considered as plant growth regulators also play vital roles in stress adaptation. Plants have evolved intricate hormone signaling networks which can crosstalk with other stress mechanisms making them ideal candidates for mediating defence responses. Here, we have presented an overview of stress, its perception and transduction in plants, also highlighting important points of interactions between various stress signaling mechanisms. We propose that stress signaling is a highly complex phenomenon where much is still needed to be deciphered to unlock the secret of robust plant defence responses.

C. Kaur · A. Pareek
Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

S. L. Singla-Pareek (✉)
Plant Stress Biology, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
e-mail: sneh@icgeb.res.in

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_7

Keywords
Abiotic stress · Biotic stress · Calcium · Crosstalk in signaling · Flooding · Pathogen perception · Reactive oxygen species (ROS) · Salt sensing · Temperature sensing · Water sensing

7.1 Concept of Stress

The concept of stress was originally developed by Hans Selye (1936). When working with rats, he showed that if an organism is severely damaged by acute noxious agents such as exposure to cold, surgical injury or intoxications with sub-lethal doses of drugs, a typical syndrome appears whose symptoms are independent of the damaging agent. This syndrome was suggested to be a generalized effort of the organism to adapt itself to new conditions and, hence, was termed as general adaptation syndrome. Selye further quoted that all agents can act as stressors, producing both stress and specific action and that there exist stressor-specific responses and non-specific general responses.

The same concept was later applied in describing unfavorable and environmental restraints in plants. Larcher (1987) described plant stress as a state in which increasing demands enforced upon a plant lead to an initial disruption of functions, followed by stabilization and improved resistance, and if the limit of tolerance is surpassed and adaptive capacity is exhausted, the result may be permanent damage or even death. Lichtenthaler (1988, 1996) extended the plant stress concept by including revival phase of plants after removal of stressors and also differentiated between eu-stress and dis-stress. Eu-stress enhances function and is a positive element for plant development, whereas dis-stress refers to persistent stress that is not resolved through adaptation and may lead to plant damage. The term "stress," however, should not be used for fast rearrangements in metabolic fluxes, photosynthetic or transpiration rates occurring due to changes in the photon flux density or trivial changes in temperature and air humidity as plants are inherently acclimatized to such steadily reoccurring changes of cell metabolism and physiological activities. In any case, stress is a dose-dependent matter (Lichtenthaler 1996). At low concentrations, a stressor can stimulate plant metabolism and growth. But high doses of all stressors are deleterious for the functioning and development of plants and demonstrate a real stress in the form of dis-stress. Thus, stress in the correct sense occurs when it exceeds a certain threshold limit and can no longer be compensated for, by the plant.

Various natural or anthropogenic stress factors exist that, depending on their intensity and duration, can impair cellular machinery of plants culminating in damage and even death. All these stress factors can be classified as abiotic or biotic stresses.

7.1.1 Abiotic Stress

Abiotic or environmental stress includes all the non-living environmental factors that can negatively or even detrimentally affect the growth and productivity of plants. Due to a constant change in climatic conditions and deterioration of environment caused by human activity, abiotic stresses are becoming a major threat to food security (Ahmad et al. 2009).

The major environmental factor that limits the productivity of plants is water stress. This happens when there isn't an adequate moisture in the soil which reduces plant water potential and turgor and, thus, affects normal functions. Water deficit or drought affects plants at several levels. The first response is stomatal closure and limitation of gas exchange along with a decline in the processes related to cell expansion and growth. As the stress prevails, photosynthesis is adversely affected. At cellular level, a reduction in hydration can lead to damage of membranes and proteins and an increase in reactive oxygen species (ROS). Desiccation is a more severe form of water stress where water loss is much more extensive causing large-scale disruptions in metabolism and cell structure and may even lead to cessation of enzyme-catalyzing reactions.

Similar to water stress, salinity is also one of the major factors severely affecting crop growth and productivity. Salinity is related to water deficit due to decrease in water status, but along with water stress, accumulation of detrimental ions also occurs, and thus, plants subjected to salinity stress appear to face two stresses at the same time. The osmotic stress component caused by a decline in the soil water potential and, therefore, restriction of water uptake by roots is a rapid and intense response of the plant to increases in external osmotic pressure and causes a stronger reduction in the growth. The second phase is a slower response and constitutes the ionic component which includes ion toxicity, nutrient imbalance and deficiencies (Munns and Tester 2008). Salinity stress, on a whole, leads to membrane damage, reduced cell expansion and division, alterations in metabolic processes, oxidative stress and genotoxicity. It affects both vegetative and reproductive plant development, with severity of response depending on the harvested organ, stem, leaf, root, shoot, fruit, fibre, or grain. However, salt stress generally reduces shoot growth more than the root growth. Plant salt tolerance is, thus, a highly complex phenomenon that involves modifications in physiological and biochemical processes, resulting in morphological and developmental changes (Singh et al. 2008).

High temperatures pose another serious threat to plant growth and productivity. When plants experience temperatures above their threshold of adaptation, changes occur in respiration and photosynthesis which cause a shortened life cycle and diminished plant productivity (Barnabás et al. 2008). Predisposition of plants to high temperatures is dependent on the developmental stage of plant though some effects certainly occur at all vegetative and reproductive stages. Further, these effects are also species- and genotype-dependent, with abundant inter- and intraspecific variations. Plants exhibit a complex response to extreme high temperatures, comprising both long-term evolutionary adaptations and short-term acclimation mechanisms, such as altering leaf orientation, transpirational cooling and alteration of membrane lipid compositions.

In addition to high temperatures, plants also experience low temperatures. Chilling stress occurs at temperatures above 0 °C but below certain threshold temperature unique for each species. However, freezing stress occurs when temperatures are below 0 °C or when radiative frosts occur with ice formation. Low temperatures pose mainly three types of problems (Ve'zina et al. 1997). First, a perturbation in membrane functions due to a decline in membrane fluidity manifested by electrolyte leakage from tissues. Second, there is a slowdown of chemical and biochemical reactions, and third, changes occur in water status and availability.

Besides these natural environmental factors which affect plant health, anthropogenic factors also pose a grave threat to plant growth and survival. Intensive methods of agriculture like wastewater irrigation, excessive use of chemical fertilizers and pesticides and industrial activities such as mining and smelting of metalliferous ores have led to accumulation of heavy metals in the environment. Plants capable of growing on soils contaminated with high levels of metals have developed three basic strategies of metal tolerance (Redondo-Gómez 2013). The first involves compartmentalization, i.e., sequestering metal ions in tissues or cellular compartments (vacuoles) which are less sensitive to metals and isolated from metabolically active compartments. The second strategy is metal excretion, as crystals through salt glands, and the third strategy is metal chelation, through organic acids, polysaccharides, phytochelatins and metallothioneins.

7.1.2 Biotic Stress

Like abiotic stresses, biotic factors also cause extensive damage to plants. Causative agents include other living organisms such as bacteria, fungi, viruses, nematodes, protists and insects. These biotic factors are termed as pathogens which account for about 15% losses in global food production. These pathogens disturb plant metabolism through secretion of enzymes, toxins, growth regulators, etc. and deprive plant of its nutrition. Some can even grow and multiply in xylem or phloem vessels, thereby blocking water or sugar transport through these tissues, in turn, causing disease.

Importantly these stress factors, both biotic and abiotic, act simultaneously on the plant. For instance, abiotic conditions such as drought, salinity and temperature variations impact the incidence and spread of biotic factors like pathogens, insects and weeds. Global warming and potential climate anomalies have, thus, led to a considerable increase in the number of such abiotic and biotic stress combinations, which is even more destructive for crops. Furthermore, the effect of combined stress factors on plants is not always additive as the outcome is mainly governed by the nature of interactions between the stress factors. Plants alter their responses to combined stress factors, exhibiting several unique as well as common responses. Therefore, to entirely understand the impact of combined abiotic and biotic stresses on plants, it is imperative to understand the nature of such interactions.

7.2 Stress Sensing and Signaling in Plants

Plants thrive in a constantly changing environment which fluctuates throughout the day due to variations in the supply and distribution of light, temperature, nutrients and minerals and even due to encounter with predators. Overall growth and development of plants is coordinately controlled by both internal factors and environmental signals, to which plants can respond either as individual cells or as whole organisms. Plants can sense and respond to these signals through a complex signaling network which often crosstalk with each other. Although there are many locations within the cell where signal integration and processing can take place, it is the plant cell plasma membrane that is considered as a primary site for the location of 'cellular computer' which computes intelligent decisions. A typical signal transduction machinery in plants comprises of three major components, signal perception, primarily through plasma membrane receptors; amplification, through second messengers; and transduction via downstream protein kinases and transcription factors, causing changes in gene expression, thereby invoking appropriate response mechanisms (Fig. 7.1).

7.2.1 Perception and Transduction of Water Stress in Plants

Plant responses to water stress are controlled by intricate regulatory events involving abscisic acid (ABA) signaling, ion transport and transcription factors (TFs). Stress sensing is facilitated by the membrane-bound receptor proteins, such as receptor-like kinases (RLKs) and histidine kinases (HKs), that transduce stress signals to inter- or intracellular signaling network (Fig. 7.2). The RLK family in Arabidopsis includes >600 members, with the leucine-rich repeat (LRR) RLKs (LRR-RLKs) forming the largest group. These LRR-RLKs have been well studied in plants and their role in response to drought, salt and cold signaling has been demonstrated (Ye et al. 2017). An LRR-type receptor-like protein kinase1 (RPK1) is known to be induced by ABA, dehydration, high salt and low temperature (Osakabe et al. 2005). In fact, RPK1 transgenic plants can improve tolerance to drought and oxidative stress. However, loss of RPK1 function leads to ABA insensitivity and reduced expression levels of various water stress-responsive genes. Likewise, GUARD CELL HYDROGEN PEROXIDE-RESISTANT1 (GHR1) has been shown to physically interact with and activate by phosphorylation the S-type anion channel SLOW ANION CHANNEL-ASSOCIATED1 (SLAC1), resulting in stomatal closure in response to drought stress (Hua et al. 2012). However, the ligands of these RLKs have not been resolved yet and require more investigations.

In addition to RLKs, sensory histidine kinases (HKs) are another class of plasma membrane proteins that play a key role in signal perception. In plants, these two-component systems are involved in the regulation of various biological processes, such as perception of plant hormones and responses to environmental cues.

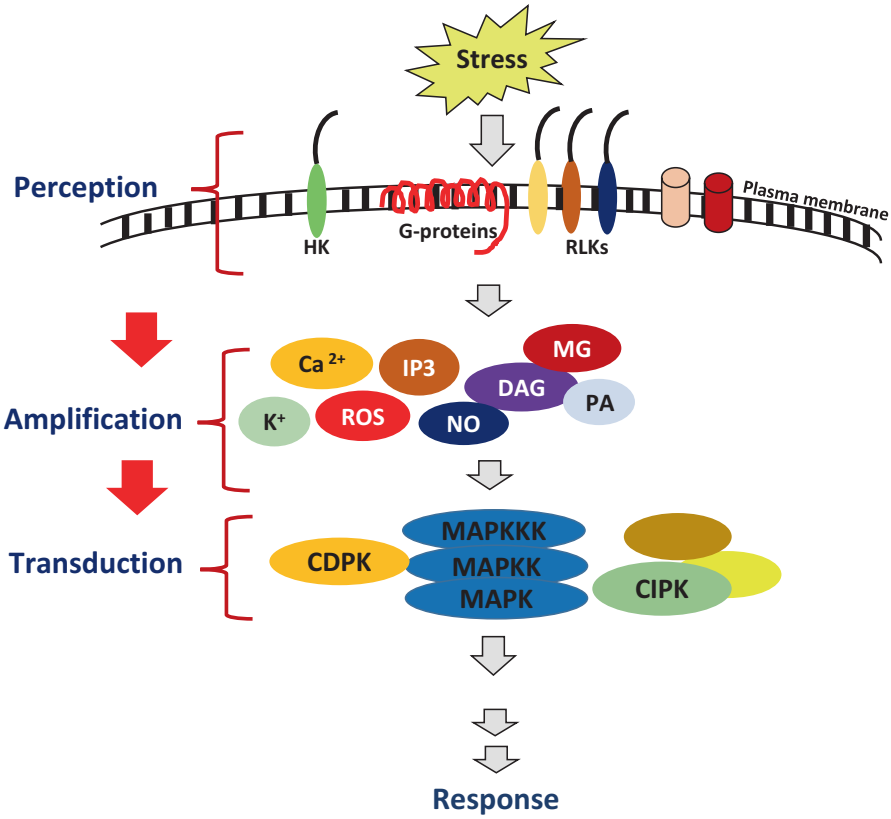

Fig. 7.1 Outline of plant signal transduction machinery in response to stress. Signal is perceived at the plasma membrane by receptors, amplified via second messengers and transduced via kinases to cause changes in gene expression in order to elicit a response. *CDPK* calcium-dependent protein kinase, *CIPK* CBL-interacting protein kinases, *DAG* diacylglycerol, *HK* histidine kinase, *IP3* inositol-1,4,5-triphosphate, *MAPK* mitogen-activated protein kinase, *MG* methylglyoxal, *NO* nitric oxide, *PA* phosphatidic acid, *ROS* reactive oxygen species, *RLK* receptor-like kinase

Arabidopsis genome contains eight HKs, of which five are involved in the perception of two plant hormones, ethylene and cytokinin (Pareek et al. 2006). Among the non-hormonal receptor HKs, AHK1 has been reported as a unique osmosensor with a positive regulatory function in the osmotic stress response (Tran et al. 2007; Wohlbach et al. 2008). Like SLN1 from yeast, AHK1 is also a hybrid HK having kinase and receiver domains within the same molecule. Overexpression of AHK1 in Arabidopsis leads to increased osmotic stress tolerance and the *ahk1* knock-out plants exhibit enhanced sensitivity to osmotic stress (Tran et al. 2007). AHK1 acts by regulating the expression of downstream genes in both an ABA-dependent and ABA-independent manner. Another HK, a plasma membrane-localized AHK5, is a ROS-dependent regulator of stomatal closure (Desikan et al. 2008). Mutants of *ahk5* show reduced sensitivity of the guard cells to ROS-induced stomatal closure

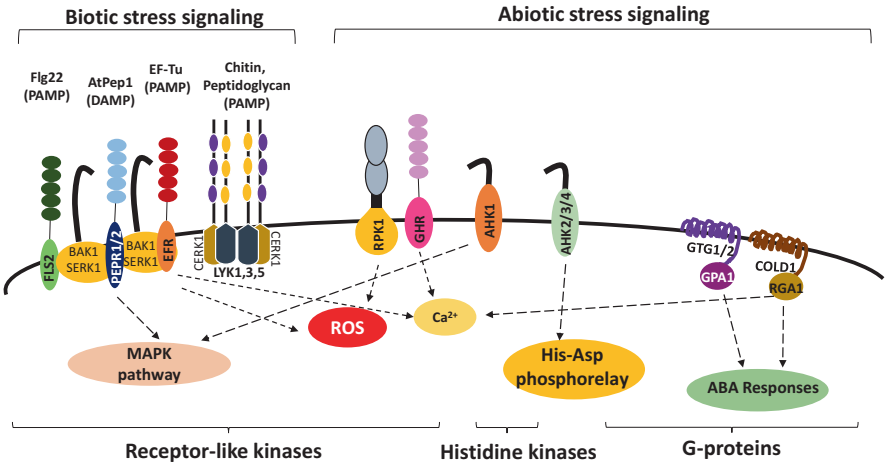

Fig. 7.2 Signaling receptors located on the plasma membrane and involved in plant stress responses. RLKs, HKs and G-proteins perceive peptidic ligands or phytohormones to trigger signaling cascades in response to stress. DAMPs (damage-associated molecular patterns) or PAMPs (pathogen-associated molecular patterns) are perceived by RLK receptors forming a part of plant immune signaling. Downstream pathways of these receptors include MAPK pathway, ABA signaling pathway, reactive oxygen species (ROS) and calcium ions

but are not affected in their response to ABA, suggesting that AHK5 acts in an ABA-independent manner. Further, some hormonal HK receptors like AHK2, AHK3 and AHK4 have been identified as negative regulators of ABA and stress signaling. Mutations of *ahk2*, *ahk3* and *ahk4* in various combinations lead to an increase in the expression of ABA-inducible genes along with enhanced tolerance to abiotic stresses including cold, salt and drought (Tran et al. 2007; Jeon et al. 2010). In addition to RLKs and HKs, cytosolic microtubules may also act as stress sensors that, by causing structural changes in the microtubular framework of the cell, control stomatal closure and turgor pressure maintenance. However, it is not yet clear as to how the sensors relay stress signals to the downstream signaling molecules.

ABA is considered to be a key player in water stress response. ABA synthesis is, in fact, one of the immediate responses of plants to abiotic stress, triggering gene expression and instigating stomatal closure to reduce water loss via transpiration. Two G-proteins (GTG1 and GTG2) from Arabidopsis have been reported be involved in ABA signaling and are probably a part of ABA receptor complexes (Pandey et al. 2009). GTG1 and GTG2 interact with GPA1 (G protein alpha subunit) and can bind ABA to mediate responses during germination, flowering, stomatal closure and root elongation. Mutants lacking both GTG1 and GTG2 have been shown to exhibit ABA-hyposensitive phenotypes. ABA-mediated abiotic stress signaling is regulated by three components, pyrabactin resistance (PYR)/PYR1-like (PYL)/regulatory component of ABA receptor (RCAR), protein phosphatase 2C (PP2C) and SNF1 (sucrose non-fermenting)-related protein kinase 2 (SnRK2). The PYR/RCAR receptors are located in the cytoplasm as inactive dimers that dissociate upon ABA binding

to inhibit PP2C activity, a negative regulator of ABA signaling, and thereby let SnRK2 to activate various downstream effectors including ion channels and transcription factors (reviewed by Upreti and Sharma 2016). Phosphatidic acid (PA) and inositol 1,4,5 triphosphate (IP3) act as second messengers in ABA signaling. In guard cells, IP3 is known to activate Ca^{2+} channels within the endoplasmic reticulum and vacuoles, resulting in the release of Ca^{2+} from internal stores into the cytosol. Increased Ca^{2+} levels inhibit the plasma membrane H^+-ATPase and prevent K^+ uptake and, in fact, drive K^+ and Cl^- efflux, thereby, closing the guard cells. Further, ABA biosynthesis is largely regulated via a positive feedback system through regulation of its own endogenous levels (Xiong and Zhu 2003). This feedback regulation of ABA synthesis can stimulate ABA accumulation and represent a critical step in stress adaptation. In this context, drought and salinity conditions are known to elevate ABA accumulation in the leaves of many plant species which is reversed once the stress is released. The promoter regions of ABA-responsive genes have been found to possess ABA-binding response elements (ABRE) which can bind basic leucine zipper transcription factors, ABRE-BINDING PROTEINS (AREBs)/ABRE-BINDING FACTORS (ABFs), and result in the upregulation of ABA-responsive genes.

After stress perception and signal relay through a composite array of signal transduction system, the effectors are finally modulated to evoke specific responses in the plant and include genes governing the accumulation of osmolytes such as proline, glycinebetaine and sugars; water transport channels like aquaporins; enzymes for ROS detoxification like catalase and superoxide dismutase; and protectants of macromolecules such as LEA proteins.

7.2.2 Perception of Flooding Stress in Plants

Flooded terrestrial plants suffer from a severe shortage of energy and carbohydrates as a result of slow gas exchange and low light levels under water, which adversely impacts photosynthesis. Endogenous levels of four gases, oxygen, carbon dioxide, ethylene and nitric oxide are altered during submergence of plant organs in water and play a key role in flooding-mediated signal transduction cascades in plants. Of these, ethylene (ET), which accumulates regardless of the water turbidity and light penetration, is considered to be the most reliable and consistent signal of early flooding stress. ET is produced by all cells of higher plants with its endogenous concentration being determined by the net outcome of its production and diffusion towards atmosphere. When surrounded by water, diffusion rate is highly reduced leading to an accumulation of ET in cells and in air spaces inside plant organs. In fact, ET levels rise within 1 h of submergence stress to around 1 $\mu l\ l^{-1}$, about 20-fold higher than in non-submerged tissues (Voesenek and Bailey-Serres 2013). Flooding-associated high levels of ethylene inhibit root elongation but can be tackled by plants through the formation of aerenchyma which removes excessive ethylene. Therefore, species ineffective in producing aerenchyma experience a strong reduction in root growth under flooded conditions. Interestingly, to avoid the detrimental effects associated with accumulation of ET levels, some aquatic or flood-prone areas inhabiting plants have reduced or even lost their ability to produce, sense and respond to ET (Voesenek et al. 2015).

As against ET which accumulates under flooding conditions, plants are rapidly depleted of oxygen in the flooded soils as water fills the existing airspaces and even respiring microorganisms consume the available oxygen. A decline in O_2 concentration from 19 to nearly 0 kPa was observed in the potting soil in 30 h upon submergence in darkness (Vashisht et al. 2011). In another study, O_2 concentration in the roots of Arabidopsis was found to decline upon complete submergence of the plant in dark from 5% to 6% in well-aerated soil conditions to nearly 0% in 15 min. Re-illumination resulted in only a trivial increase of 1% in the internal root O_2 levels suggesting that the photosynthetic O_2 diffuses from the leaves to the roots (Lee et al. 2011). Rice is a remarkably well adapted crop plant that can germinate even in the complete absence of oxygen. This anaerobic germination includes lengthening of the coleoptile for making aerial contact. However, considerable variations in the coleoptile extension have been observed among the rice genotypes during anoxia (Magneschi and Perata 2009). Unlike other cereal seeds that fail to induce α-amylases otherwise needed for starch degradation under anoxia, rice caryopses can produce them allowing starch degradation coupled to fermentative metabolism and, thereby, facilitate germination under anoxic conditions. This happens via a signaling cascade that senses the rapid depletion of soluble carbohydrates occurring during the first hours of germination under anoxia along with possible low-oxygen dependent changes in the calcium levels, leading to α-amylase formation. Activation of calcineurin B-like (CBL) marks the beginning of this signaling cascade which targets the protein kinase CIPK15, in turn triggering the SnRK1A pathway. This is followed by the induction of MYBS1 transcription factor which activates the starvation-inducible α-amylase gene RAmy3D (Lee et al. 2009). A QTL analysis identified OsTPP7, encoding for a trehalose-6-P-phosphate (T6P) phosphatase enzyme in rice, as the locus responsible for efficient anaerobic germination (Loreti et al. 2003). Non-functional OsTPP7 leads to inability of rice plants to establish themselves under submerged conditions and its presence correlates with increased sink strength of elongating coleoptiles, resulting in prolonged tolerance to complete submergence. High sucrose levels have been known to result in high T6P levels which cause a repression of SnRK1 and, hence, a downregulation of α-amylases. During anaerobic germination, OsTPP7 deludes the seedling about its sugar status by converting T6P into trehalose. Thus, rice seedlings can maintain a relative high sugar availability but low T6P levels, which, otherwise, would repress α-amylases.

The group VII ethylene response factor (ERF) TF genes, SNORKEL1 (SK1), SNORKEL2 (SK2) and SUBMERGENCE1A (SUB1A), have been termed as major regulators of the escape (morphological and anatomical traits that facilitate gas exchange between submerged organs and the aerial environment) and quiescence (traits that conserve energy and carbohydrates to extend underwater survival and enable recovery growth once floods regress) survival strategies of plants, respectively (reviewed by Voesenek and Bailey-Serres 2013). In deepwater rice, ET-induced SKs enable submergence-mediated internode elongation, allowing escape from slowly increasing floodwaters. SK1/2 possibly interacts via unknown pathways with ABA and gibberellin (GA). Very recently, a gibberellin biosynthesis gene, *SD1* (*SEMIDWARF1*) that is transcriptionally activated by an

ethylene-responsive transcription factor, *OsEIL1a*, has been shown to be responsible for the submergence-induced internode elongation (Kuroha et al. 2018). On the contrary, the ET and submergence-induced SUB1A-1 allele of SUB1A acts via transient down-regulation of GA responsiveness and suppression of genes associated with cell wall loosening, flowering and starch and sucrose catabolism (reviewed by Voesenek and Bailey-Serres 2013). In Arabidopsis, there are five group VII ERF genes (HRE1, HRE2, RAP2.2, RAP2.12, RAP2.3), of which, RAP2.12, RAP2.2 and RAP2.3 are stable only under low oxygen concentration and redundantly activate the core anaerobic response (Bui et al. 2015). In *Arabidopsis* accessions, Bay-0 and Lp2–6, a correlation between the rate of submergence recovery with submergence tolerance and productiveness has been recently demonstrated where the authors related differential recovery between the accessions to the activity of three genes: RESPIRATORY BURST OXIDASE HOMOLOG D, SENESCENCE-ASSOCIATED GENE113 and ORESARA1 (Yeung et al. 2018). These are found to function in a regulatory network involving ROS burst (upon de-submergence) and the hormones, ABA and ET, which acted to control ROS homeostasis, stomatal aperture and chlorophyll degradation during submergence recovery.

Like ET, NO also accumulates to higher concentrations in flooded tissues due to restricted gas diffusion. But as NO is highly reactive and short-lived, its accumulation is probably restricted without any additional hypoxic NO burst. Even though the exact dynamics of NO in flooded plants remains unclear, it is definite that an NO upsurge during hypoxia has functional implications for plant survival under hypoxia. This is so because chemically blocking the hypoxia-induced NO burst at the onset of hypoxia has been shown to strongly impair survival in maize root tips. Further, NO is also known to regulate ERF VII abundance and may even mediate post-translational modification of proteins via S or metal nitrosylation and Tyr nitration. Some S-nitrosylated proteins have been found to be potentially involved in flooding signaling and adaptation such as ERFVIIs, cytochrome c oxidase (COX), aconitase, phytoglobins and ascorbate peroxidase (reviewed by Sasidharan et al. 2018).

7.2.3 Salt Sensing and Signaling in Plants

All plants are known to take up Na^+ in the low-affinity range which is absolutely harmless for the plant. However, most plants can also do so in the high affinity range, the uptake being a passive process and facilitated by transporters. Some of these transporters automatically turn on, when K^+ is deficient. How Na^+ is monitored and how plants register the onset of stress due to high ambient Na^+ concentrations remain uncertain. It is, however, believed that saline conditions cause an immediate reduction in the water supply to plant tissues due to a drop in the external water potential which is recorded by plants as a stimulus in several ways. One such way is by sensing changes in the turgor which is transmitted to the membrane receptors via changes in physical forces on the membranes and the cell wall. In this context, Arabidopsis histidine kinase AtHK1 can record changes in turgor by gauging the distance between the membrane and the cell wall through its sensory domains. Activation of HK1 then

initiates a MAPK signaling cascade which ultimately alters gene expression (Urao et al. 1999). Even mechanosensitive ion channels can gauge the distortion of cell wall membrane geometry and open in response to membrane stretching. This non-selective feature of the transporters in channel opening can facilitate large membrane depolarizations which may, in turn, induce cytoplasmic Ca^{2+} levels, thereby providing a potent signal to relay further these osmolarity changes. However, these relatively speedy mechanisms are not specific to Na^+ or salt stress and respond generally to osmotic perturbations. In agreement, studies in yeast show that rapid Ca^{2+} transients (approximately 0–2 min) are utterly due to osmotic effects regardless of salts or their ionic/non-ionic nature (Matsumoto et al. 2002). Ion toxicity as a result of Na^+ or Cl^- accumulation probably occurs later. Sodium toxicity can be said to be due to the resemblance of K^+ and Na^+ ions, which affects enzymes and transporters.

Plants that have not previously been exposed to salt initially experience a large net Na^+ influx. However, exposure for longer periods reduces both net and unidirectional Na^+ influx probably due to lowering of membrane potential in response to NaCl. Second messengers are known to play important roles in regulating Na^+ uptake in plants. These include Ca^{2+}, cGMP and ROS, all of which undergo a rapid transient increase in their cytoplasmic levels in response to a surge in the salt concentration. Na^+ is known to enter plant cells through high-affinity potassium transporter (HKT) family of K^+/Na^+ transporters and non-selective cation channels (NSCCs), which include cyclic nucleotide-gated channels (CNGCs) and glutamate-activated channels (GLRs). cGMP, by exhibiting a direct inhibitory effect on NSCCs, regulates Na^+ levels (Maathuis and Sanders 2001). In addition, it also promotes K^+ uptake. Studies report a role of Ca^{2+} signaling as an intermediary process probably acting downstream of cGMP. In fact, salt stress-mediated fast and transient increases in cytosolic Ca^{2+} have been shown to trigger many signal transduction pathways, such as the salt overly sensitive (SOS) and mitogen-activated protein kinase (MAPK) pathways involved in ion channel activity, changes in enzymatic activity and gene transcription, thereby causing a wide variety of cellular responses. In Arabidopsis, a putative sensor for hyperosmotic stress OSCA1 (reduced hyperosmolality-induced calcium increase 1) has been described. Loss of its function mutant exhibits reduced calcium spike as compared to wild-type plants upon treatment with osmotic stressors, mannitol or sorbitol. Further, ambient salt concentrations are also known to induce ROS that emerges within minutes of the applied stress, mainly as H_2O_2 (Hong et al. 2009). Salt-induced ROS is also known to affect downstream pathways including MAPK and transcription factors such as ERF1. In addition, it is also known to directly influence ion fluxes such as the activation of outward rectifying K^+ channels, which is probably responsible for loss of K^+ from plant roots during salt stress (Demidchik et al. 2010). In addition to the role of Ca^{2+}, ROS and cGMP in salt stress signaling, the plant stress hormone ABA is also known to act as an endogenous messenger for osmotic imbalance generated due to severe salt and dehydration stress (Fahad et al. 2015). ABA-deficient mutants exhibit poor growth under salt stress and, hence, provide strong indications for the involvement of this hormone in regulating salinity response. ABA probably acts by controlling water loss through regulation of stomatal movements as a result of increase in Ca^{2+} levels. Further, ABA is also linked with the synthesis of osmolytes like proline and dehydrins. In addition to these

known messengers, a role of methylglyoxal (MG) has also been demonstrated under stress conditions, especially in salinity conditions (Gupta et al. 2018). MG is produced as a byproduct of glycolysis and its concentration increases under stress. It is being increasingly viewed as a stress signal molecule in plants (Kaur et al. 2015) which can affect expression of RD29B and RAB18 genes in Arabidopsis in an ABA-dependent manner. Further, MG can also regulate stomatal movements linking it to osmotic stress-related adaptation in plants (Hoque et al. 2012).

Another aspect of salinity tolerance is the extrusion of Na^+ from the cytoplasm. SOS1, which encodes a plasma membrane-located Na^+/H^+ antiporter, is a very important candidate for salt efflux from the plant cells (Ji et al. 2013). SOS1 activity is regulated via phosphorylation by the kinase CIPK24 (or SOS2) which gets activated upon association with the calcineurin B-like (CBL) calcium sensor CBL4 (or SOS3). Loss of function of any of the SOS genes results in heightened salt sensitivity along with changes in homeostasis of other cations, particularly K^+. SOS3 is activated by dimerization after binding Ca^{2+} which then allows its association with SOS2. Following the binding of SOS3 to SOS2, the C-terminal autoinhibitory domain of SOS2 is released, and SOS2-SOS3 complex can then bind and phosphorylate SOS1. The interactions of SOS2-SOS3 complex with SOS1, in turn, remove autoinhibitory domain of SOS1 and activate the antiporter which, finally, acts to limit cytoplasmic Na^+ accumulation (Ji et al. 2013). While calcium activates SOS pathway, many questions about the physiological relevance of a Ca^{2+}-initiated regulatory cascade to activate SOS1 still remain to be investigated.

Under salinity stress, cytosolic change in pH can also act as secondary signal exerting its effect via the vacuolar membrane Na^+/H^+ antiporters (Yamaguchi et al. 2005). The vacuole-localized AtCaM15 is involved in modifying the Na^+/K^+ selectivity of the tonoplast transporter AtNHX1. At the normal low vacuolar pH, AtNHX1/AtCAM15 interaction downregulates the Na^+/H^+ exchange activity. However, increase in vacuolar pH due to salt stress, signals the release of AtCAM15 in order to facilitate increase in the vacuolar compartmentation of Na^+ ions by NHX1 (Yamaguchi et al. 2005).

7.2.4 Heat Sensing in Plants

When a leaf is exposed to elevations in ambient temperatures, almost all macromolecules in the cells, including protein complexes, membranes and nucleic acid polymers, 'perceive' heat at the same time owing to the large surface-to-volume ratio of the leaf. Therefore, all macromolecules might, in principle, can be termed as thermosensors which provide output in the form of, say, a transient loss in function. However, Vu et al. (2019) have defined some criteria for terming molecules as thermosensors. Firstly, a change in temperature should directly alter either the structural feature or activity of the sensing molecule which is important for the functional module in which the thermosensor otherwise participates and efficiently conveys temperature information to the response machineries. Secondly, thermosensing capacity should impact physiological or morphological responses to temperature. In this context, there are some primary heat sensors among the many heat-responsive macromolecules which not only accurately perceive but

differentially react to various temperature increments and even elicit a distinct signaling pathway that can explicitly upregulate hundreds of heat-responsive genes.

In the moss *Physcomitrella patens*, the primary heat sensing event occurs at the plasma membrane which can sense even mild increases in temperature and consequently leads to the opening of a specific calcium channel that facilitates an influx of calcium into the cell, thereby activating the heat stress response (Saidi et al. 2009). Heat stress, in general, alters the membrane properties, and hence, chemicals that fluidize the membrane can also induce similar heat stress responses even in the absence of any actual changes in temperature. The identity of such heat stress sensors in the plasma membrane is not yet known in plants, but studies in animal systems have indicated that some ion channels, like stromal interaction molecule, transient receptor potential cation channel subfamily V and CNGCs, might function as temperature sensors (Sajid et al. 2018). The levels of cAMP and cGMP increase during heat stress which are known to facilitate the opening of CNGCs in response to heat stress. To this end, CNGC16, a pollen-expressing CNGC, has been found to be critical for heat or drought stress tolerance during the reproductive development in Arabidopsis. The *cngc16* mutant pollen shows attenuated expression of several heat stress response genes, such as *HsfA2* and *HsfB1*. These ion channels, thus, establish a link between the stress-triggered cNMP signal and a downstream transcriptional heat shock response (Tunc-Ozdemir et al. 2013). Calmodulins (CaMs) also participate in heat signaling by acting as converters of Ca^{2+} signals (Zhang et al. 2009). In Arabidopsis, the calmodulin AtCaM3 is required for the activation of different transcription factors such as heat shock factors (HSFs) and WRKY39. CaM3 interacts with calcium/calmodulin-binding protein kinase (CBK3), which phosphorylates HsfA1a, and also with a phosphatase, PP7, which dephosphorylates HsfA1a, to regulate these proteins during heat stress, indicating that Ca^{2+} induces heat stress response through the post-translational modification of HsfA1.

In addition to Ca^{2+}, ROS is also an inducer of heat stress response, being indispensable for evoking heat stress-mediated signaling (Volkov et al. 2006). Although there is no clarity as to how ROS signal is perceived and converted into transcriptional regulation, it is believed that ROS signal evokes two signaling pathways, one related to NO and the second to reactive short-chain leaf volatiles (RSLVs). The generation of ROS from RESPIRATORY BURST OXIDASE HOMOLOGUE PROTEIN, RBOHB and RBOHD, leads to NO accumulation which subsequently activates CaM3, thereby inducing heat stress response pathways. Further, RSLVs are also derived from ROS via ROS-mediated lipid peroxidation and may act as chemical signals through which plants perceive ROS generation. RSLV treatment induces the expression of many heat stress-inducible genes, some of which are induced in an HsfA1-independent manner.

Further, lipid signaling is also initiated in response to heat-induced changes in membrane fluidity through the activation of phospholipase D (PLD) and phosphatidylinositol-4-phosphate 5-kinase (PIPK). Various lipid signaling molecules such as phosphatidylinositol 4,5-bisphosphate (PIP2), IP3 and PA get accumulated during heat stress which in turn causes opening of channels and, thus, allows an influx of calcium (reviewed by Mittler et al. 2012). However, no

correlation, if any, has yet been reported between the plasma membrane channels that are activated by heat and the channels that are activated by lipid signaling in plants.

Interestingly, an unfolded protein response (UPR) pathway is also induced as a signal in response to heat stress and impairs protein stability in endoplasmic reticulum (ER) (reviewed by Mittler et al. 2012). In plants, two types of UPR are known, one in the ER and other in the cytosol. These pathways are activated in response to misfolded or unfolded proteins which accumulate during stress in plants. The ER UPR pathway in plants involves the activation of different bZIP transcription factors which are activated upon proteolytic cleavage and, thus, are released from the ER membrane. This is followed by their translocation into the nuclei where they facilitate the accumulation of ER chaperone transcripts along with the activation of brassinosteroid signaling. In contrast, the cytosolic UPR, which is induced upon accumulation of unfolded proteins in the cytosol, is primarily regulated by HSFA2, which binds to HSF-binding elements in the promoters of heat stress response genes. Notably, it is worth considering the fact that UPR may not be the primary heat sensor in plants as some heat stress-inducible chaperones can also accumulate in the absence of heat stress. Moreover, the activation of UPR even requires specific calcium signals from the plasma membrane.

7.2.5 Low-Temperature Sensing in Plants

Like heat stress, plant's perception to low temperatures also begins at the plasma membrane. Variations in membrane fluidity and modifications in the conformation of membrane proteins are considered to be the first line of physical changes occurring in the plant under low temperatures. The Arabidopsis *fad2* mutants, which are defective in oleate desaturase, have an irregular membrane composition and membrane rigidification. As a result, these mutants exhibit lethality at low temperatures (Miquel et al. 1993). In fact, several enzymes belonging to the lipid metabolism such as diacylglycerol kinase (DAGK), acyl-lipid desaturase2 (ADS2) and SFR2 (a galactolipid remodeling enzyme) have been shown to be involved in chilling or freezing responses, mediating either lipid remodeling or membrane stabilization activities at low temperatures. Plants can be said to perceive cold stress through membrane rigidification which serves as one of the primary signals for the perception of low non-freezing temperatures. In prokaryotes, the cold-induced membrane rigidification triggers autophosphorylation of the membrane-localized histidine kinases, which act as sensors of cold stress. The N-terminal domain of histidine kinase, Hik33, from *Synechocystis*, has been found to be essential for regulating homodimerization and autophosphorylation of sensory Hik33 in order to activate the expression of cold-inducible genes. Similarly in *Bacillus subtilis*, a histidine kinase, DesK, senses a decrease in membrane fluidity (Martin et al. 2009). Being a bifunctional enzyme, it possesses both kinase and phosphatase activities. The phosphoryl group of DesK, obtained after its autophosphorylation, is transferred to DesR, a DNA-binding response regulator, which then activates the acyl-lipid desaturase encoding *des* gene, leading to changes in the fluidity of membranes. Even in plants, the role of HKs in cold stress signaling is highly advocated. The gain-of-function

mutations in ethylene receptor type HKs, such as *etr1-1* and *ein4-1*, have been, in fact, shown to confer enhanced freezing tolerance in Arabidopsis (Shi et al. 2012).

Changes in membrane fluidity are usually accompanied by changes in the plant cytoskeleton, with microtubules and filaments forming bundles under low temperatures. Depolymerization of cytoskeleton is considered necessary for the induction of low-temperature-mediated gene expression in plant cells as supported by the observation that a microtubule and filament stabilizer (taxol) inhibits the expression of an otherwise cold-inducible gene, BN115, from *Brassica napus*, whereas treatment with the microfilament dispersant (colchicine) induces its expression (Sangwan et al. 2001). Initial rigidification of the plasma membrane and reorganization of the cytoskeleton, subsequently, causes an influx of calcium into the cytoplasm through CNGCs and other calcium channels. Cold stress induces a monophasic increase in cytosolic Ca^{2+} levels in the Arabidopsis root cells, without a significant temporal difference, indicating that all cells sense temperature changes instantaneously (Kiegle et al. 2000). In rice, the COLD1/RGA1 complex has been described as a cold sensor (Ma et al. 2015) and possibly represents a calcium permeable channel. COLD1 is a quantitative trait locus gene that encodes a regulator of G-protein signaling and is localized on the plasma membrane and endoplasmic reticulum. It physically interacts with RGA1 (G_{α} subunit in rice) and accelerates G-protein GTPase activity, in turn triggering Ca^{2+} signaling (Fig. 7.2). Calcium signatures generated upon cold sensing are then decrypted by calcium-binding proteins to activate downstream signals. The proteins with an EF-hand domain such as calmodulin (CaM), CaM-like (CML) proteins, Ca^{2+}-dependent protein kinases (CDPKs) and calcineurin B-like proteins (CBLs) act as Ca^{2+} sensors under cold stress.

The cold-mediated increase in cytosolic Ca^{2+} levels subsequently stimulates the expression of C-repeat (CRT) binding transcription factors, CBF/DREB1 (C-repeat-binding factor/DRE-binding protein), which, in turn, induce the expression of a subset of cold-responsive (COR) genes. CBF-dependent signaling cascade is the central cold-signaling pathway in plants. Arabidopsis genome possesses three CBF genes (CBF1, CBF2 and CBF3) which are transcriptionally regulated by a number of transcription factors. The bHLH transcription factors, ICE1 (Inducer of CBF expression 1), ICE2 (Inducer of CBF expression 2) and also CAMTA3 (Calmodulin-binding transcription activator 3), positively regulate the expression of CBFs, while MYB15 and EIN3 act as negative regulators of CBF expression in Arabidopsis. The ICE1-CBF-COR cascade is one of the main cold signaling pathways triggered in response to cold stress in plants (reviewed by Guo et al. 2018). Besides transcriptional regulation of CBF genes, CBF pathway is also regulated at the post-translational levels which affects the outcome of cold stress response. A RING finger E3 ligase HOS1 (high expression of osmotically responsive genes1) is known to ubiquitinate ICE1, leading to its degradation via the 26S proteasome pathway, while the sumoylation of ICE1 by SIZ1 (a small ubiquitin-related modifier (SUMO) E3 ligase) reduces the polyubiquitination of ICE1 and, thereby, inhibits the degradation of ICE1. Further, ICE1 also interacts with a protein kinase, OST1/SnRK2.1, which is involved in ABA signaling. OST1 is activated by cold stress and phosphorylates ICE1, thereby, repressing HOS1-mediated ICE1 degradation under cold stress which culminates

into activation of CBF/COR expression. The MAPK pathway proteins, MPK3 and MPK6, have also been shown to phosphorylate ICE1, but unlike OST1, these kinases reduce its stability and transcriptional activity which negatively regulates CBF expression and, thus, negatively impacts freezing tolerance in plants. Furthermore, jasmonate signaling also affects cold-induced expression of genes acting in the CBF/DREB signaling pathway. JAZ1 and JAZ4 interact with ICE1 and repress its transcriptional activity resulting in the attenuation of downstream gene expression (reviewed by Guo et al. 2018). ICE1, thus acts as an imperative node, integrating different signals of the network to control cold tolerance. In Arabidopsis, a plasma membrane-localized protein kinase, COLD-RESPONSIVE PROTEIN KINASE 1 (CRPK1), that functions via the conventional CBF pathway plays a negative role in regulating extreme cold responses. At freezing temperatures, CRPK1 has been found to phosphorylate 14-3-3 proteins causing their nuclear translocation in turn, destabilizing the CBF transcription factors and, thus, preventing cold stress responses.

Interestingly, the expression of CBFs is also induced by the circadian clock and light quality. Low red to far-red ratios (R:FR) and short-day conditions have been found to mimic the process of cold adaptation to increase freezing tolerance of plants. Further, two principal components of the circadian clock, CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), positively regulate CBF gene expression. Phytochrome B (PhyB), which is among the primary photoreceptors regulating photomorphogenesis in plants, has been found to function as a thermosensor (Jung et al. 2016). PhyB can directly associate with the promoters of key target genes in a temperature-dependent manner. Moreover, phytochrome-interacting factors, PIF4 and PIF7, also repress the transcription of CBF1, CBF2 and CBF3 genes during long-day conditions (reviewed by Shi et al. 2012). PIF4 is said to be a crucial integrator of light, cold and phytohormone signaling in plants.

7.2.6 Perception of Pathogen Attack Signals by Plants

Unlike animals, plants do not have a circulating immune system and, thus, they count on the capacity of each specific cell to initiate innate immune responses against impending pathogenic microorganisms. To achieve this, plants have at their cell surface RLKs and receptor-like proteins (RLPs) that function as pattern recognition receptors (PRRs) for perceiving characteristic microbial molecules, known as pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs) (Fig. 7.2). RLPs share the same basic conformation as RLKs, but they lack a kinase domain and, hence, depend on the regulatory receptor kinases known as receptor-like cytoplasmic kinases (RLCKs), to transduce perceived extracellular signals downstream. Plant PRRs can be categorized based on the nature of their ligand-binding domain. Leucine-rich repeat (LRR)-containing PRRs preferentially bind proteins or peptides, whereas lysine motifs (LysM) containing PRRs bind carbohydrate-based ligands, such as fungal chitin or bacterial peptidoglycan. Further, lectin-type PRRs bind extracellular ATP or bacterial lipopolysaccharides (LPS), and PRRs with epidermal growth factor (EGF)-like domains recognize plant cell wall-derived

oligogalacturonides. Further, ω-hydroxy fatty acid monomers (derived from plant cutin) and cellobiose (derived from cellulose) also trigger plant immunity.

Besides cell surface receptors, intracellular nucleotide-binding domain leucine-rich repeat (NLR or NBS-LRR) proteins represent another group of immune receptors that are involved in the recognition of pathogen-secreted virulence effectors (reviewed by Cesari 2018). It is believed that these effectors have evolved to suppress host immunity and/or to deploy host metabolism for virulence. However, recognition by NLRs can also betray pathogens. Recognition by NLRs may ensue either via direct binding of pathogen-secreted effectors or by sensing alterations in host components mediated by these effectors. In order to function, many NLRs require the participation of other NLR proteins. These NLR pairs often function via negative regulation, with the sensor NLR inhibiting the auto-activity of the helper NLR which is released only upon pathogen perception (reviewed by Wu et al. 2018). Some helper NLRs are known to be functionally redundant and are required by multiple sensor NLRs.

PRRs form dynamic complexes with regulatory receptor kinases at the plasma membrane to activate immune signaling. For example, LRR receptor kinases flagellin sensing 2 (FLS2), EF-TU receptor (EFR) and PEP 1 receptor (PEPR1) and PEPR2, which recognize bacterial flagellin, EF-Tu, and the endogenous AtPep1, respectively, all associate with the regulatory receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and with related somatic embryogenesis receptor kinases (SERKs) in a ligand-dependent manner (Chinchilla et al. 2007; Heese et al. 2007). Similarly, chitin elicitor receptor kinase 1 (CERK1) acts as a regulatory receptor kinase associating with different LysM-containing PRRs to activate immune signaling (Cao et al. 2014). Overall, the recruitment of regulatory receptor kinases is specified by the type of PRR ectodomain. Upon PRR complex activation following ligand binding, a downstream signaling cascade is initiated within minutes to stimulate local and systemic defence responses in the plant that can continue till several days. Quick changes in ion flux at the plasma membrane along with a rise in cytosolic Ca^{2+} levels and generation of extracellular ROS are among the first responses observed after PAMP or DAMP perception (reviewed by Couto and Zipfel 2016). The PRR-triggered ROS burst in Arabidopsis is mainly due to the activity of the NADPH oxidase (RBOHD) enzyme which associates with the PRR complex and gets phosphorylated by BOTRYTIS-INDUCED KINASE 1 (BIK1) and related PBS1-LIKE KINASE (PBL) upon PRR elicitation leading to its activation. The subsequently generated ROS burst is required for stomatal closure in order to limit pathogen entry through leaves. Besides regulating RBOHD, BIK1 and PBL1 are also required for triggering initial cytosolic Ca^{2+} burst upon sensing PAMPs and/or DAMPs. This Ca^{2+} burst, in turn, activates Ca^{2+}-dependent protein kinase (CDPK) which also regulates RBOHD and, importantly, acts as regulators of transcriptional reprogramming during plant immune responses. Further, like CDPKs, mitogen-activated protein kinase (MAPK) also leads to transcriptional reprograming upon PAMP or DAMP perception, by relaying immune signaling to the nucleus.

In this process of pathogen-triggered immunity, plant hormones make up a robust system that feedbacks on immune signaling and is capable of responding against pathogens while maintaining homeostasis. For instance, salicylic acid (SA)

positively regulates basal FLS2 levels and subsequent flg22-triggered responses. On the contrary, jasmonic acid (JA) exerts a negative effect on responses mediated by FLS2 such as ROS burst and callose deposition. Further, the third hormone, ethylene, exhibits both antagonistic and synergistic roles in its relationship with SA, while it is mostly synergistic to JA. Further, brassinosteroids (BRs) exhibit a negative effect on plant-triggered immune responses. This inhibition is mediated by the transcription factor BRASSINAZOLE-RESISTANT 1 (BZR1) which can integrate BR and gibberellin (GA) signaling and environmental cues via the activation of a set of WRKY transcription factors to negatively regulate immunity.

7.3 Specificity and Crosstalk in Stress Signaling Pathways

Crosstalk can be defined as the convergence at any instance of two signaling pathways from different stressors. This might be occurring in the form of either different pathways attaining the same end or pathways interacting and affecting each other's response in an additive or negatively regulated manner. In some cases, different stresses trigger same signaling mechanisms, as under certain conditions, these stresses cannot be distinguished from one another or it is also possible that these stresses entail the same protective action. For example, dehydration protection is necessary in plants enduring either freezing or drought conditions. Also, the production of antioxidants and scavenging enzymes is believed to be required for the protection against oxidative damage in a variety of different abiotic stresses. Typically, cross-tolerance allows plants to acclimatize to a range of different stresses after exposure to a specific stress. Interaction points exist among different abiotic stresses and between abiotic-biotic stresses (Fig. 7.3). For example, the stress hormone ABA is a critical component in defence related to cold, drought and osmotic stress but is also a regulator of defence responses against the biotic factors. Generally, several hormone signaling pathways are involved in stress interactions. The generation of ROS is also one of the key processes that is shared between different stress responses. Rapid ROS generation plays a central role in both ABA signaling and disease resistance. Evidence suggest that ABA induces NADPH-dependent respiratory burst oxidase homolog genes (*AtrbohD* and *AtrbohF*), which generate ROS in guard cells, leading to stomatal closure. Further, ROS can even lead to hypersensitive cell death in response to pathogen attack. Apart from ABA, ROS accumulation during abiotic stress also affects the level and function of other plant hormones, such as auxin, BRs, GA and NO (reviewed by Choudhury et al. 2017). The mechanisms associated with alterations in auxin homeostasis and signaling attenuation includes oxidative auxin degradation, conjugation and distribution through changes in the expression of auxin transporters. Further, BRs are also known to interact with ROS signaling through induction of RBOH gene expression and increased NADPH oxidase activity leading to concomitant increase in apoplastic H_2O_2. Even SA can also form a positive interaction loop with ROS that facilitates cell death; however, SA is required to initiate defence signaling as well. Lately, the role of ROS in direct activation of signal transduction pathways through oxidative posttranscriptional modifications and activation of kinases is also emerging (Sewelam et al. 2016).

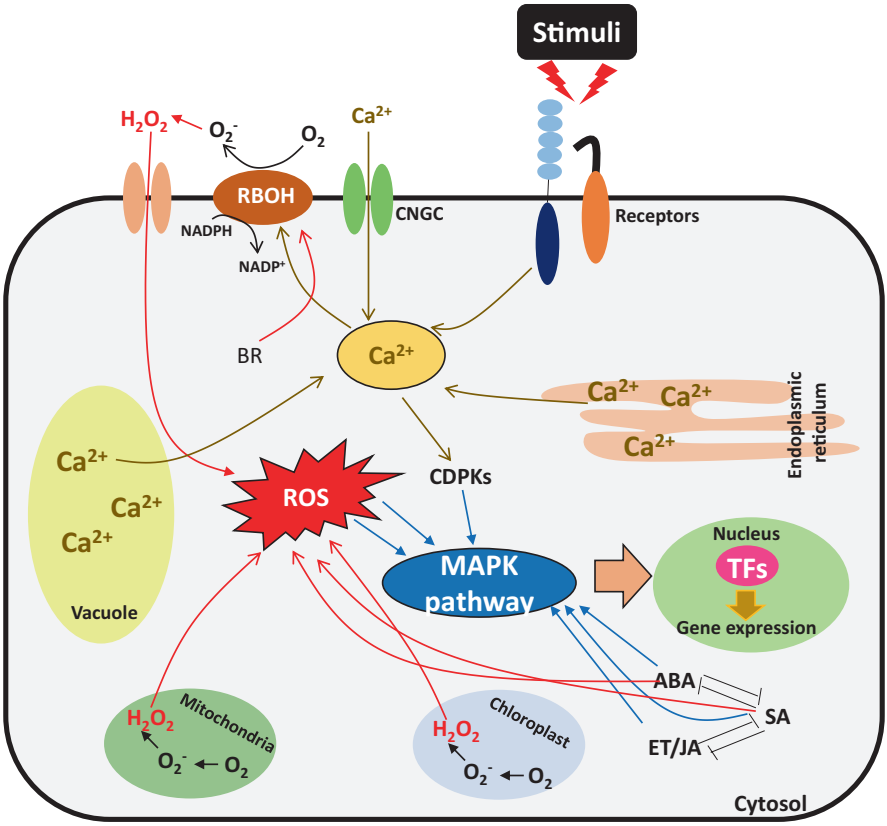

Fig. 7.3 Points of interaction in plant stress signaling networks. ROS, calcium and MAPK pathway act as three important points of convergence in stress signaling cascades. Hormonal interactions assist in modulating crosstalk at these convergence points

The other major component, besides ROS, which serves as the point of interaction among various signaling pathways is the mitogen-activated protein (MAP) kinase cascade, which transduces the perceived environmental stimuli into internal signaling pathways and consists of a MAPKKK (MAPK kinase kinase), a MAPKK (MAPK kinase) and a MAP kinase. Their increased activity in response to most stresses indicates that they execute a general function required for plant defences. During biotic stress, transmembrane receptors such as FLS2 detect PAMPs and trigger MAPK cascades in order to establish pathogen-mediated signaling (Chinchilla et al. 2007). During abiotic stress, MEKK1/MKK2/MPK4/MPK6 pathways are induced. Activated MAP kinases phosphorylate and manipulate the activity of target proteins. MAPK cascades, thus, play an important role in governing crosstalk between stress responses, as are activated by more than one type of stress or hormone, thereby integrating different signals. For instance, MPK6 from Arabidopsis is involved in response to ethylene synthesis, salt and cold stress, pathogen signaling

and stomatal control (Rodriguez et al. 2010). Further, similar to MAPK, heat shock factors (HSFs) which control the expression of heat shock proteins (HSPs) can also act as point of interactions in stress signaling. HSFs can act as molecular sensors which detect the presence of ROS and activate downstream stress-responsive genes. An *HSFA4a* gene from Arabidopsis acts as a redox sensor, due to its prompt induction in response to H_2O_2 and its ability to control the expression of ROS-scavenging enzymes (Miller and Mittler 2006). The functional diversity of HSFs, thus, reflects their critical role in allowing plants to respond to different stress conditions.

Among all, calcium is considered to be a key player in signaling cascades, but its mode of action in the context of crosstalk or specificity is ambiguous. Fluctuations in cytosolic free calcium occur during the transduction of both abiotic and biotic signals. However, the precise kinetics, amplitude and source of stimulus-induced cytosolic calcium elevations (known as 'calcium signature') suggest that it encodes information about the particular stimulus and determines the specific end response (reviewed by Knight and Knight 2001). Another opinion is that the specificity is not encoded through the calcium signature, but it is more likely that the cytosolic calcium elevation attains a minimum or maximum threshold peak value or total elevation (i.e., magnitude × time). In agreement, a variation in the timing of stimulus-induced Ca^{2+} oscillations in stomatal guard cells has been found to affect the intensity of both the stimulus and the resultant end response, and therefore, alterations in the signature lead to loss of aperture closure. Further, external Ca^{2+} or oxidative stress was found to induce Ca^{2+} oscillations resulting in stomatal closure in the wild type but not in the cells of the Arabidopsis mutant (*det3*) impaired in endomembrane energization (Allen et al. 2000). However, the mutant cells responded normally to cold and ABA stimulation, indicating different Ca^{2+}-dependent pathways for different stresses. Further, various plant abiotic stress-mediated cytosolic calcium responses use Ca^{2+} from different subcellular sources, and it is likely that the Ca^{2+} signature reflects the source used and encodes specific information relevant to the cellular machinery operating in those organelles. It is also proposed that 'effective' Ca^{2+} signatures occur only in those cell types that are meant to respond as evidenced from different cytosolic Ca^{2+} responses of epidermal, endodermal, pericycle and cortex cells within the Arabidopsis root when challenged with cold, drought and salt. Collectively, the Ca^{2+} signal is ubiquitous in stress signaling, and it is therefore a key node at which crosstalk can occur.

Specificity in signaling mechanisms, on the other hand, is also speculated and can be defined as distinction between two or more possible outcomes and, in effect, links a particular stimulus to a specific end response and not to any other end responses. Specificity might occur at the point of initial stress perception and is easy to anticipate if each stress signal has a specific sensor that can explicitly transduce the signal to cellular targets. At present, only few stress sensors have been identified and there is not enough information to assess whether cross-talk occurs at the level of sensors. A well-studied gene, *AtHK1* from Arabidopsis, is a candidate osmosensor which is up-regulated during both salt and low temperature stresses (Tran et al. 2007). Hence, it is desired to determine the in vivo role of putative sensory kinases and identification of signaling intermediates and targets to assess whether sensory kinase signaling is specific or involved in crosstalk between stress signaling pathways.

7.4 Concluding Remarks

In order to overcome sub-optimal growth conditions in the form of various abiotic and biotic stresses, perception of stress signals and their transduction is a critical step governing plant survival. An intricate interplay of signaling cascade comprising of membrane receptors, second messengers and hormones is, thus, adopted by plants to perceive, amplify and transmit stress signals in order to trigger stress responses. These signals are indeed shared in some or the other way through every constituent molecule, forming an extremely integrated regulatory network. However, a more precise knowledge on functioning and regulation of signaling networks is required to increase our ability to produce plants that exhibit high productivity even in rapidly changing and stressful environments.

Acknowledgements Charanpreet Kaur acknowledges the DST-INSPIRE Faculty Award (IFA-14/LSPA-24) received from the Department of Science and Technology (DST), Government of India. SLS-P and AP acknowledge the grant received from NWO Indo-Netherlands project.

References

Ahmad A, Diwan H, Abrol YP (2009) Global climate change, stress and plant productivity. In: Pareek A, Sopory S, Bohnert H (eds) Abiotic stress adaptation in plants. Springer, Dordrecht, pp 503–521

Allen GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, Kemper A, Hawke SD, Tallman G, Tsien RY, Harper JF, Chory J, Schroeder JI (2000) Alteration of stimulus specific guard cell calcium oscillations and stomatal closing in Arabidopsis *det3* mutant. Science 289:2338–2342

Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ 31:11–38

Bui LT, Giuntoli B, Kosmacz M, Parlanti S, Licausi F (2015) Constitutively expressed ERF-VII transcription factors redundantly activate the core anaerobic response in *Arabidopsis thaliana*. Plant Sci 236:37–43

Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. Elife 3. doi: 10.7554/eLife.03766

Cesari S (2018) Multiple strategies for pathogen perception by plant immune receptors. New Phytol 219:17–24

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500

Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J 90:856–867

Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16:537

Demidchik V, Cuin TA, Svistunenko D, Smith SJ, Miller AJ, Shabala S, Sokolik A, Yurin V (2010) Arabidopsis root K^+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. J Cell Sci 123:1468–1479

Desikan R, Horák J, Chaban C, Mira-Rodado V, Witthöft J, Elgass K, Grefen C, Cheung MK, Meixner AJ, Hooley R, Neill SJ (2008) The histidine kinase AHK5 integrates endogenous and environmental signals in Arabidopsis guard cells. PLoS One 3:e2491

Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah N, Faiq M (2015) Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul 75:391–404

Guo X, Liu D, Chong K (2018) Cold signaling in plants: insights into mechanisms and regulation. J Integr Plant Biol 60:745 60: 745–745 60: 756

Gupta BK, Sahoo KK, Ghosh A, Tripathi AK, Anwar K, Das P, Singh AK, Pareek A, Sopory SK, Singla-Pareek SL (2018) Manipulation of glyoxalase pathway confers tolerance to multiple stresses in rice. Plant Cell Environ 41:1186–1200

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci U S A 104:12217–12222

Hong CY, Chao YY, Yang MY, Cheng SY, Cho SC, Kao CH (2009) NaCl-induced expression of glutathione reductase in roots of rice (*Oryza sativa* L.) seedlings is mediated through hydrogen peroxide but not abscisic acid. Plant Soil 320:103–115

Hoque TS, Okuma E, Uraji M, Furuichi T, Sasaki T, Hoque MA, Nakamura Y, Murata Y (2012) Inhibitory effects of methylglyoxal on light-induced stomatal opening and inward K+ channel activity in Arabidopsis. Biosci Biotechnol Biochem 76:617–619

Hua D, Wang C, He J, Liao H, Duan Y, Zhu Z, Guo Y, Chen Z, Gong Z (2012) A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in Arabidopsis. Plant Cell 24:2546–2561

Jeon J, Kim NY, Kim S, Kang NY, Nova'k O, Ku SJ, Cho C, Lee DJ, Lee EJ, Strnad M, Kim J (2010) A subset of cytokinin two-component signaling system plays a role in cold temperature stress response in Arabidopsis. J Biol Chem 285:23371–23386

Ji H, Pardo JM, Batelli G, Van Oosten MJ, Bressan RA, Li X (2013) The salt overly sensitive (SOS) pathway: established and emerging roles. Mol Plant 6:275–286

Jung JH, Domijan M, Klose C, Biswas S, Ezer D, Gao M, Khattak AK, Box MS, Charoensawan V, Cortijo S, Kumar M, Grant A, Locke JC, Schafer E, Jaeger KE, Wigge PA (2016) Phytochromes function as thermosensors in Arabidopsis. Science 354:886–889

Kaur C, Kushwaha HR, Mustafiz A, Pareek A, Sopory SK, Singla-Pareek SL (2015) Analysis of global gene expression profile of rice in response to methylglyoxal indicates its possible role as a stress signal molecule. Front Plant Sci 6:682

Kiegle E, Moore CA, Haseloff J, Tester MA, Knight MR (2000) Cell type-specific calcium responses to drought, salt and cold in the Arabidopsis root. Plant J 23:267–278

Knight H, Knight MR (2001) Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci 6:262–267

Kuroha T, Nagai K, Gamuyao R, Wang DR, Furuta T, Nakamori M, Kitaoka T, Adachi K, Minami A, Mori Y, Mashiguchi K (2018) Ethylene-gibberellin signaling underlies adaptation of rice to periodic flooding. Science 361:181–186

Larcher W (1987) Streß bei Pflanzen Naturwissenschaften 74:158–167

Lee KW, Chen PW, Lu CA, Chen S, Ho TH, Yu SM (2009) Coordinated responses to oxygen and sugar deficiency allow rice seedlings to tolerate flooding. Sci Signal 2:ra61

Lee SC, Mustroph A, Sasidharan R, Vashisht D, Pedersen O, Oosumi T, Voesenek LA, Bailey-Serres J (2011) Molecular characterization of the submergence response of the *Arabidopsis thaliana* ecotype Columbia. New Phytol 190:457–471

Lichtenthaler HK (1988) In vivo chlorophyll fluorescence as a tool for stress detection in plants. In: Lichtenthaler HK (ed) Applications of chlorophyll fluorescence. Kluwer Academic, Dordrecht, pp 129–142

Lichtenthaler HK (1996) Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol 148:4–14

Loreti E, Yamaguchi J, Alpi A, Perata P (2003) Sugar modulation of α-amylase genes under anoxia. Ann Bot 91:143–148

Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X, Xu S, Niu Y, Jin J, Zhang H, Xu X, Li L, Wang W, Qian Q, Ge S, Chong K (2015) COLD1 confers chilling tolerance in rice. Cell 160:1209–1221

Maathuis FJM, Sanders D (2001) Sodium uptake in *Arabidopsis thaliana* roots is regulated by cyclic nucleotides. Plant Physiol 127:1617–1625
Magneschi L, Perata P (2009) Rice germination and seedling growth in the absence of oxygen. Ann Bot 103:181–196
Martin M, Albanesi D, Alzari PM, de Mendoza D (2009) Functional in vitro assembly of the integral membrane bacterial thermosensor DesK. Protein Expr Purif 66:39–45
Matsumoto TK, Ellsmore AJ, Cessna SG, Low PS, Pardo JM, Bressan RA, Hasegawa PM (2002) An osmotically induced cytosolic Ca2+ transient activates calcineurin signaling to mediate ion homeostasis and salt tolerance of *Saccharomyces cerevisiae*. J Biol Chem 277:33075–33080
Miller G, Mittler R (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann Bot 98:279–288
Miquel M, James D Jr, Dooner H, Browse J (1993) Arabidopsis requires polyunsaturated lipids for low-temperature survival. Proc Natl Acad Sci U S A 90:6208–6212
Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? Trends Biochem Sci 37:118–125
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Osakabe Y, Maruyama K, Seki M, Satou M, Shinozaki K, Yamaguchi-Shinozaki K (2005) Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in Arabidopsis. Plant Cell 17:1105–1119
Pandey S, Nelson DC, Assmann SM (2009) Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell 136:21–23
Pareek A, Singh A, Kumar M, Kushwaha HR, Lynn AM, Singla-Pareek SL (2006) Whole genome analysis of *Oryza sativa* L. reveals similar architecture of two-component-signaling machinery with Arabidopsis. Plant Physiol 142:380–397
Redondo-Gómez S (2013) Abiotic and biotic stress tolerance in plants. In: Rout GR, Das AB (eds) Molecular stress physiology of plants. Springer, New Delhi, pp 1–20
Rodriguez MCS, Petersen M, Mundy J (2010) Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol 61:621–649
Saidi Y, Finka A, Muriset M, Bromberg Z, Weiss YG, Maathuis FJ, Goloubinoff P (2009) The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. Plant Cell 21:2829–2843
Sajid M, Rashid B, Ali Q (2018) Mechanisms of heat sensing and responses in plants. It is not all about Ca^{2+} ions. Biol Plant 62:409
Sangwan V, Foulds I, Singh J, Dhindsa RS (2001) Cold-activation of Brassica napus *BN115* promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca^{2+} influx. Plant J 27:1–12
Sasidharan R, Hartman S, Liu Z, Martopawiro S, Sajeev N, van Veen H, Yeung E, Voesenek LACJ (2018) Signal dynamics and interactions during flooding stress. Plant Physiol 176:1106–1117
Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138:32
Sewelam N, Kazan K, Schenk PM (2016) Global plant stress signaling: reactive oxygen species at the cross-road. Front Plant Sci 7:187
Shi Y, Tian S, Hou L, Huang X, Zhang X, Guo H, Yang S (2012) Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in Arabidopsis. Plant Cell 24:2578–2595
Singh AK, Ansari MW, Pareek A, Singla-Pareek SL (2008) Raising salinity tolerant rice: recent progress and future perspectives. Physiol Mol Biol Plants 14:137–154
Tran LS, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. Proc Natl Acad Sci U S A 104:20623–20628
Tunc-Ozdemir M, Tang C, Ishka MR, Brown E, Groves NR, Myers CT, Rato C, Poulsen LR, McDowell S, Miller G, Mittler R, Harper JF (2013) A cyclic nucleotide-gated channel (CNGC16) in pollen is critical for stress tolerance in pollen reproductive development. Plant Physiol 161:1010–1020

Upreti KK, Sharma M (2016) Role of plant growth regulators in abiotic stress tolerance. In: Srinivasa Rao NK, Shivashankara KS Laxman RH (eds) Abiotic stress physiology of horticultural crops. Springer, New Delhi, pp 19–46

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell 11:1743–1754

Vashisht D, Hesselink A, Pierik R, Ammerlaan JM, Bailey-Serres J, Visser EJ, Pedersen O, Van Zanten M, Vreugdenhil D, Jamar DC, Voesenek LA (2011) Natural variation of submergence tolerance among *Arabidopsis thaliana* accessions. New Phytol 190:299–310

Ve'zina LP, Ferullo JM, Laliberté G, Laberge S, Willemot C (1997) Chilling and freezing. In: MNV P (ed) Plant ecophysiology. Wiley, New York, pp 61–100

Voesenek LACJ, Bailey-Serres J (2013) Flooding tolerance: O_2 sensing and survival strategies. Curr Opin Plant Biol 16:647–653

Voesenek LACJ, Pierik R, Sasidharan R (2015) Plant life without ethylene. Trends Plant Sci 20:1–3

Volkov RA, Panchuk II, Mullineaux PM, Schöffl F (2006) Heat stress-induced H_2O_2 is required for effective expression of heat shock genes in Arabidopsis. Plant Mol Biol 61:733–746

Vu LD, Gevaert K, De Smet I (2019) Feeling the heat: searching for plant Thermosensors. Trends Plant Sci 24:210–219

Wohlbach DJ, Quirino BF, Sussman MR (2008) Analysis of the Arabidopsis histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20:1101–1117

Wu CH, Derevnina L, Kamoun S (2018) Receptor networks underpin plant immunity. Science 360:1300–1301

Xiong L, Zhu JK (2003) Regulation of abscisic acid biosynthesis. Plant Physiol 133:29–36

Yamaguchi T, Aharon GS, Sottosanto JB, Blumwald E (2005) Vacuolar Na^+/H^+ antiporter cation selectivity is regulated by calmodulin from within the vacuole in a Ca^{2+}- and pH-dependent manner. Proc Natl Acad Sci U S A 102:16107–16112

Ye Y, Ding Y, Jiang Q, Wang F, Sun J, Zhu C (2017) The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. Plant Cell Rep 36:235–242

Yeung E, van Veen H, Vashisht D, Paiva AL, Hummel M, Rankenberg T, Steffens B, Steffen-Heins A, Sauter M, de Vries M, Schuurink RC (2018) A stress recovery signaling network for enhanced flooding tolerance in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 115:E6085–E6094

Zhang W, Zhou RG, Gao YJ, Zheng SZ, Xu P, Zhang SQ, Sun DY (2009) Molecular and genetic evidence for the key role of AtCaM3 in heat-shock signal transduction in Arabidopsis. Plant Physiol 149:1773–1784

Charanpreet Kaur – see under Chapter 1 contributions.

Ashwani Pareek – see under Chapter 10 contributions.

Sneh Lata Singla-Pareek obtained her Ph.D. from UDSC. She completed her postdoctoral work at the University of North Carolina, USA, and is currently the Group Leader of the Plant Stress Biology group at ICGEB, New Delhi, where she has been a Scientist since 2001. Her current work entails crop improvement with regard to abiotic stress tolerance, grain quality, and yield of rice. Her approaches include genetic engineering, gene pyramiding, and genome editing. She has known the Editor since her doctoral days and they have worked together.

Part II

Cellular Machinery for Decoding and Transmitting the Information

"One must ask children and birds how cherries and strawberries taste"

Johann Wolfgang Von Goethe

"A weed is a plant that has mastered every survival skill except for learning how to grow in rows"

Doug Larson

Heterotrimeric G-Protein Signaling in Plants

8

Sona Pandey

Abstract

Heterotrimeric GTP-binding proteins comprised of Gα, Gβ, and Gγ subunits are key regulators of a multitude of signaling pathways in all eukaryotes. In plants, these proteins are currently a focus of intense research due to their involvement in affecting many agronomically important traits such as seed yield, organ size, abscisic acid (ABA)-dependent signaling and stress responses, plant defense responses, symbiosis, and nitrogen use efficiency. The mechanistic details of G-protein signaling in modulating these processes remain largely unknown.

The core G-protein components and their activation/deactivation chemistries are broadly conserved all through the eukaryotic evolution; however, their regulatory mechanisms seem to have been rewired in plants to meet specific needs. A set of plant-specific G-protein components also exist that provide a new dimension to this well-characterized signaling pathway. The availability of extensive biochemical data, genetic resources, and sequence information from a variety of plant species has made it possible to compare the G-protein signaling pathways across phyla and between different plant species. Work done in the past two decades has established some of the norms of G-protein signaling in plants and sprung some surprises. This article provides a detailed account of G-protein signaling pathways in plants, their mechanistic details, how they might differ from the classical paradigm, and their importance in manipulating specific responses to generate plants for future needs.

S. Pandey (✉)
Donald Danforth Plant Science Center, St. Louis, MO, USA
e-mail: spandey@danforthcenter.org

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_8

Keywords
G-protein-coupled receptor (GPCR) · GTPase activity-accelerating protein (GAP) · GTPase · Guanine nucleotide exchange factor (GEF) · Heterotrimeric G-protein · Phospholipase · Receptor-like kinase (RLK) · Regulator of G-protein signaling (RGS)

8.1 The Heterotrimeric G-Protein Cycle

Heterotrimeric G-proteins are key plasma membrane-localized signal transducers in all eukaryotes. The heterotrimeric G-protein complex (G-proteins, hereafter) consists of three dissimilar subunits Gα, Gβ, and Gγ. The name "G-proteins" comes from the ability of the Gα protein to bind and hydrolyze guanine (G) nucleotides. As per the established paradigm of G-protein signaling, based on exhaustive studies in mammalian systems, when the Gα protein is GDP bound it is associated with the Gβγ dimer. This trimeric complex associates with an inactive G-protein-coupled receptor (GPCR) and represents the resting stage of signaling through these proteins. Signal perception or ligand binding at the GPCR causes a change in its conformation, which affects the conformation of the associated G-protein heterotrimer, resulting in a decrease in the affinity of Gα protein for the bound GDP (Fig. 8.1). Because cells have a much higher concentration for GTP in the cytosol and Gα has a significantly higher affinity for GTP compared to GDP, the GDP on Gα is replaced by GTP. The GPCRs therefore essentially act as guanine nucleotide exchange factors (GEFs), i.e., they facilitate an exchange of GTP for GDP on the Gα protein. GTP binding results in dissociation of the heterotrimeric complex into active GTP-Gα and free Gβγ. Both these entities can interact with a number of downstream effectors to transduce the signal (Gilman 1987; McCudden et al. 2005; Siderovski and Willard 2005). This represents the active stage of signaling by the G-protein complex (Fig. 8.1). The Gα proteins also possess inherent GTPase activity; therefore, the bound GTP is hydrolyzed, leading to the regeneration of GDP-Gα. GDP-Gα has a high affinity for the Gβγ dimer, resulting in the reconstitution of the inactive heterotrimeric complex, thereby completing one signaling cycle. Thus, due to its GTP- and GDP-bound states, which define the active or inactive signaling states, respectively, Gα acts as a bimodal molecular switch (McCudden et al. 2005; Oldham and Hamm 2008). The G-protein cycle is also regulated by a number of accessory proteins, e.g., regulators of G-protein signaling (RGS) proteins, that increase the GTPase activity of Gα proteins (GTPase activity accelerating proteins (GAPs)) and proteins that have guanine nucleotide dissociation inhibition activity (GDI proteins) that maintain the Gα proteins in their inactive, GDP-bound form (Siderovski and Willard 2005). The position of G-proteins in the signaling pathways is critical as they directly couple the signal perception at the plasma membrane by GPCRs to the downstream intracellular effectors. The basic biochemistry of G-proteins is conserved across phyla, although the receptors, regulators and effectors seem to have diverged significantly during the course of evolution.

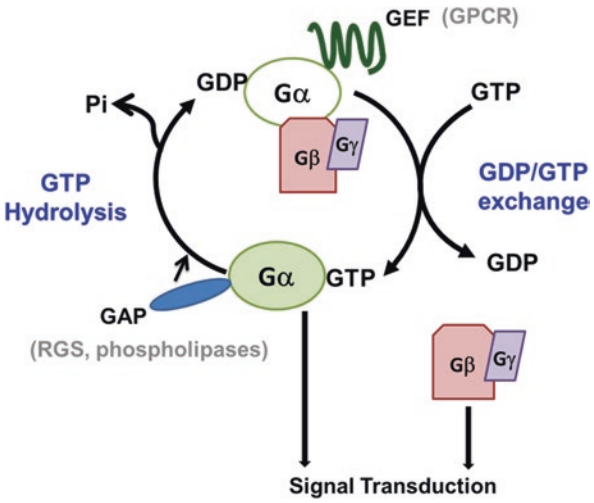

Fig. 8.1 The classical heterotrimeric G-protein cycle. In the GDP-bound form, Gα protein remains associated with the Gβγ dimer and represents the resting stage of G-protein signaling. Signal perception by a GPCR causes an exchange of GTP for GDP on Gα. This results in dissociation of the trimeric complex to active GTP-bound Gα and Gβγ, both of which can interact with downstream effectors to transduce the signal. The inherent GTPase activity of Gα protein, aided by other GTPase-activating proteins such as GS or PLCβ, promotes the hydrolysis of bound GTP and regeneration of GDP-bound Gα, which reassociates with the Gβγ dimer to complete one signaling cycle

8.2 A Brief History of G-Protein Signaling

8.2.1 Historical Accounts from Mammalian Systems

G-proteins have been a focus of intense research for a long time. The discovery of these proteins was guided by the early work of Earl Sutherland and Theodore Rall who were studying the mechanisms of hormone action in mammalian cells. This group showed conclusively that the hormones or 'the chemical signals' attach to specific molecules (receptors) on cell surfaces and the receptors transmit the information to the interior of the cell. They discovered cyclic AMP and the enzyme required for its production from ATP, adenylyl cyclase, in 1957 (Sutherland et al. 1962). Because adenylyl cyclase activity was hormone regulated, this system provided an assay to determine the hormone-induced activation of a potential receptor activity by quantifying the synthesis of an intracellular compound (cAMP). Sutherland named the hormones as 'the first messengers', which are then somehow transmitted inside the cells to produce 'the second messengers' such as cAMP and decode the information from the signal. This laid the foundation for the concept of transmembrane signaling. For his discoveries, Earl Sutherland received Nobel Prize in 1971 (Sutherland 1971).

In the late 1960s and early 1970s, Martin Rodbell and coworkers working at the National Institute of Health (USA) proposed a model comprising three functional components, which would be required for successful acquisition and transmission of biological signals such as hormones. Rodbell predicted that the first component would sense and discriminate a signal, i.e., a discriminator (receptor), whereas the last component would amplify this signal to generate a large quantity of second messenger, e.g., cAMP, inside the cell and act as an amplifier. He also predicted that the "discriminator" and "amplifiers" would be two distinct components and established the presence of a connecting component, "a transducer," that integrated the discriminator and amplifier functions. He demonstrated this "transducer" to be driven by guanosine 5′-phosphate activity (Rodbell 1992).

Meticulous work by Alfred Gilman and coworkers led to the discovery of the chemical nature of these "transducers." Using genetically altered leukemia cell lines, Gilman's group first conclusively showed the requirement of a transducer for signal transduction. They identified a mutated cell type that had a normal "receptor" and a normal "amplifier" protein but failed to respond to an outside signal. Carefully designed biochemical experiments performed over a decade by Elliott Ross and others in the Gilman lab led to the discovery of the protein that acted as a transducer. In 1980, the group purified this 'transducer protein' and named them G-proteins (Gilman 1987). When G-proteins isolated from the plasma membrane of normal cells were transferred to the defective leukemia cells that had the receptor and amplifier, they fully restored its ability to respond to external signals. For these seminal discoveries, Gilman and Rodbell received Nobel Prize in Physiology and Medicine in 1994 (Gilman 1995; Rodbell 1995). Dan Cassel and Zvi Salinger were first to report the presence of a hormone-stimulated GTPase activity which was associated with adenylyl cyclase activity in cells. In work spanning a decade (1977–1987), they confirmed the role of the GTPase activity in turning off the G-protein cycle (Selinger and Cassel 1981). At the same time, George Wheeler and Mark Bitensky established the parallels between hormone-induced G-protein signaling to light-dependent G-protein signaling, leading to the expansion of the field (Wheeler and Bitensky 1977).

The identification of potential receptors was also a very active field of research. The concept was first introduced by Paul Ehrlich (1854–1915) as specific binding sites on cell surfaces and was further developed by John Langley and Sir Henry Dale as "receptive substances." Work done in many laboratories in the 1970s and 1980s using radioisotope labeling of agonists and antagonists, quantification of binding affinities, etc., led to extensive biochemical characterization of these receptors (Gilman 1995). The first G-protein-coupled receptors (GPCRs) were purified in the laboratories of Robert Lefkowitz and Marc Caron, followed by the first cloning of a GPCR by Brian Kobilka in the Lefkowitz lab in 1986. Lefkovitz and Kolbika received the Nobel Prize in Chemistry for these and additional discoveries related to GPCR structure and function in 2012. With the availability of modern tools of cloning and purification of proteins in heterologous systems, and the availability of genome sequences and homology-based cloning, the field expanded and the G-proteins were discovered in all eukaryotic organisms regulating most aspects of growth and development (McCudden et al. 2005; Oldham and Hamm 2008). With the earlier discovery of G-proteins as targets of cholera toxin and pertussis toxin, which resulted in

major pathogenic symptoms in humans, to the current phase where GPCRs act as receptors for almost all sensory signals, neurotransmitters, and hormones, G-protein signaling is one of the most widely researched areas in human health industry. More than 60% of all pharmacological drugs available in the market are targeted at the G-protein/GPCR pathways (Siderovski and Willard 2005; Oldham and Hamm 2008).

8.2.2 History of G-Proteins in Plants

The discovery of G-proteins in the plant lineage was steered by their presence and importance in other organisms. Early on, experiments using various pharmacological compounds such as GTP and its non-hydrolyzable analog GTPγS and different G-protein agonists and antagonists, e.g., cholera and pertussis toxins or mastoparan, suggested the presence of G-protein activity in plants. Blue- and red-light-mediated responses in plants were some of the first to be proposed as controlled by G-proteins by several groups including that of the editor of this volume (Warpeha et al. 1991; Pingret et al. 1998; Raghuram et al. 1999). Several studies reported the effect of these pharmacological compounds on stomatal aperture regulation. These studies were supported by demonstrating the direct effect of these pharmacological compounds on the stomatal guard cell ion channel activities. Additional support came by quantifying gene expression changes in response to various hormones and exogenous cues (Raghuram et al. 1999). Pharmacological experiments also suggested the roles of G-proteins during nodule formation in legumes (Warpeha et al. 1991; Pingret et al. 1998).

The first G-protein genes were cloned from *Arabidopsis thaliana* by Hong Ma while working in Elliott Meyerwitz's lab in 1990 using homology-based approaches followed by the cloning of a Gβ gene in his lab (Ma et al. 1990). Soon after, G-protein sequences were cloned from multiple plant species including soybean, carrot, spinach, rice, maize and wheat, suggesting their widespread presence. The first molecular evidence for the role of G-proteins in controlling plant growth and development was reported in rice, where suppression of the *RGA* (rice Gα) gene resulted in plants with abnormal morphology and dwarfism (Fujisawa et al. 1999; Ueguchi-Tanaka et al. 2000).

The availability of sequenced genomes, first from Arabidopsis and then from rice, was an important turning point in the plant G-protein research and how it might be different from what was already known based on the mammalian systems. Even though the involvement of G-proteins was shown during regulation of a range of signaling pathways, the fully sequenced genomes of both these species showed only a single canonical Gα and Gβ gene each. This was in stark contrast to what is known from mammalian systems where each protein is present in multiple copies, e.g., 23 Gα and 5Gβ proteins in humans (McCudden et al. 2005; Siderovski and Willard 2005). The paucity of G-protein components in plants raised concerns about their suggested involvement in a multitude of plant growth and development pathways. However, the availability of critical genetic resources, especially the T-DNA knockout mutants in Arabidopsis, and additional resources in rice confirmed the pivotal roles of G-proteins in controlling almost all aspects of plant growth and development (Urano and Jones 2014). Table 8.1 summarizes the list of plant phenotypes or signaling pathways regulated by G-protein complex components. The list includes examples where genetic evidence exists.

Table 8.1 List of different biological responses regulated by G-protein complex in plants

Protein	Plant species	Pathway/phenotype	References
Gα	Rice	Suppression of the heterotrimeric G-protein resulted in abnormal morphology and dwarfism	Fujisawa et al. (1999)
Gα	Rice	Rice Gα (dwarf mutant d1) affects gibberellin signal transduction	Ueguchi-Tanaka et al. (2000)
Gα	Arabidopsis	Gα regulates ion channel activity and abscisic acid signaling in Arabidopsis guard cells	Wang et al. (2001)
Gα	Arabidopsis	Gα controls cell proliferation in Arabidopsis	Ullah et al. (2001)
Gα	Rice	Gα acts upstream of the small GTPase Rac in disease resistance of rice	Suharsono et al. (2002)
Gα	Arabidopsis	Gα regulates Arabidopsis seed germination in response to GA	Ullah et al. (2002)
RGS1	Arabidopsis	RGS protein modulates plant cell proliferation	Chen et al. (2003)
Gα GCR1	Arabidopsis	Overexpression of the putative G-protein-coupled receptor GCR1 affected DNA synthesis, seed germination, and flowering	Apone et al. (2003)
Gα	Arabidopsis	Seed germination and early seedling development was affected by interaction of a Pirin protein and Gα	Lapik and Kaufman (2003)
Gα	Arabidopsis	Role of Gα in regulating sphingolipid signaling in guard cells	Coursol et al. (2003)
Gβ	Arabidopsis	The Gβ protein regulates auxin-induced cell division and plant development	Ullah et al. (2003)
GCR1 Gα	Arabidopsis	GCR1 and Gα regulate GA- and brassinosteroid-dependent seed germination	Chen et al. (2004)
GCR1 Gα	Arabidopsis	GCR1 interacts with the Gα to regulate abscisic acid signaling	Pandey and Assmann (2004)
Gα Gβ	Arabidopsis	G-protein from Arabidopsis is required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina*; evidence for G-proteins working in a receptor-like kinase-regulated pathway	Llorente et al. (2005)
Gα	Rice	Gα is involved in rice brassinosteroid response	Wang et al. (2006)
Gα Gβ	Arabidopsis	Roles of G-proteins in modulating cell division in roots	Chen et al. (2006)
Gα	Arabidopsis	Role of Gα in ABA-dependent stomatal closure and opening	Mishra et al. (2006)
Gα	Arabidopsis	Role of Gα in a novel sugar-signaling pathway	Huang et al. (2006)

(continued)

Table 8.1 (continued)

Protein	Plant species	Pathway/phenotype	References
Gα Gβ	Arabidopsis	Role of Gα and Gβ in ABA-dependent germination and post-germination development	Pandey et al. (2006)
Gα GCR1	Arabidopsis	Role of GCR1 and Gα in blue-light-induced production of phenylalanine in etiolated seedlings	Warpeha et al. (2006)
Gα Gβ	Arabidopsis	G-proteins are involved in resistance to necrotrophic pathogens and JA signaling	Trusov et al. (2006)
Gα	Arabidopsis	Gα participates in pollen germination through modulation of a plasma membrane Ca^{2+}-permeable channel	Wu et al. (2007)
G-proteins	Pea	Role of G-protein complex in salinity and heat stress	Misra et al. (2007)
Gα Gβ	Arabidopsis	Gα and Gβ antagonistically modulate stomatal density	Zhang et al. (2008a)
G-proteins	Arabidopsis	G-proteins modulate innate immunity response in stomatal guard cells via ion channel regulation	Zhang et al. (2008b)
Gβ RGS1	Arabidopsis	Gβ and RGS regulate ion channel activity in guard cells in response to ABA	Fan et al. (2008)
Gα	Arabidopsis	Gα modulates BR signaling and biosynthesis	Gao et al. (2008)
G-proteins XLGs	Arabidopsis	G-proteins and XLGs regulate root morphogenesis	Ding et al. (2008)
G-proteins XLGs	Arabidopsis	G-proteins and XLGs regulate root-wave response	Pandey et al. (2008)
Gβγ	Arabidopsis	Regulation of auxin transport-dependent root system architecture	Mudgil et al. (2009)
XLG Gβ	Arabidopsis	Role in regulation of bacterial defense response	Zhu et al. (2009)
Gβ	Arabidopsis	Mediates pre-invasion resistance to *Magnaporthe oryzae*	Maeda et al. (2009)
Gα	Rice	Gα is required for epidermal cell death in rice	Steffens and Sauter (2009)
Gα	Arabidopsis	Gα affects jasmonate responses	Okamoto et al. (2009)
G-proteins	Arabidopsis	Role of G-proteins in NO-, H_2O_2-, and calmodulin-dependent stomatal closure	Trusov et al. (2009)
Gα	Arabidopsis	Gα suppresses the ftsh-mediated inhibition of chloroplast development	Zhang et al. (2009)
G-protein	Arabidopsis	G-proteins modulate light sensitivity during seed germination	Botto et al. (2009)
Gα	Rice	Function of Gα in BR signaling	Oki et al. (2009)
G-proteins	Arabidopsis	Auxin-mediated lateral root formation	Booker et al. (2010)

(continued)

Table 8.1 (continued)

Protein	Plant species	Pathway/phenotype	References
Gα	Arabidopsis	Gα is a regulator of transpiration efficiency	Nilson and Assmann (2010a)
Gα	Arabidopsis	Gα regulates reproductive trait plasticity in response to water availability	Nilson and Assmann (2010b)
Gβ	Rice	Suppression of the rice Gβ causes dwarfism and browning of internodes and lamina joints	Utsunomiya et al. (2011)
Gγ	Arabidopsis	Group III γ-subunit regulates guard cell K^+-channel activity and plant morphology	Chakravorty et al. (2011)
G-proteins	Arabidopsis	G-proteins regulate ROS signaling and calcium currents in guard cells	Zhang et al. (2011)
Gα	Arabidopsis	Gα interacts with cry1 in hook opening and anthocyanin synthesis	Fox et al. (2012)
Gγ	Arabidopsis	Group III γ-subunit influences organ size and shape	Li et al. (2012a)
XLG2	Arabidopsis	XLG2 regulates activation of floral integrator genes and early flowering	Heo et al. (2012)
Gα	Arabidopsis	Extracellular ATP promotes stomatal opening through Gα and ROS	Hao et al. (2012)
G-proteins	Arabidopsis	G-proteins may not have a role in ozone-induced changes in plant physiology	Hao et al. (2012)
G-proteins	Arabidopsis	G-proteins regulate cell wall defense and resistance to necrotrophic fungi	Delgado-Cerezo et al. (2012)
G-proteins	*Nicotiana benthamiana*	Regulation of plants' response to different elicitors	Zhang et al. (2012)
G-proteins	Arabidopsis	G-proteins have a role in host and nonhost resistance against *Pseudomonas syringae*	Lee et al. (2013)
Gβ	Arabidopsis	Gβ interacts with an adaptor protein AP-3μ to control ABA regulation of germination and post-germination development	Kansup et al. (2013)
Ga	Maize	Gα functions in CLAVATA signaling to control shoot meristem size	Bommert et al. (2013)
Gβ	Arabidopsis	Gβ regulates BR signaling independently of BZR1	Tsugama et al. (2013a)
G-proteins	Arabidopsis	The role of G-proteins in MLO2 function and MAMP-triggered immunity	Lorek et al. (2013)
Gα	Rice	U-box E3 ubiquitin ligase TUD1 functions with a Gα to regulate BR-regulated growth	Hu et al. (2013)
Gβ	Arabidopsis	G-proteins function with NADPH oxidases	Torres et al. (2013)

(continued)

Table 8.1 (continued)

Protein	Plant species	Pathway/phenotype	References
G-proteins	Arabidopsis	G-proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases	Liu et al. (2013)
G-proteins	Soybean	G-proteins play important roles during nodulation in soybean	Roy Choudhury and Pandey (2013)
Gα	Arabidopsis	Gα protein, H_2O_2, and NO regulate ultraviolet B-induced stomatal closure	He et al. (2013)
Gβ	Arabidopsis	G-proteins control stem cell proliferation through CLAVATA signaling	Ishida et al. (2014)
Gα	Rice and maize	Gα modulates salt-induced cellular senescence and cell division	Urano et al. (2014)
G-proteins RGS	Arabidopsis	G-protein complex mediates growth attenuation under saline stress	Colaneri et al. (2014)
G-proteins	Rice	G-proteins regulate nitrogen use efficiency in rice	Sun et al. (2014)
Gβ	Arabidopsis	Gβ interacts with NPH3 and regulates phototropism	Kansup et al. (2014)
Gγ	*Camelina sativa*	Group III Gγ overexpression results in increased seed and oil production and improved stress tolerance	Roy Choudhury et al. (2014a)
G-protein complex	soybean	Control of G-protein cycle regulates nodulation	Roy Choudhury and Pandey (2015)
Gα, GCR1	Arabidopsis	Gα and GCR1 regulate stress, nitrate and phosphate response, flavonoid biosynthesis, fruit/seed development	Chakraborty et al. (2015a)
Gβγ	Rice	Gβγ proteins play distinct roles in ABA responses and drought adaptation	Xu et al. (2015)
Gα	Maize	Gα controls prolificacy potential in maize	Urano et al. (2015b)
Gβ	Arabidopsis	Gβ controls salinity response	Yu and Assmann (2015)
Gα	Arabidopsis	G-protein mediates ethylene-induced stomatal closure via H_2O_2 synthesis	Ge et al. (2015)
Gβ	Arabidopsis	Gβ negatively regulates the ABA response and drought tolerance by modulating MAPK pathway	Xu et al. (2015)
Gβγ XLG	Arabidopsis	XLG proteins and Gβγ modulate plant immunity	Maruta et al. (2015)
Gγ	Barley	HvDep1, a group III Gγ protein regulates culm elongation and grain size	Wendt et al. (2016)
Gβ	Arabidopsis	Regulation of root system architecture via photosynthates	Mudgil et al. (2016)

(continued)

Table 8.1 (continued)

Protein	Plant species	Pathway/phenotype	References
XLG, Gβ	Moss (*P. patens*)	G-proteins regulate gametophyte growth and sporophyte formation in moss	Hackenberg et al. (2016)
G-proteins	Arabidopsis	G-proteins regulate plant immunity by directly coupling to the FLS2 receptor	Liang et al. (2016)
Group II Gγ	Tomato	G-protein γ-subunit regulates auxin and ABA signaling	Subramaniam et al. (2016)
Gα	*Chlamydomonas reinhardtii*	Gα is involved in regulation of resistance to heat and osmotic stress	Lee et al. (2017)
XLG	Arabidopsis	XLGs modulate cytokinin-dependent developmental processes	Wang et al. (2017)
Gγ	Rice	G-protein γ subunit RGG1 provides salinity stress tolerance by elevating detoxification of ROS	Swain et al. (2017)
Gα	Rice	Gα is involved in photoprotection and photoavoidance	Ferrero-Serrano et al. (2018)
Gβ	Arabidopsis	The G-protein β subunit, AGB1, interacts with FERONIA to control RALF1-regulated stomatal movement	Yu et al. (2018)
Gγ	Rice	Different alleles of group III Gγ control seed size	Sun et al. (2018)
G-proteins	Arabidopsis	Regulation of immune responses	Liang et al. (2018)

Only examples where at least some genetic or biochemical evidence exist are listed

8.3 Conserved and Novel G-Protein Complex Components in Plants

Plants represent a unique variation to the established paradigm of G-protein signaling. The core of G-protein components and their fundamental biochemistry in plants is similar to the metazoan systems. The complex contains three subunits, Gα, Gβ, and Gγ. The Gα can bind and hydrolyze GTP. The Gβ and Gγ form a non-dissociable dimer. When Gα is in its GDP-bound form, it is associated with the Gβγ dimer and represents the inactive stage of signaling. When GDP on Gα is exchanged for GTP, it dissociates from the Gβγ dimer, and both entities can interact with downstream effectors to transduce the signal, representing an active state. The GTPase activity of Gα causes hydrolysis of bound GTP to GDP and consequently its association with the Gβγ dimer to complete one signaling cycle (Fig. 8.1). However, work done in the past several years has highlighted certain differences, both in the components and in the regulation. These are discussed in the following sections.

8.3.1 Conserved G-Protein Components

One of the most obvious features of the plant G-protein signaling is the paucity of its components. Most sequenced plant genomes have a single canonical Gα and Gβ protein, with few Gγ proteins. For example, both the Arabidopsis and rice genomes encode 1 Gα, 1 Gβ and few Gγ proteins (Urano and Jones 2014). This suggests that all the diversity in the signaling pathways regulated by these proteins arises from the diversity of Gγ proteins. This was earlier thought to be true based on the analysis of the *agg1*, *agg2* and *agg3* mutants of Arabidopsis (Urano and Jones 2014). This may however be an oversimplification of the situation. The sequencing of more complex plant genomes such as soybean, wheat, and Camelina has shown that in polyploid plants, multiple G-protein genes that arose due to genome duplications have been maintained in the genome (Bisht et al. 2011; Roy Choudhury et al. 2011; Hackenberg et al. 2017). For example, the soybean genome codes for four Gα and four Gβ proteins, with additional splice variants. Because most plants are polyploids, it predicts a similar expansion of the G-protein genes in their genomes suggesting that the paucity of the G-protein subunits in model plant species may not be a true representation of the actual situation. There is already evidence that these highly similar G-proteins can result in specificity of response regulation as has been shown for the soybean Gα proteins (Roy Choudhury et al. 2014b; Roy Choudhury and Pandey 2017b).

8.3.2 Novel, Plant-Specific G-Protein Components

The presence of certain novel components in plants, which either exhibit a variation of the core G-protein components or are functionally integrated to the G-protein cycle, is an interesting feature of the plant G-protein signaling. The classic examples of plant specific G-protein components include the extra-large Gα (XLG) proteins and the group III Gγ proteins.

8.3.2.1 Extra-large Gα Protein

The extra-large Gα proteins, as the name suggests, are larger variants of the canonical Gα proteins. The C-terminal region of these proteins is similar to the Gα proteins (~22% identity with canonical Gα), but they also possess a large N-terminal extension of 300–500 amino acids (Ding et al. 2008; Pandey et al. 2008). The N-terminal region does not have any special features, except for the presence of a nuclear localization signal. XLG proteins are localized in the nucleus in addition to the plasma membrane (Ding et al. 2008; Pandey et al. 2008). The Arabidopsis genomes code for 3 XLG proteins, XLG1, XLG2, and XLG3, while 5–12 XLG proteins are present in other plants. The proteins are also found in the basal plant *Physcomitrella patens* (moss) as well as in all higher plants.

The role of XLG proteins as authentic Gα was debated for a long time mostly due to the absence of few seemingly critical amino acid residues that are required for GTP-binding and GTPase activity of canonical Gα proteins (Urano and Jones 2014). The first genetic evidence of XLG proteins working together with the established G-protein signaling components was shown using the Arabidopsis mutants lacking all three *XLG* genes (*xlg* triple). The triple mutants showed similar phenotypes as the Arabidopsis Gβ mutant (*agb1*) during root growth and in abscisic acid (ABA) sensitivity (Ding et al. 2008). One of the XLG proteins, XLG3, was shown to work with AGB1 to regulate root waving and skewing responses (Pandey et al. 2008). Recent work using biochemical analysis of XLG proteins' GTP-binding activities and their interactions with AGB1 protein has confirmed their role as a part of the G-protein trimer in plants (Zhu et al. 2009; Heo et al. 2012; Chakravorty et al. 2015; Wang et al. 2017). The most definitive evidence came from the role of XLG proteins in the moss *P. Patens*. This moss represents a unique example as it does not possess a canonical Gα gene, although the Gβ and Gγ genes similar to what is present in the other plants (and mammals) exist in its genome (Hackenberg et al. 2016). It does, however, possess an XLG gene and therefore provides an opportunity to explore the role of an XLG protein either by itself or in combination with a Gβ protein, without any interference of a canonical Gα. *P. patens* mutants lacking either the *XLG* gene or one of the two *Gβ* genes exhibited identical phenotypes, i.e., the mutants form gametophytes that do not elongate as much as the wild-type plants and have fewer leaves. Moreover, these mutants fail to form any sporophytes, the only diploid stage in moss's life cycle (Hackenberg et al. 2016). This suggests that in this basal plant, the G-proteins are required for normal life cycle completion. Orthologous genes from Arabidopsis (*AtXLG2* and *AGB1*) can completely rescue the phenotypes of the mutant moss, suggesting that the genes are true G-protein paralogs (Hackenberg et al. 2016). Because multiple copies of XLG genes are present in the genomes of all higher plants, the confirmation of their roles as a part of authentic G-protein heterotrimers has greatly expanded the number of components and diversity of G-protein networks in plants.

8.3.2.2 Novel Gγ Proteins

Plants possess both canonical (metazoan-like) and variant Gγ proteins which have been categorized in three distinct groups. The group I Gγ proteins are the classic Gγ proteins found in all organisms. These proteins are 100–120 amino acids in length and have a coiled-coil domain in the middle with a conserved DPLL motif and few conserved amino acids, which are required for their interaction with the Gβ proteins. These group I Gγ proteins also have a prenylation motif CXXL (where X is any aliphatic amino acid) at their C-terminal. Prenylation of this motif is required for the attachment of the Gγ proteins to the plasma membrane (Roy Choudhury et al. 2011). Group I Gγ proteins are represented by AGG1 and AGG2 in Arabidopsis, RGG1 in rice and GmGγ1-4 in soybean. A plant-specific variation of group I Gγ proteins is the group II Gγ proteins which are present in all plants except those of the Brassicaceae family. These proteins are very similar to the type I Gγ proteins but they lack the signature C-terminal prenylation motif. This motif has been shown to

be of critical importance in mammalian systems. Homologs of this protein are missing in Arabidopsis but are represented by RGG2 in rice and GmGγ5-7 in soybean (Roy Choudhury et al. 2011). In tomato, the group II Gγ protein has been shown to be involved in plant-microbe interaction, similar to what is reported for the group I Gγ proteins (Subramaniam et al. 2016).

The higher plant-specific group III Gγ proteins, represented by Arabidopsis AGG3, rice DEP1, GS3 and GCA2 and soybean GmGγ8-10, are at least twice as large as the group I or group II proteins and have a unique modular architecture (Roy Choudhury et al. 2011). The N-terminal region of these proteins is similar in size and sequence to the canonical Gγ proteins. This region is connected to the C-terminal with a putative transmembrane (TM) domain. The C-terminal region of these proteins is of variable length (100–500 amino acids) and is extremely rich in amino acid Cys, which can account for up to 35% of total amino acids in this region (Roy Choudhury et al. 2011). Interestingly, there is an expansion of this C-terminal region in plants that have more than one homolog of group III Gγ protein, e.g., the three soybean proteins (GmGγ 8, 9, and 10) possess almost identical Gγ-like domain, but their C-terminal region is of different lengths, respectively (Roy Choudhury et al. 2011). This unique Cys-rich region has predicted segments showing some similarity to tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) and multiple repeats of the von Willebrand factor type C modules and a Sprouty domain, which are thought to be involved in large protein complex formation. These unique proteins work together with the conventional G-protein components and are involved in regulating multiple critical plant growth, development and yield traits (discussed later). Based on their distinctive features and a predicted extracellular localization of the C-terminal region, the proteins have been hypothesized to act as a receptosome (Wolfenstetter et al. 2015; Botella 2012); however, the identity of proteins with which they might interact or the signal they might perceive is not known.

8.3.2.3 Plant-Specific Phospholipases

Phospholipases are known to be a critical regulatory part of G-protein cycle in mammalian systems. PLCβ is an established accelerator of the GTPase activity of mammalian Gα proteins (GAP), similar to the RGS proteins. Although the RGS proteins and the PLCβ bind at the distinct regions of a Gα protein, they alter its conformation that increases the rate of GTP hydrolysis by Gα (McCudden et al. 2005; Ross 2011).

Conventional PLCβ homologs are not found in plants, but another class of phospholipases, the phospholipase Dα family, has been shown to interact with and regulate the activity of Gα protein in Arabidopsis, exemplifying another variation to the established norm (Zhao and Wang 2004; Mishra et al. 2006). The role of PLDα1 to affect the GTPase activity of Gα was shown first using biochemical approaches. Additional biochemical and molecular genetic studies have confirmed the role of this unique enzyme in regulating the plant G-protein cycle. Plants have a large family of phospholipases (Pandey 2016; Roy Choudhury and Pandey 2016b), but it is not known if more of these enzymes can also modulate the G-protein cycle.

However, if it turned out to be a general regulatory mechanism, involving additional phospholipases (and not specific to PLDα1), it will provide a great degree of flexibility to the regulation of G-protein cycle in plants.

8.3.2.4 Receptor-Like Kinases (RLKs)

Work done in the last few years has provided multiple lines of evidence that the plant G-proteins may interact with the RLKs to integrate signals from multiple cues (Liu et al. 2013; Aranda-Sicilia et al. 2015; Yu et al. 2016, 2018). This is interesting as the metazoan G-proteins exclusively interact with the seven transmembrane possessing GPCRs, which are prevalent in these species, e.g., more than 800 GPCRs in humans. The activation/deactivation mechanisms of GPCRs have been studied in exquisite details, and the crystal structure of GPCRs in ligand bound and unbound form has been deciphered. Plants possess few such receptors and whether they act as GEFs is not known. Intriguingly, multiple genetic screens have uncovered specific RLKs in screens for G-protein-dependent pathways. A classic example is the identification of ERECTA as a G-protein suppressor (Llorente et al. 2005). Additional experiments using plant-microbe interactions have also suggested the involvement of RLKs in G-protein signaling (Liu et al. 2013; Aranda-Sicilia et al. 2015; Liang et al. 2016). This is tantalizing as RLKs represent one of the largest gene family in plants (~600 in Arabidopsis) and are involved in sensing a variety of environmental, chemical and developmental cues (Gish and Clark 2011).

8.3.3 Missing G-Protein Components

Another unique feature of the plant G-protein complex is a complete lack of some of the components known to be central to the G-protein cycle as per the mammalian paradigm. These include enzymes such as adenylyl cyclases, PLCβ, proteins with GDI activity, receptors with GEF activity, β-arrestins, G-protein-coupled receptor kinases (GRKs), etc. (Siderovski and Willard 2005). One explicit example includes the lack of a canonical Gα gene in the moss *P. patens*, as discussed earlier (Hackenberg et al. 2016). However, the one protein that has shown an enigmatic presence across different plant species is the RGS protein. In all eukaryotic organisms, where there is a Gα protein, there is also an RGS protein as these are required for an effective deactivation of G-protein cycle (Anantharaman et al. 2011). Basal plants such as algae and bryophytes (excluding *P. patens*, but it is also missing a canonical Gα), ferns, gymnosperms, basal angiosperms, and all dicots have gene encoding RGS proteins in their genomes. However, most monocot plants, including the model monocots such as rice, *Brachypodium*, maize, wheat, and sorghum, do not have the gene encoding RGS protein in their genomes (Urano and Jones 2014). Few studies focused on a limited number of model organisms suggested that the monocots in general have lost the RGS gene, with the exception of *Setaria* sp., which acquired it by a horizontal gene transfer from some unknown ancestor (Urano et al. 2012a, 2015a). However, exhaustive data mining and phylogenetic analysis has established that RGS proteins are present in all monocot orders and are also lost,

randomly, in many monocot plants (Hackenberg et al. 2017). No correlation could be established between the pattern of the loss of RGS and presence of specific G-protein activity. In fact, the Gα proteins from the species that have lost the RGS proteins have maintained the ability to bind and deactivated by RGS proteins from heterologous systems. The interaction interface between Gα and RGS proteins is conserved across phyla because the GTPase activity of plant Gα proteins can be accelerated by mammalian RGS proteins and vice versa (Hackenberg et al. 2017). Why then certain plants of the monocot lineage have lost this important regulatory protein and what might replace it are an active area of ongoing research.

8.4 G-Protein Interactors and Effectors

In their role as "transducers" of signals, G-proteins are expected to interact with cell-surface localized receptors and with the intracellular effectors. As has been discussed in the previous sections, in mammalian systems GPCRs are the cognate receptors for G-proteins (Siderovski and Willard 2005). The identity of G-protein-coupled receptor and their activation mechanisms are yet to be fully established in plants, although the extant data suggests the existence of nonconventional mechanisms (Pandey 2017; Pandey and Vijayakumar 2018). Similarly, the effectors of mammalian Gα proteins are well defined. In fact, the mammalian Gα proteins are classified based on the types of effectors they activate or inhibit. For example, the Gαs (stimulatory Gα) stimulate adenylyl cyclases which increase the cAMP levels, whereas Gαi (inhibitory Gα) inhibits the adenylyl cyclase activity and decreases cAMP level (Reed 1990; Spiegel et al. 1991). Others such as Gαq activate PLCβ resulting in the increased levels of IP3 and cytosolic calcium. Additional effectors such as transducins and RhoA have also been reported (Reed 1990; Spiegel et al. 1991). The most well-characterized effectors of Gβγ proteins include G-protein-coupled inwardly rectifying K^+ (GIRK) channels, voltage-gated Ca^{2+} channels, the SNARE complex, PLCβ, and phosphoinositide 3-kinase γ (PI3Kγ) (Siderovski and Willard 2005). Incidentally, many of these effectors are not found in plant systems.

Large-scale protein-protein interaction screens with specific G-protein components have identified several proteins that interact with the Gα, XLG or Gβγ proteins (Klopffleisch et al. 2011; Jones et al. 2014; Liang et al. 2017). In some cases, the interactions have been confirmed by the follow up genetic analyses of mutant combinations or by analyses of plant phenotypes. The first large scale G-protein interaction analysis was performed using multiple Arabidopsis libraries in a yeast-2-hybrid interaction system. This analysis identified some previously identified interaction partners, as well as many proteins that were not known to work in G-protein signaling pathways, which may be specific to plants. For example, a large number of G-protein interacting proteins identified in this study were related to cell wall formation (Klopffleisch et al. 2011). Another large-scale screen looking at the plasma membrane interactome (protein-protein interaction network of plasma membrane-localized proteins) also included the Arabidopsis GPA1 and AGB1 proteins as baits. Using the split-ubiquitin system (a modified version of yeast-2-hybrid,

more suitable for the membrane proteins), this screen identified 4 GPA1-interacting proteins and 39 AGB1-interacting proteins (Jones et al. 2014). Finally, a recent yeast-2-hybrid careen with the XLG proteins as bait has also uncovered several potential interactors of these proteins (Liang et al. 2017). A small subset of these proteins have been used for further characterization and seem to function in G-protein-regulated pathways. However, whether they fit the definition of G-protein effectors is still an open question.

In addition to these large-scale screens, several other studies have identified specific proteins that might interact with the Gα, XLG or Gβ proteins (Table 8.2). Only in some cases, a direct pathway from G-protein activity to response regulation via an interacting protein has been described. For example, in case of PLDα1, the protein has been shown to interact with both Gα and Gβ proteins in Arabidopsis. In case of Gα, it has been experimentally demonstrated that the protein biochemically and genetically interacts with the G-protein-dependent ABA signaling and plant development pathway (Roy Choudhury and Pandey 2016b, 2017a). Similarly, in case of ADT1 and Pirin, a small network connecting Gα, Pirin1, and ADT3 has been described for the early seedling growth and development in Arabidopsis (Warpeha et al. 2006). Additional examples where protein-protein interactions have been confirmed are listed in Table 8.2.

8.5 Regulation of G-Protein Cycle

The cyclic nature of G-protein signaling requires it to function in continuation (Fig. 8.1). There are multiple factors, which, together with the G-proteins themselves, regulate the rate and continuity of this cyclic process. The activation of the cycle depends on the rate of GTP/GDP exchange, the rate of GDP dissociation from Gα, and the rate of GTP binding on the Gα protein. The deactivation is dependent on the rate of GTP hydrolysis by Gα as well as the activity of additional accessory proteins that help accelerate this activity (Pandey 2017; Pandey and Vijayakumar 2018). Our knowledge of the activation and deactivation mechanisms of G-protein signaling is largely modeled after on what is known from the studies in the mammalian systems, but work done in the past 10 years or so has revealed the mechanistic details of G-protein signaling in plants. These data suggest that both conserved and unique signaling and regulatory mechanisms operate during plant G-protein signaling, owing to the presence of both conserved and unique G-protein signaling components and inherent properties.

8.5.1 G-Protein Activation Mechanisms in Plants

G-protein activation is a direct result of the GEF activity of GPCRs in metazoan systems, which are missing from the plant systems. There are several proteins that have seven transmembrane topology and features similar to the mammalian GPCRs, and many of these “potential” GPCRs also interact with the plant Gα proteins

Table 8.2 A list of proteins that interact with different components of heterotrimeric G-proteins

Protein	Plant species	Pathway/phenotype	References
Gα	Arabidopsis	Gα interacts with AtPirin1 during early seedling growth and development	Lapik and Kaufman (2003)
Gα	Arabidopsis	GPA1 interacts with GCR1 to regulate ABA signaling	Pandey and Assmann (2004)
Gα	Arabidopsis	Gα interacts with ABI1 phosphatase	Mishra et al. (2006)
Gα	Arabidopsis	Gα interacts with thylakoid formation 1 in sugar signaling pathway	Huang et al. (2006)
Gα	Arabidopsis	Gα interacts with PD1 during blue-light-induced phenylalanine production	Warpeha et al. (2006)
Gα	Pea	Gα interacts with phospholipase C (PLCδ) to regulate stress responses	Misra et al. (2007)
Gα	Arabidopsis	GPA1 interacts with GTG proteins to regulate ABA signaling	Pandey et al. (2009)
Gα	Rice	Gα interacts with a ubiquitin ligase TUD1 to regulate BR signaling	Hu et al. (2013)
Gα	Arabidopsis	G-proteins interact with multiple RLKs during regulation of defense-related signaling	Liu et al. (2013), Aranda-Sicilia et al. (2015), Maruta et al. (2015), Liang et al. (2016) and Tunc-Ozdemir et al. (2016)
Gα	Maize	Maize proteins interact with CLAVATA signaling pathway	Bommert et al. (2013) and Ishida et al. (2014)
Gα and RGS	Soybean	Gα and RGS interact with Nod factor receptors for regulation of nodulation	Roy Choudhury and Pandey (2015)
Gα	Arabidopsis	Gα and Gβ both interact with PLDα1	Zhao and Wang (2004) and Roy Choudhury and Pandey (2016b)
		PLDα1 also interacts with RGS1	
		Both proteins regulate each other's biochemical activity	
XLG	Arabidopsis	XLG2 interacts with RTV2 to control vernalization and flowering	Heo et al. (2012)
XLG	Arabidopsis	XLGs interact with E3 ligases PUB4 and PUB2 and function in cytokinin and developmental processes	Wang et al. (2017)
Gβ	Arabidopsis	AGB1 interacts with ERECTA for regulation of disease responses	Llorente et al. (2005)
Gβ	Arabidopsis	Gβ interacts with NDL proteins to regulate auxin transport and root architecture	Mudgil et al. (2009)

(continued)

Table 8.2 (continued)

Protein	Plant species	Pathway/phenotype	References
Gβ	Arabidopsis	ARD1 is an effector of Gβ in Arabidopsis	Friedman et al. (2011)
Gβ	Arabidopsis	Gβ interacts with BZR1 and BES1 to regulate brassinosteroid signaling and cell elongation	Zhang et al. (2017) and Tsugama et al. (2013a)
Gβ	Arabidopsis	Gβ interacts with a bZIP protein VIP1	Tsugama et al. (2013b)
Gβ	Arabidopsis	Gβ interacts with an adapter protein AP-3μ to regulate ABA-dependent germination and post-germination development	Kansup et al. (2013)
Gβ	Arabidopsis	Gβ interacts with an NPH3 to regulate phototropism	Kansup et al. (2014)
Gβ	Arabidopsis	Gβ interacts with an RLK ZAR1 (zygotic arrest 1) to regulate plant development	Yu et al. (2016)
Gβ	Arabidopsis	Gβ interacts with BBX2 transcriptional activator to promote hypocotyl elongation	Xu et al. (2017a)
Gβ	Arabidopsis	Interacts with FERONIA RLK to control RALF1-regulated stomatal movement	Yu et al. (2018)

The interactions between G-protein core components are not listed in this table

(Gookin et al. 2008; Gookin and Bendtsen 2013). At least in few cases, their involvement has also been shown in the regulation of G-protein-dependent pathways (Apone et al. 2003; Pandey and Assmann 2004; Pandey et al. 2006; Warpeha et al. 2006; Chakraborty et al. 2015a, b). However, the lack of a demonstrated GEF activity has restricted their classification as GPCRs to date, and questions the real activation mechanisms of plant Gα proteins.

There could be three possible mechanisms for the activation of G-protein cycle in plants. The simplest would be that canonical, GEF-activity-possessing GPCRs exist in plants, but have not been identified yet. GPCRs are known to have a highly conserved topology, with the classic seven transmembrane regions and N-terminal outside the cell and C-terminal inside the cell configuration, but apart from this, there are not many other sequence features to define their activities (Siderovski and Willard 2005). Several such proteins exist in plants, and they interact with the Gα proteins. However, the highly hydrophobic and multi-transmembrane nature of these proteins makes their purification and biochemical characterization extremely challenging. None of these proteins have been characterized in exquisite detail similar to the metazoan systems. Therefore, although there is no data to support their role as GEF-activity-possessing GPCRs, it has not been proved with certainty that they do not possess such an activity. At least in the case of GCR1, the most well-characterized GPCR-like protein of Arabidopsis, there is significant genetic

evidence for its role in pathways regulated by the Arabidopsis Gα protein, GPA1 (Apone et al. 2003; Pandey and Assmann 2004; Pandey et al. 2006; Warpeha et al. 2006; Chakraborty et al. 2015a, b).

Another relatively extreme possibility is that the plant Gα proteins are self-activated and do not require a GPCR for their activation (Urano et al. 2012a, b). This hypothesis is based on some unusual biochemistry of the Arabidopsis GPA1. Under in vitro conditions, Arabidopsis GPA1 exhibits an extremely high rate of GTP binding (potentially an order of magnitude higher than the metazoan Gα) and displays a very slow GTPase activity. If such a situation exists in vivo, then due to the higher concentration of GTP in cells, a Gα protein will preferably remain in a GTP-bound conformation. This would therefore suggest a scenario, where a Gα protein remains active, unless it is deactivated, which is opposite of what is known based on the established paradigm (Urano et al. 2012a, b). While in vitro data support such a hypothesis, it's in vivo significance and applicability to the plant systems in general remain to be established. Highly similar Gα proteins (e.g., the four soybean Gα proteins which are more than 90% identical and are a result of recent genome duplication) exhibit relatively subtle differences in their in vitro GTP-binding and hydrolysis activities (Bisht et al. 2011). However, these differences are relevant for the regulation of biological responses. For example, when the four soybean Gα proteins are introduced in the Arabidopsis *gpa1* null mutants, only two of them can fully complement for all mutant phenotypes whereas the other two restore only a subset of responses (Roy Choudhury and Pandey 2017b). Similar cross-species complementation experiments in yeast *gpa1* mutant also led to surprising results. Two of the soybean Gα proteins, GmGα1 and GmGα4, could fully complement the yeast mutant phenotypes, whereas the other two could only complement it only partially (Roy Choudhury et al. 2014b). Because yeast has a classical GPCR-dependent Gα activation mechanism, the complementation of a yeast Gα mutant with plant proteins is especially meaningful because it shows that at least some plant Gα proteins can be activated by a classical GEF activity of a GPCR in a heterologous system (Roy Choudhury et al. 2014b). The extent to which these proteins are self-activated in yeast system and how might that affect their ability to restore yeast Gα function is not known. These observations however suggest that it may be premature to expect all plant Gα proteins to behave identical to Arabidopsis GPA1 and the proposed self-activation of GPA1 may not be a norm in the plant kingdom.

The third possibility is the activation of Gα proteins by receptors that are not classic GPCRs. There is increasing evidence that receptor-like kinases (RLKs) interact with the G-protein-coupled signaling pathways in plants (Roy Choudhury and Pandey 2016a). The expanse of these RLKs in plants (~600 in Arabidopsis) could easily explain the integration of a variety of signals to G-proteins. However, in most cases, the interaction between an RLK and a G-protein has been demonstrated by either protein-protein interaction assays, through genetic interactions during suppression screens, pathways analysis or analyses of mutant phenotypes. The most prevalent examples are from the defense-related signaling pathways where key receptors such as flagellin-sensitive 2 (FLS2), chitin elicitor receptor kinase 1 (CERK1), BRI1-associated receptor kinase 1 (BAK1), and BAK1-interacting

receptor 1 (BIR1) have been shown to genetically interact with canonical G-proteins in Arabidopsis (Liu et al. 2013; Aranda-Sicilia et al. 2015; Liang et al. 2016; Roy Choudhury and Pandey 2016a; Tunc-Ozdemir et al. 2016; Liang et al. 2018). The involvement of RLKs and G-proteins has also been shown during plant development. In maize, the Gα protein was identified as an interactor of Fea2 (CLAVATA-2) which is a receptor-like protein of CLAVATA (an RLK) pathway (Bommert et al. 2013; Ishida et al. 2014). Additional RLKs such as ERECTA, zygotic arrest 1 (ZAR1) and receptor-like protein kinase 2 (RPK2) also interact with G-proteins to regulate specific developmental pathways in Arabidopsis (Bommert et al. 2013; Ishida et al. 2014; Maruta et al. 2015; Yu et al. 2016; Xu et al. 2017b).

Evidence for the direct modulation of G-protein cycle (but not Gα activity) by RLKs came from the studies in soybean during nodule formation (Roy Choudhury and Pandey 2015). The soybean Nod factor receptors (NFRs) are a class of lysine (Lys) motif-containing RLKs, which perceive the Nod factors secreted by rhizobia to initiate nodule formation (Broghammer et al. 2012). The NFRs interacted with both Gα and RGS proteins of soybean. Although no difference in the activity of Gα proteins was observed upon this interaction, the NFRs affected the activity of RGS proteins. NFRs, which are active kinases, phosphorylate the RGS proteins, which results in their higher GAP activity toward the Gα protein. In such a scenario, even though the Gα activity per se is not affected, an increased GAP activity of the RGS proteins leads to the faster termination of the G-protein cycle and/or less availability of the free Gβγ subunits. When a phosphomimic version of RGS proteins (a potentially activated RGS) was introduced into the soybean *nfr1* mutant plants (*nod49*), it resulted in partial restoration of nodule formation, implying the NFR-dependent nodule development is partially via the regulation of G-protein cycle in soybean (Roy Choudhury and Pandey 2015). Because G-proteins and RGS proteins are involved in a multitude of pathways regulated by RLKs, e.g., defense response, stomatal development, etc., this could potentially be a more widespread but yet unexplored regulatory mechanism in plant G-protein signaling.

It is also noteworthy that the plant-specific XLG proteins also interact with several RLKs (Maruta et al. 2015; Liang et al. 2016). Although the activation/deactivation mechanisms of XLG proteins, their biochemistries and the extent to which they are similar to the canonical Gα are not known, there is at least one example where the XLG protein's activity has been shown to be regulated by an RLK. It has been proposed that when the FLS2/BIK1 receptor complex is activated due to flg22 binding, it leads to the dissociation of XLG proteins from their Gβγ proteins. The free XLG can then be phosphorylated by BIK1 as a part of the signal transduction to the downstream effectors (Liang et al. 2016). Such a mechanism is somewhat similar to what we know from the mammalian systems where GPCR activation leads to the dissociation of the heterotrimeric G-protein complex. In the case of plant XLGs, it is not known if the dissociation of their trimeric complexes also affects the activity of XLG proteins or just availability and how the free XLG is cycled back to its trimeric complex. Nonetheless, the existence of an RLK-mediated trimeric G-protein complex dissociation is exciting and would provide critical insights if proved to be a more widespread mechanism in plants.

8.5.2 G-Protein Deactivation Mechanisms in Plants

Due to the cyclic nature of G-protein signaling, the deactivation mechanisms are as important as the G-protein activation mechanisms. Efficient and precisely regulated deactivation of G-protein cycle ensures synchronization of both parts of the cycle as well as it makes the G-proteins available for the next round of activation by a receptor, allowing for sustained signaling (Pandey 2017). Deactivation of Gα proteins, in part, is inherent to their GTPase activity. As soon as Gα is GTP-bound, it also starts to hydrolyze the bound GTP and regenerate the GDP-bound inactive form. However, the GTPase activity of Gα proteins is significantly slower than the GTP for GDP exchange on Gα in all organisms examined to date (Pandey 2017). Such a situation would result in an imbalance of the cycle. To keep the G-protein cycle synchronized, many proteins interact with the GTP-bound Gα to accelerate its GTPase activity. These proteins (GAPs) are therefore central to the G-protein deactivation mechanisms. Two types of GAPs are found in metazoan systems, RGS proteins and PLCβ homologs (Siderovski and Willard 2005). The RGS GAPs of metazoans collectively refers to a variety of proteins that have a conserved RGS domain. The RGS and PLCβ bind to the distinct regions of Gα proteins; however, the consequence is a change in Gα conformation, which allows for a faster hydrolysis of the bound GTP (Siderovski and Willard 2005; Ross 2011).

The plant Gα proteins are even slower GTPases than their metazoan counterparts, necessitating the presence of a GAP to effectively control the G-protein cycle. AtRGS1, cloned from Arabidopsis, was the first RGS GAP identified in plants (Chen et al. 2003). The protein possesses two distinct parts: the C-terminal region which has the conserved RGS domain similar to the ones found in all other eukaryotes and the N-terminal region which has a seven transmembrane (7TM) domain topology, typical of metazoan GPCRs. Intriguingly, mammalian RGS domain-containing proteins exist in multiple flavors and possess a variety of domain associations, but a 7TM domain has not been found in any mammalian RGS protein to date (Siderovski and Willard 2005) although the genomes of many basal organisms encode 7TM-containing RGS proteins. In contrast, all plant RGS proteins identified to date exhibit a very high degree of similarity to AtRGS1; they all possess an N-terminal 7TM domain fused with the C-terminal RGS domain. The 7TM domain enables the plant RGS proteins to be plasma membrane-localized and therefore in close proximity to the G-protein complex.

Biochemical experiments have confirmed the GTPase activity accelerating abilities of plant RGS proteins (Chen et al. 2003). Under in vitro assay conditions, addition of the purified RGS domain to purified Gα proteins causes an increase in its GTPase activity by at least an order of the magnitude. The *rgs1* null mutants of Arabidopsis exhibit the expected opposite phenotypes compared to the Arabidopsis *gpa1* null mutants in multiple hormonal and developmental signaling pathways, confirming the in planta role of RGS proteins as deactivators of G-protein signaling (Chen et al. 2003; Fan et al. 2008).

In all species that possess a Gα protein, it is expected that an RGS protein will exist as well. This relationship holds true for all metazoans. In fact, there is a direct

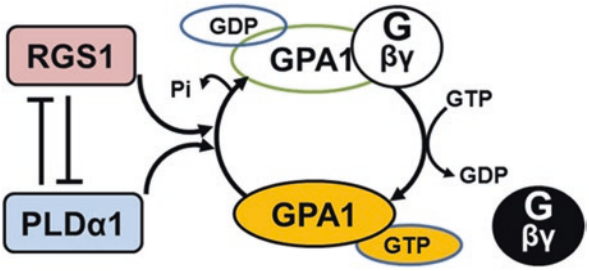

Fig. 8.2 In Arabidopsis, the GTPase activity of Gα is accelerated by the RGS1 protein and by PLDα1. Both these regulators interact with each other and affect their activities. PLDα1 interacts with RGS1, and its product, PA (not shown), binds and inhibits its GAP activity of RGS1. Conversely, RGS1 interacts with PLDα1 and inhibits its phospholipase activity, thereby influencing PA production. A net result of this complex regulation is to provide strictly controlled levels and duration of active Gα protein under any given condition

correlation between the number of Gα proteins and the number of RGS proteins in any given species (Anantharaman et al. 2011). However, the sequencing of the rice genome sprung a surprise when a canonical Gα protein was identified but no RGS protein homolog could be detected. In fact, almost all monocot genomes that were sequenced first including maize, *Brachypodium*, sorghum, wheat, etc., showed an absence of the RGS homolog in their genomes. This led to the initial hypothesis that the monocot plants do not contain an RGS protein (Urano et al. 2012a). Given the importance of these proteins in regulation of G-protein signaling, it was an astonishing observation. Few follow up studies using a small subset of plant species erroneously suggested that the RGS genes are lost in the genomes of monocot plants due to certain evolutionary co-adaptation (Urano et al. 2012a, b, 2015a). Detailed analysis of all sequenced monocot genomes however has confirmed that most monocots do possess an RGS protein-coding gene in their genomes, even though it is lost from a subset of species (Hackenberg et al. 2017). There is no evidence for the theory of adaptive coevolution of the Gα:RGS protein pair in plants. It is not known why some monocot species have lost RGS genes, but when present it is functional. Incidentally, the Gα proteins from the plant species that do not have an inherent RGS protein have retained their ability to be affected by a heterologous RGS protein, i.e., Gα proteins from rice or maize exhibit an increase in their GTPase activity when incubated with an RGS protein from Arabidopsis (or other sources) (Hackenberg et al. 2017).

Other established GAPs from mammalian systems, the PLCβ homologs, are not found in plants. However, recent work suggests that phospholipase D (PLD) proteins can act as GAPs in plants. In Arabidopsis, PLDα1, the most highly expressed PLD, has been shown to increase the GTPase activity of GPA1 (Pandey 2016; Roy Choudhury and Pandey 2016b). Genetic interactions combined with biochemical analysis have demonstrated that PLDα1 works in a small double negative regulatory loop with the Gα and RGS proteins to regulate signaling via developmental and environmental cues (Fig. 8.2). PLDα1 acts as a GAP for the Gα protein. Additionally, PLDα1 also interacts with the RGS1, and RGS1 inhibits the phospholipase activity

of PLDα1 (Roy Choudhury and Pandey 2016b). Furthermore, phosphatidic acid, a product of PLDα1 phospholipase activity, binds with and inhibits the GAP activity of RGS1 protein (Roy Choudhury and Pandey 2017a). The net effect of these biochemical and physical interactions is possibly translated to the availability of free active Gα protein, which would determine the amplitude and duration of the G-protein cycle and thereby provide for the specificity of response regulation (Pandey 2016, 2017; Roy Choudhury and Pandey 2016b, 2017a; Pandey and Vijayakumar 2018).

The PLDα proteins and its orthologs are present in all plants, so how might this regulation work in plants that do not have an RGS protein? At present, the cause of the random loss of RGS proteins from certain monocots is not known. Given the importance of this protein in regulating plant signaling, it is also surprising why it is under a relaxed selection in monocot genomes (Hackenberg et al. 2017). As mentioned previously, the plant Gα proteins have retained the ability to be affected by an RGS protein, regardless of its presence in the genome. It may be that there are additional proteins, which do not show an overall sequence similarity with RGS proteins but have a similar interaction interface to regulate the GTPase activity of Gα. One such example could be the COLD1 protein in rice, which is reported act as a GAP for the rice Gα but its homologs in Arabidopsis, the GTG proteins, do not have such an activity (Ma et al. 2015; Pandey et al. 2009).

The XLG proteins and the G-protein cycle regulated by them must also have a deactivation mechanism, but currently there is no information on their mechanistic details from any species. Conventional RGS proteins do not have any effect on the GTPase activity of XLG proteins (unpublished data from the author's lab). Future research combining biochemistry, genetic and plant physiology and development is needed to decipher these mechanisms.

8.6 Roles of G-Proteins in Influencing Plant Growth and Development and in Improving Plant Productivity

G-proteins regulate critical processes to affect almost all aspects of plant life. Biochemical experiments performed using G-protein agonists and antagonists identified from studies in metazoan systems suggested the involvement of G-proteins in hormone responses, light signaling, stress response, and plant-microbe interactions. The availability of gene knockout mutants and their detailed physiological analyses under a variety of conditions has firmly established the pivotal roles of G-proteins in almost every aspect of plant growth and development.

8.6.1 Role of G-Proteins in Plant Hormone Signaling

Pharmacological and physiological data had long predicted the role of G-proteins in controlling plant hormone signaling. Analysis of Arabidopsis, rice, maize, soybean, pea, and Camelina mutants has confirmed these roles. The most elaborate data are

available from Arabidopsis where G-proteins mutants have been characterized in detail in response to almost all plant hormones at the phenotypic and physiological levels as well as large-scale omics levels (Pandey et al. 2010; Wang et al. 2011; Li et al. 2012b; Alvarez et al. 2011; Urano and Jones 2014; Urano et al. 2016b). Arabidopsis *gpa1*, *agb1* and *agg3* mutants show altered sensitivity to ABA in a tissue and developmental stage-dependent manner. For seed germination, early seedling growth and development and root growth responses, the mutants exhibit hypersensitivity to ABA (Pandey et al. 2006). This response is intricately controlled by the RGS1 protein and the PLDα1 protein as well as their interaction (Roy Choudhury and Pandey 2016b, 2017a). For stomatal responses, the same *gpa1*, *agb1*, and *agg3* mutants exhibit hyposensitivity, and the extent to which it is controlled by RGS1 and PLDα1 is not well defined (Fan et al. 2008; Xu et al. 2015; Liu et al. 2017; Mishra et al. 2006). The *xlg* triple mutants of Arabidopsis are also hypersensitive to ABA for the seed germination and early seedling growth response but not during the stomatal responses (Ding et al. 2008).

G-protein mutants also show altered sensitivity to GA and BR. During seed germination and seedling development, the *gpa1* and *agb1* mutants show hyposensitivity to GA and BR (Chen et al. 2004; Gao et al. 2008; Tsugama et al. 2013a; Zhang et al. 2017). The altered GA and BR sensitivity has also been shown for the rice Gα mutants. The *d1* mutant of rice (a Gα mutant) shows less sensitivity to GA and BR and that has been proposed to be the cause of defect in its internode elongation and dwarfism (Wang et al. 2006; Oki et al. 2009; Hu et al. 2013). The altered sensitivity to ABA has been also observed in soybean hairy roots transgenic for *Gα* and *Gβ* genes (Roy Choudhury and Pandey 2013). Similarly, the Camelina and Setaria plants overexpressing the Arabidopsis *AGG3* gene and the rice mutants defective in *DEP1* gene (an *AGG3* homolog) or overexpressing *DEP1* gene also show altered sensitivity to ABA (Roy Choudhury et al. 2014a; Kaur et al. 2018). The altered sensitivity to ABA, GA, and BR in G-protein mutants therefore seems to be conserved in all higher plants examined to date. However, the XLG and Gβ mutants of the moss *P. patens* did not show any defects in their hormone sensitivity under the conditions tested (Hackenberg et al. 2016).

The Arabidopsis *agb1* mutants also show hypersensitivity to auxins (Ullah et al. 2003). Altered auxin sensitivity has been proposed to be the cause of higher rates of cell division in these plants. The *agb1* mutants also have significantly higher density of lateral roots and have more root mass, a phenotype attributed to its hypersensitivity to auxins or by altered auxin transport (Mudgil et al. 2009; Booker et al. 2010; Subramaniam et al. 2016). The altered ABA and to some extent the altered auxin responses of G-protein mutants have also been characterized at the transcriptome and proteome levels (Ullah et al. 2003; Alvarez et al. 2011). These large-scale datasets reveal massive changes caused due to the absence of specific G-proteins (Pandey et al. 2010; Wang et al. 2011; Li et al. 2012b; Chakraborty et al. 2015b).

The ethylene sensitivity of G-protein mutants has been explored in some detail in Arabidopsis. It appears that for the regulation of ethylene-dependent phenotypes, AGB1 works with the XLG3 proteins and not with the canonical Gα or other XLG proteins. Both *agb1* and *xlg3* mutants exhibit hypersensitivity to ethylene precursor ACC in the classic triple response pathway by developing exaggerated hook angles,

super short and thick hypocotyls, and very small roots with lots of root hairs (Pandey et al. 2008; Ge et al. 2015). Finally, there is some evidence for the regulation of cytokinin responses by the XLG proteins. The XLG proteins have been shown to work with a class of ubiquitin ligases (PUB2 and PUB4). The *xlg* triple mutant and the *pub2/4* double mutant exhibit defects in cytokinin responses, stamen development, tapetum development, and male fertility (Wang et al. 2017). There are few studies suggesting the role of G-proteins in JA signaling and responses during plant-microbe interactions (Trusov et al. 2006; Okamoto et al. 2009).

8.6.2 Role of G-Proteins in Plant Development

Analysis of G-protein mutants from various plant species has revealed alteration of many developmental programs. In Arabidopsis, the *gpa1* and *agb1* mutants show developmental defects from early on. There are clear differences in their shoot apical meristem development (Urano et al. 2016b) The hypocotyls of G-protein mutants are significantly shorter compared to the WT plants, when grown in the darkness. The leaves of G-protein mutant plants are rounder and crinkly in appearance (Urano et al. 2016b). An alteration in cell division rate is thought to be the basis of these phenotypes. The *rgs1* mutants exhibit phenotypes opposite of the *gpa1* mutants (longer hypocotyl in darkness, elongated leaves), suggesting that these phenotypes are regulated by classic G-protein signaling mechanisms (Chen et al. 2003). Another subset of phenotypes are altered in only a subset of the mutants. For example, the *agb1* mutant and the triple Gγ mutants (plants lacking *agg1*, *agg2*, and *agg3* genes) show short and blunt siliques, a phenotype not seen in the *gpa1* or *xlg* triple mutants (Urano et al. 2016b). In addition, the *Gα* and *Gβγ* genes regulate many phenotypes in opposite manner, for example, the root mass and stomatal density. For both these phenotypes, the Gα protein is a positive regulator of response; therefore the *gpa1* mutants have less root mass and lower stomatal density than the wild-type plants, whereas the Gβ proteins are negative regulators, i.e., the *agb1* mutant has more root mass and higher stomatal density compared to the wild-type plants (Chen et al. 2006; Zhang et al. 2008a). These distinct regulations have been explained on the basis of the requirement of both Gα and Gβγ proteins for signaling versus only one of the subunits. It has been proposed that if both Gα and Gβγ entities are involved in signal transduction, the lack of either one of them will make the pathway non-functional and result in identical or similar phenotypes. Alternatively, if only Gβγ is responsible for response regulation and the role of Gα is to keep it in its inactive, trimeric conformation, then lack of Gα will result in abundance of free Gβγ and more signaling output, whereas a lack of Gβγ will result in no signaling output (Pandey et al. 2010).

Contrary to the G-protein regulation of plant hormone signaling pathways, which seem to be generally conserved, the development phenotypes are quite distinct when comparing different plant lineages. As has been mentioned previously, the XLG and Gβ mutants of moss *P. patens* not only show defects in gametophyte elongation but fail to produce any sporophyte, confirming that the genes are essential for life cycle completion in this species (Hackenberg et al. 2016). The extent to which

such a requirement is conserved in other plants is unknown as this moss is the only basal organism where the G-protein signaling has been examined in detail.

There are clear differences in developmental phenotypes when comparing the G-protein mutants of dicot versus monocot plants. The Gα mutants of rice (Ueguchi-Tanaka et al. 2000), maize (Bommert et al. 2013), Setaria, and *Brachypodium* (unpublished data from the author's lab) are all severely dwarf. Moreover, a monocot Gβ null mutant could never be obtained, suggesting it might be essential for plant survival (Utsunomiya et al. 2011). There are no published reports of the *xlg* mutants from any monocot plants to date, but it is possible that the Gβ protein is working with the XLG proteins to regulate plant survival in monocots. Regardless of such possible interactions, it is clear that in dicots, every possible combination of G-protein mutants (including those with *XLG* genes) are viable which is not the case with monocot G-proteins (Urano et al. 2016a).

Another developmental pathway regulated by a subset of G-proteins is the reproductive organ size, seed size and seed numbers. In Arabidopsis the *AGG3* gene was initially also identified as an organ size regulator (Li et al. 2012a). The *agg3* mutants have smaller reproductive organs and smaller seeds. Similar defects were also seen in the *agb1* mutant, suggesting that these developmental pathways are specifically regulated by the AGB1.AGB3 (Gβγ3) combinations (Chakravorty et al. 2011). Overexpression of *AGG3* gene led to larger flowers, fruits, and seeds in Arabidopsis as well as in Camelina (Li et al. 2012a; Roy Choudhury et al. 2014a). These plants also produced more seeds. Incidentally, one of the rice homologs of *AGG3* gene, *Grain Size 3* (*GS3*), was initially identified as a major quantitative trait locus (QTL) for grain size regulation in rice (Fan et al. 2006; Sun et al. 2018). However, contrary to a direct positive regulation of seed size by *AGG3* gene in dicots, the situation in monocots is extremely complicated (Botella 2012; Sun et al. 2018). Although the gene is responsible for grain size determination, different mutations in the same gene result in shorter or longer grains. Furthermore, there is a huge effect on environment, as depending on the growth conditions larger or smaller seeds as well as yields have been reported by the overexpression of *AGG3* gene homologs of rice and barley (Botella 2012; Sun et al. 2018; Wendt et al. 2016). Targeted overexpression of the *AGG3* gene in Setaria (a model monocot) also revealed that the effect of this gene on seed size and number determination is complicated and is highly affected by growth conditions (Kaur et al. 2018).

Another homolog of *AGG3* gene, named *Dense and Erect Panicle 1* (*DEP1*), was initially identified as a major QTL for panicle erectness and branching in rice (Huang et al. 2009). Similar to the situation with *GS3*, this allele also seems to have complex regulation (Botella 2012; Sun et al. 2018). While the overexpression of the Arabidopsis homolog in Camelina resulted in significantly more branching (Roy Choudhury et al. 2014a), Setaria plants overexpressing this gene did not show a significant change in either branching or panicle erectness under greenhouse growth conditions (Kaur et al. 2018). The lineage specific regulation of G-protein pathways in plants is an active area of future research.

Interestingly, the same *DEP1* gene, which is responsible for panicle branching, density and erectness, was also identified as a major QTL for nitrogen use efficiency

(NEU) in rice (Sun et al. 2014). Furthermore, the role of *DEP1* in controlling nitrogen use was dependent on the G-protein cycle. A role of G-proteins in regulating NEU has not been explored for the dicot G-protein mutants; however, Setaria plants overexpressing the *AGG3* gene did exhibit better growth in low nitrogen conditions during early development (Kaur et al. 2018).

8.6.3 Role of G-Proteins in Abiotic Stress Tolerance

Many of the abiotic stress responses of plants are mediated via ABA signaling pathways. Therefore, it was not surprising that the G-protein mutants exhibit differences in their abiotic stress responses owing to their altered ABA signaling. Some of these responses are mediated by their altered water loss regulation via stomata. G-proteins directly regulate ABA-dependent ion channel regulation in stomatal guard cells (Wang et al. 2001; Coursol et al. 2003; Fan et al. 2008; Zhang et al. 2011). In addition, G-proteins also control stomata number per se, thereby affecting the transpiration rates and water use efficiency (Zhang et al. 2008a; Nilson and Assmann 2010a). Different G-protein subunits have been shown to be involved in regulating salt stress tolerance in Arabidopsis, rice, Camelina, and pea (Colaneri et al. 2014; Urano et al. 2014; Yu and Assmann 2015). Recent evidence also suggests the involvement of G-proteins in controlling stress responses by modulating the redox status of the cells (Torres et al. 2013; Liu et al. 2017; Swain et al. 2017). The involvement of G-proteins during stress responses of early seedling emergence has been suggested via the regulation of certain metabolic networks encompassing phenyl alanine production (Warpeha et al. 2006).

8.6.4 Role of G-Proteins During Defense Responses

The role of G-proteins in modulating biotic stress responses was initially reported from rice, where the *RGA1* gene was proposed to act with small GTPases to control disease resistance (Suharsono et al. 2002). The discovery of Gγ proteins in Arabidopsis and generation of multiple single- or higher-order mutants followed by the phenotypic analysis of *gpa1*, *agb1*, *agg1*, and *agg2* mutants uncovered the roles of G-proteins in controlling both bacterial and fungal diseases in Arabidopsis. Interestingly, in Arabidopsis the Gγ proteins show selectivity when regulating biotic versus abiotic responses. In general, the abiotic responses are regulated by the AGB1/AGG3 combination, whereas the biotic responses are regulated by AGB1/AGG1 or AGG2 combination (Trusov et al. 2007).

Studies done over a decade suggest that the G-protein-mediated regulation of defense responses are widespread as its involvement has been shown in responses again both host and nonhost bacterial pathogens including agrobacterium, a variety of biotrophic and necrotrophic fungi and viruses (Zhu et al. 2009; Delgado-Cerezo et al. 2012; Lee et al. 2013; Liu et al. 2013; Lorek et al. 2013; Aranda-Sicilia et al. 2015; Liang et al. 2016, 2018; Trusov et al. 2006; Maruta et al. 2015; Zhang et al. 2012). The molecular basis of G-protein action has been explored in some of these interactions.

One general theme is that G-proteins interact with various RLKs, which are involved in sensing pathogenic signals. In addition, the involvement of G-proteins has also been shown in modifying cell wall components in response to an infection, affecting reactive oxygen species production, and interacting with the jasmonic acid and MAP kinase signaling network, all of which are well-established components of plant response to pathogens (Delgado-Cerezo et al. 2012; Torres et al. 2013). Furthermore, the involvement of G-proteins has been shown in controlling stomatal aperture during pathogen infection by directly affecting ion channel activities, essentially controlling the severity of infection at the pathogen entry point (Zhang et al. 2008b; He et al. 2013).

8.6.5 Role of G-Proteins During Nodule Formation in Legumes

Nodule formation on leguminous plants' roots is the main source of atmospheric nitrogen fixation. The role of G-proteins during nodule formation was reported in one of the earlier studies where the use of various pharmacological compounds suggested the involvement of G-proteins during this process (Pingret et al. 1998). Recent work in soybean using elegant biochemical and molecular genetic approaches has uncovered a pathway connecting G-protein complex to the Nod factor receptors (NFRs) and the regulation of the G-protein cycle by receptor activity. These studies have shown that the Gα proteins are the negative regulators of nodule formation, whereas the Gβγ and the RGS proteins are positive regulators (Roy Choudhury and Pandey 2013). RNAi-mediated inhibition or constitutive overexpression of Gα proteins resulted in the development of more or fewer nodules per plant, respectively, compared to the wild-type controls. The trend was opposite in plants expressing lower of higher levels of Gβγ or RGS proteins, i.e., overexpression led to more and RNAi-mediated inhibition led to fewer nodules per root (Roy Choudhury and Pandey 2013, 2015). The NFR1 receptors interacted with the Gα proteins as well as with the RGS proteins of soybean and phosphorylated the RGS proteins. Phosphorylation resulted in the activation of RGS proteins' GAP activity, which promoted formation of inactive Gα and consequently more nodules. This phosphorylation-dependent regulation of G-protein cycle was verified in planta by overexpressing a phosphomimic version of RGS protein in plants lacking an active NFR1α receptor, which does not form nodules. Phosphomimic RGS was able to partially restore nodule formation confirming that at least one of the pathways connecting signal perception at the plasma membrane to the downstream cytosolic and nuclear components is via G-proteins (Roy Choudhury and Pandey 2015).

8.6.6 G-Proteins and Sugar Sensing in Plants

Sugar sensing has been dealt with in detail in Chap. 13. Briefly a number of studies link sugar sensing in plants to G-protein-dependent pathway. The RGS1 mutants of Arabidopsis were hyposensitive to high glucose concentrations (6% glucose). This has led to the hypothesis that the 7TM domain of the plant RGS proteins could possibly be a receptor for sugars, in addition to the other well-characterized sugar

sensors and receptors. In the presence of high sugar, RGS1 seems to re-localize from the plasma membrane to internal membranes. There is also some evidence of sugar-induced phosphorylation of RGS protein in Arabidopsis (Colaneri et al. 2014; Urano et al. 2012a, b).

8.6.7 G-Proteins and Light Signaling

The pivotal role of mammalian G-proteins in light perception (rhodopsin, the photoreceptor in humans, is a GPCR) prompted the plant scientists to explore the role of G-proteins in light sensing and signaling during the earlier stages of G-protein research when these were not characterized at the molecular basis. Pharmacological experiments suggested the involvement of G-proteins in light signaling in plants (Warpeha et al. 1991; Raghuram et al. 1999). However, later work using molecular genetic analysis has failed to identify a role of G-proteins in light perception per se. Nevertheless, G-protein mutants do respond differently to light by altering their developmental programming (Wei et al. 2008; Botto et al. 2009). All G-protein mutants have skotomorphogenetic phenotypes during seedling development (Botto et al. 2009). The G-protein mutants also respond differently to blue light during seedling emergence (Warpeha et al. 2006) as well as have been reported to interact with cry1 and NPH3 to modulate different blue-light-dependent responses (Fox et al. 2012; Kansup et al. 2014). G-proteins are also required for protection of plants against UV damage (He et al. 2013). Recently the role of rice Gα protein has been shown during photo-protection and photo-avoidance (Ferrero-Serrano et al. 2018).

8.7 G-Proteins and Plasticity

Details in the previous sections confirm without doubt the involvement of G-proteins in controlling almost all aspects of plant growth and development. However, the availability of complete gene knockout mutants of Arabidopsis G-proteins in all possible combinations and their ability to survive, grow, and successfully complete the life cycle appeared to be a great paradox. If G-proteins are truly so important for the plant life, how do the plants lacking them survive? Furthermore, most phenotypes of Arabidopsis G-protein mutants seem to suggest that even though the proteins modulate the severity of a given response, they are not essential for response regulation, i.e., the plant lacking G-proteins shows more or less sensitivity to any given response compared to the wild-type plants, but they do not completely eliminate it. For example, the Gα and Gβ mutants of Arabidopsis are hypersensitive to ABA or hyposensitive to gibberellic acid or brassinosteroid, but the responses are not completely abolished. This was explained on the basis of phenotypic plasticity in plants and the role of G-protein in modulating it (Assmann 2004). Following multiple studies using Arabidopsis mutants, a consensus emerged that the plant G-protein signaling has evolved to suit the sedentary lifestyle of plants, and contrary to the non-plant systems where it works more like an on/off switch, plant G-proteins titrate the overall response to a given growth and development condition (Pandey

2017). This could be due to existence of multiple, interconnected signaling networks where the lack of one pathway or one network allows for activation of another parallel network, generating excessive redundancy during signal transduction (Pandey 2017; Pandey and Vijayakumar 2018). Overall, this hypothesis may still hold true but several recent studies have uncovered specific pathways where G-proteins are essential.

As has been mentioned earlier, in addition to their modulatory role during gametophyte elongation, the G-proteins are also essential for the completion of moss life cycle (Hackenberg et al. 2016). Whether the nonessential nature of G-protein regulation in higher plants is an evolutionary development that coincides with the predominance of diploid stage in plants' life cycle is not known at this time. Additional studies with more basal plants expanding to different clades of evolutionary branches will help solve this enigma. Similarly, the lack of a viable Gβ null mutant in any monocot plant species to date, also implies the gene is essential for plant survival. It is also possible that additional combinations of XLG genes or Gβ genes are functional and not yet explored. Furthermore, there may exist in the genomes other genes, which might be plant specific and integral components of plant G-protein complex. These potential scenarios change our overall perception of modulatory versus controlling roles of G-protein signaling in plants. However, it is certain that the work done in model plants such as Arabidopsis may not fully represent the importance of G-protein signaling in plants and future work in additional agronomically important species will uncover many surprises.

8.8 Conclusions and Perspective

Almost three decades of active research since the discovery of heterotrimeric G-proteins in plants has established their critical roles in modulating plant growth, development, survival, and yield. Overall, the existing data suggest that while the core components, basic biochemistry, and key interactions of G-protein components are conserved across kingdoms, plants represent a unique variation to the theme. The same components seem to have been wired differently to suit the plant's way of life. It could be due to the fact that in contrast to metazoan responses which are extremely fast (light or neurotransmitter perception, which happens in the millisecond time scale), most plant G-protein-regulated responses are slower, taking place over days, weeks, or even during the lifespan of the plant. Plants have therefore acquired new G-protein partners, effectors, regulators or repurposed the ones already available to modulate such responses. The plant G-protein research has possibly entered into the most exciting phase now, as potentially all the components have been discovered, the involvement of G-proteins in regulating various processes has been established, the biochemistries have been elucidated, and novel interactors have been identified. Furthermore, the availability of thousands of sequenced genomes, new gene-editing technologies, and, most importantly, the role of G-proteins in directly and indirectly affecting plant yield have made it critical to carefully and diligently explore their activation/deactivation mechanisms, their regulations, and their precise manipulation to serve the need for the future generation.

Acknowledgments The author sincerely thanks several colleagues for multiple rounds of discussion during the writing of this book chapter. We also apologize to the colleagues whose work could not be cited due to space constraint. Research in the Pandey lab is supported by NIFA/AFRI (2015-67013-22964) and NSF (IOS-1557942 and MCB-1714693) grants to SP.

References

Alvarez S, Hicks LM, Pandey S (2011) ABA-dependent and -independent G-protein signaling in Arabidopsis roots revealed through an iTRAQ proteomics approach. J Proteome Res 10:3107–3122

Anantharaman V, Abhiman S, de Souza RF, Aravind L (2011) Comparative genomics uncovers novel structural and functional features of the heterotrimeric GTPase signaling system. Gene 475:63–78

Apone F, Alyeshmerni N, Wiens K, Chalmers D, Chrispeels MJ, Colucci G (2003) The G-protein-coupled receptor GCR1 regulates DNA synthesis through activation of phosphatidylinositol-specific phospholipase C. Plant Physiol 133:571–579

Aranda-Sicilia MN, Trusov Y, Maruta N, Chakravorty D, Zhang Y, Botella JR (2015) Heterotrimeric G proteins interact with defense-related receptor-like kinases in Arabidopsis. J Plant Physiol 188:44–48

Assmann SM (2004) Plant G proteins, phytohormones, and plasticity: three questions and a speculation. Sci STKE 2004:re20

Bisht NC, Jez JM, Pandey S (2011) An elaborate heterotrimeric G-protein family from soybean expands the diversity of plant G-protein networks. New Phytol 190:35–48

Bommert P, Je BI, Goldshmidt A, Jackson D (2013) The maize Galpha gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. Nature 502:555–558

Booker KS, Schwarz J, Garrett MB, Jones AM (2010) Glucose attenuation of auxin-mediated bimodality in lateral root formation is partly coupled by the heterotrimeric G protein complex. PLoS One 5:e12833

Botella JR (2012) Can heterotrimeric G proteins help to feed the world? Trends Plant Sci 17:563–568

Botto JF, Ibarra S, Jones AM (2009) The heterotrimeric G-protein complex modulates light sensitivity in Arabidopsis thaliana seed germination. Photochem Photobiol 85:949–954

Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ, Roepstorff P, Thirup S, Ronson CW, Thygesen MB, Stougaard J (2012) Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. Proc Natl Acad Sci U S A 109:13859–13864

Chakraborty N, Sharma P, Kanyuka K, Pathak RR, Choudhury D, Hooley R, Raghuram N (2015a) G-protein alpha-subunit (GPA1) regulates stress, nitrate and phosphate response, flavonoid biosynthesis, fruit/seed development and substantially shares GCR1 regulation in A. thaliana. Plant Mol Biol 89:559–576

Chakraborty N, Sharma P, Kanyuka K, Pathak RR, Choudhury D, Hooley RA, Raghuram N (2015b) Transcriptome analysis of Arabidopsis GCR1 mutant reveals its roles in stress, hormones, secondary metabolism and phosphate starvation. PLoS One 10:e0117819

Chakravorty D, Trusov Y, Zhang W, Acharya BR, Sheahan MB, McCurdy DW, Assmann SM, Botella JR (2011) An atypical heterotrimeric G-protein gamma-subunit is involved in guard cell K(+)-channel regulation and morphological development in Arabidopsis thaliana. Plant J 67:840–851

Chakravorty D, Gookin TE, Milner MJ, Yu Y, Assmann SM (2015) Extra-large G proteins expand the repertoire of subunits in Arabidopsis heterotrimeric G protein signaling. Plant Physiol 169:512–529

Chen JG, Willard FS, Huang J, Liang J, Chasse SA, Jones AM, Siderovski DP (2003) A seven-transmembrane RGS protein that modulates plant cell proliferation. Science 301:1728–1731

Chen JG, Pandey S, Huang J, Alonso JM, Ecker JR, Assmann SM, Jones AM (2004) GCR1 can act independently of heterotrimeric G-protein in response to brassinosteroids and gibberellins in Arabidopsis seed germination. Plant Physiol 135:907–915

Chen JG, Gao Y, Jones AM (2006) Differential roles of Arabidopsis heterotrimeric G-protein subunits in modulating cell division in roots. Plant Physiol 141:887–897

Colaneri AC, Tunc-Ozdemir M, Huang JP, Jones AM (2014) Growth attenuation under saline stress is mediated by the heterotrimeric G protein complex. BMC Plant Biol 14:129

Coursol S, Fan LM, Le Stunff H, Spiegel S, Gilroy S, Assmann SM (2003) Sphingolipid signalling in Arabidopsis guard cells involves heterotrimeric G proteins. Nature 423:651–654

Delgado-Cerezo M, Sanchez-Rodriguez C, Escudero V, Miedes E, Fernandez PV, Jorda L, Hernandez-Blanco C, Sanchez-Vallet A, Bednarek P, Schulze-Lefert P, Somerville S, Estevez JM, Persson S, Molina A (2012) Arabidopsis heterotrimeric G-protein regulates cell wall defense and resistance to necrotrophic fungi. Mol Plant 5:98–114

Ding L, Pandey S, Assmann SM (2008) Arabidopsis extra-large G proteins (XLGs) regulate root morphogenesis. Plant J 53:248–263

Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Fan LM, Zhang W, Chen JG, Taylor JP, Jones AM, Assmann SM (2008) Abscisic acid regulation of guard-cell K+ and anion channels in Gbeta- and RGS-deficient Arabidopsis lines. Proc Natl Acad Sci U S A 105:8476–8481

Ferrero-Serrano A, Su Z, Assmann SM (2018) Illuminating the role of the Galpha heterotrimeric G protein subunit, RGA1, in regulating photoprotection and photoavoidance in rice. Plant Cell Environ 41:451–468

Fox AR, Soto GC, Jones AM, Casal JJ, Muschietti JP, Mazzella MA (2012) cry1 and GPA1 signaling genetically interact in hook opening and anthocyanin synthesis in Arabidopsis. Plant Mol Biol 80:315–324

Friedman EJ, Wang HX, Jiang K, Perovic I, Deshpande A, Pochapsky TC, Temple BR, Hicks SN, Harden TK, Jones AM (2011) Acireductone dioxygenase 1 (ARD1) is an effector of the heterotrimeric G protein beta subunit in Arabidopsis. J Biol Chem 286:30107–30118

Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, Sasaki T, Asahi T, Iwasaki Y (1999) Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice. Proc Natl Acad Sci U S A 96:7575–7580

Gao Y, Wang S, Asami T, Chen JG (2008) Loss-of-function mutations in the Arabidopsis heterotrimeric G-protein alpha subunit enhance the developmental defects of brassinosteroid signaling and biosynthesis mutants. Plant Cell Physiol 49:1013–1024

Ge XM, Cai HL, Lei X, Zhou X, Yue M, He JM (2015) Heterotrimeric G protein mediates ethylene-induced stomatal closure via hydrogen peroxide synthesis in Arabidopsis. Plant J 82:138–150

Gilman AG (1987) G proteins: transducers of receptor-generated signals. Annual Review of Biochemistry 56:615–649

Gilman AG (1995) Nobel Lecture. G proteins and regulation of adenylyl cyclase. Biosci Rep 15:65–97

Gish LA, Clark SE (2011) The RLK/Pelle family of kinases. Plant J 66:117–127

Gookin TE, Bendtsen JD (2013) Topology assessment, G protein-coupled receptor (GPCR) prediction, and in vivo interaction assays to identify plant candidate GPCRs. Methods Mol Biol 1043:1–12

Gookin TE, Kim J, Assmann SM (2008) Whole proteome identification of plant candidate G-protein coupled receptors in Arabidopsis, rice, and poplar: computational prediction and in-vivo protein coupling. Genome Biol 9:R120

Hackenberg D, Perroud PF, Quatrano R, Pandey S (2016) Sporophyte formation and life cycle completion in moss requires heterotrimeric G-proteins. Plant Physiol 172:1154–1166

Hackenberg D, McKain MR, Lee SG, Roy Choudhury S, McCann T, Schreier S, Harkess A, Pires JC, Wong GK, Jez JM, Kellogg EA, Pandey S (2017) Galpha and regulator of G-protein signaling (RGS) protein pairs maintain functional compatibility and conserved interaction

interfaces throughout evolution despite frequent loss of RGS proteins in plants. New Phytol 216:562–575
Hao LH, Wang WX, Chen C, Wang YF, Liu T, Li X, Shang ZL (2012) Extracellular ATP promotes stomatal opening of Arabidopsis thaliana through heterotrimeric G protein alpha subunit and reactive oxygen species. Mol Plant 5:852–864
He JM, Ma XG, Zhang Y, Sun TF, Xu FF, Chen YP, Liu X, Yue M (2013) Role and interrelationship of Galpha protein, hydrogen peroxide, and nitric oxide in ultraviolet B-induced stomatal closure in Arabidopsis leaves. Plant Physiol 161:1570–1583
Heo JB, Sung S, Assmann SM (2012) Ca2+-dependent GTPase, extra-large G protein 2 (XLG2), promotes activation of DNA-binding protein related to vernalization 1 (RTV1), leading to activation of floral integrator genes and early flowering in Arabidopsis. J Biol Chem 287:8242–8253
Hu X, Qian Q, Xu T, Zhang Y, Dong G, Gao T, Xie Q, Xue Y (2013) The U-box E3 ubiquitin ligase TUD1 functions with a heterotrimeric G alpha subunit to regulate Brassinosteroid-mediated growth in rice. PLoS Genet 9:e1003391
Huang J, Taylor JP, Chen JG, Uhrig JF, Schnell DJ, Nakagawa T, Korth KL, Jones AM (2006) The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. Plant Cell 18:1226–1238
Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X (2009) Natural variation at the DEP1 locus enhances grain yield in rice. Nat Genet 41:494–497
Ishida T, Tabata R, Yamada M, Aida M, Mitsumasu K, Fujiwara M, Yamaguchi K, Shigenobu S, Higuchi M, Tsuji H, Shimamoto K, Hasebe M, Fukuda H, Sawa S (2014) Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. EMBO Rep 15:1202–1209
Jones AM, Xuan Y, Xu M, Wang RS, Ho CH, Lalonde S, You CH, Sardi MI, Parsa SA, Smith-Valle E, Su T, Frazer KA, Pilot G, Pratelli R, Grossmann G, Acharya BR, Hu HC, Engineer C, Villiers F, Ju C, Takeda K, Su Z, Dong Q, Assmann SM, Chen J, Kwak JM, Schroeder JI, Albert R, Rhee SY, Frommer WB (2014) Border control – a membrane-linked interactome of Arabidopsis. Science 344:711–716
Kansup J, Tsugama D, Liu S, Takano T (2013) The Arabidopsis adaptor protein AP-3mu interacts with the G-protein beta subunit AGB1 and is involved in abscisic acid regulation of germination and post-germination development. J Exp Bot 64:5611–5621
Kansup J, Tsugama D, Liu S, Takano T (2014) Arabidopsis G-protein beta subunit AGB1 interacts with NPH3 and is involved in phototropism. Biochem Biophys Res Commun 445:54–57
Kaur J, Roy Choudhury S, Vijayakumar A, Hovis L, Rhodes Z, Polzin R, Blumenthal D, Pandey S (2018) Arabidopsis Type III Ggamma protein AGG3 is a positive regulator of yield and stress responses in the model monocot Setaria viridis. Front Plant Sci 9:109
Klopffleisch K, Phan N, Augustin K, Bayne RS, Booker KS, Botella JR, Carpita NC, Carr T, Chen JG, Cooke TR, Frick-Cheng A, Friedman EJ, Fulk B, Hahn MG, Jiang K, Jorda L, Kruppe L, Liu C, Lorek J, McCann MC, Molina A, Moriyama EN, Mukhtar MS, Mudgil Y, Pattathil S, Schwarz J, Seta S, Tan M, Temp U, Trusov Y, Urano D, Welter B, Yang J, Panstruga R, Uhrig JF, Jones AM (2011) Arabidopsis G-protein interactome reveals connections to cell wall carbohydrates and morphogenesis. Mol Syst Biol 7:532
Lapik YR, Kaufman LS (2003) The Arabidopsis cupin domain protein AtPirin1 interacts with the G protein alpha-subunit GPA1 and regulates seed germination and early seedling development. Plant Cell 15:1578–1590
Lee S, Rojas CM, Ishiga Y, Pandey S, Mysore KS (2013) Arabidopsis heterotrimeric G-proteins play a critical role in host and nonhost resistance against Pseudomonas syringae pathogens. PLoS One 8:e82445
Lee CS, Ahn W, Choi YE (2017) The G-protein alpha-subunit gene CGA1 is involved in regulation of resistance to heat and osmotic stress in Chlamydomonas reinhardtii. Cell Mol Biol (Noisy-le-grand) 63:29–39
Li S, Liu Y, Zheng L, Chen L, Li N, Corke F, Lu Y, Fu X, Zhu Z, Bevan MW, Li Y (2012a) The plant-specific G protein gamma subunit AGG3 influences organ size and shape in Arabidopsis thaliana. New Phytol 194:690–703

Li S, Pandey S, Gookin TE, Zhao Z, Wilson L, Assmann SM (2012b) Gene-sharing networks reveal organizing principles of transcriptomes in Arabidopsis and other multicellular organisms. Plant Cell 24:1362–1378

Liang X, Ding P, Lian K, Wang J, Ma M, Li L, Li L, Li M, Zhang X, Chen S, Zhang Y, Zhou JM (2016) Arabidopsis heterotrimeric G proteins regulate immunity by directly coupling to the FLS2 receptor. Elife 5:e13568

Liang Y, Gao Y, Jones AM (2017) Extra large G-protein interactome reveals multiple stress response function and partner-dependent XLG subcellular localization. Front Plant Sci 8:1015

Liang X, Ma M, Zhou Z, Wang J, Yang X, Rao S, Bi G, Li L, Zhang X, Chai J, Chen S, Zhou JM (2018) Ligand-triggered de-repression of Arabidopsis heterotrimeric G proteins coupled to immune receptor kinases. Cell Res. 28: 529–543

Liu J, Ding P, Sun T, Nitta Y, Dong O, Huang X, Yang W, Li X, Botella JR, Zhang Y (2013) Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. Plant Physiol 161:2146–2158

Liu C, Xu Y, Long D, Cao B, Hou J, Xiang Z, Zhao A (2017) Plant G-protein beta subunits positively regulate drought tolerance by elevating detoxification of ROS. Biochem Biophys Res Commun 491:897–902

Llorente F, Alonso-Blanco C, Sanchez-Rodriguez C, Jorda L, Molina A (2005) ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. Plant J 43:165–180

Lorek J, Griebel T, Jones AM, Kuhn H, Panstruga R (2013) The role of Arabidopsis heterotrimeric G-protein subunits in MLO2 function and MAMP-triggered immunity. Mol Plant Microbe Interact 26:991–1003

Ma H, Yanofsky MF, Meyerowitz EM (1990) Molecular cloning and characterization of GPA1, a G protein alpha subunit gene from Arabidopsis thaliana. Proc Natl Acad Sci U S A 87:3821–3825

Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X, Xu S, Niu Y, Jin J, Zhang H, Xu X, Li L, Wang W, Qian Q, Ge S, Chong K (2015) COLD1 confers chilling tolerance in rice. Cell 160:1209–1221

Maeda K, Houjyou Y, Komatsu T, Hori H, Kodaira T, Ishikawa A (2009) AGB1 and PMR5 contribute to PEN2-mediated preinvasion resistance to *Magnaporthe oryzae* in Arabidopsis thaliana. Mol Plant Microbe Interact 22:1331–1340

Maruta N, Trusov Y, Brenya E, Parekh U, Botella JR (2015) Membrane-localized extra-large G proteins and Gbg of the heterotrimeric G proteins form functional complexes engaged in plant immunity in Arabidopsis. Plant Physiol 167:1004–1016

McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS (2005) G-protein signaling: back to the future. Cell Mol Life Sci 62:551–577

Mishra G, Zhang W, Deng F, Zhao J, Wang X (2006) A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science 312:264–266

Misra S, Wu Y, Venkataraman G, Sopory SK, Tuteja N (2007) Heterotrimeric G-protein complex and G-protein-coupled receptor from a legume (Pisum sativum): role in salinity and heat stress and cross-talk with phospholipase C. Plant J 51:656–669

Mudgil Y, Uhrig JF, Zhou J, Temple B, Jiang K, Jones AM (2009) Arabidopsis N-MYC DOWNREGULATED-LIKE1, a positive regulator of auxin transport in a G protein-mediated pathway. Plant Cell 21:3591–3609

Mudgil Y, Karve A, Teixeira PJ, Jiang K, Tunc-Ozdemir M, Jones AM (2016) Photosynthate regulation of the root system architecture mediated by the heterotrimeric G protein complex in Arabidopsis. Front Plant Sci 7:1255

Nilson SE, Assmann SM (2010a) The alpha-subunit of the Arabidopsis heterotrimeric G protein, GPA1, is a regulator of transpiration efficiency. Plant Physiol 152:2067–2077

Nilson SE, Assmann SM (2010b) Heterotrimeric G proteins regulate reproductive trait plasticity in response to water availability. New Phytol 185:734–746

Okamoto H, Gobel C, Capper RG, Saunders N, Feussner I, Knight MR (2009) The alpha-subunit of the heterotrimeric G-protein affects jasmonate responses in Arabidopsis thaliana. J Exp Bot 60:1991–2003

Oki K, Inaba N, Kitagawa K, Fujioka S, Kitano H, Fujisawa Y, Kato H, Iwasaki Y (2009) Function of the alpha subunit of rice heterotrimeric G protein in brassinosteroid signaling. Plant Cell Physiol 50:161–172

Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9:60–71

Pandey S (2016) Phospholipases as GTPase activity accelerating proteins (GAPs) in plants. Plant Signal Behav 11:e1176821

Pandey S (2017) Heterotrimeric G-protein regulatory circuits in plants: conserved and novel mechanisms. Plant Signal Behav 12:e1325983

Pandey S, Assmann SM (2004) The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein alpha subunit GPA1 and regulates abscisic acid signaling. Plant Cell 16:1616–1632

Pandey S, Vijayakumar A (2018) Emerging themes in heterotrimeric G-protein signaling in plants. Plant Sci 270:292–300

Pandey S, Chen JG, Jones AM, Assmann SM (2006) G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol 141:243–256

Pandey S, Monshausen GB, Ding L, Assmann SM (2008) Regulation of root-wave response by extra large and conventional G proteins in Arabidopsis thaliana. Plant J 55:311–322

Pandey S, Nelson DC, Assmann SM (2009) Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell 136:136–148

Pandey S, Wang RS, Wilson L, Li S, Zhao Z, Gookin TE, Assmann SM, Albert R (2010) Boolean modeling of transcriptome data reveals novel modes of heterotrimeric G-protein action. Mol Syst Biol 6:372

Pingret JL, Journet EP, Barker DG (1998) Rhizobium nod factor signaling. Evidence for a G-protein-mediated transduction mechanism. Plant Cell 10:659–672

Raghuram N, Chandok MR, Sopory SK (1999) Light regulation of nitrate reductase gene expression in maize involves a G-protein. Mol Cell Biol Res Commun 2:86–90

Reed RR (1990) G protein diversity and the regulation of signaling pathways. New Biol 2:957–960

Rodbell M (1992) The role of GTP-binding proteins in signal transduction: from the sublimely simple to the conceptually complex. Curr Top Cell Regul 32:1–47

Rodbell M (1995) Nobel Lecture. Signal transduction: evolution of an idea. Biosci Rep 15:117–133

Ross EM (2011) Galpha(q) and phospholipase C-beta: turn on, turn off, and do it fast. Sci Signal 4:pe5

Roy Choudhury S, Pandey S (2013) Specific subunits of heterotrimeric G proteins play important roles during nodulation in soybean. Plant Physiol 162:522–533

Roy Choudhury S, Pandey S (2015) Phosphorylation-dependent regulation of G-protein cycle during nodule formation in soybean. Plant Cell 27:3260–3276

Roy Choudhury S, Pandey S (2016a) Interaction of heterotrimeric G-protein components with receptor-like kinases in plants: an alternative to the established signaling paradigm? Mol Plant 9:1093–1095

Roy Choudhury S, Pandey S (2016b) The role of PLDalpha1 in providing specificity to signal-response coupling by heterotrimeric G-protein components in Arabidopsis. Plant J 86:50–61

Roy Choudhury S, Pandey S (2017a) Phosphatidic acid binding inhibits RGS1 activity to affect specific signaling pathways in Arabidopsis. Plant J 90:466–477

Roy Choudhury S, Pandey S (2017b) Recently duplicated plant heterotrimeric Galpha proteins with subtle biochemical differences influence specific outcomes of signal-response coupling. J Biol Chem 292:16188–16198

Roy Choudhury S, Bisht NC, Thompson R, Todorov O, Pandey S (2011) Conventional and novel Ggamma protein families constitute the heterotrimeric G-protein signaling network in soybean. PLoS One 6:e23361

Roy Choudhury S, Riesselman AJ, Pandey S (2014a) Constitutive or seed-specific overexpression of Arabidopsis G-protein gamma subunit 3 (AGG3) results in increased seed and oil production and improved stress tolerance in Camelina sativa. Plant Biotechnol J 12:49–59

Roy Choudhury S, Wang Y, Pandey S (2014b) Soya bean Galpha proteins with distinct biochemical properties exhibit differential ability to complement Saccharomyces cerevisiae gpa1 mutant. Biochem J 461:75–85

Selinger Z, Cassel D (1981) Role of guanine nucleotides in hormonal activation of adenylate cyclase. Adv Cyclic Nucleotide Res 14:15–22

Siderovski DP, Willard FS (2005) The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 1:51–66

Spiegel AM, Backlund PS Jr, Butrynski JE, Jones TL, Simonds WF (1991) The G protein connection: molecular basis of membrane association. Trends Biochem Sci 16:338–341

Steffens B, Sauter M (2009) Heterotrimeric G protein signaling is required for epidermal cell death in rice. Plant Physiol 151:732–740

Subramaniam G, Trusov Y, Lopez-Encina C, Hayashi S, Batley J, Botella JR (2016) Type B heterotrimeric G Protein gamma-subunit regulates auxin and ABA signaling in tomato. Plant Physiol 170:1117–1134

Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K (2002) The heterotrimeric G protein alpha subunit acts upstream of the small GTPase Rac in disease resistance of rice. Proc Natl Acad Sci U S A 99:13307–13312

Sun H, Qian Q, Wu K, Luo J, Wang S, Zhang C, Ma Y, Liu Q, Huang X, Yuan Q, Han R, Zhao M, Dong G, Guo L, Zhu X, Gou Z, Wang W, Wu Y, Lin H, Fu X (2014) Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. Nat Genet 46:652–656

Sun S, Wang L, Mao H, Shao L, Li X, Xiao J, Ouyang Y, Zhang Q (2018) A G-protein pathway determines grain size in rice. Nat Commun 9:851

Sutherland EW (1971) Nobel prize in physiology or medicine 1971: the action of hormones outlined. Lakartidningen 68(44):4991–4995

Sutherland EW, Rall TW, Menon T (1962) Adenyl cylase. I. Distribution, preparation, and properties. J Biol Chem 237:1220–1227

Swain DM, Sahoo RK, Srivastava VK, Tripathy BC, Tuteja R, Tuteja N (2017) Function of heterotrimeric G-protein gamma subunit RGG1 in providing salinity stress tolerance in rice by elevating detoxification of ROS. Planta 245:367–383

Torres MA, Morales J, Sanchez-Rodriguez C, Molina A, Dangl JL (2013) Functional interplay between Arabidopsis NADPH oxidases and heterotrimeric G protein. Mol Plant Microbe Interact 26:686–694

Trusov Y, Rookes JE, Chakravorty D, Armour D, Schenk PM, Botella JR (2006) Heterotrimeric G proteins facilitate Arabidopsis resistance to necrotrophic pathogens and are involved in jasmonate signaling. Plant Physiol 140:210–220

Trusov Y, Rookes JE, Tilbrook K, Chakravorty D, Mason MG, Anderson D, Chen JG, Jones AM, Botella JR (2007) Heterotrimeric G protein gamma subunits provide functional selectivity in Gbetagamma dimer signaling in Arabidopsis. Plant Cell 19:1235–1250

Trusov Y, Sewelam N, Rookes JE, Kunkel M, Nowak E, Schenk PM, Botella JR (2009) Heterotrimeric G proteins-mediated resistance to necrotrophic pathogens includes mechanisms independent of salicylic acid-, jasmonic acid/ethylene- and abscisic acid-mediated defense signaling. Plant J 58:69–81

Tsugama D, Liu S, Takano T (2013a) Arabidopsis heterotrimeric G protein beta subunit, AGB1, regulates brassinosteroid signalling independently of BZR1. J Exp Bot 64:3213–3223

Tsugama D, Liu S, Takano T (2013b) A bZIP protein, VIP1, interacts with Arabidopsis heterotrimeric G protein beta subunit, AGB1. Plant Physiol Biochem 71:240–246

Tunc-Ozdemir M, Urano D, Jaiswal DK, Clouse SD, Jones AM (2016) Direct modulation of heterotrimeric G protein-coupled signaling by a receptor kinase complex. J Biol Chem 291:13918–13925

Ueguchi-Tanaka M, Fujisawa Y, Kobayashi M, Ashikari M, Iwasaki Y, Kitano H, Matsuoka M (2000) Rice dwarf mutant d1, which is defective in the alpha subunit of the heterotrimeric G protein, affects gibberellin signal transduction. Proc Natl Acad Sci U S A 97:11638–11643

Ullah H, Chen JG, Young JC, Im KH, Sussman MR, Jones AM (2001) Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science 292:2066–2069

Ullah H, Chen JG, Wang S, Jones AM (2002) Role of a heterotrimeric G protein in regulation of Arabidopsis seed germination. Plant Physiol 129:897–907
Ullah H, Chen JG, Temple B, Boyes DC, Alonso JM, Davis KR, Ecker JR, Jones AM (2003) The beta-subunit of the Arabidopsis G protein negatively regulates auxin-induced cell division and affects multiple developmental processes. Plant Cell 15:393–409
Urano D, Jones AM (2014) Heterotrimeric G protein-coupled signaling in plants. Annu Rev Plant Biol 65:365–384
Urano D, Jones JC, Wang H, Matthews M, Bradford W, Bennetzen JL, Jones AM (2012a) G protein activation without a GEF in the plant kingdom. PLoS Genet 8:e1002756
Urano D, Phan N, Jones JC, Yang J, Huang J, Grigston J, Taylor JP, Jones AM (2012b) Endocytosis of the seven-transmembrane RGS1 protein activates G-protein-coupled signalling in Arabidopsis. Nat Cell Biol 14:1079–1088
Urano D, Colaneri A, Jones AM (2014) G alpha modulates salt-induced cellular senescence and cell division in rice and maize. J Exp Bot 65:6553–6561
Urano D, Dong T, Bennetzen JL, Jones AM (2015a) Adaptive evolution of signaling partners. Mol Biol Evol 32:998–1007
Urano D, Jackson D, Jones AM (2015b) A G protein alpha null mutation confers prolificacy potential in maize. J Exp Bot 66:4511–4515
Urano D, Maruta N, Trusov Y, Stoian R, Wu Q, Liang Y, Jaiswal DK, Thung L, Jackson D, Botella JR, Jones AM (2016a) Saltational evolution of the heterotrimeric G protein signaling mechanisms in the plant kingdom. Sci Signal 9:ra93
Urano D, Miura K, Wu Q, Iwasaki Y, Jackson D, Jones AM (2016b) Plant morphology of heterotrimeric G protein mutants. Plant Cell Physiol 57:437–445
Utsunomiya Y, Samejima C, Takayanagi Y, Izawa Y, Yoshida T, Sawada Y, Fujisawa Y, Kato H, Iwasaki Y (2011) Suppression of the rice heterotrimeric G protein beta-subunit gene, RGB1, causes dwarfism and browning of internodes and lamina joint regions. Plant J 67:907–916
Wang XQ, Ullah H, Jones AM, Assmann SM (2001) G protein regulation of ion channels and abscisic acid signaling in Arabidopsis guard cells. Science 292:2070–2072
Wang L, Xu YY, Ma QB, Li D, Xu ZH, Chong K (2006) Heterotrimeric G protein alpha subunit is involved in rice brassinosteroid response. Cell Res 16:916–922
Wang RS, Pandey S, Li S, Gookin TE, Zhao Z, Albert R, Assmann SM (2011) Common and unique elements of the ABA-regulated transcriptome of Arabidopsis guard cells. BMC Genomics 12:216
Wang Y, Wu Y, Yu B, Yin Z, Xia Y (2017) EXTRA-LARGE G PROTEINs interact with E3 ligases PUB4 and PUB2 and function in cytokinin and developmental processes. Plant Physiol 173:1235–1246
Warpeha KM, Hamm HE, Rasenick MM, Kaufman LS (1991) A blue-light-activated GTP-binding protein in the plasma membranes of etiolated peas. Proc Natl Acad Sci U S A 88:8925–8929
Warpeha KM, Lateef SS, Lapik Y, Anderson M, Lee BS, Kaufman LS (2006) G-protein-coupled receptor 1, G-protein Galpha-subunit 1, and prephenate dehydratase 1 are required for blue light-induced production of phenylalanine in etiolated Arabidopsis. Plant Physiol 140:844–855
Wei Q, Zhou W, Hu G, Wei J, Yang H, Huang J (2008) Heterotrimeric G-protein is involved in phytochrome A-mediated cell death of Arabidopsis hypocotyl. Cell Res 18:949–960. PMID: 19160542
Wendt T, Holme I, Dockter C, Preuss A, Thomas W, Druka A, Waugh R, Hansson M, Braumann I (2016) HvDep1 is a positive regulator of culm elongation and grain size in barley and impacts yield in an environment-dependent manner. PLoS One 11:e0168924
Wheeler GL, Bitensky MW (1977) A light-activated GTPase in vertebrate photoreceptors: regulation of light-activated cyclic GMP phosphodiesterase. Proc Natl Acad Sci U S A 74:4238–4242
Wolfenstetter S, Chakravorty D, Kula R, Urano D, Trusov Y, Sheahan MB, McCurdy DW, Assmann SM, Jones AM, Botella JR (2015) Evidence for an unusual transmembrane configuration of AGG3, a class C Ggamma subunit of Arabidopsis. Plant J 81:388–398
Wu Y, Xu X, Li S, Liu T, Ma L, Shang Z (2007) Heterotrimeric G-protein participation in Arabidopsis pollen germination through modulation of a plasmamembrane hyperpolarization-activated Ca2+-permeable channel. New Phytol 176:550–559

Xu DB, Chen M, Ma YN, Xu ZS, Li LC, Chen YF, Ma YZ (2015) A G-protein beta subunit, AGB1, negatively regulates the ABA response and drought tolerance by down-regulating AtMPK6-related pathway in Arabidopsis. PLoS One 10:e0116385

Xu DB, Gao SQ, Ma YN, Wang XT, Feng L, Li LC, Xu ZS, Chen YF, Chen M, Ma YZ (2017a) The G-Protein beta subunit AGB1 promotes hypocotyl elongation through inhibiting transcription activation function of BBX21 in Arabidopsis. Mol Plant 10:1206–1223

Xu L, Yao X, Zhang N, Gong BQ, Li JF (2017b) Dynamic G protein alpha signaling in Arabidopsis innate immunity. Biochem Biophys Res Commun. pii: S0006-291X(17)31382-7

Yu Y, Assmann SM (2015) The heterotrimeric G-protein beta subunit, AGB1, plays multiple roles in the Arabidopsis salinity response. Plant Cell Environ 38:2143–2156

Yu TY, Shi DQ, Jia PF, Tang J, Li HJ, Liu J, Yang WC (2016) The Arabidopsis receptor kinase ZAR1 is required for zygote asymmetric division and its daughter cell fate. PLoS Genet 12:e1005933

Yu Y, Chakravorty D, Assmann SM (2018) The G protein beta subunit, AGB1, interacts with FERONIA in RALF1-regulated stomatal movement. Plant Physiol 176(3):2426–2440

Zhang L, Hu G, Cheng Y, Huang J (2008a) Heterotrimeric G protein alpha and beta subunits antagonistically modulate stomatal density in Arabidopsis thaliana. Dev Biol 324:68–75

Zhang W, He SY, Assmann SM (2008b) The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. Plant J 56:984–996

Zhang L, Wei Q, Wu W, Cheng Y, Hu G, Hu F, Sun Y, Zhu Y, Sakamoto W, Huang J (2009) Activation of the heterotrimeric G protein alpha-subunit GPA1 suppresses the ftsh-mediated inhibition of chloroplast development in Arabidopsis. Plant J 58:1041–1053

Zhang W, Jeon BW, Assmann SM (2011) Heterotrimeric G-protein regulation of ROS signalling and calcium currents in Arabidopsis guard cells. J Exp Bot 62:2371–2379

Zhang H, Wang M, Wang W, Li D, Huang Q, Wang Y, Zheng X, Zhang Z (2012) Silencing of G proteins uncovers diversified plant responses when challenged by three elicitors in Nicotiana benthamiana. Plant Cell Environ 35:72–85

Zhang T, Xu P, Wang W, Wang S, Caruana JC, Yang HQ, Lian H (2017) Arabidopsis G-protein beta subunit AGB1 interacts with BES1 to regulate Brassinosteroid signaling and cell elongation. Front Plant Sci 8:2225

Zhao J, Wang X (2004) Arabidopsis phospholipase Dalpha1 interacts with the heterotrimeric G-protein alpha-subunit through a motif analogous to the DRY motif in G-protein-coupled receptors. J Biol Chem 279:1794–1800

Zhu H, Li GJ, Ding L, Cui X, Berg H, Assmann SM, Xia Y (2009) Arabidopsis extra large G-protein 2 (XLG2) interacts with the Gbeta subunit of heterotrimeric G protein and functions in disease resistance. Mol Plant 2:513–525

Sona Pandey is a Principal Investigator at the Donald Danforth Plant Science Center in St. Louis, Missouri, USA. She is also an Adjunct Professor in the Department of Biology at Washington University, St. Louis. She is the Director of the Research Experience for Undergraduate (REU) program at the Danforth Center and a member of the Minority Affairs Committee at the American Society of Plant Biology. She received a BSc (Hons.) in Chemistry and MSc in Biotechnology from Banaras Hindu University, Varanasi, India. She received her Ph.D. in Life Sciences from JNU, New Delhi, India, with the Editor and Prof. Neera B. Sarin. She worked as a Research Scientist in the Center for Plant Molecular Biology, New Delhi, India, before starting a postdoctoral career at the Pennsylvania State University, University Park, PA, USA. Her area of specialization is molecular and cellular plant biology, with special focus on understanding the signaling mechanisms that are operative during plant development and stress tolerance using model systems such as *Arabidopsis*, *Brachypodium*, *Setaria*, and moss as well as crops such as soybeans and *Camelina*. Her lab uses the state-of-the-art phenotyping, genomics, proteomics, and classical molecular-genetic and biochemical approaches to address some of the most important unanswered questions in the field of plant growth and development.

Plant Hormones: Some Glimpses on Biosynthesis, Signaling Networks, and Crosstalk

9

Autar K. Mattoo and Rakesh K. Upadhyay

Abstract

Plant hormones are major cellular signaling molecules that modulate growth and development and respond to internal and external cues in plants although differently than is understood about hormones specific to animals. The fortuitous discovery of hormones in animal/human systems and plants occurred around the similar time span. Hormones are also functional in the same cells where they are synthesized as well as in the neighboring or distant cell. Although at least nine plant hormones are now recognized, many more could be discovered and characterized in the future. Their perception, intra- and intercellular movement/communication, and interaction with receptors and gene regulators are better understood now; however, the intimate details are yet to be discovered. Each plant hormone has a unique/specific function and also regulates networks of other hormones via crosstalks involving specific transcription factors and small RNAs. This new knowledge has brought to light the fact that the regulation of plant physiological processes involves a complex crosstalk among different hormones. The new developments in various technologies, including forward genetics, ease of plant transformation systems, and the gain-of-function and loss-of-function model systems, have contributed to the progress made thus far. This chapter provides salient features on hormone biology and selected crosstalks between hormones impacting various plant processes and the responses to abiotic stresses.

A. K. Mattoo (✉) · R. K. Upadhyay
The Henry A. Wallace Beltsville Agricultural Research Center, Sustainable Agricultural Systems Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA
e-mail: autar.mattoo@usda.gov; rakesh.upadhyay@usda.gov

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_9

Keywords
Abiotic stress · Auxin · Biosynthesis · Cytokinin · Fruit ripening · Gibberellins · Hormone crosstalk · Jasmonic acid · Leaf development · Root elongation · Seed germination · Strigolactones · Wounding

9.1 Introduction

The science about "hormones" has far advanced since the word "hormone" was first coined for a molecule, synthesized in small intestinal glands, which stimulated pancreatic secretion and was named "secretin," over 110 years ago to date by Wm. M. Bayliss and Ernest H. Starling (1902). Interestingly prior to this discovery, Charles Darwin had described in 1880 properties of a plant substance named "auxein" (Greek) that enhanced growth in canary grass (Phalaris canariensis) and which became the first plant hormone named "auxin" to be discovered and defined. In 1935, auxin/IAA was defined as a signaling molecule in plants (Thimann and Koepfli 1935). Another observation made in 1864 by a German scientist Girardin (1864) about shade trees that were defoliating near a leaking illuminating gas (from gas mains) led 42 years later (in 1901) to the demonstration by Russian Plant Physiologist Dimitry Nikolayevich Neljubov that the active component of the illuminating gas was the gaseous ethylene (Neljubov 1901). Ethylene was later coined as a gaseous hormone in plants. Ethylene became established as the second plant hormone after the English scientist Gane showed 33 years later that plants actually synthesize ethylene (Gane 1934).

In 1898, a Japanese scientist Hori discovered that rice disease called "Bakanae" with symptoms of infertility and excessive growth promotion of rice seedlings caused by a fungus was due to a product synthesized by the fungus *Gibberella fujikuroi* (Hori 1898). The active principle was named after the fungus as "gibberellin" (GA), and gibberellins were later found to be synthesized also by plants and found to regulate plant development processes from stem elongation, seed germination, floral development, to plant senescence. GAs remained to be part of classical plant growth hormones (Phinney 1983).

In the 1960s, two of the five classical plant hormones were discovered, namely, cytokinin and abscisic acid. Cytokinin (CK) structurally resembles adenine because it is biosynthesized by a modification of adenine and shares properties of "kinetin," and its commonly found form is zeatin. Zeatin was simultaneously discovered by Miller (1961) and Letham (1967). CKs prevail in meristematic tissues, are synthesized in the root tissue, and then translocate to the plant shoots. A myriad of plant processes in growth and development are regulated by CK including antisenescence (Nooden et al. 1979) and N signaling (Sakakibara et al. 1998) functions.

Abscisic acid (ABA) was the fifth classical plant hormone discovered by Frederick Addicott during studies related to abscission in cotton fruit (Addicott et al. 1968; Addicott and Lyon 1969). ABA synthesis was found to be initiated in the photosynthesizing organelle chloroplasts. ABA freely moves in the stem, is transported in xylem and phloem, and moves also via parenchyma.

Growth promoter brassin (brassinosteroid) was discovered in 1979 through studies with rape pollen (Grove et al. 1979), and early information about brassin roles/function was reviewed by one of its discoverers (Mandava 1988). Subsequently, a lot of research has been published about its role in a number of biological processes including growth, cell division, flowering, photomorphogenesis, and others (Clouse 2011). Similarly, a strigolactone, (+)-strigol, was identified earlier in cotton root exudates (Cook et al. 1966) and by now other strigolactones have been identified: sorgolactone (Hauck et al. 1992), orobanchol (Yokota et al. 1998), and solanocol (Xie et al. 2007).

The fact remains that fortuitous discovery of hormones in animal/human systems and plants occurred around the similar time span. However, initially hormones were considered to be produced in one organ and then moved via blood stream to the destination of its cellular function (O'Malley 1989). The latter fact about hormone moving to another cellular/organelle destination, however, could not be as easily demonstrated in plants since they do not have a similar "blood" flow. Nonetheless, movements/transport of small molecules/solutes along plant architecture from root to shoot to flowers to grain (or fruit) is well known. In recent years, newer developments have shown that hormones are also functional in the same cells where they are synthesized as well as in the neighboring or distant cells (Finch and Rose 1995). Thus the debate on whether hormones act distantly rather than in cells where their synthesis occurs was resolved.

Today at least nine major plant hormones are recognized whose origin and functional aspects have produced valuable information. These are, in no particular order, "auxins," "ethylene," "cytokinin," "gibberellins," "abscisic acid," "brassinosteroids," "jasmonates," "strigolactones," and "salicylic acid." Florigen (flowering), nitric oxide (NO), and polyamines (PAs) (mainly putrescine, spermidine, and spermine) as plant growth regulators are additions to this elite group of plant regulators. The current understanding of the plant hormones is that each of them plays a critical role in almost every part of the plant not only specifically and singularly but also via interaction with other hormone(s). For many of them, tremendous progress about their perception, intra- and intercellular movement/communication, and interaction with receptors and gene regulation has been made mainly due to the developments in technology, including forward genetics, ease of plant transformation systems, and the gain-of-function and loss-of-function model systems.

9.2 Biosynthesis of Plant Hormones

Precursors of a majority of established plant hormones fall into three main classes: those synthesized from amino acids, auxin (IAA) (from tryptophan), and ethylene (from methionine)—IAA is also synthesized via a tryptophan-independent pathway (Fig. 9.1). Methionine (Met) is the precursor of ethylene and higher polyamines. Ethylene pathway initiates with formation of S-adenosyl methionine (SAM) from Met which is then converted to ethylene via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC) (Fig. 9.2). Polyamine (PA) biosynthesis initiates from arginine (ARG)/ornithine (ORN) to synthesize putrescine which in the presence of decarboxylated SAM is converted to spermidine (SPD) (Fig. 9.2). In turn, an additional molecule

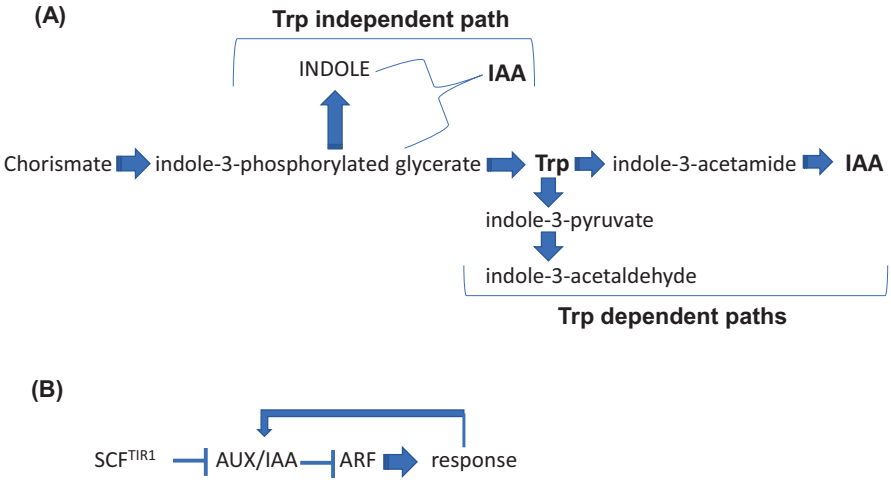

Fig. 9.1 Simplified biosynthesis (**a**) and signaling pathways (**b**) for IAA/auxin

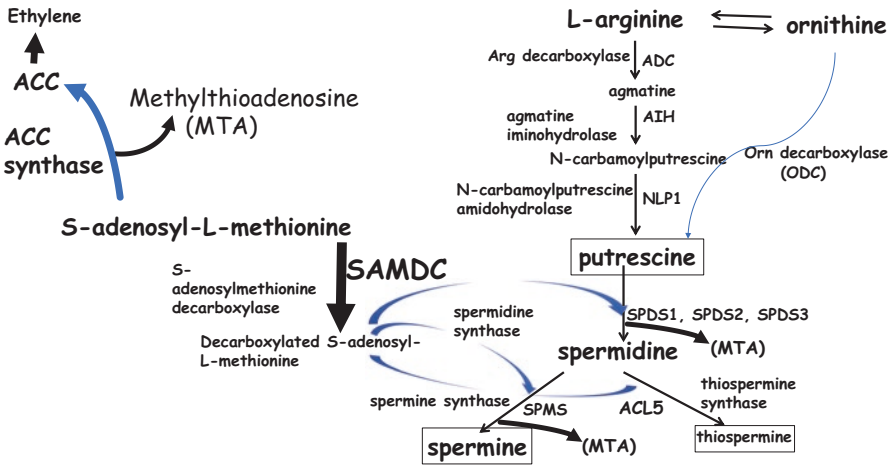

Fig. 9.2 Biosynthetic pathways for the synthesis of ethylene and polyamines putrescine, spermidine, spermine, and thiospermine

of decarboxylated SAM and SPD as substrates is converted to spermine (SPM). SPD is also a substrate for thiospermine catalyzed by thiospermine synthase (Fig. 9.2). Plants utilize both ARG and ORN pathways except for *Arabidopsis* in which, genomic studies showed, ornithine decarboxylase (ODC) gene is missing in this plant.

Isoprenoid pathway (including carotenoids) is responsible for the synthesis of a number of plant hormones, namely, abscisic acid (ABA), brassinosteroids (brassins), cytokinins (CKs), gibberellins (GAs), and strigolactones, while the jasmonate (JAs) family of hormones is derived from lipids (α-linolenic acid) (Fig. 9.3a, b). SA is synthesized from chorismate or arogenate (via shikimate/phenylalanine pathway). The second messenger

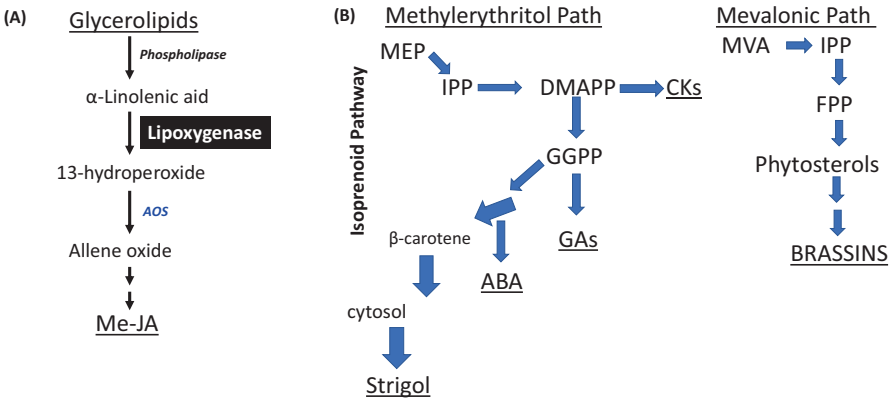

Fig. 9.3 Biosynthetic pathways for jasmonates from glycerolipids (**a**) and isoprenoid-derived hormones CKs, GAs, ABA, strigol, and brassins (**b**)

NO is derived from the amino acid arginine, which is also a precursor of PAs. Nitrate reductase (NR) is a confirmed plant enzyme that catalyzes NO synthesis. Unlike the animal systems where NO synthase is well characterized, its plant counterpart is yet to be identified and characterized. Florigen known as a floral program activator is a protein derived from *FLOWERING LOCUS T* (*FT*) gene (Samach et al. 2000).

Further, plants also synthesize small peptides that seem to work as hormones as in the case of florigen. These other peptide hormones include 18-amino acids long systemin known for its role in plant defense, such as against insect attack. Similarly, another peptide hormone called RALF, made up of 49 amino acids, is expressed in plant organs and influences cellular function. The early narrow view of plant hormones and their action has drastically changed with the recognition that special and diverse hormone-like molecules are synthesized by plants and their function consigns hormonal action to them.

9.3 Modes of Interaction Among Hormones

As stated in previous sections, plant hormones are structurally diverse and their biosynthetic precursors have been mostly identified and characterized. While it is understood that each hormone has a specific, independent mode of action, recent developments/advances in plant biology have also brought a new reality to fore on the complexity in hormonal involvement in plant biological processes. Hormone homeostasis involves concerted crosstalk between synthesis, degradation, and conjugation and how each step is regulated. Specifically, several hormones singly and/or in combination with other hormone(s) interact to positively or negatively regulate a certain mode of action and thereby impact diverse plant processes, including growth and development and multiphasic signaling. Namely, IAA, BRs, CKs, ABA, JAs, ethylene, and GAs impact aspects of seed dormancy/germination, leaf development, reproduction, growth, fruit

development and ripening, senescence, and cell death (Linkies and Leubner-Metzger 2012; Miransari and Smith 2014; Buchanan et al. 2015). Polyamine spermine interacts with ABA, IAA, and ethylene to regulate ripening of strawberry (Guo et al. 2018).

Asymmetric IAA gradients regulate plant developmental processes, for instance, embryo-/organogenesis, apical hook formation (apical dominance), fruit development, and pattern of roots (Weijers et al. 2018). Root cell type-specific responses of IAA at the gene level analyzed by transcriptomics showed discrete response competence by different cell types (Bargmann et al. 2013), further attesting to the fact that auxin/IAA regulation of plant development is complex. Polyamine SPM negatively regulates auxin carrier (*Aux/IAA*, *ARF*, and *SAUR*) genes in *Arabidopsis* (Gonzalez et al. 2011), while in tomato fruit higher SPD/SPM stimulate expression of auxin-regulated genes (Kolotilin et al. 2011).

Likewise, ABA, ethylene, and IAA enhance hypocotyl elongation via DOF transcription factor DAG1 (Lorrai et al. 2018). Ethylene, ABA, BRs, SA, and JAs are considered as promoters while CKs and GAs as inhibitors of leaf senescence. CK receptor kinase (CKR) likely regulates CK transport into leaves and may be involved in maintaining homeostasis of CK in the leaf tissue (Sugiyama and Sakakibara 2002; Kumar et al. 2004). Thus, shoot to the root translocation of CK via the phloem seems to control vascular patterning in the root apex (Bishopp et al. 2011).

Ethylene is a gaseous hormone with simplest structure, and like ABA it is the singleton of its class. Ethylene is produced in all higher plants and fungi, being more studied for its role as a fruit ripening hormone, abscission, dormancy, and the triple response.

Modulation of ethylene signaling by polyamines and quantification of endogenous ACC and NO demonstrated that NO and ACC are inversely correlated in impacting olive fruit abscission (Parra-Lobeto and Gomez-Jimenez 2011). Tomato germplasm with SPD and SPM accumulation trait can substitute for ethylene deficiency and modulate primary metabolism (Sobolev et al. 2014). Signaling pathways of PAs, GAs, and ethylene were shown to be prominently upregulated during dark-induced senescence of barley concomitant with the downregulation of CK, JA, and IAA signaling pathways (Sobieszczuk-Nowicka et al. 2018). SPD/SPM regulate GA's conjugation into inactive forms. Evidence for PAs in altering GA signaling gene(s) in *Arabidopsis* or tomato remains to be ascertained (Anwar et al. 2015).

Arabidopsis mutants deficient in polyamine oxidase PAO4 are unable to back convert SPM to SPD to PUT and therefore do not produce the oxidation product hydrogen peroxide. However, these mutants accumulate NO (Sequera-Mutiozabal et al. 2016). High NO and low PAO4 (and likely high PAs) become causative thereof in delaying senescence. Some of these aspects have been reviewed (Mattoo and Sobieszczuk-Nowicka 2019).

Brassins have attracted a lot of attention due to their involvement in diverse physiological processes and are considered as master regulators of GAs synthesis, thereby making them relevant to plant growth and development (Unterholzner et al. 2015). Brassin-specific transcription factors involved in regulation of plant growth have been found localized to nucleus (Yin et al. 2005; Wang et al. 2002). Strigolactones have been characterized but less are known about their roles in plant biology and less so about their interactions with other plant hormones (Zwanenburg

and Blanco-Ania 2018). Their notable activities include involvement in architecture of plants, inhibition of shoot branching, and as being anti-carcinogenic (Mayzlish-Gati et al. 2015; Zwanenburg and Blanco-Ania 2018).

Tomato genotypes deficient in ethylene or JAs are deficient in accumulating organic acids, while their genetic crosses with high PAs can reverse this deficiency; moreover, a cross of PAs X ethylene-deficient lines caused severe loss in the accumulation of amino acids (Fatima et al. 2016). These findings demonstrated that a robust and metabolism-based crosstalk exists between plant hormones in regulating plant metabolism.

JA was shown to inhibit tobacco shoot formation and upregulate *ADC*, *ODC,* and *SAMDC* expression (Biondi et al. 2001). It was shown to induce PA conjugation via a JA-responsive transcription factor R2R3-MYB8 (Kaur et al. 2010). PAs also stimulate JA conjugation (Gonzalez et al. 2011). JA signaling and SA-upregulated genes seem to work in concert to activate pathology defense in rice (Tamaoki et al. 2013). It has also been shown that PAs weaken ethylene-mediated plant defense against certain tomato pathogens such as *Botrytis cinerea* (Nambeesan et al. 2012).

Significant developments in our understanding of plant hormonal signal transduction mechanisms have occurred for most hormones. However, less so is known about molecular aspects underlying PA action. PAs act as "rejuvenator molecules" and are antagonistic to aging of normal plant cells (Handa and Mattoo 2010; Mattoo et al. 2010; Sobieszczuk-Nowicka et al. 2018, 2019). Not surprisingly, recent studies indicate that each PA—putrescine (PUT), spermidine (SPD), spermine (SPM), and thermo-SPM—independently and specifically regulate diverse plant processes (Mattoo et al. 2010; Anwar et al. 2015). A complex relationship among the three PAs in regulating gene medleys involved in the biosynthesis and signaling pathways of other plant hormones has been documented (Anwar et al. 2015).

9.4 Crosstalk Between Hormones in Plant Development

The sessile nature of plants has made them able to either continuously cease or resume growth. Plant hormones play a major role in this flexible architecture and growth patterns involving a delegated single hormone and via crosstalk with other hormones and growth factors. As stated above, previous studies greatly advanced our knowledge of how each plant hormone individually affected plant growth and development and stress responses. To this knowledge, new advances have made it evident that plant physiological processes are regulated in a complex crosstalk among different hormones. How hormonal crosstalk coordinates processes during plant growth and development as well as in response to changing environment is a major challenge to plant biologists. Thus, biosynthetic pathways of hormones are getting clearer, novel signaling mechanisms have been identified and proposed, and several biochemical processes involved have been unearthed (Murphy 2015). Broadly, crosstalk with other pathways is mediated by transcription factors, small RNAs, or long noncoding RNAs (LncRNAs) as well as protein: protein interactions with or without involving protein promoters. Several crosstalk networks involving different hormonal regulation of

Table 9.1 Selected hormonal crosstalk examples

Process	Crosstalk genes	Integrating hormonal pathways
Seed germination	*ETO3, CTR1, ETR1, EIN2, EIN6, ACO2*	ABA, gibberellic acid, and ethylene
Root growth and development	*PIN1, PIN2, AUX1, VAS1, ACS, ACO, ERF1, ASA1*	Ethylene and auxin/IAA
Leaf development	*KNOX1, IPT7, SPY, TCP*	Gibberellic acid, cytokinins, and brassinosteroids
Fruit development and ripening	*MADS-RIN, ARF2A, PHY, LOX, SlSAMDC, FaSAMDC*	Ethylene, auxin/IAA, JA/MeJA, ABA, and polyamines
Abiotic stress	*ERF1*, *JERF3*, *PDF1.2, PR*	Ethylene, JA/MeJA, ABA, cytokinins, polyamines, and salicylic acid
Biotic stress	*ERF1, ORA59, MYC*	Ethylene, JA/MeJA, and salicylic acid

plant processes are now known as summarized in Table 9.1, and these will be highlighted below.

9.4.1 Crosstalk in Root Development and Elongation

A complex molecular interaction between ethylene and auxin/IAA is known to regulate root elongation (Benková and Hejátko 2009; Muday et al. 2012; Van de Poel et al. 2015; Hu et al. 2017). Ethylene stimulates IAA biosynthesis and upregulates several IAA transporters, namely, *PIN1, PIN2, and AUX1,* in *Arabidopsis* (Ruzicka et al. 2007; Stepanova et al. 2007; Swarup et al. 2007). Ethylene-induced IAA production is localized in the root tip (Swarup et al. 2007). This IAA signal is redirected by polar transport toward the root elongation zone inhibiting in turn the cell elongation (Ruzicka et al. 2007). Studies using IAA transport mutants (*pin2* and *aux1*) indicated an ethylene-insensitive root growth in the absence of ethylene and IAA crosstalk (Ruzicka et al. 2007). Another enzyme known as VAS1 regulates IAA and ethylene production which leads to synergistic coordination in the biosynthesis of both hormones (Zheng et al. 2013; Pieck et al. 2015).

IAA is known to regulate ethylene biosynthesis during root development (Benková and Hejátko 2009; Muday et al. 2012). Exogenous application of IAA induces the expression of ethylene pathway enzymes ACC synthase (ACS) and ACC oxidase (ACO) in pea and *Arabidopsis roots* (Peck and Kende 1995, 1998; Tsuchisaka and Theologis 2004; Stepanova et al. 2007). Several processes in root biology, for example, root gravitropism, root hair initiation and elongation, hypocotyl growth, and apical hook formation, are regulated by ethylene and IAA crosstalk (Lee et al. 1990; Tanimoto et al. 1995; Pitts et al. 1998; Collett et al. 2000; Rahman et al. 2002; Lehman et al. 1996). The role of IAA-ethylene crosstalk in

orchestrating primary root elongation in sugar beet (*Beta vulgaris* L.) has also been demonstrated (Abts et al. 2017). Many of these crosstalks are mediated by transcription factors; for example, *Arabidopsis* ERF1 mediates such a crosstalk by regulating *ASA1* expression (Mao et al. 2016).

9.4.2 Crosstalk During Seed Germination

The roles of ABA and GAs in the regulation of seed germination are well known. Primarily, ABA initiates and maintains seed dormancy while GAs are known to release dormancy and initiate seed germination. Ethylene and ABA work antagonistically in regulating seed germination; however, ethylene effects on seed dormancy and germination is based on reciprocal effects on both ABA and GA biosynthesis and signaling (Arc et al. 2013; Corbineau et al. 2014; Miransari and Smith 2014). Ethylene and NO counteract ABA-mediated seed dormancy and, in turn, enhance germination in Arabidopsis (Arc et al. 2013; Corbineau et al. 2014). In ethylene mutants, *eto3* and *ctr1*, ABA perception is significantly reduced, but this is significantly enhanced in the ethylene-insensitive alleles of *etr1*, *ein2,* and *ein6* (Subbiah and Reddy 2010). Far-red light-based loss of the ethylene receptor, ETR1, was demonstrated to affect ABA and GA biosynthesis and signaling during seed germination (Wilson et al. 2014). In ethylene biosynthesis mutant, *aco2*, ethylene production by *ACO2* blocks ABA-controlled inhibition of endosperm rupture (Linkies et al. 2009; Linkies and Leubner-Metzger 2012). At the molecular level, a number of plant hormones (ABA, IAA, ethylene, GA, CKs, and BRs) could impact germination, with opposite effects between ethylene and BRs, IAA and JAs, and ABA and GAs (Corbineau et al. 2014; Miransari and Smith 2014).

9.4.3 Crosstalk During Leaf Development

Leaf development is divided into several important events: initiation, maintenance, and regulation of shoot apical meristem, leaf maturation, and differentiation (Veit 2004; Braybrook and Kuhlemeier 2010). Each of these activities is regulated by a set of hormones and their crosstalk (Shwartz et al. 2016; Bar and Ori 2014). The homeostatic equilibrium between hormones together with the nature of their interactions seems to impact all stages of leaf development. A coordination between IAA and CKs regulates leaf initiation. In tomato, light was found to be essential for both IAA and CK to regulate leaf initiation (Yoshida et al. 2011).

Regulation of shoot apical meristem by GA is controlled by a plethora of transcription factors and proteins. For example, class I KNOTTED LIKE HOMEOBOX (KNOXI) and TCP proteins regulate GA dynamics. KNOXI maintains GA levels by repressing the GA biosynthesis gene GA20ox and activating the GA inactivation gene GA2ox. These effects on GA homeostasis by KNOXI in tuning the shoot apical meristem and leaf boundary and in modulating compound leaf development

have been investigated in *Arabidopsis*, maize, tobacco, and tomato (Sakamoto et al. 2001; Hay et al. 2002; Jasinski et al. 2005; Bolduc and Hake 2009). The rate at which a leaf matures is also known to be regulated by several plant hormones. Among these, GA regulates cell proliferation and expansion rate in Arabidopsis (Achard et al. 2009). Also, GA negatively regulates leaf complexity in tomato. Only primary leaflets with smooth margins are formed and the leaves mature faster than the wild-type leaves with increased GA accumulation or GA response/signaling (Gray 1957; Jones 1987; Chandra-Shekhar and Sawhney 1991; Van Tuinen et al. 1999; Hay et al. 2002; Bassel et al. 2008; Jasinski et al. 2008; Fleishon et al. 2011).

KNOX1 regulated crosstalk between CK and GA biosynthesis/signaling pathways by triggering CK biosynthesis via the activation of CK biosynthetic enzyme isopentenyltransferase (*IPT7*) and repression of GA biosynthetic genes GA20-oxidase transcription (Sakamoto et al. 2001; Jasinski et al. 2005). Overexpression of Arabidopsis KNOXI gene in lettuce (Lactuca sativa) leaves leads to indeterminate growth due to accumulation of specific type of CK (Frugis et al. 2001). KNOXI proteins also affect the BR hormone signaling (Farquharson 2014; Tsuda et al. 2014). These studies made it clear that KNOXI proteins coordinate the activity of several plant hormones during leaf development.

The *Arabidopsis* GA response inhibitor SPINDLY (SPY) interacts with transcription factor TEOSINTE BRANCHED1 (TCP) and positively regulates CK signaling (Greenboim-Wainberg et al. 2005; Steiner et al. 2012). Notably, overexpression of the *Arabidopsis* class I TCPs, AtTCP14 and AtTCP15, in tomato resulted in a fewer leaflets, smooth leaflet margins, and ectopic meristems on leaf petioles, thus impacting leaf morphology (Steiner et al. 2016).

Hormonal crosstalk between different hormones during leaf development can be species specific. For example, GA influences leaf expansion in pea but not so in tomato. Further, while IAA promotes leaf simplification in tomato, it promotes indeterminate growth in pea (DeMason and Chetty 2011). Interestingly, in the absence of an auxin response, cytokines are unable to significantly prolong tomato leaf morphogenesis (Shani et al. 2010).

9.4.4 Crosstalk During Fruit Development and Ripening

Ethylene is established as the primary ripening hormone in climacteric fruits. Its role in the ripening of non-climacteric fruits seems minimal. The development of fruit ripening tomato mutants, viz., *rin*, *nor*, and *Nr*, enabled studies that unraveled a central role of transcription factors and ethylene in ripening (Giovannoni 2007). The MADS-box gene *SlMADS-RIN* seems to repress rather than activate ethylene responses. Other hormones also seem to play a role in fruit ripening, a field of research that is growing. An integration of other hormonal pathways is now known to be a part of fruit ripening process. For example, IAA-responsive transcription factor, AUXIN RESPONSE FACTOR 2A (ARF2A), which regulates crosstalk between ethylene and IAA, seems to play a critical role in the ripening process (Hao et al. 2015; Breitel et al. 2016). *SlZFP2* (*tomato zinc finger protein*) transcription

factor modulates crosstalk between ABA and ethylene during fruit development and ripening in tomato (Weng et al. 2015).

Light plays a dual role during plant development, providing energy for photosynthesis and modulating overall plant growth and development. Light is a stimulus for seed germination, seedling de-etiolation, phototropism, flowering, fruit ripening and pigmentation, and circadian rhythms (Giovannoni 2004; Azari et al. 2010; Llorente et al. 2016; Cruz et al. 2018). Research utilizing photomorphogenic tomato mutants helped decipher the importance of light signaling in fruit biology and quality traits (Levin et al. 2006; Azari et al. 2010). Tomato *high pigment* (*hp*) mutants, *hp1* and *hp2*, have higher light responsiveness, over-accumulate chlorophyll and chloroplasts in leaves, but have immature fruits with an intense red pigmentation (Mustilli et al. 1999; Levin et al. 2003, 2006). These mutants accumulate carotenoids, flavonoids, tocopherol, and ascorbic acid—nutritional molecules—as compared to their wild relatives (Yen et al. 1997; Liu et al. 2004; Kolotilin et al. 2007). Thus, carotenogenesis is particularly upregulated in *hp* mutants, supporting the positive influence of light on isoprenoid metabolism in both fruit and vegetative tissues (Piringer and Heinze 1954; Alba et al. 2000; Schofield and Paliyath 2005). Two negative regulators of light signal transduction pathway, namely, UV-Damaged DNA Binding Protein1 (DDB1) for *hp1* mutation and Deetiolated1 (DET1) for *hp2*, caused these mutations (Mustilli et al. 1999; Schroeder et al. 2002; Levin et al. 2003; Lieberman et al. 2004; Liu et al. 2004). Fruit-specific silenced phytochrome (PHY)-encoding genes (Bianchetti et al. 2018), cryptochrome1a (CRY1a)-deficient mutants, and *CRY1a*-overexpressing lines (Liu et al. 2018) all have significant alterations in carotenoid biosynthesis. PHY is also controlled by RIN, a master regulator of ethylene, since *rin* tomato mutants do not develop carotenoids, indicating a crosstalk between ethylene and light (Martel et al. 2011).

Interestingly, all fruit metabolic processes influenced by light are also strictly controlled by an integrated, multi-hormonal signaling network (Giovannoni 2004; Karlova et al. 2011; Liu et al. 2015). Ethylene regulates multiple ripening-related physiological, biochemical, and molecular processes (Barry and Giovannoni 2007; Pech et al. 2012). Therefore, interference with ethylene biosynthesis, perception, or signal transduction can directly impact fruit ripening initiation and progression (Liu et al. 2015). IAA too has been shown to interfere with fruit ripening and carotenoid accumulation, since IAA-treated tomato fruits have delayed ripening phenotype along with downregulation of carotenoid biosynthesis (Su et al. 2015). The involvement of ethylene and IAA in the light-mediated regulation of tomato fruit ripening and carotenogenesis was investigated by comparing the impact of light and dark treatments together with loss of *Sl-DET1*/*HP2* function (Cruz et al. 2018). Also, upregulation of polyamine pathway to upregulate SPD and SPM led to higher lycopene accumulation and ethylene evolution in tomato, further suggesting a crosstalk between polyamines, lycopene accumulation, and ethylene (Mehta et al. 2002). In this context, the observation that polyamines play a crucial role in strawberry fruit ripening via a crosstalk with ethylene, ABA, and IAA is very pertinent (Guo et al. 2018).

9.5 Hormonal Crosstalk During Abiotic Stress

Combating environmental stresses is crucial to maintain a crop or to enhance it. During such adverse conditions, crosstalk between hormones may be a deciding factor to combat the stress and maintain the crop. Defense responses involving crosstalk between several hormones during abiotic stress have been highlighted. *Arabidopsis ERF1,* a downstream component of the ethylene signaling pathway that integrates JA and ethylene signaling pathways, has been shown to regulate defense-responsive genes *β-CH I* (*basic chitinase*) and *PDF1.2* (*plant defensin 1.2*) (Solano et al. 1998; Lorenzo et al. 2003). *ERF1* integrates various abiotic stress pathways and regulates stress-responsive gene expression by binding to different *cis*-acting elements in response to different stress signals (Cheng et al. 2013). An activator-type and jasmonate-induced ERF protein, *JERF3*, binds to the ethylene-responsive *cis*-element (GCC box), JA-responsive *cis*-element, and dehydration-responsive *cis*-element (DRE) to mediate crosstalk between dehydration, high salt, and low temperature. Expression of *JERF3* is mainly induced by ethylene, JA, cold, salt, or ABA in tomato. Constitutive expression of *JERF3* in transgenic tobacco significantly activated expression of pathogenesis-related genes (basic chitinase and PR proteins) that harbor the GCC box, resulting in enhanced tolerance to pathogens and salt. Thus, *JERF3* functions as a linker in ethylene and osmotic stress-signaling pathways (Wang et al. 2004).

Interaction between ethylene and ABA regulates stomatal closure under drought stress. For example, high ethylene concentrations inhibit ABA-induced stomatal closure in leaves (Tanaka et al. 2005). Also, it is known that ABA-deficient maize seedlings have increased ethylene production, which indicates that ABA inhibits ethylene production (Sharp 2002). Therefore, the increase in ABA concentration under drought stress may cause a reduction in ethylene production.

Expression of Brassin receptor, BRL3, at high levels in Arabidopsis promoted resistance to drought (Fabregas et al. 2018). Moreover, the drought resistance was not accompanied by negating the growth of the transgenic plants.

9.6 Hormonal Crosstalk During Wounding Stress

Wounding is a special, site-specific stress which has been examined in terms of hormonal involvement. Wound-induced gene expression accompanies upregulation of JA and ethylene biosynthesis and response-associated genes in *Arabidopsis* suggesting a possible crosstalk (Cheong et al. 2002; Reymond et al. 2000). The observation of ABA accumulation at the wounded site is also considered a response to dehydration due to wounding (León et al. 2001). Wounding also led to high expression of CPK32 (Chotikacharoensuk et al. 2006), which is known to phosphorylate the ABA-responsive transcription factor ABF4 (Choi et al. 2005). During wounding, JA treatment upregulates numerous ERF genes (McGrath et al. 2005). *Arabidopsis* ERF1 has been shown to be the master regulator of ethylene and JA crosstalk (Lorenzo et al. 2003), while ERF4 is a negative regulator of ABA and ethylene responses and could be an integrator of both pathways (Yang et al. 2005).

9.7 Conclusions

Significant progress has been made in delineating the biosynthetic pathways for each plant hormone, as newer hormone-like molecules are discovered and identified. Each plant hormone seems to have a singularly specific and characteristic function in plant biological processes, but it is more and more recognized that each of them is also specifically connected to specific crosstalk(s) with other hormones. Such interactions result in either positive or antagonistic effects on one or more plant biological process(es). As we understand more about how plant hormones walk, talk, and interact in plant cells, the intricate details of their impact in modulating plant growth, development, and death should become clearer. It is not surprising that plant hormones seem to have a say in which direction a plant will eventually proceed/progress, right from seed germination to root development/elongation, leaf development, fruit set, fruit development/ripening, and finally to senescence and death, or may even rejuvenate briefly before the final end. We anticipate that the hormonal biological interactions will further deepen our understanding of normal plant life processes as well as how plants utilize hormonal crosstalk for sustenance of life and productivity during environmental extremes.

Further Reading

Abeles F, Morgan P, Saltviet N Jr (1992) Ethylene in plant biology. Elsevier, San Diego

Buchanan BB, Gruissem W, Jones RL (eds) (2015) Biochemistry & molecular biology of plants, 2nd edn. Wiley Blackwell, New York

Dharmasiri NDharmasiri S, Estelle M (2005) Nature 435:441–445

Lifschitz E, Ayre BG, Eshed Y (2014) Florigen and anti-florigen – a systemic mechanism for coordinating growth and termination in flowering plants. Front Plant Sci 5:465

Mattoo AK, Suttle J (eds) (1991) The plant hormone ethylene. CRC Press, Boca Raton

Mockaitis K, Estelle M (2008) Auxin receptors and plant development: a new signaling paradigm. Annu Rev Cell Dev Biol.

References

Abts W, Vandenbussche B, De Proft MP, Van de Poel B (2017) The role of auxin-ethylene crosstalk in orchestrating primary root elongation in sugar beet. Front Plant Sci 8:444

Achard P, Gusti A, Cheminant S, Alioua M, Dhondt S, Coppens F (2009) Gibberellin signaling controls cell proliferation rate in Arabidopsis. Curr Biol 19:1188–1193

Addicott FT, Lyon JL (1969) Physiology of abscisic acid and related substances. Annu Rev Plant Physiol 20:139–164

Addicott FT, Lyon JL, Ohkuma K, Thiessen WE, Carns HR, Smith OE, Cornforth JW, Milborrow BV, Ryback G, Wareing PF (1968) Abscisic acid: a new name for Abscisin II (Dormin). Science 159:1493

Alba R, Cordonnier-Pratt MM, Pratt LH (2000) Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. Plant Physiol 123:363–370

Anwar R, Mattoo AK, Handa AK (2015) Polyamine interactions with plant hormones: crosstalk at several levels. In: Kusano T, Suzuki H (eds) Polyamines, vol 22. Springer, Tokyo, pp 267–302

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A (2013) ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci 4:63

Azari R, Reuveni M, Evenor D, Nahon S, Shlomo H, Chen L (2010) Overexpression of UV-damaged DNA binding protein 1 links plant development and phytonutrient accumulation in high pigment-1 tomato. J Exp Bot 61:3627–3637

Bar M, Ori N (2014) Leaf development and morphogenesis. Development 141:4219–4230

Bargmann BOR, Vanneste S, Krouk G, Nawy T, Efroni I, Shani E, Choe G, Friml J, Bergmann DC, Estelle M, Birnbaum KD (2013) A map of cell type-specific auxin responses. Mol Syst Biol 9:688

Barry CS, Giovannoni JJ (2007) Ethylene and fruit ripening. J Plant Growth Regul 26:143–159

Bassel GW, Mullen RT, Bewley JD (2008) *Procera* is a putative DELLA mutant in tomato (*Solanumlycopersicum*): effects on the seed and vegetative plant. J Exp Bot 59:585–593

Bayliss WM, Starling EH (1902) Mechanism of pancreatic secretion. J Physiol 28:325–353

Benková E, Hejátko J (2009) Hormone interactions at the root apical meristem. Plant Mol Biol 69:383–396

Bianchetti RE, Lira BS, Moneiro SS, DeMarco D, Purgatto E, Rossi M (2018) Fruit-localized phytochromes regulate plastid biogenesis, starch synthesis and carotenoid metabolism in tomato. J Exp Bot 69:3573–3586

Biondi S, Scaramagli S, Capitani F, Altamura MM, Torrigiani P (2001) Methyl jasmonate upregulates biosynthetic gene expression, oxidation and conjugation of polyamines, and inhibits shoot formation in tobacco thin layers. J Exp Bot 52:231–242

Bishopp A, Lehesranta S, Vaten V, Help H, El-Showk E, Scheres B, Helariutta K, Mahonen AP, Sakakibara H, Helariutta Y (2011) Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. Curr Biol 21:927–932

Bolduc N, Hake S (2009) The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene *ga2ox1*. Plant Cell 21:1647–1658

Braybrook SA, Kuhlemeier C (2010) How a plant builds leaves. Plant Cell 22:1006–1018

Breitel DA, Chappell-Maor L, Meir S, Panizel I, Puig CP, Hao Y, Yifhar T, Yasuor H, Zouine M, Bouzayen M, Granell Richart A, Rogachev I, Aharoni A (2016) AUXIN RESPONSE FACTOR 2 intersects hormonal signals in the regulation of tomato fruit ripening. PLoS Genet 12:e1005903

Cook CE, Whichard LP, Turner B, Wall ME, Egley GH (1966) Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. Science 154:1189–1190

Chandra-Shekhar KN, Sawhney VK (1991) Regulation of leaf shape in the *solanifolia* mutant of tomato (*Lycopersiconesculentum*) by plant growth substances. Ann Bot 67:3–6

Cheng MC, Liao PM, Kuo WW, Lin TP (2013) The *Arabidopsis ETHYLENE RESPONSE FACTOR1* regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. Plant Physiol 162:1566–1582

Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. Plant Physiol 129:661–677

Choi HI, Park HJ, Park JH, Kim S, Im MY, Seo HH, Kim YW, Hwang I, Kim SY (2005) *Arabidopsis* calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. Plant Physiol 139:1750–1761

Chotikacharoensuk T, Arteca RN, Arteca JM (2006) Use of differential display for the identification of touch-induced genes from an ethylene-insensitive *Arabidopsis* mutant and partial characterization of these genes. J Plant Physiol 163:130–1320

Clouse SD (2011) Brassinosteroids. Arabidopsis Book 9:e0151

Collett CE, Harberd NP, Leyser O (2000) Hormonal interactions in the control of *Arabidopsis* hypocotyl elongation. Plant Physiol 124:553–562

Corbineau F, Xia Q, Bailly C, El-Maarouf-Bouteau H (2014) Ethylene, a key factor in the regulation of seed dormancy. Front Plant Sci 5:539

Cruz AB, Bianchetti RE, Alves FRR, Purgatto E, Peres LEP, Rossi M, Freschi L (2018) Light, ethylene and auxin signaling interaction regulates carotenoid biosynthesis during tomato fruit ripening. Front Plant Sci 9:1370

DeMason DA, Chetty VJ (2011) Interactions between GA, auxin, and UNI expression controlling shoot ontogeny, leaf morphogenesis, and auxin response in Pisum sativum (Fabaceae): or how the uni-tac mutant is rescued. Am J Bot 98:775–791

Fabregas N, Lozano-Elena F, Blasco-Escamez D, Tohge T, Martinez-Andujar C, Albacete A, Osorio S, Bustamante M, Riechmann JL, Nomura T, Yokota T, Conesa A, Alfocea FP, Fernie AR, Cano-Delgado AI (2018) Overexpression of the vascular brassinosteroid receptor BRL3 confers drought resistance without penalizing plant growth. Nat Commun 9:4680

Farquharson KL (2014) A rice KNOX transcription factor represses brassinosteroid production in the shoot apical meristem. Plant Cell 26:3469

Fatima T, Sobolev A, Teasdale J, Kramer M, Bunce J, Handa A, Mattoo AK (2016) Fruit metabolite networks in engineered and non-engineered tomato genotypes reveal fluidity in a hormone and agroecosystem specific manner. Metabolomics 12:103

Finch CE, Rose MR (1995) Hormones and the physiological architecture of life history evolution. Q Rev Biol 70:1–52

Fleishon S, Shani E, Ori N, Weiss D (2011) Negative reciprocal interactions between gibberellin and cytokinin in tomato. New Phytol 190:609–617

Frugis G, Giannino D, Mele G, Nicolodi C, Chiappetta A, Bitonti MB, Innocenti AM, Dewitte W, Van Onckelen H, Mariotti D (2001) Overexpression of KNAT1 in lettuce shifts leaf determinate growth to a shoot-like indeterminate growth associated with an accumulation of isopentenyl-type cytokinins. Plant Physiol 126:1370–1380

Gane R (1934) Production of ethylene by some ripening fruits. Nature (London) 134:1008

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:170–181

Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 10:283–289

Girardin JPL (1864) Jahresber. Agrikult Chem Versuchssta Berlin 7:199

Gonzalez ME, Marco F, Minguet EG, Carrasco-Sorli P, Blázquez MA, Carbonell J, Ruiz OA, Pieckenstain FL (2011) Perturbation of spermine synthase gene expression and transcript profiling provide new insights on the role of the tetra-amine spermine in *Arabidopsis* defense against *Pseudomonas viridiflava*. Plant Physiol 156:2266–2277

Gray RA (1957) Alteration of leaf size and shape and other changes caused by gibberellins in plants. Am J Bot 44:674–682

Greenboim-Wainberg Y, Maymon I, Borochov R, Alvarez J, Olszewski N, Ori N, Eshed Y, Weiss D (2005) Cross talk between gibberellin and cytokinin: the *Arabidopsis* GA response inhibitor *SPINDLY* plays a positive role in cytokinin signaling. Plant Cell 17:92–102

Grove MD, Spencer GF, Rohwedder WK, Mandava NB, Worley JF, Warthen JD, Steffens GL, Flippen-Anderson JL, Cook JC (1979) Brassinolide, a plant growth-promoting steroid isolated from Brassica napus pollen. Nature 281:216–217

Guo J, Wang S, Yu X, Dong R, Li Y, Mei X, Shen Y (2018) Polyamines regulate strawberry fruit ripening by abscisic acid, auxin, and ethylene. Plant Physiol 177:339–351

Handa AK, Mattoo AK (2010) Differential and functional interactions emphasize the multiple roles of polyamines in plants. Plant Physiol Biochem 48:540–546

Hao Y, Hu G, Breitel D, Liu M, Mila I, Frasse P, Fu Y, Bouzayen M, Zouine M (2015) Auxin Response Factor SlARF2 is an essential component of the regulatory mechanism controlling fruit ripening in tomato. PLoS Genet 11:e1005649

Hauck C, Muller S, Schildknecht H (1992) A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. J Plant Physiol 139:474–478

Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M (2002) The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Curr Biol 12:155–1565

Hori S (1898) Some observations on 'Bakanae' disease of the rice plant. Mem Agric Res Sta (Tokyo) 12:110–119

Hu Y, Vandenbussche F, Van Der Straeten D (2017) Regulation of seedling growth by ethylene and the ethylene-auxin crosstalk. Planta 245:467–489

Jasinski S, Piazza P, Craft J, Hay A, Woolley L, Rieu I, Phillips A, Hedden P, Tsiantis M (2005) KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr Biol 15:1560–1565

Jasinski S, Tattersall A, Piazza P, Hay A, Martinez-Garcia JF, Schmitz G, Theres K, McCormick S, Tsiantis M (2008) PROCERA encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant J 56:603–612

Jones GM (1987) Gibberellins and the *procera* mutant of tomato. Planta 172:280–284

Karlova R, Rosin FM, Busscher-Lange J, Parapunova V, Do PT, Fernie AR et al (2011) Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. Plant Cell 23:923–941

Kaur H, Heinzel N, Schöttner M, Baldwin IT, Gális I (2010) R2R3-NaMYB8 regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in Nicotiana attenuata. Plant Physiol 152:1731–1747

Kolotilin I, Koltai H, Tadmor Y, Bar-Or C, Reuveni M, Meir A et al (2007) Transcriptional profiling of high pigment-2dg tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. Plant Physiol 145:389–401

Kolotilin I, Koltai H, Bar-Or C, Chen L, Nahon S, Shlomo H, Levin I, Reuveni M (2011) Expressing yeast SAMdc gene confers broad changes in gene expression and alters fatty acid composition in tomato fruit. Physiol Plant 142:211–223

Kumar V, Mills DJ, Anderson JD, Mattoo AK (2004) An alternative agriculture system is defined by a distinct expression profile of select gene transcripts and proteins. Proc Natl Acad Sci USA 101:10535–10540

Lee JS, Chang WK, Evans ML (1990) Effects of ethylene on the kinetics of curvature and auxin redistribution in gravistimulated roots of Zea mays. Plant Physiol 94:1770–1775

Lehman A, Black R, Ecker JR (1996) HOOKLESS1, an ethylene response gene, is required for differential cell elongation in Arabidopsis hypocotyls. Cell 85:183–194

León J, Rojo E, Sa'nchez-Serrano JJ (2001) Woundsignalling in plants. J Exp Bot 52:1–9

Letham DS (1967) Regulators of cell division in plant tissue: a comparison of the activities of zeatin and other cytokinins in five bioassays. Planta 74:228–242

Levin I, Frankel P, Gilboa N, Tanny S, Lalazar A (2003) The tomato dark green mutation is a novel allele of the tomato homolog of the deetiolated1 gene. Theor Appl Genet 106:454–460

Levin I, de Vos C, Tadmor Y, Bovy A, Lieberman M, Oren-Shamir M et al (2006) High pigment tomato mutants—more than just lycopene (a review). Isr J Plant Sci 54:179–190

Lieberman M, Segev O, Gilboa N, Lalazar A, Levin I (2004) The tomato homolog of the gene encoding UV-damaged DNA binding protein 1 (DDB1) underlined as the gene that causes the *high pigment-1*mutant phenotype. Theor Appl Genet 108:1574–1581

Linkies A, Leubner-Metzger G (2012) Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. Plant Cell Rep 31:253–270

Linkies A, Müller K, Morris K, Turecková V, Wenk M, Cadman CSC, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE et al (2009) Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidiumsativum* and Arabidopsis thaliana. Plant Cell 21:3803–3822

Liu Y, Roof S, Ye Z, Barry C, Van Tuinent A, Vrebalov J et al (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proc Natl Acad Sci USA 101:9897–9902

Liu M, Pirrello J, Chervin C, Roustan J-P, Bouzayen M (2015) Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol 169:2380–2390

Liu CC, Ahammed GJ, Wang GT, Xu CJ, Chen KS, Zhou YH et al (2018) Tomato CRY1a plays a critical role in the regulation of phytohormone homeostasis, plant development, and carotenoid metabolism in fruits. Plant Cell Environ 41:354–366

Llorente B, D'Andrea L, Ruiz-Sola MA, Botterweg E, Pulido P, Andilla J et al (2016) Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism. Plant J 85:107–119

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15:165–178

Lorrai R, Gandolfi F, Boccaccini A, Ruta V, Possenti M, Tramontano A, Costantino P, Lepore R, Vittorioso P (2018) Genome-wide RNA-seq analysis indicates that the DAG1 transcription factor promotes hypocotyl elongation acting on ABA, ethylene and auxin signaling. Sci Rep 8:15895

Mandava NB (1988) Plant growth-promoting brassinosteroids. Ann Rev Plant Physiol Plant Mol Biol 39:23–52

Mao JL, Miao ZQ, Wang Z, Yu LH, Cai XT, Xiang CB (2016) *Arabidopsis* ERF1 mediates cross-talk between ethylene and auxin biosynthesis during primary root elongation by regulating *ASA1* expression. PLoS Genet 12:e1006076

Martel C, Vrebalov J, Tafelmeyer P, Giovannoni JJ (2011) The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a colorless non-ripening dependent manner. Plant Physiol 157:1568–1579

Mattoo AK, Sobieszczuk-Nowicka E (2019) Polyamine as signaling molecules and leaf senescence. In: Sarwat M, Tuteja N (eds) Senescence signalling and control in plants. Elsevier, Academic, pp 125–138

Mattoo AK, Minocha SC, Minocha R, Handa AK (2010) Polyamines and cellular metabolism in plants: transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. Amino Acids 38:405–413

Mayzlish-Gati E, Laufer D, Grivas CF et al (2015) Strigolactone analogs act as new anti-cancer agents in inhibition of breast cancer in xenograft model. Cancer Biol Ther 16:1682–1688

McGrath KC, Dombrecht B, Manners JM, Schenk PM, Edgar CI, Maclean DJ, Scheible WR, Udvardi MK, Kazan K (2005) Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiol 139:949–959

Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat Biotechnol 20:613–618

Miller CO (1961) A kinetin-like compound in maize. Proc Natl Acad Sci USA 47:170–174

Miransari M, Smith DL (2014) Plant hormones and seed germination. Environ Exp Bot 99:110–121

Muday GK, Rahman A, Binder BM (2012) Auxin and ethylene: collaborators or competitors? Trends Plant Sci 17:181–195

Murphy A (2015) Hormone crosstalk in plants. J Exp Bot 66:4853–4854

Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C (1999) Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. Plant Cell 11:145–157

Nambeesan S, AbuQamar S, Laluk K, Mattoo AK, Mickelbart MV, Ferrnuzzi MG, Mengiste T, Handa AK (2012) Polyamines attenuate ethylene-mediated defense responses to abrogate resistance to *Botrytis cinerea* in tomato. Plant Physiol 158:1034–1045

Neljubov D (1901) Uber die horizontale nutation der stengel von Pisum sativum und einigerandererpflanzen. Pflanzen Beih Bot Zentralbl 10:128–138

Nooden LD, Kahanak GM, Okatan Y (1979) Science 206:841–843

O'Malley BW (1989) Editorial: did eukaryotic steroid receptors evolve from intracrine gene regulators? Endocrinology 125:1119–1120

Parra-Lobeto MC, Gomez-Jimenez MC (2011) Polyamine-induced modulation of genes involved in ethylene biosynthesis and signaling pathways and nitric oxide production during olive mature fruit abscission. J Exp Bot 62:4447–4465

Pech JC, Purgatto E, Bouzayen M, Latché A (2012) Ethylene and fruit ripening. Annu Plant Rev 44:275–304

Peck SC, Kende H (1995) Sequential induction of the ethylene biosynthesis enzymes by indole-3-acetic acid in etiolated peas. Plant Mol Biol 28:293–301

Peck SC, Kende H (1998) Differential regulation of genes encoding 1-aminocyclopropane-carboxylate (ACC) synthase in etiolated pea seedlings: effects of indole-3-acetic acid, wounding, and ethylene. Plant Mol Biol 38:977–982

Phinney BO (1983) The history of gibberellins. In: Crozier A (ed) The biochemistry and physiology of gibberellins. Praeger, New York, pp 19–52

Pieck M, Yuan Y, Godfrey J, Fisher C, Zolj S, Vaughan D et al (2015) Auxin and tryptophan homeostasis are facilitated by the ISS1/VAS1 aromatic aminotransferase in Arabidopsis. Genetics 201:185–199

Piringer AA, Heinze PH (1954) Effect of light on the formation of a pigment in the tomato fruit cuticle. Plant Physiol 29:467–472

Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in Arabidopsis. Plant J 16:553–560

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S (2002) Auxin and ethylene response interactions during Arabidopsis root hair development dissected by auxin influx modulators. Plant Physiol 130:1908–1917

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12:707–720

Ruzicka K, Ljung K, Vanneste S, Podhorská R, Beeckman T, Friml J et al (2007) Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell 19:2197–2212

Sakakibara H, Suzuki M, Takei K, Deji A, Taniguchi M, Sugiyama T (1998) A response-regulator homolog possibly involved in nitrogen signal transduction mediated by cytokinin in maize. Plant J 14:337–344

Sakamoto T, Kamiya N, Ueguchi-Tanaka M, Iwahori S, Matsuoka M (2001) KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. Genes Dev 15:581–590

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF et al (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science 288:1613–1616

Schofield A, Paliyath G (2005) Modulation of carotenoid biosynthesis during tomato fruit ripening through phytochrome regulation of phytoene synthase activity. Plant Physiol Biochem 43:1052–1060

Schroeder DF, Gahrtz M, Maxwell BB, Cook RK, Kan JM, Alonso JM et al (2002) De-etiolated 1 and damaged DNA binding protein 1 interact to regulate Arabidopsis photomorphogenesis. Curr Biol 12:1462–1472

Sequera-Mutiozabal MI, Erban A, Kopka J, Atanasov KE, Bastida J, Fotopoulos V, Alcázar R, Tiburcio AF (2016) Global metabolic profiling of Arabidopsis polyamine oxidase 4 (AtPAO4) loss-of-function mutants exhibiting delayed dark-induced senescence. Front Plant Sci 7:173

Shani E, Ben-Gera H, Shleizer-Burko S, Burko Y, Weiss D, Ori N (2010) Cytokinin regulates compound leaf development in tomato. Plant Cell 22:3206–3217

Sharp RE (2002) Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant Cell Environ 25:211–222

Shwartz I, Levy M, Ori N, Bar M (2016) Hormones in tomato leaf development. Dev Biol 419:132–142

Sobieszczuk-Nowicka E, Paluch-Lubawa E, Mattoo AK, Arasimowicz-Jelonek M, Gregersen PL, Pacak A (2019) Polyamines – a new metabolic switch: crosstalk with networks involving senescence, crop improvement, and mammalian cancer therapy. Front Plant Sci 10:859

Sobieszczuk-Nowicka E, Wrzesiński T, Bagniewska-Zadworna A, Sz K, Rucińska-Sobkowiak R, Polcyn W, Misztal L, Mattoo AK (2018) Physio-genetic signatures of dark-induced leaf senescence and its reversal in the monocot barley. Plant Physiol 178:654–671

Sobolev A, Neelam A, Fatima T, Shukla V, Handa AK, Mattoo AK (2014) Genetic introgression of ethylene-suppressed transgenic tomatoes with higher-polyamines trait overcomes many unintended effects due to reduced ethylene on the primary metabolome. Front Plant Sci 5:632

Solano R, Stepanova A, Chao QM, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714

Steiner E, Efroni I, Gopalraj M, Saathoff K, Tseng TS, Kieffer M, Eshed Y, Olszewski N, Weiss D (2012) The Arabidopsis O-linked N-acetylglucosaminetransferase SPINDLY interacts with class I TCPs to facilitate cytokinin responses in leaves and flowers. Plant Cell 24:96–108

Steiner E, Livne S, Kobinson-Katz T, Tal L, Pri-Tal O, Mosquna A, Tarkowská D, Muller B, Tarkowski P, Weiss D (2016) SPINDLY inhibits class I TCP proteolysis to promote sensitivity to cytokinin. Plant Physiol 171:1485–1494

Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in Arabidopsis roots. Plant Cell 19:2169–2185

Su L, Diretto G, Purgatto E, Danoun S, Zouine M, Li Z et al (2015) Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. BMC Plant Biol 15:114

Subbiah V, Reddy KJ (2010) Interactions between ethylene, abscisic acid and cytokinin during germination and seedling establishment in Arabidopsis. J Biosci 35:451–458

Sugiyama T, Sakakibara H (2002) Regulation of carbon and nitrogen assimilation through gene expression. In: Foyer CH, Noctor G (eds) Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism. Kluwer, Dordrecht, pp 227–238

Swarup R, Perry P, Hangenbeek D, Van Der Straeten D, Beemster GT, Sandberg G et al (2007) Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. Plant Cell 19:2186–2196

Tamaoki D, Seo S, Yamada S, Kano A, Miyamoto A, Shishido H, Miyoshi S, Taniguchi S, Akimitsu K, Gomi K (2013) Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signal Behav 8:e24260

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S (2005) Ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol 138:2337–2343

Tanimoto M, Roberts K, Dolan L (1995) Ethylene is a positive regulator of root hair development in Arabidopsis thaliana. Plant J 8:943–948

Thimann KV, Koepfli JB (1935) Identity of the growth-promoting and root-forming substances of plants. Nature 135:101

Tsuchisaka A, Theologis A (2004) Unique and overlapping expression patterns among the Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene family members. Plant Physiol 136:2982–3000

Tsuda K, Kurata N, Ohyanagi H, Hake S (2014) Genome-wide study of KNOX regulatory network reveals brassinosteroid catabolic genes important for shoot meristem function in rice. Plant Cell 26:3488–3500

Unterholzner SJ, Rozhon W, Papacek M, Ciomas J, Lange T, Kugler KG, Mayer KF, Sieberer T, Poppenberger B (2015) Brassinosteroids are master regulators of gibberellin biosynthesis in Arabidopsis. Plant Cell 27:2261–2272

Van de Poel B, Smet D, Van Der Straeten D (2015) Ethylene and hormonal crosstalk in vegetative growth and development. Plant Physiol 169:61–72

Van Tuinen A, Peters AHLJ, Kendrick RE, Zeevart JAD, Koornneef M (1999) Characterization of the *procera* mutant of tomato and the interaction of gibberellins with end-of-day far-red light treatment. Physiol Plant 106:121–128

Veit B (2004) Determination of cell fate in apical meristems. Curr Opin Plant Biol 7:57–64

Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T, Chory J (2002) Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell 2:505–513

Wang H, Huang Z, Chen Q et al (2004) Ectopic overexpression of tomato *JERF3* in tobacco activates downstream gene expression and enhances salt tolerance. Plant Mol Biol 55:183

Weijers D, Nemhauser J, Yang Z (2018) Auxin: small molecule, big impact. J Exp Biol 69:133–136

Weng L, Zhao F, Li R, Xiao H (2015) Cross-talk modulation between ABA and ethylene by transcription factor SlZFP2 during fruit development and ripening in tomato. Plant Signal Behav 10:e1107691

Wilson RL, Bakshi A, Binder BM (2014) Loss of the ETR1 ethylene receptor reduces the inhibitory effect of far-red light and darkness on seed germination of Arabidopsis thaliana. Front Plant Sci 5:433

Xie X, Kusumoto D, Takeuchi Y, Yoneyama K, Yamada Y, Yoneyama K (2007) 2′-epi-orobanchol and solanacol, two unique strigolactones, germination stimulants for root parasitic weeds, produced by tobacco. J Agric Food Chem 55:8067–8072

Yang Z, Tian L, Latoszek-Green M, Brown D, Wu K (2005) *Arabidopsis* ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. Plant Mol Biol 58:585–596

Yen HC, Shelton BA, Howard LR, Lee S, Vrebalov J, Giovannoni JJ (1997) The tomato high-pigment (hp) locus maps to chromosome 2 and influences plastome copy number and fruit quality. Theor Appl Genet 95:1069–1079

Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J (2005) A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell 120:249–259

Yokota T, Sakai H, Okuno K, Yoneyama K, Takeuchi Y (1998) Alectrol and orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. Phytochemistry 49:1967–1973

Yoshida S, Mandel T, Kuhlemeier C (2011) Stem cell activation by light guides plant organogenesis. Genes Dev 25:1439–1450

Zheng Z, Guo Y, Novak O, Dai Z, Zhao Y, Ljung K et al (2013) Coordination of auxin and ethylene biosynthesis by the aminotransferase VAS1. Nat Chem Biol 9:244–246

Zwanenburg B, Blanco-Ania D (2018) Strigolactones: new plant hormones in the spotlight. J Exp Bot 69:2205–2218

Autar K. Mattoo obtained his doctoral degree from the Maharaja Sayajirao University of Baroda, India, and later joined as a Faculty in the same department. He did his postdoctoral work with Dr. D. Bruce Keech at the Department of Biochemistry, The University of Adelaide, South Australia. Moonlighting as a Visiting Scientist in Dr. Robert S. Vickery (University of New South Wales, Kensington) lab, he characterized for the first time the subcellular distribution of isoenzymes in normal tomato cultivar and *rin* mutant. He held a 1-year Visiting Faculty appointment in Dr. Morris Lieberman's lab at USDA's Beltsville Agricultural Research Centre. Early 1979, after resigning from Baroda University, he worked in the lab of Prof. Marvin Edelman, Weizmann Institute of Science (Israel). During this tenure, he also moonlighted at the Volcani Center, Hebrew University of Jerusalem at Rehovot, and Technion University. He returned for good to USDA's BARC at the end of 1980 where he served as Research Leader of the Plant Molecular Biology Laboratory and Vegetable Laboratory at USDA-ARS. In 2002, he became ST Level Supergrade Scientist, and in 2005, he returned to bench at the Sustainable Agricultural Systems Lab. The Editor carried out his sabbatical in 1989 in Dr. Mattoo's lab. Dr. Mattoo's interests are on hormone biology especially ethylene and polyamines and also in the dynamics of proteins in photosystem I.

Dr. Rakesh K. Upadhyay obtained his Ph.D. from CSIR-National Botanical Research Institute and University of Lucknow, India, on the Role of Ethylene Response Factors in tomato. After his Ph.D., he joined the laboratory of Dr. Autar K. Mattoo where as a postdoctoral Plant Molecular Physiologist his research concerns molecular crosstalk of polyamine-ethylene-jasmonic acid nexus in fruit ripening and delineating abiotic stress response in tomato.

The Two-Component System: Transducing Environmental and Hormonal Signals

10

Ramsong Chantre Nongpiur, Priyanka Gupta, Ashutosh Sharan, Deepti Singh, Sneh Lata Singla-Pareek, and Ashwani Pareek

Abstract

In response to external stimuli, protein phosphorylation plays a significant role in signal transduction which regulates growth and development in plants. Histidine and aspartate phosphorylation (multistep phosphorelay) operating in two-component system (TCS) is one of the signalling mechanisms which regulate a plethora of processes in plants. The two-component system members in plants have been found to function in the perception of phytohormones such as cytokinins and ethylene as well as subsequent downstream signalling. In addition, the TCS members have also been shown to regulate the responses to various environmental stresses. The TCS is at the heart of the crosstalk between development and environmental stress responses. In this chapter, we describe the TCS and the role of its various members in plants towards growth and controlling development as influenced by internal (hormones) and external (environmental stress) signals.

Keywords

Abiotic stress · Histidine kinase · Hormone signalling · Multistep phosphorelay · Two-component system

R. C. Nongpiur · P. Gupta · A. Sharan · D. Singh · A. Pareek (✉)
Stress Physiology and Molecular Biology Laboratory, Jawaharlal Nehru University, New Delhi, India
e-mail: ashwanip@mail.jnu.ac.in

S. L. Singla-Pareek
Plant Stress Biology, International Centre for Genetic Engineering and Biotechnology, New Delhi, India

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_10

10.1 Introduction

Plant growth and development is a complex process involving various cell-to-cell signalling effectors such as phytohormones, small RNAs, peptides as well as intracellular signalling pathways. In addition, since plants are sessile organisms, environmental cues such as light, temperature, soil pH, water availability and humidity play a pivotal role in regulating these effectors and, consequently, overall plant growth and development. As a result, plant cells have developed extensive and intriguing cell signalling circuitries which enable them to sense these environmental cues and effectors and transduce this signal, which eventually results in regulation of growth and development. On the other hand, when exposed to unfavourable environmental conditions, plants resort to strategies which initiate appropriate cellular responses enabling them to adapt to these conditions. Among these strategies, intracellular signal transduction pathways constitute one of the most important aspects of cell signalling for plant growth and development and response to environmental stresses. Signal transduction in plants is comprised primarily of three major steps: (1) the perception of the signal (chemical or environmental); (2) downstream signalling through protein-protein interactions, post-translational modifications or secondary messengers; and (3) regulation of gene expression mediated by transcription factors, ultimately leading to altered physiology and developmental profiles (Zhu 2001). This structure in signalling is not exclusive to plants, but rather conserved in eukaryotes. However, McCarty and Chory (2000) stated that the signalling pathways involved in development are unique for plants, animals and fungi despite the shared principles. In contrast, it is also known that the major elements of signalling pathways related to stress response, defence and sugar metabolism are, at least, partially conserved in all eukaryotes (McCarty and Chory 2000). One of the major mechanisms for cell signalling in both prokaryotes and eukaryotes is protein phosphorylation. Various proteins can be phosphorylated on specific amino acid residues, and the phosphorylation status (phosphorylated or dephosphorylated) of the proteins usually determines whether the proteins are active or not. In mammals, about one-third of the total proteins are thought to be phosphorylated at one time or the other. Though the percentage of phosphorylated proteins in plants is not known, the importance of protein phosphorylation in various processes in plant system has been covered in various reviews. Cellular proteins get phosphorylated by the action of kinases, which, in turn, can be categorized based on the amino acid residue they phosphorylate. The most common phosphorylation sites on proteins are serine/threonine residues, tyrosine residues and histidine residues. The kinases which phosphorylate these residues are designated as serine/threonine kinases, tyrosine kinases and histidine kinases, respectively. Though in eukaryotes, the serine/threonine and tyrosine kinases are the predominant kinases, the histidine kinases (HKs) are the most prevalent kinases in prokaryotes. HKs have also been reported in eukaryotes such as yeast, slime moulds, fungi and plants. These histidine kinases have been reported to function mostly as the sensor molecules of a signal transduction system termed as ‘two-component system’ (TCS).

10.2 The Two-Component System (TCS)

From bacteria to higher eukaryotes such as plants, the two-component system is a highly conserved signalling pathway which has been reported to function in a wide array of essential developmental processes as well as response to environmental stimuli. Interestingly, the TCS has not been reported in most of the higher eukaryotes and metazoans. In prokaryotes, the TCS is the dominant signalling machinery involved in a large subset of essential functions such as cell cycle regulation, sensing of changes in extracellular physiochemical conditions, nitrogen metabolism and resistance to antimicrobial peptides (Quon et al. 1996; Bekker et al. 2006; Monedero et al. 2017). Typically, in prokaryotes, the TCS is comprised of two distinct proteins, a sensor histidine kinase (HK) and a corresponding response regulator (RR) protein, which mediates downstream signalling and hence the name 'two-component system'. Based on the difference in the number of steps involved in the phosphotransfer, which arises due to the occurrence of different types of histidine kinases as well as the presence of a third or fourth protein, the TCS has been characterized into two different classes. Figure 10.1 presents a highly simplified view of the structure of

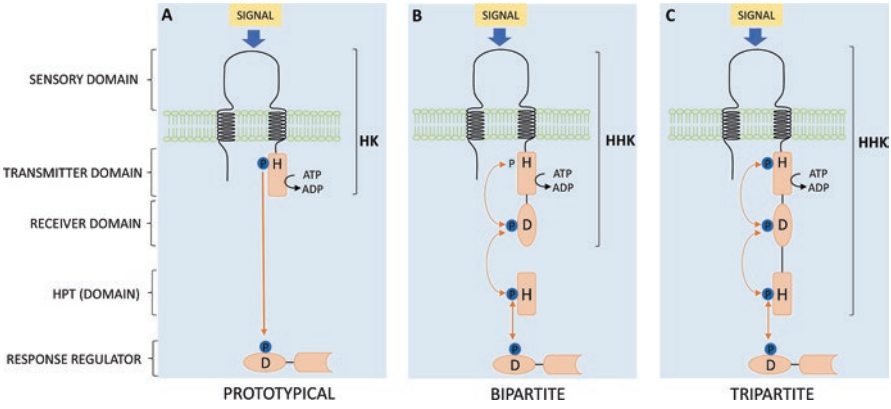

Fig. 10.1 Two-component system (TCS) signal transduction. (a) Prototypical TCS signal transduction. The HK gets autophosphorylated at a conserved His-residue on the transmitter domain, and this phosphoryl group gets transferred to a conserved Asp-residue on the receiver domain of a response regulator (RR) protein. (**b**) **TCS His-Asp phosphorelay involving a bipartite hybrid histidine kinase (HHK).** The hybrid histidine kinase gets autophosphorylated at a conserved His-residue on its transmitter domain, and this phosphoryl group gets transferred to a conserved Asp-residue on the receiver domain of the HHK. This phosphoryl group is then transferred to a conserved histidine residue on a histidine phosphotransfer protein (HPT) and ultimately to a conserved Asp-residue to on a response regulator protein. (**c**) **TCS His-Asp phosphorelay involving a tripartite hybrid-histidine kinase (HHK).** The hybrid histidine kinase gets autophosphorylated at a conserved His-residue on its transmitter domain, and this phosphoryl group gets transferred to a conserved Asp-residue on the receiver domain on the HHK. This phosphoryl group is then transferred to a conserved histidine residue on histidine phosphotransferase domain present on the HHK and ultimately to a conserved Asp-residue on a receiver domain of a response regulator protein. In this case, the response regulator would have to be localized in the cytosol for signalling to proceed. Downstream signalling for all three TCS is mediated through the output domain of the RR

various types of TCS and their mode of action through the phosphorylation process in living organisms. These are briefly described as follows.

10.2.1 The Canonical Two-Component System

Also known as the prototypical TCS, this type of TCS is comprised of two components only, viz. the histidine kinase (HK) and the cognate response regulator (RR). In this type of TCS, a typical HK would usually be a membrane-bound protein consisting of a sensory domain and a transmitter domain harbouring a conserved histidine residue as a phosphorylation target. The RR would be comprised of a receiver domain, which contains a conserved aspartate residue for phosphorylation, and an effector domain which facilitates its function once phosphorylated or active (Stock et al. 2000). Signal is perceived by the sensory domain of the HK, and this results in physiochemical changes that, in turn, stimulate the ATP-dependent autophosphorylation at the conserved histidine residue in its transmitter domain (Alvarez et al. 2016). This phosphoryl group on the HK is then transferred to the conserved aspartate residue on the receiver domain of the cognate RR, resulting in modulation (activation/deactivation) of the function of the RR. A very well-known example of the canonical TCS is the EnvZ-Ompr system which mediates osmosensing in Gram-negative bacteria (Forst and Roberts 1994). The canonical TCS has not been reported in eukaryotes so far, but it is the predominant type of TCS in prokaryotes. Almost all of the sequenced bacterial genomes possess one or more cognate pairs of HK and RR of a prototypical TCS.

10.2.2 The Two-Component System Multistep Phosphorelay

This type of TCS is characterized by the presence of a different type of histidine kinase called the hybrid histidine kinase (HHK). The HHKs are found in both prokaryotes and eukaryotes. In eukaryotes, the HHKs are the predominant form of HKs. As far as we know, only HHKs have been reported in higher eukaryotes such as plants. The signalling mediated by the HHK is more complex as compared to that of its canonical counterpart. The signalling involves the autophosphorylation at a conserved histidine residue. Subsequent transfer of this phosphate to the cognate RR occurs via a multistep relay involving an additional receiver domain (RD) and a phosphotransfer (HPT) protein/domain (Stock et al. 2000). Thus, in the multistep phosphorelay, the phosphoryl group is transferred from His-Asp-His-Asp. Apart from the RR which is involved at the end of the phosphorelay, the three conserved domains (TD, RD and HPT) containing the three phosphorylation sites (H, D, H) could be part of a single protein or divided into two or three separate proteins, respectively (Burbulys et al. 1991; Posas et al. 1996; Kwon et al. 2000). Thus, the HHKs can also be bipartite and tripartite based on the number of domains they possess (Alvarez et al. 2016). The bipartite HHKs are predominant in eukaryotes, while the tripartite HHKs are found in prokaryotes with very limited reports of their

occurrence in eukaryotes. Thus, in the TCS multistep phosphorelay, the signal would be perceived by the sensory domain of the HHK, and this results in the ATP-dependent autophosphorylation at the conserved His residue in the transmitter domain, and the phosphate would get transferred to the conserved Asp residue in the receiver domain of the protein. The phosphate group is then transferred to the conserved His residue in the HPT and finally to the conserved Asp residue of the cognate response regulator (Appleby et al. 1996; Alvarez et al. 2016).

Histidine kinases (prototypical and hybrid) have been shown to function as either homodimers or heterodimers, and autophosphorylation can occur through intermolecular and intramolecular reactions (Levit et al. 1996; Tanaka et al. 1998; Dutta et al. 1999; Cotter and Jones 2003). However, it should be noted that in HKs which dimerize, the monomeric unit is usually inactive (Levit et al. 1996). Once the signal stops, the HK and its cognate RR undergo dephosphorylation. Interestingly, it has been shown that in multistep phosphorelays, the HHK also mediates the dephosphorylation of the cognate RR (Alvarez et al. 2016).

10.3 Origins of the Two-Component System

Among the superfamily genes in bacteria, two-component system genes are perhaps one of the most predominant. In certain species such as *Geobacter sulfurreducens*, the histidine kinases alone can constitute 2.7% of the total number of proteins encoded by the organism (Galperin 2005). Most of the evolutionary data about the origins of HKs, HPTs and RRs has been obtained from sequence similarity, mechanism of action, domain architecture, abundance and distribution and tree-based methods (Wuichet et al. 2010). The TCS is found in over 98% of the sequenced bacterial genomes but has not been identified in most archaea genomes and is absent in *Crenarchaeota*, *Korarchaeota* and *Nanoarchaeota* (Wuichet et al. 2010; Galperin et al. 2018). Based on its wide distribution in diverse bacterial species and phylogenetic analysis, the TCS is presumed to have originated during early bacterial evolution and subsequently inherited by some archaea through independent lateral gene transfer events (Koretke et al. 2000). It has been proposed that TCS originated in bacteria through the insertion of histidine kinase domain and receiver domain into one-component regulators and the eventual fragmentation of these domains into two separate proteins (HK and RR) (Ulrich et al. 2005). One-component regulators are proteins which contain both the input and output domains similar to those of the HKs and RRs of TCS (Ulrich et al. 2005). It was further discussed that since the one-component regulators were predominantly cytosol localized, they were involved in sensing of cytosolic signals (Ulrich et al. 2005). Since the majority of the HKs that evolved was plasma membrane localized, the evolution of TCS facilitated for extracellular sensing in bacteria, which provided a significant advantage as compared to intracellular sensing (Ulrich et al. 2005). Thus, the evolution of TCS enhanced the sensory capabilities of bacteria without much alteration to the responses mediated by the conserved output domain of the RR, a majority of which function as transcriptional regulators. One interesting aspect about TCS is that

majority of the genes encoding the HK and its cognate RR are usually present either as part of an operon or 20 bases apart (Koretke et al. 2000). This facilitates the duplication or lateral gene transfer of intact signalling pathway (Koretke et al. 2000). This provides further evidence that a particular HK-RR pair emerged from the division of a single one-component protein. These cognate TCS genes are thus very closely linked and would co-evolve through duplication of all their components and subsequent modification and differentiation (Koretke et al. 2000). An analysis of the phylogenetic relationship of histidine kinases from 206 prokaryotic genomes brings out that the new histidine kinases were introduced into the genome either through lineage-specific expansion or horizontal gene transfer, where the genes acquired through horizontal gene transfer were more likely to retain their original function, and those that were formed through lineage-specific expansion were more likely to attain new functions (Alm et al. 2006). There are reports which show that the ATP-binding domain of HKs shares distant homology with proteins such as heat-shock protein 90 (Hsp 90), the DNA repair protein MutL and type II topoisomerases (Dutta and Inouye 2000). These proteins share distinct structural conservation, mode of ATP binding and, in some cases, similar ATP hydrolysis mechanism with the HKs (Dutta et al. 1999). Thus, the origins of HKs may be from one or more of such ATPases. The hybrid HKs probably emerged from gene fusion, duplication and rearrangements of HKs and RRs (Capra and Laub 2012). The HPTs may have evolved from a range of other proteins or through the degradation of HKs (Capra and Laub 2012). However, the origins of RRs are still unknown.

It is proposed that TCS radiated from bacteria to Archaea and Eukarya through multiple horizontal gene transfer events well after these groups have emerged as separate kingdoms and were well into their speciation phases (Koretke et al. 2000). The evolution of the TCS signalling pathways in eukaryotes is quite interesting. TCS are present in multiple genera in Eukarya such as diatoms, fungi, slime mould, green algae, moss and higher plants. However, the TCS are not found in metazoans. It has been stated that since the eukaryotic two-component signalling elements are lineage specific, they probably were acquired from lateral gene transfer events occurring after the mitochondrial endosymbiosis that resulted in the last eukaryotic common ancestor (Anantharaman et al. 2007). They further stated that the eukaryotic TCS were acquired through lateral gene transfer events resulting from endosymbiosis with cyanobacteria, host-parasite interactions and bacterial phagocytosis (Anantharaman et al. 2007). The distribution of TCS genes in representative species from various kingdoms is provided in Table 10.1. It is interesting to note that in prokaryotes, free-living species have a higher number of TCS genes in comparison with those that live with an organismic host (Koretke et al. 2000). The wide array of environmental signals to which free-living bacteria and archaea are exposed to probably resulted in the acquisition of higher number of TCS genes for mediating appropriate responses to these signals.

Schaller et al. (2011) have comprehensively reviewed the acquisition and evolution of TCS in eukaryotes, plants in particular. They reported that the composition of plant TCS was different from that of prokaryotes. Plant HKs were hybrid HKs, and RRs have acquired domains such as the Myb domain, which in turn support the

Table 10.1 TCS genes of representative organisms from bacteria, archaea and lower eukaryotes

Organism	No. of TCS genes				References
	HK	HPT	RR	Total	
Mycobacterium tuberculosis	12	–	12	24	Parish (2014)
Synechosystis sp.	42	3*	38	80	Mizuno et al. (1996)
Nostoc sp.	131	3*	80	211	Wang et al. (2002)
Myxococcus xanthus	163	–	119	272	Shi et al. (2008)
Aciduliprofundum boonei	2	–	4	6	Galperin et al. (2018)
Halobacterium salinarum	11	–	9	20	Galperin et al. (2018)
Methanospirillum hungatei	46	–	87	133	Galperin et al. (2018)
Phaeodactylum tricornutum	11	–	3	14	Bowler et al. (2008)
Thalassiosira pseudonana	3	–	8	11	Montsant et al. (2007)
Dictyostelium discoideum	15	1	4	20	Thomason and Kay (2000)
Saccharomyces cerevisiae	1	1	2	4	Brown et al. (1993); Ota and Varshavsky (1993); Maeda et al. (1994)

*denotes HPT domain present within a hybrid HK

continued evolution of the plant TCS after their acquisition from prokaryotes (Schaller et al. 2011). In addition, the plant TCS included HPTs and a much higher number of RRs which probably resulted from lineage-specific expansion of the RRs (Schaller et al. 2011). Interestingly, plant TCS also contains diverged two-component elements or families such as the ethylene receptors, phytochromes and pseudo-RRs (Bleecker 1999; Makino et al. 2000; Rockwell et al. 2006). These diverged elements are basically genes and gene families which have evolved from TCS in such a way that their TCS structure was more or less maintained but was functionally divergent from the classical histidine phosphorylation and phosphotransfer. Among the TCS families in plants, only the sensory histidine kinases comprising the cytokinin receptors and a few other hybrid HKs involved in various developmental processes and responses to external stimuli have been reported to operate through the multi-step His-Asp phosphorelay (Héricourt et al. 2016; Pekárová et al. 2016; Yuan et al. 2016). Thus, after the acquisition of TCS from prokaryotes, the TCS in plants has evolved a long way and is now composed of classical and diverged elements. Its functions therefore range from the perception of phytohormones, light and environmental stresses to various developmental processes and regulation of the circadian clock. For a more detailed description of the evolution of each of these signalling elements in plants, a few recent articles are recommended (Pils and Heyl 2009; Takata et al. 2010; Gallie 2015a; Li et al. 2015a; Inoue et al. 2017). In the following sections, we discuss the plant TCS with respect to each of these functions.

10.4 The Two-Component System in Plants

Over the past few decades, genome sequencing of many plant species has been completed. This has led to the identification of putative TCS members in various plant species such as *Arabidopsis*, rice, poplar, soybean, maize, tomato, etc. (Hwang et al. 2002; Pareek et al. 2006; Chu et al. 2011; Singh and Kumar 2012; He et al. 2016a). Based on these reports in general, the TCS in plants is composed primarily of bipartite hybrid histidine kinases, a lower number of histidine phosphotransfer proteins (HPTs) and a relatively large assortment of response regulators. The number and composition of the different components of TCS in different plant species are provided in Table 10.2. However, TCS in many plant species has been identified based on similarities in sequence and domain architecture of the proteins to their well-characterized counterparts in prokaryotes and other eukaryotes. Functional validation of histidine kinase activity and phosphorelay activity is yet to be ascertained in most of the species and hence these are considered putative TCS genes. Due to the fact that the TCS system of *Arabidopsis* is the most well studied, it will be used for detailed discussion, and examples from other species will be included wherever applicable.

The genome of *Arabidopsis* is comprised of 54 TCS genes encoding putative histidine kinases (AHKs), histidine phosphotransfer proteins (AHPs) and response regulators (ARRs) (Hwang et al. 2002). This suggests that the TCS could be involved in various roles in plant growth and development. The earliest studies carried out on

Table 10.2 Two-component system in representative plant species

Organism	No. of TCS genes				References
	HK	HPT	RR	Total	
Arabidopsis thaliana	11	6	32	49	Hwang et al. (2002)
Oryza sativa	11	5	36	52	Pareek et al. (2006)
Lotus japonicus	15	6	28	49	Ishida et al. (2009)
Glycine max	21	13	47	81	Mochida et al. (2010)
Zea mays	11	9	28	48	Chu et al. (2011)
Populus trichocarpa	12	12	25	49	Singh and Kumar (2012)
Triticum aestivum	7	10	45	62	Gahlaut et al. (2014)
Brassica rapa	20	8	57	85	Liu et al. (2014)
Solanum lycopersicum	20	6	39	65	He et al. (2016a)
Cucumis sativus	18	7	21	46	He et al. (2016b)
Citrullus lanatus	19	6	24	49	He et al. (2016b)

the TCS genes in *Arabidopsis* were centred around the function of the putative histidine kinases. Interestingly, phytochromes were also characterized as AHKs suggesting that the TCS might be involved in light-mediated signalling, although it was later found that the phytochromes were not canonical histidine kinases (Hughes and Lamparter 1999). Not only this, the AHKs were found to be receptors of cytokinin and ethylene, and one of the AHKs (AHK1) was found to function as a putative osmosensor as well (Urao et al. 1999; Bleecker and Kende 2000; Inoue et al. 2001). Other studies have also shown the involvement of the AHPs and ARRs in cytokinin signalling, light signalling and drought stress response (Hwang and Sheen 2001; Sakai et al. 2001; Nishiyama et al. 2013). These findings strongly indicate the varied functions of the TCS in plants. Since the *Arabidopsis* TCS is a superfamily comprising different types of proteins, they were categorized accordingly based on structure, domain architecture as well as putative function (Heyl et al. 2013). The AHKs were divided into four sub-groups comprising the HK for canonical HKs, the CHK for CHASE domain-containing HKs, the ETR/ERS for ethylene receptors and the PHY for phytochromes (Heyl et al. 2013). The HPTs are grouped together, and the RRs were further sub-divided into five different groups designated as type-A RRs, type-B RRs (contain MYB DNA-binding domain), type-C RRs, pseudo-RRs or PsRRs for clock genes and an additional group designated simply as RR for potentially new clades (Heyl et al. 2013). Figure 10.2 shows the domain organization of representative sensory HKs from *Arabidopsis*.

10.5 Mediating Light Sensing via Phytochromes: A Case of Divergence

Phytochromes are a superfamily of photoreceptors which perceive the red/far-red spectrum. They play an integral role in the growth and development of plants. A detailed description of light sensing is covered in Chap. 2. It was shown that phytochromes are serine/threonine kinases (Yeh and Lagarias 1998). Yet in phylogenetic

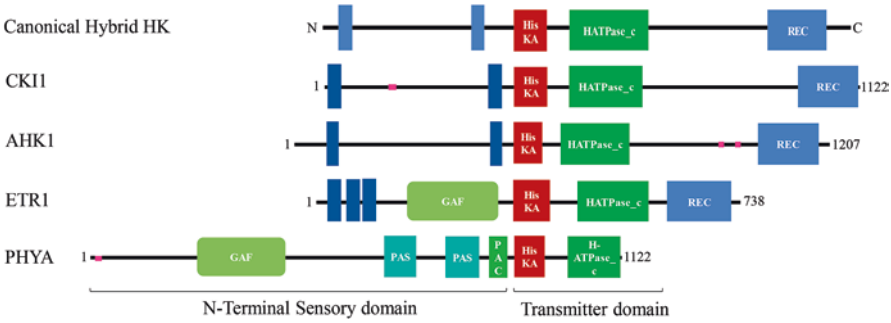

Fig. 10.2 Domain organization of representative *Arabidopsis* sensory HKs (CKI1 and AHK1), ethylene receptor (ETR1) and phytochrome (PHYA) illustrating their resemblance to the canonical hybrid HKs of prokaryotes, further indicating their ancestry as hybrid HKs. All HKs have been drawn to scale so that the exact size and position of each of the domains on the proteins are depicted

analyses, plant phytochromes show a clear resemblance with hybrid histidine kinases (Hwang et al. 2002; Pareek et al. 2006). Thus, phytochromes form a distinct superfamily of proteins, where structurally they resemble histidine kinases but functionally do not possess histidine kinase activity and do not participate in a conventional multistep his-asp phosphorelay (Li et al. 2015a). Signalling mediated by phytochromes proceeds via light-dependent autophosphorylation and subsequent phosphorylation of other proteins, ultimately resulting in the degradation or repression of negative regulators of photomorphogenesis such as constitutive photomorphogenic 1 (COP1) and phytochrome-interacting factors (PIFs) (Li et al. 2011). Thus, phytochromes are a perfect example of the evolutionary divergence of hybrid histidine kinases in plants. Typically, the canonical plant phytochrome domain assembly includes an N-terminal photosensory domain comprising three conserved domains (the Per/Arnt/Sim [PAS], cGMP phosphodiesterase/adenylate cyclase/FhlA [GAF] and phytochrome [PHY] domains) and a C-terminal regulatory domain comprising a PAS-PAS repeat along with a histidine kinase (HK) or histidine kinase-related domain (HKRD) which lacks the conserved histidine residue and has serine/threonine kinase activity (Yeh and Lagarias 1998; Rockwell et al. 2006; Shin et al. 2016). Li et al. (2015a) reported that the canonical plant phytochrome evolved from a non-cyanobacterial precursor shared with Archaeplastida and placed the origin of canonical plant phytochromes in a common ancestor of extant streptophytes. Additionally, the latest phylogenetic analyses elucidating the evolution of plant phytochromes have pointed that they have evolved from cyanobacterial phytochromes, which are histidine kinases (Kooß and Lamparter 2017). Thus, both studies reveal that prior to them attaining canonical structure and function, the phytochromes were histidine kinases. The verification of histidine kinase activity and phosphotransfer in cyanobacterial and bacterial phytochromes further substantiate these claims (Yeh et al. 1997; Davis et al. 1999).

10.6 Mediating Hormonal Signalling

10.6.1 Cytokinin Signalling: Canonical Multistep Phosphorelay in Plants

Cytokinins have been shown to regulate cell cycle, cell proliferation in shoot and root apical meristems, circadian rhythm, leaf senescence, responses to biotic and abiotic stresses, nutrient uptake and the development of lateral roots, leaves, vascular tissues and gametophyte (Kieber and Schaller 2014). Here we will discuss the TCS with respect to its role in phytohormone signalling (cytokinin signalling and ethylene signalling in particular), where histidine kinases play a major role as receptors. The signalling of other phytohormones does not proceed through HK activity or phosphorelay.

In *Arabidopsis*, four HKs, viz. AHK2, AHK3, AHK4 and CKI1, have been shown to be responsive to cytokinin. AHK4 was the first HK to be identified as a cytokinin receptor and later followed by the identification of AHK2, AHK3 and CKI1, although the mechanism of action for CKI1 appears to be different from the other three HKs (Kakimoto 1996; Hwang and Sheen 2001; Inoue et al. 2001). Among the proposed cytokinin receptors in *Arabidopsis*, CKI1 is perhaps unique in the sense that it is responsive to cytokinin but can function independent of cytokinin (Hwang and Sheen 2001). Interestingly, CKI1 has been found to act upstream of the cytokinin-responsive AHPs to mediate its functions in embryogenesis. CKI1 has been shown to have integral roles in reproductive development, and there are multiple reports which show that CKI1 is involved in female gametophyte development as well as vegetative growth (Pischke et al. 2002; Hejatko et al. 2003; Deng et al. 2010; Yuan et al. 2016). Since CKI1 can function independent of cytokinin, it is still unclear as to whether it can be considered as a full-fledged cytokinin receptor. The lack of a CHASE domain (cyclases/histidine-kinase-associated sensory extracellular) indicates that it probably is not. On the other hand, AHK2, AHK3 and AHK4 have all been shown to be cytokinin receptors. These three cytokinin receptors are characterized by the presence of a CHASE domain, which have been shown to contain the ligand-binding sites for cytokinins (Stolz et al. 2011). It has been shown that the binding of various natural and synthetic cytokinins to the membrane distal PAS domain located within the CHASE domain of AHK4 (Hothorn et al. 2011). Although crystal structures of AHK2 and AHK3 have not been reported, the characterization of these two HKs as cytokinin receptors is supported through cytokinin-binding assays and cytokinin response assays (Stolz et al. 2011). In these assays, AHK4 can functionally replace AHK2 but not AHK3 indicating a differential ligand specificity for AHK3 (Spíchal et al. 2004; Stolz et al. 2011). Nevertheless, there is some degree of redundancy in the functions of these three cytokinin receptors. Through the use of various assays in bacteria, yeast and *Arabidopsis* protoplast, cytokinin signalling has been shown to proceed through a canonical multistep His-Asp phosphorelay (Inoue et al. 2001; Suzuki et al. 2001; Ueguchi et al. 2001; Yamada et al. 2001; Heyl and Schmülling 2003; Kakimoto 2003; Ferreira and Kieber 2005; Müller and Sheen 2007; Maxwell and Kieber 2010; Hwang et al. 2012; Kieber and Schaller 2014). It

is interesting to note that cytokinin perception occurs at both the plasma membrane and the ER membrane (Caesar et al. 2011; Wulfetange et al. 2011; Romanov et al. 2018). In *Arabidopsis*, the binding of cytokinin to its receptors (AHK2, AHK3 and AHK4) results in autophosphorylation of the AHK at the conserved histidine residue and multistep phosphorelay to the AHPs (AHP1-5) which transfer the phosphoryl group to and activate type-B RRs (Kieber and Schaller 2014). The type-B RRs act as transcription factors and induce the expression of a number of cytokinin-responsive genes including type-A RRs. The type-A RRs have been shown to function as partially redundant negative regulators of cytokinin signalling (Kiba et al. 2003; Jennifer et al. 2004; Lee et al. 2007). Unlike the type-B RRs, the type-A RRs do not contain a DNA-binding domain and hence do not function as transcriptional regulators. Thus, the type-A RRs mediate the negative feedback of cytokinin signalling, primarily through protein-protein interactions, probably through competing with the type-B RRs for interaction with AHPs or through the interaction with and dephosphorylation of type-B RRs (Schaller et al. 2008; Kieber and Schaller 2014; Sharan et al. 2017). Cytokinin signalling is also inhibited by the pseudo-HPT, AHP6 (Mahonen et al. 2006). So far, cytokinin signalling is the only signal transduction pathway in plants where the canonical multistep His-Asp phosphorelay of prokaryotes has been preserved, with very few evolutionary alterations to the domain structure and organization in the proteins. Interestingly, it is known that the genes necessary for cytokinin synthesis and signalling were present in cyanobacteria (Frebort et al. 2011; Spíchal 2012). Another report revealed that cyanobacteria could regulate cytokinin metabolism and signalling in a light-dependent manner, indicating the origins of functional cytokinin signalling as early as cyanobacteria (Frébortová et al. 2017). This supports the current theory for the acquisition of cytokinin signalling in land plants through lateral gene transfer from cyanobacteria during primary endosymbiosis (Spíchal 2012). However, the complete set of genes for canonical cytokinin signalling of land plants was only obtained in the predecessors of charophyte algae and land plants, with the moss *Physcomitrella patens* being the most basal land plant known (to date) to encode a complete set of canonical cytokinin signalling gene families (HKs, HPT, type-A RRs and type-B RRs) (Gruhn and Heyl 2013; Gruhn et al. 2014). While a majority of the TCS machinery such as the phytochromes have diverged through the course of evolution of land plants, the reasons for the conservation of the canonical multistep phosphorelay for cytokinin signalling are still not clearly defined. Perhaps immediately after the primary endosymbiosis, the cytokinin signalling predecessors possessed an alternative function and hence were retained. Gradually, as a result of functional diversification through lineage-specific expansion, accompanied by independent lateral gene transfer events, the complete set of canonical cytokinin signalling genes were eventually attained in bryophytes.

In higher plants, cytokinin signalling downstream of the of the HKs and HPTs is quite complex and involves a varied plethora of proteins, particularly the type-B RRs being the transcription factors (Lohrmann et al. 2001; Sakai et al. 2001; Imamura et al. 2003; Mason et al. 2004; Mason et al. 2005; Rashotte et al. 2006; Zubo et al. 2017). Through the use of T-DNA insertion mutants, five RRs which belong to subfamily-1 type-B RRs (ARR1, ARR2, ARR10, ARR11 and ARR12)

have been shown to be involved in cytokinin signalling in *Arabidopsis* (Mason et al. 2005; Yokoyama et al. 2006; Argyros et al. 2008; Ishida et al. 2008; Gruhn and Heyl 2013; Hill et al. 2013; Kurepa et al. 2014; Shull et al. 2016). It has even been proposed that the remaining subfamily-1 type-B RRs might have more specific spatial or temporal roles in mediating cytokinin responses (Ishida et al. 2008). These RRs regulate the expression of multiple genes with one report showing that in the *arr1arr10arr12* triple mutant, cytokinin treatment resulted in 62 out of 71 cytokinin-inducible genes to be expressed at least two folds lower than in the wild-type plants treated with cytokinin (Argyros et al. 2008). This indicates that these three type-B RRs regulate the expression of a large subset of genes which, in turn, regulate the downstream cytokinin responses. Additionally, it has been shown that another type-B RR, ARR18, also mediates cytokinin signalling in *Arabidopsis* (Veerabagu et al. 2012). Interestingly, there is a study which shows that ARR2 mediates cytokinin responses through proteolysis (Kim et al. 2012). Cytokinin treatment resulted in the degradation of ARR2 through the 26S proteasome pathway, and this degradation was dependent on the cytokinin-induced phosphorylation at the conserved asp-80 residue in the receiver domain, ultimately resulting in the attenuation of the cytokinin signalling (Kim et al. 2012). Thus, cytokinin signalling is tightly regulated with multiple levels of controls and checks in place. A simplified representation of cytokinin signalling in *Arabidopsis* is provided in Fig. 10.3.

10.6.2 Ethylene Signalling: A Combination of Serine and Histidine Kinase Signalling

Ethylene (C_2H_4), a simple hydrocarbon, is a small gaseous molecule with great significance as a major phytohormone. It mediates several developmental responses (Abeles et al. 1992; Mcmanus 2012) as it also imparts adaptive responses towards several stresses. However, it is widely known for its significant role in the ripening of climacteric fruits, such as bananas, pears, tomatoes and apples. Blocking ethylene perception in crops can prevent yellowing of vegetables and abscission of leaves and flowers (Mcmanus 2012). Contrarily, intentional application of ethylene is practiced to induce pre- or post-harvest fruit ripening.

Identification of *Arabidopsis* ethylene response mutants in the late 1980s gave an insight of the ethylene signalling pathway (Bleecker et al. 1988; Guzmán and Ecker 1990). Etiolated *Arabidopsis* seedlings showed a short and thick hypocotyl, a short root with an exaggerated apical hook in response to ethylene (phenotype called 'triple response'). The 'triple response' is highly specific to ethylene. Map-based methods like chromosome walking were used to clone the corresponding genes and identify several key components of this pathway which includes the very first known plant hormone receptor, ETR1 (Chang et al. 1993). At present, all central elements involved in ethylene signalling in *Arabidopsis* have been identified, and their mechanistic aspects have been elucidated using genetics, molecular biology, biochemistry and cell biology. These findings have been supported and further elaborated with studies in other plant species, especially tomato (Klee 2004).

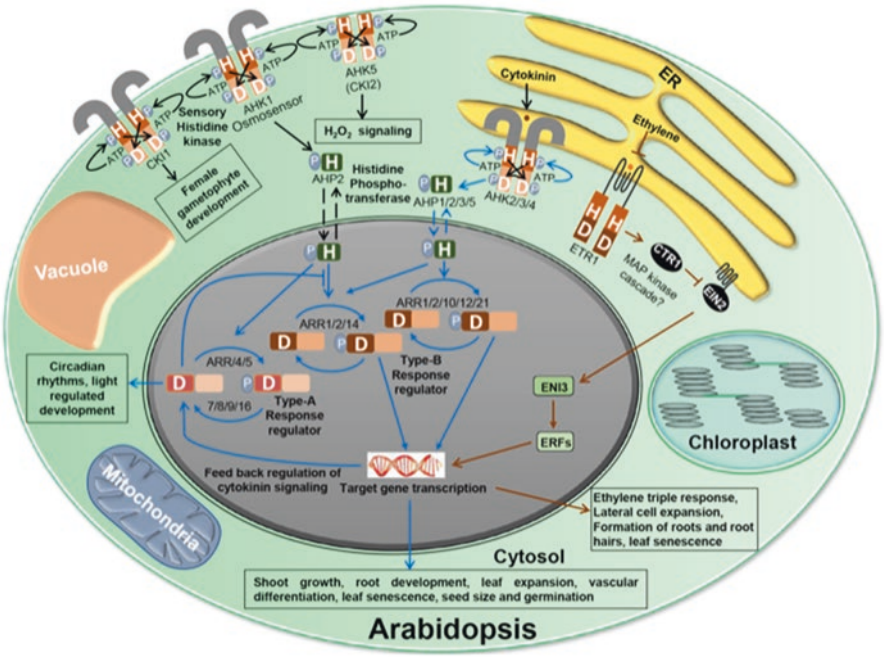

Fig. 10.3 Diagram showing the two-component system circuitry and downstream signalling in response to cytokinin, ethylene and osmotic stress in Arabidopsis. Cytokinin perception by AHK2, AHK3 and AHK4 occurs either at the endoplasmic reticulum (ER) or the plasma membrane (PM), which then phosphorylate AHPs 1, 2, 3 and 5. The phosphorylated AHPs then phosphorylate their cognate type-B RRs, which regulate transcription of target genes, including type-A RRs. The type-A RRs function to negatively regulate the phosphorelay in a negative feedback loop. AHK1 is a putative osmosensor, and under osmotic stress, it is presumed to activate AHP2, which in turn activates both type-B and type-A RRs. Binding of ethylene to ETR1 leads to its deactivation, which in turn leads to the inactivation of CTR1. This leads to the derepression of EIN2, which gets proteolytically cleaved, and the C-terminal end enters the nucleus to activate EIN3. EIN3 activates the ERFs, which in turn regulate gene expression

In plants, the ethylene signalling pathway is highly conserved and dates back to an algal ancestor (Klee 2004; Rzewuski and Sauter 2008; Ju et al. 2015). Ethylene signalling consists of mainly four steps: (1) perception of ethylene by an ethylene receptor complex present at the endoplasmic reticulum (ER) membrane; (2) cleavage of ETHYLENE-INSENSITIVE2 (EIN2) is triggered by ethylene detection; (3) the cleaved soluble part of EIN2 suppresses the expression of two regulatory F-box proteins, whose function is to degrade two master transcription factors through 26S proteasome; and (4) stabilization of these two transcription factors leads to the downstream gene expression (Merchante et al. 2013). The pathway basically depends on negative regulation and post-translational controls. In absence of ethylene, the responses are repressed, and the repression involves protein phosphorylation and protein turnover (Merchante et al. 2013).

Plants have small family of ethylene receptors (e.g., *Arabidopsis* with five and tomato with six ethylene receptors) having overlapping as well as distinct functions (Guo and Ecker 2004; Shakeel et al. 2013). The ethylene receptors are structurally similar to the prokaryotic two-component receptors, having an N-terminal ligand-binding domain, a GAF domain followed by a histidine kinase domain. Some isoforms also consist of a C-terminal receiver domain, which serves as the second element in the two-component system (Bleecker et al. 1998; Müller-Dieckmann et al. 1999). The ethylene-binding domain of the ethylene receptors lies within the ER membrane and the GAF, histidine kinase and receiver domains are placed in the cytoplasm (Müller-Dieckmann et al. 1999; Chen et al. 2002; Grefen et al. 2008). As ethylene can diffuse across membranes, its receptor has no obligation to be present at the cell surface. The preferential solubility of ethylene in hydrophobic environments justifies the localization of the ethylene-binding pocket to the membrane. The ethylene receptors form dimers with the help of disulphide bonds; each dimer can bind to a single ethylene molecule with a copper ion serving as a cofactor (Schaller and Bleecker 1995; Rodríguez et al. 1999). These dimers are present in clusters within the ER membrane and interact with downstream proteins of the pathway (Grefen et al. 2008). The GAF domain facilitates protein-protein interactions between monomers as well as isomers of ethylene receptors (Merchante et al. 2013).

In *Arabidopsis*, the five ethylene receptors are ETR1, ETR2 ERS1, ERS2 and EIN4. Although histidine kinase activity has been reported in ETR1, it has been shown that the canonical histidine kinase activity does not appear to play a major role in ethylene receptor signalling (Gamble et al. 1998; Wang et al. 2003; Merchante et al. 2013; Shakeel et al. 2013). One study has shown that out of the five ethylene receptors, only ETR1 has retained its histidine kinase activity, while the other four receptors have diverged and phosphorylated on serine residues. Nevertheless, ethylene does mediate the autokinase activity of ETR1 (Moussatche and Klee 2004). In another study, ethylene completely inhibited the intrinsic kinase activity of ETR1 in vitro; however, the nature of the kinase activity was not defined (Voet-van-Vormizeele and Groth 2008). It was later reported that, although the HK activity of ETR1 is not required for ethylene signalling, it does play a modulating role in the regulation of ethylene responses (Hall et al. 2012). In addition, ethylene has been shown to regulate cold signalling through transcriptional regulation of ARRs, indicating a crosstalk with canonical multistep phosphorelay mediated by cytokinin. There is also ample evidence that TCS is not the primary mode of ethylene signalling. Rather than a canonical multistep phosphorelay involving HPTs and RRs, ethylene receptors function through the activation of CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1; a serine/threonine kinase having sequence similarity to the Raf kinase family), which is a negative regulator of the downstream ethylene response (Kieber et al. 1993). Binding of ethylene to the receptor results in the deactivation of CTR1 and, hence the downstream ethylene signalling can proceed. This model is based on the fact that null mutations in multiple ethylene receptor genes display constitutive ethylene responses similar to *ctr1* loss-of-function mutants, whereas dominant, gain-of-function receptor mutations confer ethylene insensitivity (Hua and Meyerowitz 1998). Ethylene signalling downstream of CTR1

depends on the phosphorylation status of EIN2, a central regulator of the ethylene-signalling pathway (Alonso et al. 1999). The N-terminal domain of EIN2 is tethered to the ER membrane, whereas the C-terminal portion (C-END) has a novel plant-specific domain which is cytosolic, and its expression is sufficient for the activation of ethylene responses (Alonso et al. 1999; Wen et al. 2012). The CTR1 kinase, in absence of ethylene, phosphorylates the EIN2 C-END and prevents it from signalling (Ju et al. 2012). In the presence of ethylene, CTR1 remains inactive and thus the unphosphorylated EIN2 C-END is then proteolytically released from the ER-anchored domain (Ju et al. 2012; Wen et al. 2012). The cleaved C-END binds to the 3' untranslated regions of mRNA meant for the expression of two F-box proteins, EIN3-binding F-BOX 1 and 2 (EBF1/2), and represses the translation of the same (Merchante et al. 2013; Li et al. 2015b). This repression is very critical in ethylene signalling, as in the nucleus, the proteolytic degradation of two master transcription factors, EIN3/EIL1, is controlled by EBF1/2 proteins. EIN3/EIL1 are essentially required for all known ethylene responses (An et al. 2010). In the absence of ethylene, EBF1/2 target EIN3/EIL1 for ubiquitylation and degradation, in an SCFEBF1/EBF2 ubiquitin-ligating complex (An et al. 2010). When ethylene is bound to the receptors, EIN2 C-END represses translation of EBF1/2, thereby permitting the quick accumulation of EIN3/EIL1 transcription factors in the nucleus that leads to rapid responses to ethylene (Li et al. 2015b). The primary targets of EIN3 include transcription factor genes in the APETELA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) family, like ERF1, regulating further expression in a transcriptional cascade of ethylene signalling (Solano et al. 1998; Chang et al. 2013). These global changes in gene expression lead to a diverse array of cellular, metabolic and physiological responses (An et al. 2010). A simplified version of ethylene signalling in *Arabidopsis* is provided in Figure 10.3. For a more detailed description of the ethylene signalling pathway, a few extensive reviews are recommended (Klee 2004; Merchante et al. 2013; Gallie 2015b; Ju and Chang 2015; Chang 2016).

10.7 Two-Component System Members in Context with Abiotic Stress Response in Plants

TCS is one of the several signalling pathways involved in various stress responses. Various individual members of these TCS systems, viz. HK, HPT and RR, are known to regulate abiotic stress signalling in plants either in positive or negative manner. *Arabidopsis* TCS members are the most explored in terms of their role in abiotic stresses and ABA (Abscisic Acid) signalling. However, recent reports also provide information about the active involvement of the rice TCS members in abiotic stress as well as ABA signalling.

Various genome-wide approaches and transcriptome analysis have identified TCS members from different plants and their possible role in abiotic stresses. Histidine kinases from two of the Medicago species MsHK1 (*Medicago sativa*) and MtHK2 (*Medicago truncatula*) are induced in response to salinity stress (Merchan et al.

2007; Coba de la Peña et al. 2008). The tomato genome encodes for 65 TCS members, and most of them are stress inducible as well as participate in hormone signalling (He et al. 2016a). Similar analysis carried out on Chinese cabbage identified 85 TCS members which showed variable expression pattern under drought, salinity and ABA (Liu et al. 2014). Similarly, in another study, the genomes of cucumber and watermelon were analysed for putative TCS members, and it was found that most of the identified TCS genes were differentially expressed in response to abiotic stresses as well as ABA (He et al. 2016b). In rice, the differential expression of TCS genes under various abiotic stresses has also been reported (Karan et al. 2009; Singh et al. 2015). Rice and *Arabidopsis* show similarity in terms of the type and number of histidine kinases encoded by their respective genomes (Pareek et al. 2006). Among the 11 histidine kinases (HK), each in rice and *Arabidopsis* (phytochromes excluded), 5 are ethylene receptors, while 6 are characterized as either cytokinin receptors or putative osmosensor (Hwang et al. 2002; Pareek et al. 2006). Among these non-ethylene receptors, *Arabidopsis* possess one putative osmosensor AtHK1/AHK1; one ETR1-dependent histidine kinase AHK5/CKI2, which also functions in ABA signalling; and, as has already been mentioned, four cytokinin receptors (CKI1, AHK2, AHK3 and AHK4/CRE1/WOL1) (Hwang et al. 2002). Among the AHKs, AHK1, AHK2, AHK3 and AHK4 are the most characterized through the use of genetically engineered transgenic plants and mutants. These proteins have been found to be directly or indirectly involved in abiotic stress regulation. On the other hand, AHK5 is the least characterized histidine kinase in *Arabidopsis*.

The *Arabidopsis* HKs differ in their responses to the environmental stresses. AtHK1 functions as a positive regulator of osmotic stress as well as ABA signalling and was the first identified putative osmosensor in *Arabidopsis* (Urao et al. 1999; Tran et al. 2007). Role of the AHK1/AtHK1 has been identified in mitigation of desiccation and water stress during early seed germination as well as vegetative growth, respectively (Tran et al. 2007). The regulation of stress tolerance mediated by AHK1 is both by ABA-dependent and ABA-independent pathways (Tran et al. 2007). In ATHK1 overexpression transgenic plants, osmotic stress tolerance is conferred by the accumulation of ABA level by up-regulation of ABA biosynthetic genes such as ABA1, ABA2 and AAO3 via Abscisic acid responsive element (ABRE)-binding proteins, while transcription factors such as MYB/MYC are responsible for induction of stress-responsive genes. Moreover, ABA-independent pathways of AHK1 involves dehydration responsive element binding (DREB) protein-mediated induction of stress-regulated genes (Tran et al. 2007). *Arabidopsis* mutant plants defective in AHK1 are sensitive to water stress and are poor in solute accumulation (Tran et al. 2007). Interestingly, the *loss*- and *gain-of-function* analysis of AHK2, AHK3 and AHK4 in *Arabidopsis* revealed that these genes act as negative regulators of drought, salinity and ABA signalling, but they function as positive regulators of cold and high-light stress responses (Tran et al. 2007; Jeon et al. 2010). Another HK, AHK5, has been found to negatively regulate osmotic stress tolerance and possibly functions through direct interactions with three HPTs – AHP1, AHP2 and AHP5 (Mira-Rodado et al. 2012; Pham et al. 2012). In rice, HKs have been shown to be responsive to salinity stress (Karan et al. 2009). One of the

rice histidine kinases, OsHK3, has been reported to be a mediator of antioxidant defence response in an ABA-dependent manner (Wen et al. 2015). OsHK3 is induced in response to drought, ABA and H_2O_2, and it functions downstream of H_2O_2 in the ABA signalling transduction pathway to regulate responses to abiotic stress (Wen et al. 2015). In Poplar, one study has shown that a hybrid histidine kinase, PtHK1, could function as an osmosensor in yeast (Héricourt et al. 2013). They have also shown that PtHK1 dimerizes at the plasma membrane and proposed that HK1 may have an osmosensory role in Populus cells (Héricourt et al. 2013).

HPTs are a very crucial mediator of TCS signalling as they shuttle between the receptor and response regulator and hence assist in signal amplification. Since the HKs are involved in the responses to various abiotic stresses, the downstream HPTs are implicated in these responses as well. Reports reveal that drought stress represses the expression of AHPs and the *ahp2/3/5* triple mutants are drought tolerant, suggesting that AHP2, AHP3 and AHP5 are probably negative regulators of drought stress signalling (Nishiyama et al. 2013). The triple mutant plants are also sensitive to ABA at the seed germination stage and exhibit improved tolerance towards salinity stress as well (Nishiyama et al. 2013). Thus, Ahp2, Ahp3 and Ahp5 are negative regulators of the salinity stress response as well, albeit in a functionally redundant manner. Another group also demonstrated that the AHP2, AHP3 and AHP5 are involved in cold stress signalling and directly regulate ARR1 (Jeon and Kim 2013). In rice, two of the authentic HPTs, OsAHP1 and OsAHP2, are involved in abiotic stress signalling. Similar to their *Arabidopsis* orthologs, the rice AHPs were also found to be negative regulators of drought stress tolerance, as the OsAHP2/3 underexpression transgenic rice plants were tolerant to drought (Sun et al. 2014). Unlike their *Arabidopsis* counterparts, the OsAHPs (AHP1 and AHP2) are positive regulators of salinity stress tolerance as the OsAHP2/3 underexpression transgenic rice plants were found to be hypersensitive to salinity stress as compared to the WT (Sun et al. 2014). It has been proposed that the regulation of salinity stress signalling by OsAHPs is done through the up-regulation of some of the key Na^+ transporters and exchangers such as OsNHX1, OsSOS1 and OsHKT1;1 (Sun et al. 2014).

As mentioned, RRs are the last component of the TCS signalling but not the ultimate target of this signal cascade. In *Arabidopsis*, drought and cold stress induce a set of RR genes including ARR5, ARR15 (type-A RRs) and RR22 (type-C RR) (Kang et al. 2013). Though these RRs function downstream to the cytokinin signalling, they also function independent of the cytokinin receptors suggesting that they are also regulated by other signalling molecules outside the TCS (Kang et al. 2013). In 2010, one study demonstrated that arr1arr12 (type-B RR) double mutants are salt tolerant (Mason et al. 2010). They also showed that a quadruple mutant of type-A RRs, viz. arr3, arr4, arr5 and arr6, is also salt tolerant (Mason et al. 2010). This reveals that a few selected RRs in *Arabidopsis* function to negatively regulate salinity tolerance. Another report further demonstrated that transcript levels of ARR1, ARR10 and ARR12 are reduced under drought stress (Nguyen et al. 2016). *Arabidopsis* triple mutants for these genes are drought tolerant and show increased sensitivity to ABA, suggesting that these ARRs act as negative regulators of drought stress signalling (Nguyen et al. 2016). Knockdown of these sets of RRs promotes

the ABA response and lead to the higher accumulation of LEA (late embryogenesis abundant) proteins, as well as osmoprotectant biosynthetic genes such as P5CS1 and SUS1 as a protective mechanism under drought (Nguyen et al. 2016). On the other hand, freezing stress has a mixed effect on RR genes. Some of the RRs are positive regulators, while some are negative regulators of cold stress (Jeon et al. 2010; Jeon and Kim 2013; Kang et al. 2013). Cold stress induces the expression of a set of type-A RRs such as ARR5, ARR6, ARR7 and ARR15 (Jeon et al. 2010). Expectedly, different phytohormones like ethylene and cytokinin modulate the expression of these genes as well (Shi et al. 2012). Prolonged exposure to cold stress leads to down-regulation of ARR5, ARR7 and ARR15 in EIN3-dependent manner which in turn can be overcome by cytokinin treatment (Shi et al. 2012). Moreover, these cold-induced ARRs function as negative regulator of cold stress signalling. In addition, the *Arabidopsis* triple mutant plants (*arr5,6,7*) are cold stress tolerant implicating a negative regulatory role for these three RRs in cold stress response (Jeon et al. 2010). This was further validated through the overexpression of ARR7, which rendered the transgenic *Arabidopsis* plants hypersensitive to cold stress as well as ABA-insensitive (Jeon et al. 2010). In contrast, a cold stress tolerance phenotype was observed in *Arabidopsis* overexpressing ARR1, and cytokinin treatment improved the cold tolerance in the transgenic plants (Kang et al. 2013). This indicates that ARR1 is a positive regulator of cold tolerance in *Arabidopsis*. In the same study, one of the type-C RRs of *Arabidopsis*, ARR22, was found to be cold and dehydration inducible (Kang et al. 2013). Overexpression of the ARR22 promotes transgenic *Arabidopsis* plant survival under dehydration, drought and cold stresses highlighting its importance in the abiotic stress response (Kang et al. 2013). In rice, the RR genes have not been characterized with regard to their involvement in abiotic stress signalling. In one study, it was reported that a rice type-A RR gene, OsRR6, is induced in response to salinity, dehydration and cold stress indicating that OsRR6 may play an important role in abiotic stress signalling in rice (Jain et al. 2006). More recently, one study revealed that a nonsense mutation, leading to the premature termination of OsRR22 (A type-B RR) translation, resulted in improved salinity tolerance in the mutant rice (Takagi et al. 2015). Hence, OsRR22 functions to negatively regulate salinity tolerance in rice.

10.8 Role of Two-Component System in Biotic Stress Response

Plants are exposed to a wide array of pathogens and pests. Biotic agents include viruses, bacteria, phytoplasmas, oomycetes, fungi, nematodes and insect pests. Plant interaction with pathogens and the consequent response have been covered in Chap. 21.

Till date, only a few of the histidine kinases in plants have been shown to be implicated in the biotic stress response. In 2012, Pham et al. showed that mutation in AHK5 resulted in accelerated disease progression in *Arabidopsis* mutants upon *Pseudomonas syringae* DC 3000 (PstDC3000) infection (Pham et al. 2012).

Interestingly, they also reported that AHK5 mediates responses to bacterial infection through the regulation of phytohormone [Salicylic acid (SA), Jasmonic acid (JA) and ABA] levels, all of which play an integral role in plant immunity (Pham et al. 2012). Interestingly, they also found that the *Arabidopsis ahk-5* mutant was also more susceptible to infection by the necrotrophic fungus, *Botrytis cinerea*, and this susceptibility was correlated to a decreased early reactive oxygen species (ROS) production (Pham et al. 2012). Thus, AHK5 plays an integral role in the resistance to both bacterial and fungal infection in *Arabidopsis*. AHK5 contributes towards salinity tolerance as well, making AHK5 (or it's orthologs in crop plants) an interesting target for crop improvement.

Besides AHK5, the *Arabidopsis* cytokinin receptors – AHK2, AHK3 and AHK4/CRE1/WOL1 – have been reported to mediate responses to infection. Plant cytokinin receptors have particularly been exploited by gall-forming bacteria and biotrophic fungi which produce cytokinins and auxins to enhance pathogenicity and alter host physiology (Choi et al. 2011). However, resistance (R) proteins have been shown to increase endogenous cytokinin levels, which then results in the downstream signalling through cytokinin receptors AHK2 and AHK3. This results in multistep phosphorelay and activation of ARR2 transcription leading to enhancement of the SA-dependent expression of defence-related genes such as pathogenesis-related 1 and 2 (PR1 and PR2) and WRKY18 (Choi et al. 2011). Additionally, cytokinin signalling also activates the cytokinin response factor 5 (CRF5), which acts as a transcription factor for many PR genes and as such is involved in many pathogen response pathways (Liang et al. 2010). CRF5 contains the ARR1/ARR2-binding motif in the upstream region of its promoter and acts as a downstream partner of type-B ARRs and as negative regulators of cytokinin signalling similar to the type-A ARRs (Liang et al. 2010). Thus, cytokinin signalling and plant immunity are interconnected, and further studies are required to dissect the complexity of this crosstalk. Apart from bacterial and fungal pathogens, the cytokinin receptors have also been implicated in the plant responses to *Heterodera* spp. of nematodes. *Heterodera* spp. of nematodes infect plant roots and induce syntium, a feeding tissue which is formed by the nematode through the modulation of plant phytohormones such as cytokinins. In one study, various cytokinin- signalling defective mutants of *Arabidopsis* (all single-gene or multiple-gene mutants of the TCS involved in cytokinin-mediated canonical multistep phosphorelay) were subjected to *Heterodera schachtii* infection, and it was found that all of the mutant lines were less susceptible to infection as compared to the wild type (Shanks et al. 2016). This indicated a role for the cytokinin receptors; the cytokinin-responsive HPTs and the cytokinin-responsive type-B RRs are required for nematode parasitism (Shanks et al. 2016). Additional analysis using multigene mutants of type-A RRs revealed that the type-A RRs function to reduce the infection of *H. schachtii* and thus play an integral role in the resistance of *Arabidopsis* to nematode infection (Shanks et al. 2016).

In addition to TCS involved in cytokinin signalling, the ethylene receptors also play a major role in the responses to biotic stresses. Ethylene synthesis is up-regulated in response to necrotrophic pathogens and herbivorous insect attack in plants. In *Arabidopsis* ethylene-insensitive mutants (*etr-1* and *ein-2)*, it was found

that fln22 (a bacterial PAMP)-triggered ROS production was reduced (Mersmann et al. 2010). It was also reported that in *Arabidopsis* plants with mutated EIN2, all the FLS-2-mediated responses were drastically impaired implicating a role of EIN2 in mediating plant innate immunity (Boutrot et al. 2010). Ethylene has a role in the infection by the necrotrophic fungi, *Alternaria brassicicola* and *Botrytis cinerea* (Thomma et al. 1999). There was a significant reduction in the pathogen-induced levels of resistance genes in *Arabidopsis ein2* mutants subjected to *A. brassicicola* and *B. cinerea* (Thomma et al. 1999). Since the binding of ethylene to its receptors results in the derepression of EIN2 and subsequent activation of FLS-2 transcription, this implies that the ethylene receptors play a key role in regulating the innate immunity against bacterial and fungal pathogens. Interestingly, it has also been shown that the tomato ethylene-insensitive mutant (never ripe) displayed comprehensively reduced disease symptoms when subjected to virulent bacterial pathogens (*Xanthomonas campestris* pv. vesicatoria and *Pseudomonas syringae* pv. tomato) (Lund et al. 1998; Ciardi et al. 2000). Thus, the role of ethylene in regulating plant immune response may vary depending upon the species. While, in *Arabidopsis*, ethylene binding would result in the activation of EIN2 and subsequent immune response and resistance, in tomato, mutation in the receptors leading to ethylene insensitivity results in bacterial resistance. Recently, it has been shown that EIN2 mutation in rice also resulted in increased susceptibility to the blast fungus *Magnaporthe oryzae* suggesting a role for EIN2 in fungal resistance (Yang et al. 2017). They further revealed that EIN2 plays an integral part in pathogen resistance through induction of ROS production, jasmonic acid production and accumulation of phytoalexins. Phytoalexins are low molecular weight secondary metabolites which possess antimicrobial activity and play an integral role in plant defence against pathogen infection (Ahuja et al. 2012). Thus, ethylene receptors, through the ethylene-dependent regulation of EIN2 activity, mediate the immune responses of various plant species through the induction of a wide array of defence-associated genes. Interestingly, the *Arabidopsis* ETR1 also regulates ARR2, a response regulator implicated in the cytokinin-dependent induction of SA during pathogen infection (Hass et al. 2004). Thus, various hormone signalling pathways are connected in the biotic stress response of plants, with the TCS forming an integral component of this increasingly complex signalling network.

10.9 Pseudo-response Regulators (PsRRs) and the Regulation of Circadian Clock

Similar to how the phytochromes have diverged to become serine/threonine kinases, the PsRRs are a unique subset of TCS RRs which have lost their conserved aspartate residue that is necessary for phosphorylation through two-component signalling (Hwang et al. 2002; Pareek et al. 2006). In *Arabidopsis*, the PsRRs have been shown to regulate the circadian clock. In fact, PsRR1 (TOC1) is an integral component of the central clock in *Arabidopsis* (Makino et al. 2002). In addition, PsRRs 5, 7 and 9 also play a key role in the regulation of the central circadian clock in *Arabidopsis*

(Farré et al. 2005; Nakamichi et al. 2005; Salome and McClung 2005; Nakamichi et al. 2010; Salome et al. 2010). For a comprehensive understanding of the *Arabidopsis* circadian clock, an excellent review by Norihito Nakamichi is recommended (Nakamichi 2011). In the review, the integral roles of all the five *Arabidopsis* PsRRs in the regulation of the central clock have been well described. In short, the PsRRs play a key role in regulating the expression of two myb-transcription factors, CCA1 and LHY, which regulate a large subset of genes. PsRR1 (TOC1) promotes the expression of CCA1 and LHY, while PsRRs 5, 7 and 9 are negative regulators (Nakamichi 2011). PsRR3 functions to stabilize TOC1 in the evening (Nakamichi 2011). As components of the central clock, the PsRRs are thus implicated in a wide array of biological processes and form an extremely important diverged group of the TCS in plants.

10.10 Conclusions and Perspectives

The two-component system seems to have evolved in bacteria primarily to enable the monitoring of the external environment. While a majority of the sensory histidine kinases are membrane localized, a few cytosolic kinases are also present, which shows that the TCS also plays a role in sensing of intracellular signals. In plants, the two-component system is a complex superfamily of genes with an extremely diverse set of functions. It is interesting that the TCS also functions to monitor external as well as internal signals in plants. What is even more interesting is that most of these processes are mostly interconnected. For example, cytokinin and ethylene signalling converge at the level of the RRs and further mediate salicylic acid responses. The same TCS members involved in phytohormone perception and signalling are regulating responses to abiotic and biotic stresses. With new roles and mechanisms of action for proteins being uncovered regularly, new functions for the TCS in plants are anything but inevitable. Nevertheless, this chapter has highlighted the various processes in which the TCS in plants is involved in. The cross-talk among various TCS signalling and other signalling pathways is quite complex and requires an in-depth analysis. Perhaps with new genetic resources and tools for the functional characterization of the TCS genes, we would be able to obtain a more holistic view of exact mechanisms underlying their various functions. For now, we can conclude that the TCS in plants function to mediate not only responses to biotic and abiotic stresses but also phytohormone and light responses to regulate plant growth and development.

Acknowledgement RCN and PG acknowledge Council for Scientific and Industrial Research (CSIR) for their research fellowship. AS acknowledges University Grants Commission (UGC), while DS acknowledges Department of Biotechnology (DBT) for her research fellowship. SLS-P and AP would like to thank Indo-US Science and Technology Forum (IUSSTF) for the grant of funds via Indo-US Advanced Bioenergy Consortium (IUABC). Research in the lab of AP is also supported from funds received from Department of Biotechnology, Government of India, International Atomic Energy Agency (Vienna) and Department of Science and Technology (DST-PURSE) through Jawaharlal Nehru University.

References

Abeles FB, Morgan PW, Saltveit ME, Mikal E (eds) (1992) Ethylene in plant biology. Academic Press, San Diego

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17:73–90

Alm E, Huang K, Arkin A (2006) The evolution of two-component systems in bacteria reveals different strategies for niche adaptation. PLoS Comput Biol 2:e143

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science 284:2148–2152

Alvarez AF, Barba-Ostria C, Silva-Jiménez H, Georgellis D (2016) Organization and mode of action of two component system signaling circuits from the various kingdoms of life. Environ Microbiol 18:3210–3226

An F, Zhao Q, Ji Y, Li W, Jiang Z, Yu X, Zhang C, Han Y, He W, Liu Y, Zhang S, Ecker JR, Guo H (2010) Ethylene-induced stabilization of ETHYLENE INSENSITIVE3 and EIN3-LIKE1 is mediated by proteasomal degradation of EIN3 Binding F-Box 1 and 2 that requires EIN2 in Arabidopsis. Plant Cell 22:2384–2401

Anantharaman V, Iyer LM, Aravind L (2007) Comparative Genomics of Protists: New Insights into the Evolution of Eukaryotic Signal Transduction and Gene Regulation. Annu Rev Microbiol 61:453–475

Appleby JL, Parkinson JS, Bourret RB (1996) Signal Transduction via the Multi-Step Phosphorelay: Not Necessarily a Road Less Travelled. Cell 86:845–848

Argyros RD, Mathews DE, Chiang Y-H, Palmer CM, Thibault DM, Etheridge N, Argyros DA, Mason MG, Kieber JJ, Schaller GE (2008) Type B response regulators of Arabidopsis play key roles in cytokinin signaling and plant development. Plant Cell 20:2102–2116

Bekker M, de MJ T, Hellingwerf KJ (2006) The role of two-component regulation systems in the physiology of the bacterial cell. Sci Prog 89:213–242

Bleecker AB (1999) Ethylene perception and signaling: an evolutionary perspective. Trends Plant Sci 4:269–274

Bleecker AB, Kende H (2000) Ethylene: A gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Bleecker AB, Estelle MA, Somerville C and Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in Arabidopsis thaliana. Science (80-) 241: 1086–1089

Bleecker AB, Esch JJ, Hall AE, Rodriguez FI, Binder BM (1998) The ethylene-receptor family from Arabidopsis: structure and function. Philos Trans R Soc B Biol Sci 353:1405–1412

Boutrot F, Segonzac C, Chang KN, Qiao H, Ecker JR, Zipfel C, Rathjen JP (2010) Direct transcriptional control of the Arabidopsis immune receptor FLS2 by the ethylene-dependent transcription factors EIN3 and EIL1. Proc Natl Acad Sci U S A 107:14502–14507

Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, Maheswari U, Martens C, Maumus F, Otillar RP, Rayko E, Salamov A, Vandepoele K, Beszteri B, Gruber A, Heijde M, Katinka M, Mock T, Valentin K, Verret F, Berges JA, Brownlee C, Cadoret JP, Chiovitti A, Choi CJ, Coesel S, De Martino A, Detter JC, Durkin C, Falciatore A, Fournet J, Haruta M, Huysman MJ, Jenkins BD, Jiroutova K, Jorgensen RE, Joubert Y, Kaplan A, Kröger N, Kroth PG, La Roche J, Lindquist E, Lommer M, Martin-Jézéquel V, Lopez PJ, Lucas S, Mangogna M, McGinnis K, Medlin LK, Montsant A, Oudot-Le Secq MP, Napoli C, Obornik M, Parker MS, Petit JL, Porcel BM, Poulsen N, Robison M, Rychlewski L, Rynearson TA, Schmutz J, Shapiro H, Siaut M, Stanley M, Sussman MR, Taylor AR, Vardi A, von Dassow P, Vyverman W, Willis A, Wyrwicz LS, Rokhsar DS, Weissenbach J, Armbrust EV, Green BR, Van de Peer Y, Grigoriev IV (2008) The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 456:239–244

Brown JL, North S, Bussey H (1993) SKN7, a yeast multicopy suppressor of a mutation affecting cell wall beta-glucan assembly, encodes a product with domains homologous to prokaryotic two-component regulators and to heat shock transcription factors. J Bacteriol 175:6908–6915

Burbulys D, Trach KA, Hoch JA (1991) Initiation of sporulation in B. subtilis is controlled by a multicomponent phosphorelay. Cell 64:545–552

Caesar K, Thamm AMK, Witthöft J, Elgass K, Huppenberger P, Grefen C, Horak J, Harter K (2011) Evidence for the localization of the Arabidopsis cytokinin receptors AHK3 and AHK4 in the endoplasmic reticulum. J Exp Bot 62:5571–5380

Capra EJ, Laub MT (2012) Evolution of two-component signal transduction systems. Annu Rev Microbiol 66:325–347

Chang C (2016) Q&A: How do plants respond to ethylene and what is its importance? BMC Biol 14:7

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. Science 262:539–544

Chang KN, Zhong S, Weirauch MT, Hon G, Pelizzola M, Li H, Huang SC, Schmitz RJ, Urich MA, Kuo D, Nery JR, Qiao H, Yang A, Jamali A, Chen H, Ideker T, Ren B, Bar-Joseph Z, Hughes TR, Ecker JR (2013) Temporal transcriptional response to ethylene gas drives growth hormone cross-regulation in Arabidopsis. elife 2:e00675

Chen Y-F, Randlett MD, Findell JL, Schaller GE (2002) Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of Arabidopsis. J Biol Chem 277:19861–19866

Choi J, Choi D, Lee S, Ryu C-M, Hwang I (2011) Cytokinins and plant immunity: old foes or new friends? Trends Plant Sci 16:388–394

Chu ZX, Ma Q, Lin YX, Tang XL, Zhou YQ, Zhu SW, Fan J, Cheng BJ (2011) Genome-wide identification, classification, and analysis of two-component signal system genes in maize. Genet Mol Res 10:3316–3330

Ciardi JA, Tieman DM, Lund ST, Jones JB, Stall RE, Klee HJ (2000) Response to *Xanthomonas campestris* pv. vesicatoria in tomato involves regulation of ethylene receptor gene expression. Plant Physiol 123:81–92

Coba de la Peña T, Cárcamo CB, Almonacid L, Zaballos A, Lucas MM, Balomenos D, Pueyo JJ (2008) A salt stress-responsive cytokinin receptor homologue isolated from *Medicago sativa* nodules. Planta 227:769–779

Cotter PA, Jones AM (2003) Phosphorelay control of virulence gene expression in Bordetella. Trends Microbiol 11:367–373

Davis SJ, Vener AV, Vierstra RD (1999) Bacteriophytochromes: Phytochrome-Like photoreceptors from nonphotosynthetic. Science 286:2517–2520

Deng Y, Dong H, Mu J, Ren B, Zheng B, Ji Z, Yang W-C, Liang Y, Zuo J (2010) Arabidopsis histidine kinase CKI1 acts upstream of histidine phosphotransfer proteins to regulate female gametophyte development and vegetative growth. Plant Cell 22:1232–1248

Dutta R, Inouye M (2000) GHKL, an emergent ATPase/kinase superfamily. Trends Biochem Sci 25:24–28

Dutta R, Qin L, Inouye M (1999) Histidine kinases: diversity of domain organization. Mol Microbiol 34:633–640

Farré EM, Harmer SL, Harmon FG, Yanovsky MJ, Kay SA (2005) Overlapping and distinct roles of PRR7 and PRR9 in the Arabidopsis circadian clock. Curr Biol 15:47–54

Ferreira FJ, Kieber JJ (2005) Cytokinin signaling. Curr Opin Plant Biol 8:518–525

Forst SA, Roberts DL (1994) Signal transduction by the EnvZ-OmpR phosphotransfer system in bacteria. Res Microbiol 145:363–373

Frebort I, Kowalska M, Hluska T, Frebortova J, Galuszka P (2011) Evolution of cytokinin biosynthesis and degradation. J Exp Bot 62:2431–2452

Frébortová J, Plíhal O, Florová V, Kokáš F, Kubiasová K, Greplová M, Šimura J, Novák O, Frébort I (2017) Light influences cytokinin biosynthesis and sensing in Nostoc (cyanobacteria). J Phycol 53:703–714

Gahlaut V, Mathur S, Dhariwal R, Khurana JP, Tyagi AK, Balyan HS, Gupta PK (2014) A multi-step phosphorelay two-component system impacts on tolerance against dehydration stress in common wheat. Funct Integr Genomics 14:707–716

Gallie DR (2015a) Appearance and elaboration of the ethylene receptor family during land plant evolution. Plant Mol Biol 87:521–539

Gallie DR (2015b) Ethylene receptors in plants – why so much complexity? F1000Prime Rep 7: 39–50
Galperin MY (2005) A census of membrane-bound and intracellular signal transduction proteins in bacteria: bacterial IQ, extroverts and introverts. BMC Microbiol 5:35–53
Galperin MY, Makarova KS, Wolf YI, Koonin EV (2018) Phyletic distribution and lineage-specific domain architectures of archaeal two-component signal transduction systems. J Bacteriol 200:e00681–e00617
Gamble RL, Coonfield ML, Schaller GE (1998) Histidine kinase activity of the ETR1 ethylene receptor from Arabidopsis. Proc Natl Acad Sci 95:7825–7829
Grefen C, Städele K, Růzicka K, Obrdlik P, Harter K, Horák J (2008) Subcellular localization and in vivo interactions of the Arabidopsis thaliana ethylene receptor family members. Mol Plant 1:308–320
Gruhn N, Heyl A (2013) Updates on the model and the evolution of cytokinin signaling. Curr Opin Plant Biol 16:569–574
Gruhn N, Halawa M, Snel B, Seidl MF, Heyl A (2014) A subfamily of putative cytokinin receptors is revealed by an analysis of the evolution of the two-component signaling system of plants. Plant Physiol 165:227–237
Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7:40–49
Guzmán P, Ecker JR (1990) Exploiting the triple response of Arabidopsis to identify ethylene-related mutants. Plant Cell 2:513–523
Hall BP, Shakeel SN, Amir M, Haq NU, Qu X, Schaller GE (2012) Histidine kinase activity of the ethylene receptor etr1 facilitates the ethylene response in Arabidopsis. Plant Physiol 159:682–695
Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, Hwang I, Zhu T, Schäfer E, Kudla J, Harter K (2004) The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis. EMBO J 23:3290–3302
He Y, Liu X, Ye L, Pan C, Chen L, Zou T, Lu G (2016a) Genome-wide identification and expression analysis of two-component system genes in tomato. Int J Mol Sci 17:1204–1224
He Y, Liu X, Zou T, Pan C, Qin L, Chen L, Lu G (2016b) Genome-wide identification of two-component system genes in cucurbitaceae crops and expression profiling analyses in cucumber. Front Plant Sci 7:899
Hejatko J, Pernisoa M, Eneva T, Palme K, Brzobohaty B (2003) The putative sensor histidine kinase CKI1 is involved in female gametophyte development in Arabidopsis. Mol Gen Genomics 269:443–453
Héricourt F, Chefdor F, Bertheau L, Tanigawa M, Maeda T, Guirimand G, Courdavault V, Larcher M, Depierreux C, Bénédetti H, Morabito D, Brignolas F, Carpin S (2013) Characterization of histidine-aspartate kinase HK1 and identification of histidine phosphotransfer proteins as potential partners in a Populus multistep phosphorelay. Physiol Plant 149:188–199
Héricourt F, Chefdor F, Djeghdir I, Larcher M, Lafontaine F, Courdavault V, Auguin D, Coste F, Depierreux C, Tanigawa M, Maeda T, Glévarec G, Carpin S (2016) Functional divergence of poplar histidine-aspartate kinase hk1 paralogs in response to osmotic stress. Int J Mol Sci 17:2061
Heyl A, Schmülling T (2003) Cytokinin signal perception and transduction. Curr Opin Plant Biol 6:480–488
Heyl A, Brault M, Frugier F, Kuderova A, Lindner A-C, Motyka V, Rashotte AM, Schwartzenberg KV, Vankova R, Schaller GE (2013) Nomenclature for members of the two-component signaling pathway of plants. Plant Physiol 161:1063–1065
Hill K, Mathews DE, Kim HJ, Street IH, Wildes SL, Chiang Y-H, Mason MG, Alonso JM, Ecker JR, Kieber JJ, Schaller GE (2013) Functional characterization of type-B response regulators in the Arabidopsis cytokinin response. Plant Physiol 162:212–224
Hothorn M, Dabi T, Chory J (2011) Structural basis for cytokinin recognition by Arabidopsis thaliana histidine kinase 4. Nat Chem Biol 7:766–768

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell 94:261–271
Hughes J, Lamparter T (1999) Prokaryotes and phytochrome. The connection to chromophores and signaling. Plant Physiol 121:1059–1068
Hwang I, Sheen J (2001) Two-component circuitry in Arabidopsis cytokinin signal transduction. Nature 413:383–389
Hwang I, Chen H-C, Sheen J (2002) Two-component signal transduction pathways in Arabidopsis. Plant Physiol 129:500–515
Hwang I, Sheen J, Müller B (2012) Cytokinin Signaling Networks. Annu Rev Plant Biol 63:353–380
Imamura A, Kiba T, Tajima Y, Yamashino T, Mizuno T (2003) In vivo and in vitro characterization of the arr11 response regulator implicated in the his-to-asp phosphorelay signal transduction in Arabidopsis thaliana. Plant Cell Physiol 44:122–131
Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from Arabidopsis. Nature 409:1060–1063
Inoue K, Nishihama R, Kohchi T (2017) Evolutionary origin of phytochrome responses and signaling in land plants. Plant Cell Environ 40:2502–2508
Ishida K, Yamashino T, Yokoyama A, Mizuno T (2008) Three Type-B Response Regulators, ARR1, ARR10 and ARR12, Play Essential but Redundant Roles in Cytokinin Signal Transduction Throughout the Life Cycle of Arabidopsis thaliana. Plant Cell Physiol 49:47–57
Ishida K, Niwa Y, Yamashino T, Mizuno T (2009) A genome-wide compilation of the two-component systems in Lotus japonicus. DNA Res 16:237–247
Jain M, Tyagi A, Khurana J (2006) Molecular characterization and differential expression of cytokinin-responsive type-A response regulators in rice (Oryza sativa). BMC Plant Biol 6:1
Jennifer W, To PC, Haberer G, Ferreira FJ, Deruè J, Mason MG, Schaller GE, Alonso JM, Ecker JR, Kieber JJ (2004) Type-A Arabidopsis Response Regulators Are Partially Redundant Negative Regulators of Cytokinin Signaling. Plant Cell 16:658–671
Jeon J, Kim J (2013) Arabidopsis response Regulator1 and Arabidopsis histidine phosphotransfer Protein2 (AHP2), AHP3, and AHP5 function in cold signaling. Plant Physiol 161:408–424
Jeon J, Kim NY, Kim S, Kang NY, Novák O, Ku S-J, Cho C, Lee DJ, Lee E-J, Strnad M, Kim J (2010) A subset of cytokinin two-component signaling system plays a role in cold temperature stress response in Arabidopsis. J Biol Chem 285:23371–23386
Ju C, Chang C (2015) Mechanistic Insights in Ethylene Perception and Signal Transduction. Plant Physiol 169:85–95
Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, Garrett WM, Kessenbrock M, Groth G, Tucker ML, Cooper B, Kieber JJ, Chang C (2012) CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc Natl Acad Sci 109:19486–19491
Ju C, Van de Poel B, Cooper ED, Thierer JH, Gibbons TR, Delwiche CF, Chang C (2015) Conservation of ethylene as a plant hormone over 450 million years of evolution. Nat Plants 1:14004
Kakimoto T (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. Science 274:982–985
Kakimoto T (2003) Perception and signal transduction of cytokinins. Annu Rev Plant Biol 54:605–627
Kang NY, Cho C, Kim J (2013) Inducible Expression of Arabidopsis Response Regulator 22 (ARR22), a Type-C ARR, in Transgenic Arabidopsis Enhances Drought and Freezing Tolerance. PLoS One 8:e79248
Karan R, Singla-Pareek SL, Pareek A (2009) Histidine kinase and response regulator genes as they relate to salinity tolerance in rice. Funct Integr Genomics 9:411–417
Kiba T, Yamada H, Sato S, Kato T, Tabata S, Yamashino T, Mizuno T (2003) The type-A response regulator, ARR15, acts as a negative regulator in the cytokinin-mediated signal transduction in Arabidopsis thaliana. Plant Cell Physiol 44:868–874

Kieber JJ, Schaller GE (2014) Cytokinins. Arab B 12:e0168
Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell 72:427–441
Kim K, Ryu H, Cho Y-H, Scacchi E, Sabatini S, Hwang I (2012) Cytokinin-facilitated proteolysis of Arabidopsis response regulator 2 attenuates signaling output in two-component circuitry. Plant J 69:934–945
Klee HJ (2004) Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 135:660–667
Kooß S, Lamparter T (2017) Cyanobacterial origin of plant phytochromes. Protoplasma 254:603–607
Koretke KK, Lupas AN, Warren PV, Rosenberg M, Brown JR (2000) Evolution of two-component signal transduction. Mol Biol Evol 17:1956–1970
Kurepa J, Li Y, Smalle JA (2014) Cytokinin signaling stabilizes the response activator ARR1. Plant J 78:157–168
Kwon O, Georgellis D, Lin EC (2000) Phosphorelay as the sole physiological route of signal transmission by the arc two-component system of Escherichia coli. J Bacteriol 182:3858–3862
Lee DJ, Park J-Y, Ku S-J, Ha Y-M, Kim S, Kim MD, Oh M-H, Kim J (2007) Genome-wide expression profiling of ARABIDOPSIS RESPONSE REGULATOR 7(ARR7) overexpression in cytokinin response. Mol Gen Genomics 277:115–137
Levit M, Liu Y, Surette M, Stock J (1996) Active site interference and asymmetric activation in the chemotaxis protein histidine kinase CheA. J Biol Chem 271:32057–32063
Li J, Li G, Wang H, Wang Deng X (2011) Phytochrome signaling mechanisms. Arab B 9:e0148
Li F-W, Melkonian M, Rothfels CJ, Villarreal JC, Stevenson DW, Graham SW, Wong GK-S, Pryer KM, Mathews S (2015a) Phytochrome diversity in green plants and the origin of canonical plant phytochromes. Nat Commun 6:7852–7863
Li W, Ma M, Feng Y, Li H, Wang Y, Ma Y, Li M, An F, Guo H (2015b) EIN2-directed translational regulation of ethylene signaling in Arabidopsis. Cell 163:670–683
Liang YS, Ermawati N, Cha J-Y, Jung MH, Su'udi M, Kim MG, Ha S-H, Park C-G, Son D (2010) Overexpression of an AP2/ERF-type Transcription factor CRF5 confers pathogen resistance to Arabidopsis plants. J Korean Soc Appl Biol Chem 53:142–148
Liu Z, Zhang M, Kong L, Lv Y, Zou M, Lu G, Cao J, Yu X (2014) Genome-wide identification, phylogeny, duplication, and epression analyses of two-component system genes in chinese cabbage (*Brassica rapa* ssp. pekinensis). DNA Res 21:379–396
Lohrmann J, Sweere U, Zabaleta E, Bäurle I, Keitel C, Kozma-Bognar L, Brennicke A, Schäfer E, Kudla J, Harter K (2001) The response regulator ARR2: a pollen-specific transcription factor involved in the expression of nuclear genes for components of mitochondrial complex I in Arabidopsis. Mol Gen Genomics 265:2–13
Lund ST, Stall RE, Klee HJ (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. Plant Cell 10:371–382
Maeda T, Wurgler-Murphy SM, Saito H (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast. Nature 369:242–245
Mahonen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, Törmäkangas K, Ikeda Y, Oka A, Kakimoto T, Helariutta Y (2006) Cytokinin signaling and its inhibitor ahp6 regulate cell fate during vascular development. Science 311:94–98
Makino S, Kiba T, Imamura A, Hanaki N, Nakamura A, Suzuki T, Taniguchi M, Ueguchi C, Sugiyama T, Mizuno T (2000) Genes encoding pseudo-response regulators: insight into his-to-asp phosphorelay and circadian rhythm in Arabidopsis thaliana. Plant Cell Physiol 41:791–803
Makino S, Matsushika A, Kojima M, Yamashino T, Mizuno T (2002) The APRR1/TOC1 quintet implicated in circadian rhythms of Arabidopsis thaliana: I. Characterization with APRR1-overexpressing plants. Plant Cell Physiol 43:58–69
Mason MG, Li J, Mathews DE, Kieber JJ, Schaller GE (2004) Type-B response regulators display overlapping expression patterns in Arabidopsis. Plant Physiol 135:927–937

Mason MG, Mathews DE, Argyros DA, Maxwell BB, Kieber JJ, Alonso JM, Ecker JR, Schaller GE (2005) Multiple Type-B response regulators mediate cytokinin signal transduction in Arabidopsis. Plant Cell 17:3007–3018

Mason MG, Jha D, Salt DE, Tester M, Hill K, Kieber JJ, Eric Schaller G (2010) Type-B response regulators ARR1 and ARR12 regulate expression of AtHKT1;1 and accumulation of sodium in Arabidopsis shoots. Plant J 64:753–763

Maxwell BB, Kieber JJ (2010) Cytokinin signal transduction. In: Davies P.J. (ed) Plant Hormones. Springer, Dordrecht, pp 329–357

McCarty DR, Chory J (2000) Conservation and innovation in plant signaling pathways. Cell 103:201–209

Mcmanus MT (ed) (2012) Annual plant reviews, vol. 44: the plant hormone ethylene. Wiley, New York

Merchan F, de Lorenzo L, Rizzo SG, Niebel A, Manyani H, Frugier F, Sousa C, Crespi M (2007) Identification of regulatory pathways involved in the reacquisition of root growth after salt stress in Medicago truncatula. Plant J 51:1–17

Merchante C, Alonso JM, Stepanova AN (2013) Ethylene signaling: simple ligand, complex regulation. Curr Opin Plant Biol 16:554–560

Mersmann S, Bourdais G, Rietz S, Robatzek S (2010) Ethylene signaling regulates accumulation of the fls2 receptor and is required for the oxidative burst contributing to plant immunity. Plant Physiol 154:391–400

Mira-Rodado V, Veerabagu M, Witthöft J, Teply J, Harter K, Desikan R (2012) Identification of two-component system elements downstream of AHK5 in the stomatal closure response of Arabidopsis thaliana. Plant Signal Behav 7:1467–1476

Mizuno T, Kaneko T, Tabata S (1996) Compilation of all genes encoding bacterial two-component signal transducers in the genome of the cyanobacterium, Synechocystis sp. strain PCC 6803. DNA Res 3:407–414

Mochida K, Yoshida T, Sakurai T, Yamaguchi-Shinozaki K, Shinozaki and Tran L-SP (2010) Genome-wide analysis of two-component systems and prediction of stress-responsive two-component system members in soybean. DNA Res 17: 303–324

Monedero V, Revilla-Guarinos A, Zúñiga M (2017) Physiological role of two-component signal transduction systems in food-associated lactic acid bacteria. Adv Appl Microbiol 99:1–51

Montsant A, Allen AE, Coesel S, De Martino A, Falciatore A, Mangogna M, Siaut M, Heijde M, Jabbari K, Maheswari U, Rayko E, Vardi A, Apt KE, Berges JA, Chiovitti A, Davis AK, Thamatrakoln K, Hadi MZ, Lane TW, Lippmeier JC, Martinez D, Parker MS, Pazour GJ, Saito MA, Rokhsar DS, Armbrust EV, Bowler C (2007) Identification and comparative genomic analysis of signaling and regulatory components in the diatom *thalassiosira pseudonana*. J Phycol 43:585–604

Moussatche P, Klee HJ (2004) Autophosphorylation activity of the Arabidopsis ethylene receptor multigene family. J Biol Chem 279:48734–48741

Müller B and Sheen J (2007) Arabidopsis cytokinin signaling pathway. Sci STKE 2007: cm5

Müller-Dieckmann H-J, Grantz AA, Kim S-H (1999) The structure of the signal receiver domain of the Arabidopsis thaliana ethylene receptor ETR1. Structure 7:1547–1556

Nakamichi N (2011) Molecular mechanisms underlying the Arabidopsis circadian clock. Plant Cell Physiol 52:1709–1718

Nakamichi N, Kita M, Ito S, Yamashino T, Mizuno T (2005) PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of Arabidopsis thaliana. Plant Cell Physiol 46:686–698

Nakamichi N, Kiba T, Henriques R, Mizuno T, Chua NH, Sakakibara H (2010) PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. Plant Cell 22:594–605

Nguyen KH, Van Ha C, Nishiyama R, Watanabe Y, Leyva-González MA, Fujita Y, Tran UT, Li W, Tanaka M, Seki M, Schaller GE, Herrera-Estrella L, Tran LS (2016) Arabidopsis type B cytokinin response regulators ARR1, ARR10, and ARR12 negatively regulate plant responses to drought. Proc Natl Acad Sci U S A 113:3090–3095

Nishiyama R, Watanabe Y, Leyva-Gonzalez MA, Van Ha C, Fujita Y, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K, Herrera-Estrella L, Tran LS (2013) Arabidopsis AHP2, AHP3, and AHP5 histidine phosphotransfer proteins function as redundant negative regulators of drought stress response. Proc Natl Acad Sci U S A 110:4840–4845

Ota IM, Varshavsky A (1993) A yeast protein similar to bacterial two-component regulators. Science 262:566–569

Pareek A, Singh A, Kumar M, Kushwaha HR, Lynn AM, Singla-Pareek SL (2006) Whole-genome analysis of Oryza sativa reveals similar architecture of two-component signaling machinery with Arabidopsis. Plant Physiol 142:380–397

Parish T (2014) Two-Component Regulatory Systems of Mycobacteria. Microbiol Spectr 2:0010–2013

Pekárová B, Szmitkowska A, Dopitová R, Degtjarik O, Žídek L, Hejátko J (2016) Structural aspects of multistep phosphorelay-mediated signaling in plants. Mol Plant 9:71–85

Pham J, Liu J, Bennett MH, Mansfield JW, Desikan R (2012) Arabidopsis histidine kinase 5 regulates salt sensitivity and resistance against bacterial and fungal infection. New Phytol 194:168–180

Pils B, Heyl A (2009) Unraveling the evolution of cytokinin signaling. Plant Physiol 151:782–791

Pischke MS, Jones LG, Otsuga D, Fernandez DE, Drews GN, Sussman MR (2002) An Arabidopsis histidine kinase is essential for megagametogenesis. Proc Natl Acad Sci U S A 99:15800–15805

Posas F, Wurgler-Murphy SM, Maeda T, Witten EA, Thai TC, Saito H (1996) Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1–YPD1–SSK1 "Two-Component" osmosensor. Cell 86:865–875

Quon KC, Marczynski GT, Shapiro L (1996) Cell cycle control by an essential bacterial two-component signal transduction protein. Cell 84:83–93

Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ (2006) A subset of Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. Proc Natl Acad Sci U S A 103:11081–11085

Rockwell NC, Su Y-S, Lagarias JC (2006) Phytochrome structure and signaling mechanisms. Annu Rev Plant Biol 57:837–858

Rodríguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB (1999) A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science 283:996–998

Romanov GA, Lomin SN, Schmülling T (2018) Cytokinin signaling: from the ER or from the PM? That is the question! New Phytol 18:41–53

Rzewuski G, Sauter M (2008) Ethylene biosynthesis and signaling in rice. Plant Sci 175:32–42

Sakai H, Honma T, Aoyama T, Sato S, Kato T, Tabata S, Oka A (2001) ARR1, a transcription factor for genes immediately responsive to cytokinins. Science 294:1519–1521

Salome PA, McClung CR (2005) PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. Plant Cell 17:791–803

Salome PA, Weigel D, McClung CR (2010) The role of the Arabidopsis morning loop components cca1, lhy, prr7, and prr9 in temperature compensation. Plant Cell 22:3650–3661

Schaller GE, Bleecker AB (1995) Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. Science 270:1809–1811

Schaller GE, Kieber JJ, Shiu S-H (2008) Two-component signaling elements and histidyl-aspartyl phosphorelays. Arab B 6:e0112

Schaller GE, Shiu S-H, Armitage JP (2011) Two-component systems and their co-option for eukaryotic signal transduction. Curr Biol 21:R320–R330

Shakeel SN, Wang X, Binder BM and Schaller GE (2013) Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family. AoB Plants 5: plt010

Shanks CM, Rice JH, Zubo Y, Schaller GE, Hewezi T, Kieber JJ (2016) The role of cytokinin during infection of Arabidopsis thaliana by the cyst nematode *Heterodera schachtii*. Mol Plant-Microbe Interact 29:57–68

Sharan A, Soni P, Nongpiur RC, Singla-Pareek SL, Pareek A (2017) Mapping the 'Two-component system' network in rice. Sci Rep 7:9287–9299

Shi X, Wegener-Feldbrügge S, Huntley S, Hamann N, Hedderich R, Søgaard-Andersen L (2008) Bioinformatics and experimental analysis of proteins of two-component systems in *Myxococcus xanthus*. J Bacteriol 190:613–624

Shi Y, Tian S, Hou L, Huang X, Zhang X, Guo H, Yang S (2012) Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in Arabidopsis. Plant Cell 24:2578–2595

Shin A-Y, Han Y-J, Baek A, Ahn T, Kim SY, Nguyen TS, Son M, Lee KW, Shen Y, Song P-S, Kim JI (2016) Evidence that phytochrome functions as a protein kinase in plant light signalling. Nat Commun 7:11545

Shull TE, Kurepa J, Smalle JA (2016) Cytokinin signaling promotes differential stability of type-B ARRs. Plant Signal Behav 11:e1169354

Singh G, Kumar R (2012) Genome-wide *insilico* analysis of plant two component signaling system in woody model plant *Populus trichocarpa*. Res Plant Biol 2:13–23

Singh A, Kushwaha HR, Soni P, Gupta H, Singla-Pareek SL, Pareek A (2015) Tissue specific and abiotic stress regulated transcription of histidine kinases in plants is also influenced by diurnal rhythm. Front Plant Sci 6:711

Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714

Spíchal L (2012) Cytokinins - recent news and views of evolutionally old molecules. Funct Plant Biol 39:267–284

Spíchal L, Rakova NY, Riefler M, Mizuno T, Romanov GA, Strnad M, Schmülling T (2004) Two cytokinin receptors of Arabidopsis thaliana, CRE1/AHK4 and AHK3, differ in their ligand specificity in a bacterial assay. Plant Cell Physiol 45:1299–1305

Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Annu Rev Biochem 69:183–215

Stolz A, Riefler M, Lomin SN, Achazi K, Romanov GA, Schmülling T (2011) The specificity of cytokinin signalling in Arabidopsis thaliana is mediated by differing ligand affinities and expression profiles of the receptors. Plant J 67:157–168

Sun L, Zhang Q, Wu J, Zhang L, Jiao X, Zhang S, Zhang Z, Sun D, Lu T, Sun Y (2014) Two rice authentic histidine phosphotransfer proteins, OsAHP1 and OsAHP2, mediate cytokinin signaling and stress responses in rice. Plant Physiol 165:335–345

Suzuki T, Miwa K, Ishikawa K, Yamada H, Aiba H, Mizuno T (2001) The Arabidopsis sensor histidine kinase, AHK4, can respond to cytokinins. Plant Cell Physiol 42:107–113

Takagi H, Tamiru M, Abe A, Yoshida K, Uemura A, Yaegashi H, Obara T, Oikawa K, Utsushi H, Kanzaki E, Mitsuoka C, Natsume S, Kosugi S, Kanzaki H, Matsumura H, Urasaki N, Kamoun S, Terauchi R (2015) MutMap accelerates breeding of a salt-tolerant rice cultivar. Nat Biotechnol 33:445–449

Takata N, Saito S, Saito C, Uemura M (2010) Phylogenetic footprint of the plant clock system in angiosperms: evolutionary processes of Pseudo-Response Regulators. BMC Evol Biol 10:126–139

Tanaka T, Saha SK, Tomomori C, Ishima R, Liu D, Tong KI, Park H, Dutta R, Qin L, Swindells MB, Yamazaki T, Ono AM, Kainosho M, Inouye M, Ikura M (1998) NMR structure of the histidine kinase domain of the *E. coli* osmosensor EnvZ. Nature 396:88–92

Thomason P, Kay R (2000) Eukaryotic signal transduction via histidine-aspartate phosphorelay. J Cell Sci 113:3141–3150

Thomma BP, Eggermont K, Tierens KF, Broekaert WF (1999) Requirement of functional ethylene-insensitive 2 gene for efficient resistance of Arabidopsis to infection by *Botrytis cinerea*. Plant Physiol 121:1093–1102

Tran L-SP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. Proc Natl Acad Sci U S A 104:20623–20628

Ueguchi C, Sato S, Kato T, Tabata S (2001) The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in Arabidopsis thaliana. Plant Cell Physiol 42:751–755

Ulrich LE, Koonin EV, Zhulin IB (2005) One-component systems dominate signal transduction in prokaryotes. Trends Microbiol 13:52–56

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell 11:1743–1754

Veerabagu M, Elgass K, Kirchler T, Huppenberger P, Harter K, Chaban C, Mira-Rodado V (2012) The Arabidopsis B-type response regulator 18 homomerizes and positively regulates cytokinin responses. Plant J 72:721–731

Voet-van-Vormizeele J, Groth G (2008) Ethylene controls autophosphorylation of the histidine kinase domain in ethylene receptor ETR1. Mol Plant 1:380–387

Wang L, Sun Y-P, Chen W-L, Li J-H, Zhang C-C (2002) Genomic analysis of protein kinases, protein phosphatases and two-component regulatory systems of the cyanobacterium *Anabaena sp*. strain PCC 7120. FEMS Microbiol Lett 217:155–165

Wang W, Hall AE, O'Malley R, Bleecker AB (2003) Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from Arabidopsis is not required for signal transmission. Proc Natl Acad Sci U S A 100:352–357

Wen X, Zhang C, Ji Y, Zhao Q, He W, An F, Jiang L, Guo H (2012) Activation of ethylene signaling is mediated by nuclear translocation of the cleaved EIN2 carboxyl terminus. Cell Res 22:1613–1616

Wen F, Qin T, Wang Y, Dong W, Zhang A, Tan M, Jiang M (2015) OsHK3 is a crucial regulator of abscisic acid signaling involved in antioxidant defense in rice. J Integr Plant Biol 57:213–228

Wuichet K, Cantwell BJ, Zhulin IB (2010) Evolution and phyletic distribution of two-component signal transduction systems. Curr Opin Microbiol 13:219–225

Wulfetange K, Lomin SN, Romanov GA, Stolz A, Heyl A, Schmü T (2011) The cytokinin receptors of Arabidopsis are located mainly to the endoplasmic reticulum. Plant Physiol 156:1808–1818

Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, Miwa K, Yamashino T, Mizuno T (2001) The Arabidopsis AHK4 histidine kinase is a cytokinin-binding receptor that transduces cytokinin signals across the membrane. Plant Cell Physiol 42:1017–1023

Yang C, Li W, Cao J, Meng F, Yu Y, Huang J, Jiang L, Liu M, Zhang Z, Chen X, Miyamoto K, Yamane H, Zhang J, Chen S, Liu J (2017) Activation of ethylene signaling pathways enhances disease resistance by regulating ROS and phytoalexin production in rice. Plant J 89:338–353

Yeh K-C, Lagarias JC (1998) Eukaryotic phytochromes: Light-regulated serine/threonine protein kinases with histidine kinase ancestry. Proc Natl Acad Sci U S A 95:13976–13981

Yeh K-C, Wu S-H, Murphy J, Lagarias C (1997) A cyanobacterial phytochrome two-component light sensory system. Science 277:1505–1508

Yokoyama A, Yamashino T, Amano Y-I, Tajima Y, Imamura A, Sakakibara H, Mizuno T (2006) Type-B ARR transcription factors, ARR10 and ARR12, are implicated in cytokinin-mediated regulation of protoxylem differentiation in roots of Arabidopsis thaliana. Plant Cell Physiol 48:84–96

Yuan L, Liu Z, Song X, Johnson C, Yu X, Sundaresan V (2016) The CKI1 histidine kinase specifies the female gametic precursor of the endosperm. Dev Cell 37:34–46

Zhu J-K (2001) Plant salt tolerance. Trends Plant Sci 6:66–71

Zubo YO, Blakley IC, Yamburenko MV, Worthen JM, Street IH, Franco-Zorrilla JM, Zhang W, Hill K, Raines T, Solano R, Kieber JJ, Loraine AE, Schaller GE (2017) Cytokinin induces genome-wide binding of the type-B response regulator ARR10 to regulate growth and development in Arabidopsis. Proc Natl Acad Sci U S A 114:E5995–E6004

Ramsong Chantre Nongpiur completed his Ph.D. from JNU, with Prof. Ashwani Pareek. His doctoral work entailed the detailed functional characterization of two members of the two-component system of rice. He is currently working as a Research Fellow in ICGEB where he is trying to develop osmotic stress-tolerant rice through genetic engineering.

Priyanka Gupta completed her Ph.D. from JNU with Prof. Ashwani Pareek. Priyanka's doctoral work involves the characterization of one of the putative cytokinin receptors in rice.

Ashutosh Sharan obtained his Ph.D. from JNU under Prof. Ashwani Pareek. Ashutosh's work involves mapping the interactome of the TCS in rice as well as functional characterization of one of the response regulators.

Deepti Singh is pursuing Ph.D. from JNU under Prof. Ashwani Pareek. She is working towards elucidating the role of one of the rice HKs and HPTs with regard to osmotic stress response.

Sneh Lata Singla-Pareek – see under Chapter 7 contributions.

Ashwani Pareek obtained his Ph.D. from the UDSC. His doctoral work comprised the analysis of heat-shock proteins and salt stress proteins in rice. He carried out his postdoctoral work in the Laboratory of Prof. Ralph S Quatrano, University of North Carolina, USA. He is currently a Professor of Life Sciences at the JNU, New Delhi. His current research comprises forward and reverse genetics as well as systems biology approaches for understanding abiotic stress tolerance in plants, with the ultimate goal of generating abiotic stress tolerant crops. He has published jointly with the Editor including a book by Springer, and they have worked on some of the projects together.

Calcium Signaling: A Communication Network that Regulates Cellular Processes

11

Sibaji Kumar Sanyal, Swati Mahiwal, and Girdhar Kumar Pandey

Abstract

Calcium (Ca^{2+}), which regulates diverse signaling networks, is one of the most important second messengers in plants. A typical signal is generated by the influx of Ca^{2+} into the cytosol through influx channel proteins, which then is decoded by Ca^{2+} binding proteins followed by maintenance of Ca^{2+} homeostasis driven by efflux transporters. The plant Ca^{2+} signaling system seems to have evolved differently than Ca^{2+} signaling pathway in the animal system, yet there is a high level of overlap in functional aspects and processes it modulates. In plants, Ca^{2+} signaling participates actively to transduce signals for physiological (biotic and abiotic stresses and nutrient sensing) and developmental processes. Recently involvement of Ca^{2+} in plant memory mechanisms is also being reported. In this chapter, we will describe the significance of the Ca^{2+} signaling in plants and how it brings specificity in regulating different physiological processes in plants.

Keywords

Calcium channels · Calcium · Memory · Signal transduction · Stress · Symbiosis · Transporters

11.1 Why Calcium Is Selected as a Signaling Molecule?

For maintaining cellular activity and homeostasis, mainly four major ions [sodium (Na^+), potassium (K^+), magnesium (Mg^+), and calcium (Ca^{2+})] are very important, but out of all, only Ca^{2+} fits well into the role of a biological messenger due to rapid and reversible change in its concentration in the cytosol as well as in the Ca^{2+}

S. K. Sanyal · S. Mahiwal · G. K. Pandey (✉)
Department of Plant Molecular Biology, University of Delhi, New Delhi, India
e-mail: gkpandey@south.du.ac.in

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_11

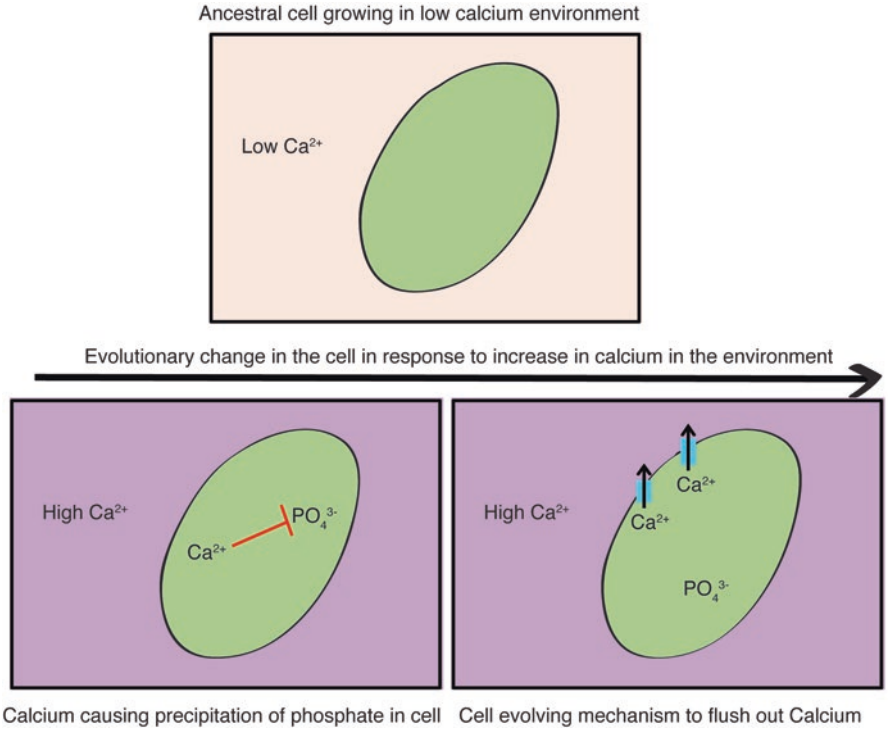

Fig. 11.1 A hypothetical model depicting how Ca^{2+} extrusion system provides the basis for the evolution of Ca^{2+} signaling. The ancestral cell initially grew in a low Ca^{2+} environment. As cell moved from low external Ca^{2+} environment to high external Ca^{2+} environment, it accumulated Ca^{2+} inside. The mechanism that developed for Ca^{2+} extrusion later resulted in the evolution of Ca^{2+} homeostasis and signaling

storehouse (Brini and Carafoli 2000; Kudla et al. 2010). The change in the concentration of Ca^{2+} at the cytosol (keeping it very low at resting state) is important as it protects the cell from the cytotoxic effect of Ca^{2+} because at elevated levels Ca^{2+} precipitates cellular phosphate (Hepler and Wayne 1985; Sanders et al. 1999). Therefore, the cell has devised ways to sequester a large quantity of cytosolic Ca^{2+}, and this effort by the cell was probably the evolutionary basis of the beginning of the Ca^{2+} homeostasis and signaling mechanism (Sanders et al. 1999; Plattner and Verkhratsky 2015). Figure 11.1 describes a hypothetical situation that might have led to the genesis of Ca^{2+} signaling. Another reason for nature to choose Ca^{2+} over its other contemporaries was because it can fit into binding cavities having irregular shape, whereas closely related ions such as Mg^{2+} attract coordinated oxygen of the binding cavity with greater affinity, resulting in requirement of perfectly octahedral cavities, which never exist in proteins (Brini and Carafoli 2000; Clapham 2007). Ca^{2+} interacts variably with coordinating oxygen atoms of the binding site in complex protein, which leads to conformational changes in the active site of the protein (a very important feature for protein activation discussed later in the chapter) and

also affects the charge of a protein, which helps in triggering signal transduction (Brini and Carafoli 2000; Clapham 2007). Moreover, Ca^{2+} sheds water at a rate of approximately 10^9 water molecules per second compared to Mg^{2+} (approximately 10^5 water molecules per second). Hence, Ca^{2+} can control fast reactions compared to Mg^{2+} and hence is more suitable as a signaling molecule (Hepler and Wayne 1985).

11.2 The Paradigm of Ca^{2+} Signaling

As already explained above, the toxic nature of higher Ca^{2+} concentration in the cytosol resulted in the evolution of a cellular machinery, which kept the concentration of Ca^{2+} very low in the cytoplasm (approx. 100 nM). The abundance of Ca^{2+} in a very high amount in cellular organelles and comparatively low abundance in the cytosol tempted researchers to think on the lines that this phenomenon probably was linked to the generation of Ca^{2+}-mediated signals (Kudla et al. 2010). Working to prove this hypothesis, researchers could show that in plants the stress stimuli (abiotic and biotic factors), hormones (Kudla et al. 2010), and other cellular second messengers [nicotinic acid adenine dinucleotide phosphate (NAADP) (Navazio et al. 2000), inositol-1,4,5-triphosphate (IP_3) (Drobak and Ferguson 1985; Schumaker and Sze 1987; Blatt et al. 1990; Gilroy et al. 1990), inositol hexakis phosphate (IP_6) (Lemtiri-Chlieh et al. 2003), sphingosine-1-phosphate (S1P) (Spiegel and Milstien 2003), and cyclic ADP ribose (cADPR) (Allen et al. 1995)] resulted in changes of the cytosolic Ca^{2+} levels (Trewavas and Malho 1998; Trewavas 1999). This gave rise to a new line of thought, which indicated that each specific external or internal perturbation led to a very discrete change in the cellular Ca^{2+} dynamics (McAinsh et al. 1995; Kudla et al. 2010). So Hethrington and colleagues formulated the concept of "Ca^{2+} signatures," which defined that each signal (perturbations that a cell faces) results in the generation of a specific Ca^{2+} signature at a very specific location in the cell (spatial aspect) and for a certain period of time (the temporal aspect) (Kudla et al. 2010). The generation of a "Ca^{2+} signature" is dependent on three major events to fulfill its function, viz., (a) the influx of Ca^{2+} in the cytoplasm from external and internal stores to increase the cellular concentration and genesis of the signature, (b) the binding of this suddenly enhanced Ca^{2+} to Ca^{2+} binding proteins or Ca^{2+} sensors that propagate the signal in the signaling pathway, and (c) finally, efflux of this Ca^{2+} from the cytosol to maintain a pre-signature state, i.e., resting Ca^{2+} concentration (Sanders et al. 2002; Dodd et al. 2010; Kudla et al. 2010). Each of the events involves a large array of proteins that play a very important role in the proper execution of the signal transduction. Figure 11.2 briefly presents an overview of the Ca^{2+} signaling event.

11.2.1 Ca^{2+} Influx Channels: The Sentries that Let Ca^{2+} in the Cell

In a resting plant cell (without any stimuli), Ca^{2+} level in the cytosol is in the submicromolar range (100–200 nM), whereas it is maintained mainly in the millimolar range in the apoplast, endoplasmic reticulum (ER), and vacuoles (the storehouse of

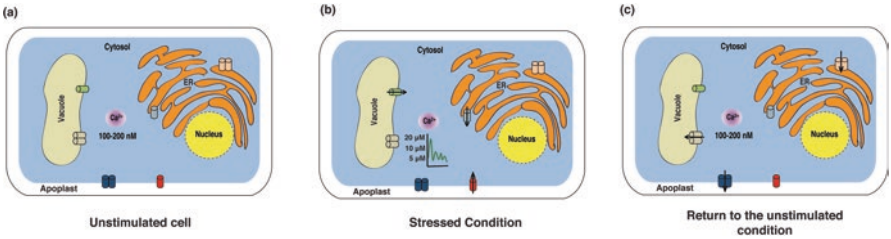

Fig. 11.2 A hypothetical situation depicting the current understanding of Ca^{2+} homeostasis in a cell that gives rise to a Ca^{2+} signature. (**a**) Under normal conditions, the cell maintains a majority of Ca^{2+} in the apoplast and in the cellular stores (vacuole and ER). (**b**) Perturbations due to stress or any other stimuli lead to the release of Ca^{2+} from these stores through Ca^{2+} influx channels into the cytosol resulting in the transient rise of Ca^{2+}, and as a result, a Ca^{2+} signature is formed. (**c**) At the end of the cue, the Ca^{2+} is pushed back into these stores through different sets of channels/transporters to maintain the cytosolic low Ca^{2+} levels

Ca^{2+}) (Stael et al. 2012; Himschoot et al. 2017). Some other organelles can also serve as cellular stores besides the abovementioned (Stael et al. 2012). A plant cell uses different channels to increase the Ca^{2+} concentration in the cytoplasm in response to different stimuli (McAinsh and Pittman 2009). The mode of functioning of channels and place of action are quite different from each other, and this forms the basis of their classification. At the plasma membrane (PM) voltage-gated channels and ligand-gated channels are functional (Kudla et al. 2010). The same type of channels is seen at the vacuolar membrane (VM) although their molecular identity is different from the ones present at the PM (Kudla et al. 2010). The ER majorly has the ligand-gated channels (Kudla et al. 2010).

The voltage-gated channels of the PM can be broadly classified as depolarization-activated Ca^{2+}-permeable channels (DACC), hyperpolarization-activated Ca^{2+}-permeable channels (HACC), and voltage-independent Ca^{2+} channels (VICCs) (they are also known as cation channels) (White and Broadley 2003; Kudla et al. 2010). The hypothesis on the functioning of these channels assumes that DACC perform short transient influx of Ca^{2+} in the cell (Thion et al. 1998) and HACC perform the sustained Ca^{2+} influx (Hamilton et al. 2000). The VICCs uptake Ca^{2+} at physiological voltages or using very weak voltage (White and Broadley 2003). It is thought that the VICCs maintain Ca^{2+} homeostasis in an unstimulated plant cell (White and Broadley 2003). The molecular identity of these channels are yet to be determined (Tang and Luan 2017). Moving away from the PM, the tonoplast has slow vacuolar (SV)-type channels for the transport of Ca^{2+} from the vacuole to the cytosol (Johannes et al. 1992; Allen and Sanders 1994). Elevated Ca^{2+} concentration on each side of the channel controls its activity, i.e., the cytosolic side activates it and the vacuolar side inactivates the channel (Pottosin et al. 2005; Pottosin and Schonknecht 2007). The two-pore channel1 (TPC1) is also localized at the tonoplast and can generate SV current resulting in its classification as an SV channel (Guo et al. 2016b). TPC1 requires both Ca^{2+} and voltage gating for activation (Guo et al. 2016b). There are also reports on the existence of fast vacuolar channel and

Ca^{2+}-insensitive vacuolar channels, but these are not very well characterized (Kudla et al. 2010). Annexins, which are actually membrane trafficking proteins, can form channels of the class of voltage-gated cation channel that can transport Ca^{2+} when they annex membranes (Kudla et al. 2010; Laohavisit et al. 2010; Laohavisit and Davies 2011; Swarbreck et al. 2013). Annexins can also sense the rise in the cytosolic Ca^{2+} using their Ca^{2+} binding motif and then go on to bind and annex membranes using their phospholipid binding motifs to bind to the phospholipids present in the membranes (Konopka-Postupolska and Clark 2017). Although annexins are ubiquitous, they can function at the PM to transport Ca^{2+} (Davies 2014).

The ligand-gated channels form the second major group that aid Ca^{2+} influx in the cell. The cyclic nucleotide-gated channels (CNGC) were majorly found in the PM (DeFalco et al. 2016). There are also reports of their presence at the vacuole, ER, and nuclear envelope (DeFalco et al. 2016). They are activated by the binding of cAMP and cGMP and inactivated by Ca^{2+}/CaM binding (Jha et al. 2016). The glutamate receptors of the PM are activated by amino acids (Glu, Gly, Ala, Asn, Cys, and Ser) (Qi et al. 2006) and help to bring Ca^{2+} into the cytoplasm (Dodd et al. 2010; Kudla et al. 2010). It has already been discussed that IP_3 and IP_6 can initiate Ca^{2+} release by acting on ligand-gated channels present in the ER (Kudla et al. 2010). Contrasting view on this is discussed in a later section in the chapter. The ER membrane also has a ligand-gated channel that is activated by NAADP (Navazio et al. 2000).

There has been a recent entry into the group of plant Ca^{2+} influx channels. The newly identified reduced hyperosmolality-induced Ca^{2+} increase (OSCA) channels are thought to be osmosensors (Yuan et al. 2014). The OSCAs help in sensing osmotic stress and in maintaining overall cell physiology. It is a PM-localized protein with nine transmembrane domains (Yuan et al. 2014). The mechanistic control of Ca^{2+} influx by this channel needs to be further elucidated. Mechanosensitive channel mid-complementing activity (MCA) has also been implicated in Ca^{2+} influx at the PM (Nakagawa et al. 2007; Yamanaka et al. 2010; Rosa et al. 2017). Overview of Ca^{2+} influx channels is summarized in Fig. 11.3.

11.2.2 Ca^{2+} Signature Decoding Proteins in the Plant Cell

The enhanced Ca^{2+} in the cytosol has to be detected by cellular proteins to decode the stimuli that had in the first place caused the transient rise of cytosolic Ca^{2+} (Dodd et al. 2010; Kudla et al. 2010). For this, proteins have some special structural motifs that aid in the fast binding of Ca^{2+} (DeFalco et al. 2010). Nature evolved the helix-loop-helix structural motif (commonly termed as EF-hand) where two α-helices are bridged by a Ca^{2+}-chelation loop (Gifford et al. 2007). The chelation loop has negatively charged amino acids placed at important locations in the loop to fulfill two important requirements for Ca^{2+} binding – (a) providing oxygen through negatively charged amino acids (through either their side chain or their backbone) to ionically bind Ca^{2+} and (b) placing these amino acids at positions in the loop where it can fulfill the pentagonal bipyramidal geometry required by Ca^{2+} (Gifford et al. 2007).

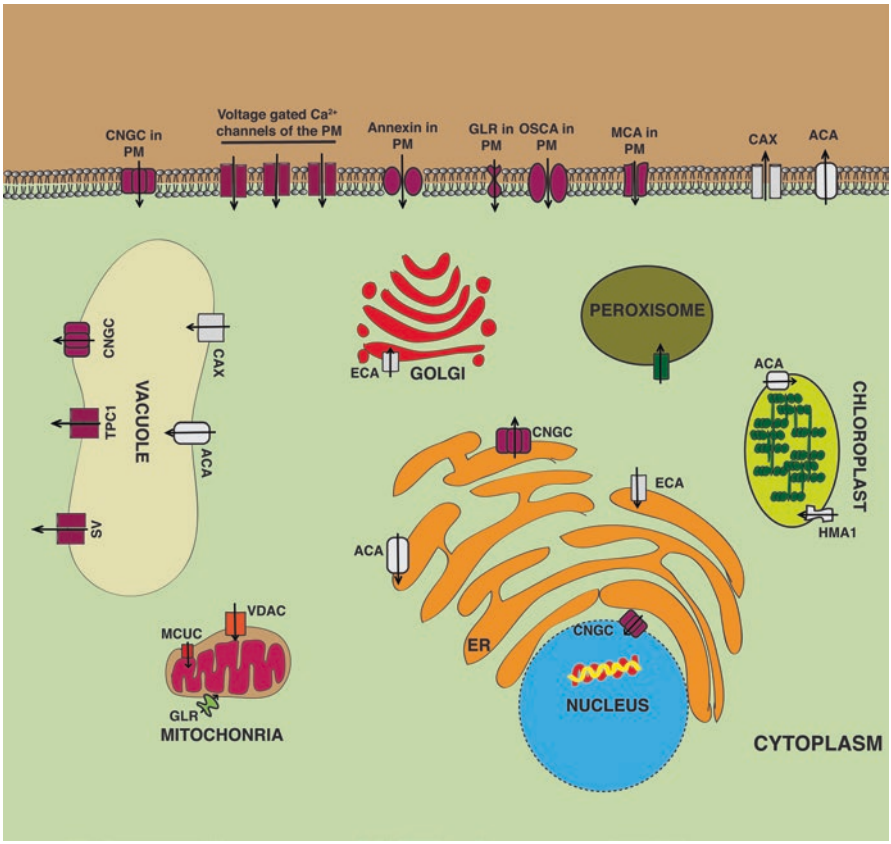

Fig. 11.3 A model depicting the Ca^{2+} transport elements working to achieve homeostasis in a cell. The elements colored in maroon are the proteins responsible for the influx of Ca^{2+} in a cell from the Ca^{2+} stores after they have been activated by either voltage or their respective ligands. The CNGCs, voltage-gated channels, annexins, GLR, OSCAs, and MCA are functional at the PM. At the vacuole, TPC1, SV, and a different CNGC are functional. A CNGC transports Ca^{2+} into the cytosol from ER. The elements colored in silver are those responsible for Ca^{2+} extrusion from a cell. At PM and vacuole, CAX and ACA function to extrude Ca^{2+} out of cytoplasm. At the ER, ACA and ECA function for Ca^{2+} sequestration. Besides the cytoplasm, chloroplast, mitochondria, and nucleus are the other sites where Ca^{2+} signaling occurs. In the chloroplast, ACA and HMA1 help in the uptake of Ca^{2+}. Mitochondria have two distinct mechanisms for Ca^{2+} uptake; VDAC takes up Ca^{2+} to the inner membrane from where Ca^{2+} is pumped into the matrix by MCUC and a GLR. The nucleus has a CNGC, which imports Ca^{2+} from the nuclear envelope to the nucleoplasm. ER is the probable supplier of Ca^{2+} to the nuclear envelope. Not much is known about the peroxisomal Ca^{2+} transport, and the identity of the Ca^{2+} importer is unknown. Golgi, ER, and vacuole probably act only as reservoirs of Ca^{2+}. All details are provided in the text

The positioning of these amino acids in the loop is marked by both their linear position and tertiary geometry due to their alignment on the axes of the pentagonal bipyramid: 1(+X), 3(+Y), 5(+Z), 7(−Y), 9(−X), and 12 (−Z) (Gifford et al. 2007). Usually, Asp and Glu are the most commonly occurring amino acids at these positions, and

this type of organization of the loop is termed as being canonical in nature (Gifford et al. 2007). The noncanonical EF-hand-containing proteins have variations in this typical structure but are still able to bind Ca^{2+} (Gifford et al. 2007). However, the basic ideology that drives the canonical, as well as noncanonical Ca^{2+} sensors, is that Ca^{2+} binding causes a structural change in the protein that makes it ready to transduce a signal. Plants have calmodulin (CaM) (Du et al. 2011), CaM-like proteins (CML) (McCormack and Braam 2003), Ca^{2+}-dependent protein kinases (CDPKs) (Harmon et al. 2000), and calcineurin B-like (CBL) proteins as the major Ca^{2+} binding proteins, which act as Ca^{2+} sensors to propagate signals (Kudla et al. 1999).

CaM is a small protein with two globular domains; each domain has two EF-hands, joined by a central region (Bouche et al. 2005). Ca^{2+} binding changes the conformation of CaM to expose its hydrophobic clefts that can now interact with downstream targets that include kinases, enzymes, transcription factors, and channels/transporters (Zeng et al. 2015). The CMLs are larger than CaMs and have variable EF-hands (McCormack and Braam 2003; DeFalco et al. 2010; Hashimoto and Kudla 2011). They have less than 50% sequence identity at amino acid level with CaMs and no other functional domains besides the EF-hands (Zeng et al. 2015). The variation in the amino acids is also observed in the position of critical amino acids in the loop. Like CaMs, CMLs can also bind to kinases and transcription factors to propagate signals (Zeng et al. 2015). The CBL proteins in addition to the EF-hands bind to a specific target kinase, CBL-interacting protein kinase (CIPK) (Sanyal et al. 2015). Although the CBL also has four EF-hands, most of them are noncanonical (Sanyal et al. 2015). As a result, the Ca^{2+} binding affinity of CBL is lower than the CaM (Sanchez-Barrena et al. 2013). CIPKs have a Ser/Thr kinase domain, a NAF/FISL motif for CBL interaction, and a PPI domain for phosphatase interaction (Sanyal et al. 2016). CIPKs are activated only when a Ca^{2+}-bound CBL interacts with them at their NAF domain (Albrecht et al. 2001). This interaction removes the block from the kinase domain of CIPK. The CBL-CIPK module is formed after interaction, and this module together can control the activity of downstream targets that are primarily transcription factors, channels, and transporters (Albrecht et al. 2001; Sanyal et al. 2016). Together the CaM, CML, and CBL belong to the Ca^{2+} sensor relay group of Ca^{2+} sensors in the plant (Hashimoto and Kudla 2011). The other group of proteins that fulfill the dual role of sensing and relaying the Ca^{2+} signals, hence termed sensor responders, are CDPKs (Harper et al. 2004). These proteins contain two distinct domains, the Ca^{2+} binding CaM-like domain with four EF-hands and the Ser/Thr kinase domain (Harper et al. 2004). In CDPKs, like the CIPKs, the kinase domain is blocked under normal condition, and Ca^{2+} binding unblocks the kinase domain and hence activates the enzyme (Takahashi and Ito 2011). Taken together, the CBL-CIPK module (not the individual proteins) is also grouped in the sensor responder group (Hashimoto and Kudla 2011).

Besides these core groups of proteins, there are certain other protein kinases that can act to relay the Ca^{2+} signals. The common feature of these proteins is that they are all Ser/Thr kinases and are normally inactive under low cytosolic Ca^{2+} concentration (Chae et al. 2010). These are activated either by CaM or by direct Ca^{2+} binding (Sanyal et al. 2015). The CDPK-related protein kinases (CRK) (Lindzen and

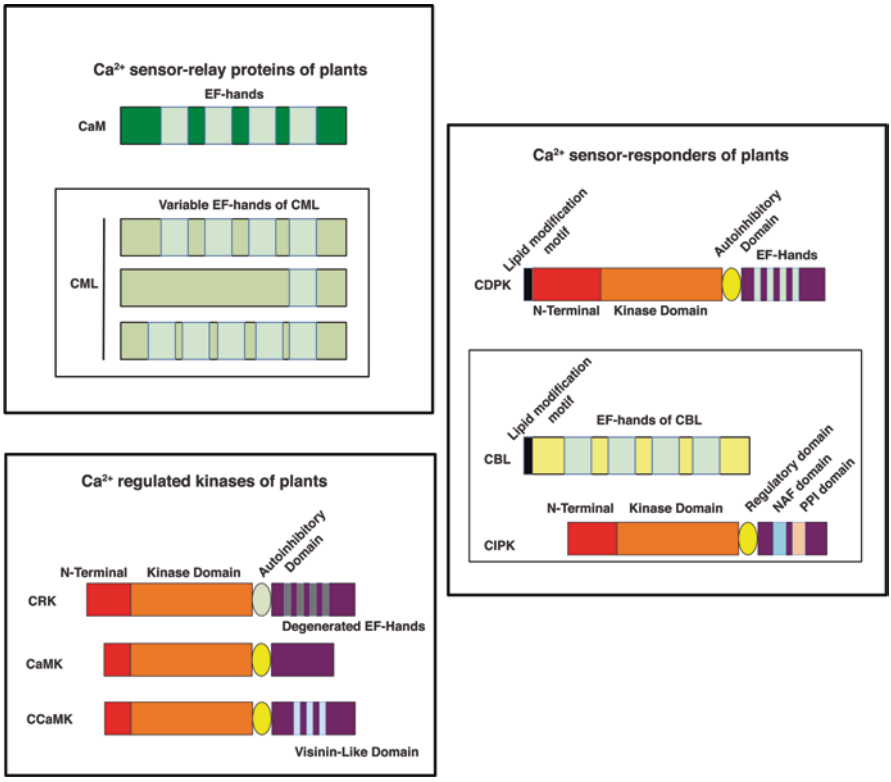

Fig. 11.4 Ca^{2+} decoders present in a plant cell. A plant cell has sensor relay proteins that can sense Ca^{2+} and bind to other proteins to relay the signal. The CaM and CML fall in that group. CDPKs form a different class, sensor responder. CBL-CIPK module is also grouped as sensor responders. Both CDPK and CBLs have lipid modification motifs that help in their cellular localization. The other kinases are Ca^{2+}-regulated kinases, and their mode of function is summarized in the text

Choi 1995) and CaM-activated kinases (CaMK) are activated by CaM binding (Patil et al. 1995). In CRKs, the EF-hands are degenerated, and in CaMK, EF-hands are absent. The Ca^{2+} and CaM-activated kinases (CCaMK) on the other hand have visinin-like domains that can bind Ca^{2+} (Chae et al. 2010). A summary of Ca^{2+} decoding tools is depicted in Fig. 11.4.

11.2.3 Ca^{2+} Efflux Channels: The Ca^{2+} Emigration Centers of Cell

Once the Ca^{2+}-mediated signaling is executed, the cytotoxic increase in Ca^{2+} concentration needs to be reduced by pumping out from the cytosol or sequestered into endomembrane reservoir like ER and vacuoles to prevent any detrimental effect. Plant cell probably maintains two different mechanisms to achieve this – (a) Ca^{2+}/

proton antiporter/cation exchangers immediately lower the Ca^{2+} level inside the cell as soon as the signaling event is over and then (b) the P-type Ca^{2+} ATPases take over to maintain the low resting concentration of Ca^{2+} (Kudla et al. 2010).

The Ca^{2+}/cation antiporter (CaCA) family members can transport Ca^{2+} out of the cytosol using a counter exchange of ions (Emery et al. 2012). They are classified into five groups: (i) YRBG (found in bacteria and archaea), (ii) NCKX (K^+-dependent Na^+/Ca^{2+} exchanger) (found in eukaryotes excluding land plants), (iii) NCX (Na^+/Ca^{2+} exchangers), (iv) CCX (cation/Ca^{2+} exchanger), and (v) CAX (Ca^{2+}/H^+ exchanger) (Emery et al. 2012). Plants encode MHX (Mg^{2+}/H^+), which is homologous to group NCX (Emery et al. 2012). The CAX family is the only one that functionally shows Ca^{2+} exchange activity in plants (Emery et al. 2012). There are six CAX genes in the *Arabidopsis* genome, and majority of them are reported to be localized on the tonoplast indicating that they might extrude the cytosolic Ca^{2+} to be stored in the vacuole (Maser et al. 2001; Shigaki et al. 2006; Pittman and Hirschi 2016). However some antiporter activity was also reported at the plasma membrane, and so it can be inferred that they might also function to flush out Ca^{2+} from the cell (Luo et al. 2005). The CAX proteins normally remain in an auto-inhibited state due to the binding of the N-terminal regulatory domain to an adjacent region within the N-terminus of the same protein (Pittman and Hirschi 2001; Pittman et al. 2002; Mei et al. 2007). The CAX proteins get rid of this auto-inhibition either by forming heteromers (Zhao et al. 2009; Hocking et al. 2017) or by interacting with other proteins (like a kinase that can phosphorylate them) to modulate their activity (Cheng and Hirschi 2003; Cheng et al. 2004a, b). Besides CAX, there is another group reported to exist in plants, which has similarity with CAX and contains additional EF-hands, but not much is known about their Ca^{2+} transportability (Emery et al. 2012).

The P-type ATPases, named so as they form phosphorylated intermediates, are energized by hydrolysis of ATP and extrude cations (Pedersen et al. 2012). They are divided into five subfamilies (P1–P5) depending on ions they transport (Pedersen et al. 2012). P1 (A and B) transports heavy metals, P2 (A and B) transports Ca^{2+} and P2 (C and D) transports Na^+ or K^+, P3A transports H^+, P4 transports phospholipids, and P5 has no assigned specificity (Axelsen and Palmgren 1998; Pedersen et al. 2012). A P1B-type heavy metal ATPase 1 (HMA1) can transport Ca^{2+} along with other heavy metals and is located in the chloroplast (Seigneurin-Berny et al. 2006; Moreno et al. 2008). The P2A-type ATPases are also known as ER-type Ca^{2+}-ATPases (ECA) and characterized by having phosphorylation sites at the cytosolic region (Geisler et al. 2000). The ECAs are present at the ER, Golgi, and endosomes (Kudla et al. 2010). The P2B-type ATPases are activated by binding of CaM to an auto-inhibitory domain present in them and hence are known as auto-inhibited Ca^{2+}-ATPases (ACA) (Geisler et al. 2000). They also have a phosphorylation site at their cytosolic region like the ECA (Geisler et al. 2000). The ACAs are present in ER, vacuole, PM, and chloroplast (Kudla et al. 2010). Overview of Ca^{2+} efflux channels is summarized in Fig. 11.3.

11.3 Role of Ca^{2+} in Chloroplast, Mitochondria, Peroxisome, and Nucleus

Another concept has emerged recently that Ca^{2+} signaling can occur independently in the organelles (Stael et al. 2012; Kudla et al. 2018). The chloroplast can have Ca^{2+} in the range of 15 mM or higher (Stael et al. 2012). The channels or transporters responsible for chloroplastic Ca^{2+} entry remain elusive except for HMA1 mentioned in the previous section and ACA1 (Huang et al. 1993; Johnson et al. 2006). However it is known that light plays a critical role in chloroplast Ca^{2+} uptake, and like the sequestration system of the cytosol, excess Ca^{2+} is pumped into the thylakoid membrane or stromal proteins or a yet-unidentified chloroplastic Ca^{2+} store (Stael et al. 2012). Light to dark transition also leads to Ca^{2+} accumulation in the stroma (Johnson et al. 1995; Sai and Johnson 2002; Sello et al. 2016). Ca^{2+} can affect the photosynthesis, as it is an important structural component of photosystem II (PSII) (Stael et al. 2012; Kudla et al. 2018). Ca^{2+} is required by the chloroplast ATP-synthase to regulate photosynthetic proton flow and ATP production (Zakharov et al. 1993; Stael et al. 2012). The PSII metal cluster Mn_4CaO_5 is responsible for the efficient oxidation of H_2O (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011; Kudla et al. 2018). Besides, Ca^{2+} is important for electron flow during photosynthesis and photoprotection (Hochmal et al. 2015). Another important component that governs the generation of ATP is the proton motive force (PMF). PMF, in the chloroplast, is modulated by TPK3, an EF-hand containing transmembrane two-pore K^+ channel, dependent on Ca^{2+} for its activity (Carraretto et al. 2013; Hochmal et al. 2015). The next important protein in the chloroplast is the versatile chloroplast Ca^{2+} sensor (CAS) protein. The CAS protein thus far has been implicated in initiating stomatal closure and CO_2 availability (Nomura et al. 2008; Hochmal et al. 2015; Wang et al. 2016), photoacclimation (Petroutsos et al. 2011), and photosynthetic efficiency (Wang et al. 2014). Besides these, Ca^{2+} and CAS have a direct role in signaling as certain environmental perturbations are known to generate Ca^{2+} signatures inside the chloroplast stroma (Loro et al. 2016). CAS helps in the generation of Ca^{2+} signature in the stroma in response to pathogen-associated molecular patterns (PAMPs) (Nomura et al. 2012). CAS uses the $^1O_2$-mediated retrograde signaling to suppress chloroplast gene expression and transcriptional reprogramming for immune response (Nomura et al. 2012). As a concurrent approach, the CAS can also activate MAPK pathway for plant defense (Guo et al. 2016a). The newest member of the chloroplast Ca^{2+} sensor is calredoxin (CRX), a protein with four EF-hands and a thioredoxin domain (Hochmal et al. 2016). It needs Ca^{2+} for its thioredoxin activity, and binding to chloroplast 2-cys peroxiredoxin is responsible for stress acclimation (Hochmal et al. 2016).

The plant mitochondria have a free Ca^{2+} concentration of about 200 nM (Stael et al. 2012). In animals, it has been established that mitochondria can influence the Ca^{2+} signature by closely interacting with the ER (Clapham 2007). Also, mitochondria can sequester Ca^{2+} and generate special mitochondrial signals to regulate ATP production in animals (Jouaville et al. 1999). Ca^{2+} regulation is also seen in plant mitochondria in response to stimuli (Logan and Knight 2003). Ca^{2+} is accumulated

in the mitochondria of plants prior to the induction of programmed cell death (Arpagaus et al. 2002; Tiwari et al. 2002; Virolainen et al. 2002). The knowledge on the mitochondrial Ca^{2+} transporters of plants is still at a very nascent stage. Ca^{2+} is believed to be taken into the mitochondrial inner membrane space by voltage-gated anion channels (VDACs) (Wagner et al. 2016). From there, it is taken inside the matrix by mitochondrial Ca^{2+} uniporter complexes (MCUC) (Stael et al. 2012; Wagner et al. 2015; Teardo et al. 2017) and GLR3.5 (Wagner et al. 2016). The Ca^{2+} extrusion mechanism of plant mitochondria is still only speculative; however, animals have a very well-worked out system (Wagner et al. 2016).

Although the exact plant peroxisomal concentration is not known, in animals, it is predicted to be either 150 nM or 2 μM (Stael et al. 2012). Again information on the uptake and extrusion machinery is not available, but it is known that independent Ca^{2+} fluxes occur in the peroxisome, and it enhances the detoxification of ROS by using *Arabidopsis* catalase 3 (CAT3) (Costa et al. 2010). It is believed that CaM modulates the activity of CAT3 (Yang and Poovaiah 2002).

The nucleus serves as the center where regulation of gene expression takes place in response to Ca^{2+} signals. The nucleus can autonomously maintain Ca^{2+} signals, which are not dependent on cytosolic Ca^{2+} fluxes occurring at the cytosol (Xiong et al. 2004; Walter et al. 2007; Xiong et al. 2008). The free nucleolar Ca^{2+} concentration is about 100 nM, and probably, the flux in the Ca^{2+} is generated due to the release of Ca^{2+} from the nuclear envelope (Stael et al. 2012). Two important nuclear envelope K^+ channels are CASTOR and POLLUX that help in the Ca^{2+} release into the nucleus from the nuclear envelope (Charpentier et al. 2008). POLLUX-mediated control of CNGC15 is responsible for Ca^{2+} influx into the cytoplasm (Charpentier et al. 2016). Ca^{2+} influences the gene expression inside the nucleus by modulating the CaM binding transcription activator (CAMTA) and CDPKs (Bouche et al. 2002; Boudsocq et al. 2010). All information on Ca^{2+} uptake machinery of organelles is summarized in Fig. 11.3.

11.4 Differences Between Plant and Animal Ca^{2+} Signaling

The basic paradigm of a Ca^{2+} signaling event is the same in animals and plants. During the initiation of a signaling event in animals, Ca^{2+} enters into the cytosol from an external source and internal sources (ER and sarcoplasmic reticulum (SR)) (Berridge et al. 2000). Ca^{2+} itself and second messengers generated due to several stimuli induce Ca^{2+} release from these stores also known as Ca^{2+}-induced Ca^{2+} release (CICR) (Berridge et al. 2000). The buffers present in the cytosol bind to the excess Ca^{2+} and leave only a small amount of Ca^{2+} molecules to bind to Ca^{2+}-sensing proteins to elicit the signal response (Berridge et al. 2000). At the termination of the signal, Ca^{2+} dislodges from the buffers and the proteins and is removed from the cytosol by exchangers (e.g., Na^+/Ca^{2+} exchangers (NCX)) and ATPases (e.g., plasma membrane Ca^{2+}-ATPases (PMCA)) (Berridge et al. 2000). The ER and SR sequester Ca^{2+} by using the sarcoplasmic reticulum Ca^{2+}-ATPases (SERCA) pumps (Berridge et al. 2000). Mitochondria rapidly sequester Ca^{2+} using a uniporter but later release

it when the Ca^{2+} concentration in the cytosol is lowered. This Ca^{2+} is then either pumped out of the cytosol or sequestered into ER (Berridge et al. 2000).

From an evolutionary perspective, it is reported that the proteins involved in Ca^{2+} signaling were expanded more by genome duplication and recombination in eukaryotes (Marchadier et al. 2016) The Ca^{2+} signaling elements have evolved differentially in animals and plants (Edel et al. 2017). The animals have predominantly lost efflux proteins and plants influx proteins during evolution (Marchadier et al. 2016; Edel et al. 2017). The rise of Ca^{2+} in the plant cytosol after the challenge of stimuli is comparatively slower than the mammalian cell (Edel et al. 2017). Also, animals have a more diverse array of Ca^{2+} binding proteins than plants (Marchadier et al. 2016; Edel et al. 2017).

The triggering machinery that leads to a Ca^{2+} influx in the animal cell is very well-worked out. The IP_3 second messenger is generated when a stimulus activates phospholipase C, and it leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate. The IP_3 then can act on several Ca^{2+} receptors leading to the opening of Ca^{2+}-specific channels and increase of the cytosolic Ca^{2+} (Berridge et al. 2000). In the plant system, these receptors are still unknown (Berridge et al. 2000; Kudla et al. 2010; Singh et al. 2015; Singh and Pandey 2016). But surprisingly there are pieces of evidence that suggest that IP_3 can induce Ca^{2+} release in plants (mentioned at the beginning of this chapter). It is speculated that plants have evolved a mechanism to use IP_3 in a way which is different from animals. But the fact remains that till date no IP_3 receptor has been identified in plants (Kudla et al. 2010). Only algae species of *Volvox* and *Chlamydomonas* display the presence of IP_3 receptor channels, which seem to be absent in higher plants (Wheeler and Brownlee 2008). Similarly, the other prominent animal second messenger cADPR (Berridge et al. 2000) is also absent in plants as the gene ADP ribosyl cyclase is not present in plants (Kudla et al. 2010). Also, targets of cADPR in animals, the ryanodine receptors (RYR), are also absent in plants (Kudla et al. 2010).

The higher plants do not possess canonical voltage-gated Ca^{2+} channels like the animals (Verret et al. 2010). These are present mostly in the lower plant forms (Verret et al. 2010). Also, ATP-gated purinergic channels (P2XRs) and Cys loop superfamily of channels are present in lower plants (Verret et al. 2010). The TPC and GLR Ca^{2+} channels of plants and animals are comparable in number (Verret et al. 2010). In contrast, the plants have a higher number of CNGCs and mechanosensitive channels than animals (Verret et al. 2010). The plant CNGCs have a different structure than the animal CNGCs probably to facilitate a cross talk between CaM and cyclic nucleotide signaling (Jha et al. 2016). In animals during an immune response, the stromal interaction molecules (STIMs) and pore-forming Orai proteins (that form the Ca^{2+} release-activated Ca^{2+} (CRAC) channels) help in Ca^{2+} release in the cytosol. The STIMs are EF-hand-containing ER-localized Ca^{2+} sensor and Orai form Ca^{2+} channel at the PM. When the STIMs sense drop in the Ca^{2+} concentration in the ER, they activate the Orai, and the STIM-activated Orai lets the Ca^{2+} into the cytosol (Derler et al. 2016). In plants, it appears as if only one of the partners (STIM or Orai) is present at a time (Edel et al. 2017). So probably plants

have evolved Ca^{2+} transport machinery without the requirement of this additional STIM-Orai Ca^{2+} channel.

Perhaps, the most distinguishing feature of animal and plant Ca^{2+} signaling is the evolution of the effector proteins in both systems. The animal system has a plethora of enzymes that are regulated by Ca^{2+} (Berridge et al. 2003). Among them, there are kinases and phosphatases that can bind to CaM or other Ca^{2+}-generated second messengers (Berridge et al. 2003). One of the Ca^{2+}-regulated enzymes, calcineurin, which can directly bind to Ca^{2+} and enzymatically, is a phosphatase (Berridge et al. 2003). Calcineurin is composed of two subunits, calcineurin A (CnA) (the phosphatase) and its activator calcineurin B (CnB). The CnB subunit has four EF-hands and can directly bind to Ca^{2+}. Along with Ca^{2+}-CaM, CnB binds and activates CnA to make it a functional calcineurin phosphatase that transduces Ca^{2+}-mediated signal (Luan 2009). The search for a similar phosphatase in plants resulted in the identification of CBLs, which had the EF-hands (Kudla et al. 1999). The partner of this CBL led to the discovery of CIPK (Shi et al. 1999). However, in contrast to the calcineurin phosphatase, the plant CBL interacts with CIPK, which is a kinase. Till date, no calcineurin-like molecule has been identified in plants, but the presence of a structurally similar signaling system (CBL-CIPK) indicates that plants have taken a different route to transduce the Ca^{2+} signal. Presence of two different subgroups of kinases (CDPK and CBL-CIPK) that can directly bind to Ca^{2+}, provide plants with a selective advantage so that they can surpass the two-step activation process required by animals. This probably allows plants to develop a more rapid and robust signaling architecture. Other important additions to the plant genome are the CMLs and CDPK, which are absent in animals (Chae et al. 2010; Virdi et al. 2015). However, there are reports of CDPKs being found in protozoa, and this phenomenon could be explained by horizontal gene transfer (Wernimont et al. 2011; Edel et al. 2017). The CaMs/CML, CDPKs, and CBL-CIPKs are the most dominant EF-hand-containing proteins in plants in terms of their number in the plant cell (Edel et al. 2017). So, what plants lost in terms of diversity (Ca^{2+} binding protein) was made up by increasing the number of members in the respective gene family (Edel et al. 2017). These proteins, in turn, adopted newer and diverse functions (Edel et al. 2017). Moreover, plants also lack the typical cyclic nucleotide signaling system (Edel et al. 2017), but again, this might be compensated by using the typical Ca^{2+} signaling pathway to cross talk with other signaling pathways (abscisic acid (ABA) and reactive oxygen species (ROS)) [discussed later in the chapter].

Another feature of the animal cell is the generation of an action potential (AP), which is dependent on a Na^+/Ca^{2+} voltage-dependent cation channel (Edel et al. 2017). In higher plants, the absence of voltage-gated anion channels led to the evolution of an alternative method for the generation of AP (Edel et al. 2017). The fast AP in Venus flytrap employs Cl^- in place of Na^+ for the generation of an AP (Bemm et al. 2016). The quick-activating anion channel (QUAC1), which might be activated by the Ca^{2+} influx, may have a role in long-range AP propagation (Hedrich et al. 2016; Edel et al. 2017). These in the case of plants are probably propagated through the phloem to make up for the absence of nerves present in plants (Edel et al. 2017).

11.5 Physiological Role of Ca^{2+} Signaling in Plants

11.5.1 Abiotic Stress, ABA Signaling, and the Role of Ca^{2+}

The majority of abiotic stress (cold, salt, osmotic stress, and drought) signals are propagated by two very important mediators at a cellular level – Ca^{2+} and ABA. Abiotic stresses lead to an immediate increase in plant ABA levels (Raghavendra et al. 2010). It is believed that stress perception leads to ABA synthesis in vascular tissues and it is transported to neighboring tissues where it is taken up by the cell using specialized ATP-dependent transporters (Raghavendra et al. 2010). However, in the guard cells (stomata), the local production of ABA is sufficient to elicit a response (Bauer et al. 2013). Similar to Ca^{2+} as a signaling molecule, ABA also regulates signaling processes where (a) ABA generated by stress signals is sensed by RCAR/PYR1/PYLs and (b) these ABA-bound receptors bind to PP2C phosphatase and remove it from SnRK2, which results in (c) autophosphorylation and activation of SnRK2 that lead to phosphorylation and activation of transcription factors and/or channels to produce a response. Some of the downstream targets of ABA signaling reported till date are transporters and channels (AKT1, AKT2, NPF6.3, SLAC1/SLAH3), superoxide generators (RBOHD and RBOHF), and transcription factors (ABI5 and ABF1/4) (Edel and Kudla 2016). These abovementioned proteins are also targets of Ca^{2+}-mediated kinases (CDPKs and CBL-CIPK module) (Edel and Kudla 2016). The SLAC1 channel, a very important player in the guard cell regulation, is controlled by SnRK2 (ABA regulated kinase) and CDPK and CBL-CIPK. It is also hypothesized that probably the regulation of SLAC1, for stomatal closure, requires both CDPKs and SnRKs (Edel and Kudla 2016). The PP2Cs (ABI1 and PP2CA) dephosphorylate these kinases (SnRKs, CDPKs, and CIPKs) to counteract their control of SLAC1. As such the PP2Cs serve to prevent stomatal closure by keeping SLAC1 dephosphorylated (Edel and Kudla 2016). These facts suggest that the Ca^{2+} signaling components are very intricately woven together with ABA signaling components, and so, there is an integration of these two signaling system at the cellular level (Edel and Kudla 2016). However, there is another question on the linearity of the signaling architecture – whether (i) ABA signaling extends the message to Ca^{2+} signaling pathway or (ii) there could be some other mechanism. In guard cell, the first scenario is operational (Munemasa et al. 2015). In other cells (other than the guard cell), the situation is not very clear, and in root cells, new reports suggest that Ca^{2+} signaling prevents ABA signaling (Edel and Kudla 2016). Besides, the Ca^{2+} signaling pathway can modulate ABA signaling by using the C2-domain ABA-related (CAR) proteins (with functional Ca^{2+} binding C2 domain) to mediate the Ca^{2+}-dependent recruitment of ABA receptors to PM (Rodriguez et al. 2014; Diaz et al. 2016; Edel and Kudla 2016).

Plant can respond to stress stimuli by reprogramming itself for mid- and long-term adaptations (Kudla et al. 2018). The fast adaptation of plants can be seen in the rapid closure of guard cells (stomata) to stop transpiration and resultant water loss. The immediate closure is probably elicited by using the existing cellular pool of proteins and ions, and for long-term control (i.e., inhibition of stomatal reopening),

Ca^{2+}-mediated processes are used (reviewed in Kudla et al. 2010). ABA itself can also trigger rise in cytosolic Ca^{2+} levels, and it results in the activation of an SV channel slow anion channel-1 (SLAC1) and rapid transient anion channels. The resulting anion release from these two channels causes depolarization of guard cell and causes an outflow of K^+ ion from the guard cell due to activation of outward rectifying K^+ channels. The loss of anions and K^+ causes the closure of stomata. As already stated, the SLAC1 channel is very important in the entire process and is therefore targeted by both ABA and Ca^{2+}-regulated kinases. To maintain kinase specificity, SLAC1 probably is phosphorylated at different residues. Once the signal is over (to return to the normal stage), the SLAC1 is dephosphorylated by PP2Cs to make it inactive (Edel and Kudla 2016). A SLAC1 homolog 3 (SLAH3) also needs to be phosphorylated for stomatal closure (Maierhofer et al. 2014; Edel and Kudla 2016). These channels (SLAC1 and SLAH3) can also be controlled via phosphorylation by CDPKs, CIPK, and SNF1-related kinases 2.6 (SnRK2.6) (Edel and Kudla 2016). For controlling gene expression, ABA responsive element (ABRE) binding transcription factors (TFs) turn out to be a very important target of SnRKs, CDPKs, and CIPKs (Edel and Kudla 2016).

To maintain proper functioning, cellular machinery must return to their original state once a stress condition is over. Plants maintain a feedback loop system to stop ABA-related gene expression. The ABI4 and Yin Yang1 (YY1) transcription factors are expressed in response to ABA signal (Li et al. 2016). The ABI4 can also modulate the expression of other ABA responsive genes. Probably to counteract the response to ABA and negatively regulate ABA signaling, YY1 regulates abscisic acid repressor 1 (ABR1) expression by binding to YY1 sites in the ABR1 promoter (Li et al. 2016). The CBL9-CIPK3 module also negatively regulates ABA signaling by phosphorylating ABR1 (Sanyal et al. 2017) and results in the activation of the ABR1, which represses ABI4, YY1, and other ABA responsive genes, thus allowing the plant to return to normal growth and development. The CIPK15-ERF7 pathway is another negative regulator of plant ABA signaling (Song et al. 2005). This pathway targets GCC box-containing genes and the repressor complex of Sin3 and HDA19 to suppress gene transcription (Song et al. 2005). To counteract negative response, plants also have positive regulators of ABA signaling. The redundant pathway of CIPK11 and CIPK26 converges into ABI5 and regulates ABI5-mediated gene expression (Lyzenga et al. 2013; Zhou et al. 2015b).

11.5.2 Ca^{2+} Influences Ion Sensing and Signaling in Plants

For optimal growth and development, plants require mineral nutrients such as ions, and a fine-tuned homeostasis of these ions governs the cellular physiology. Ca^{2+} can mediate this by using its various protein decoders to control the plethora of channels/transporters and maintain a proper cellular balance. The CBL-CIPK23 module has lately become one of the master regulators involved in plant nutrient sensing and uptake. The first report of CBL1/CBL9-CIPK23 indicated that it could mediate a phosphorylation-dependent K^+ uptake by modulating the activity of AKT1 channel

(Li et al. 2006; Xu et al. 2006). Later, HAK5 channel was also reported to be controlled by CIPK23 (Ragel et al. 2015). Similarly, CIPK23 modulates the cellular uptake of iron (Fe^{2+}) by modulating a yet-unknown iron transporter (Tian et al. 2016). Moving away from the acquisition, Ca^{2+}-mediated signals can cause CBL-CIPK23 to inhibit ion uptake. CBL1/CBL9-CIPK23 can modulate the dual-affinity nitrate transporter (CHL1/NRT1.1/NPF6.3) by phosphorylation. This modulation prevents the entry of excess nitrate into the cytoplasm when external nitrate concentration is high (Liu and Tsay 2003). Similarly, CBL1-CIPK23 can phosphorylate ammonium transporter (AMT1;2) to block the accumulation of NH_4^+ (Straub et al. 2017).

There is another unique situation where SnRKs and the CBL-CIPK module in combination prevent the excess Mg^{2+} accumulation in the cell. Mg^{2+} is an important cellular ion as it takes part in important enzymatic reactions, but in excess, it can be cytotoxic to the plants (Gao et al. 2015). So, plants have adopted convergence of SnRK2 (SnRK2 subclass III) and Ca^{2+} signaling mediated by CBLs (CBL2/CBL3)-CIPKs (CIPK3/CIPK9/CIPK23/CIPK26)) to instigate a tonoplast sequestration of excess cytosolic Mg^{2+} (Mogami et al. 2015; Tang et al. 2015).

11.5.3 Ca^{2+} Signaling, ROS, and Biotic Stress

The pathogens (biotic stress) cause equal havoc on plants as abiotic stress that ultimately results in cell death and affecting plant growth and development. Therefore, to protect itself from biotic stress, plant cells deploy several reactive species (reactive nitrogen and reactive oxygen) and concomitant activation of several signaling pathways (Boudsocq and Sheen 2013; Frederickson Matika and Loake 2014). A pathogen can be sensed by plants by recognition of either microbe-associated molecular patterns (MAMPs)/PAMPs or effector proteins. The former is recognized by cell surface pattern-recognition receptors (PRRs) and the latter by nucleotide binding leucine-rich repeat (NB-LRR) (Boudsocq and Sheen 2013). The recognition event triggers the internal system in plants, whereupon Ca^{2+} is accumulated in the cytosol to initiate Ca^{2+} signaling and ROS is produced that cross talks with the MAPK pathway and the hormone signaling pathway (ABA, salicylic acid (SA), and jasmonic acid (JA)/ethylene (ET)), which finally results in the response by modulating gene expression and callose deposition (Kissoudis et al. 2014). The callose deposition, which defends the cell against pathogen invasion, is controlled by ABA (Kissoudis et al. 2014). Pathogen attack initiates SA and JA/ET signaling that provides plants further resistance against pathogens (Kissoudis et al. 2014). The ROS generated can produce a hypersensitive response (HR)-mediated cell death in cases where symptoms of stress are not produced (Kissoudis et al. 2014).

The recognition of MAMPs causes alteration of nuclear Ca^{2+} concentration (Dodd et al. 2010). This leads to the activation of protein kinases that are not directly Ca^{2+} signal decoders (e.g., MAPKs, wound-activated kinases, etc.) (Lecourieux et al. 2005; Ma and Berkowitz 2007). Among the Ca^{2+} signal decoders, the role of CDPKs till date has been significantly worked out (Boudsocq et al. 2010; Boudsocq and Sheen 2013). CDPKs phosphorylate the transcription factors that influence the

early gene regulation (Boudsocq and Sheen 2013). CDPKs can also phosphorylate phenylalanine ammonia-lyase (PALs) and ACC synthase to control the SA accumulation and ET production, respectively (Boudsocq and Sheen 2013). But the most important nodes are the respiratory burst homologs (RBOHs), which are converging point of both CDPKs and CBL-CIPKs (Boudsocq and Sheen 2013; Drerup et al. 2013). The RBOHs are important as they produce ROS following pathogen recognition by plants (Torres and Dangl 2005). Modulation of this target by both CDPK and CBL-CIPKs makes it an important component for plant signal transduction following pathogen interaction. The CaMs/CMLs are also activated by elevated Ca^{2+} and leads to reactive nitrogen production, which further aid in plant defense (Frederickson Matika and Loake 2014).

11.5.4 Ca^{2+} in Pollen Tube Development

The plant sexual reproduction is heavily dependent on Ca^{2+} for the germination, elongation, and guidance of pollen tube by employing Ca^{2+}-mediated processes (Steinhorst and Kudla 2013). Ca^{2+} impacts the growth direction of the pollen and directs it toward a zone having a higher Ca^{2+} concentration (Malho and Trewavas 1996). The apical tip of the pollen has a very high cytosolic Ca^{2+} concentration (2–10 μM) (may also be called the clear zone) and is followed by the shank of the tube with a Ca^{2+} concentration of 20–200 nM (Steinhorst and Kudla 2013). The clear zone is devoid of any large organelles (vacuoles, nucleus, amyloplast) as Ca^{2+} interacts and disables the actin filaments so that these organelles are blocked from moving into the clear zone (Cai and Cresti 2009; Steinhorst and Kudla 2013). Several studies have proved the importance of this very steep Ca^{2+} gradient helps in the elongation of the pollen tube (Obermeyer and Weisenseel 1991; Rathore et al. 1991; Miller et al. 1992; Pierson et al. 1994). The speed at which pollen tubes can grow has been reported at the range of 1000 μm/h (in lily) to 14,400 μm/h (in Tradescantia or Hemerocallis) (Michard et al. 2009; Qin and Yang 2011). There is evidence that there is a synchronous oscillation of cytosolic Ca^{2+} and the growth rate of pollen tube (Steinhorst and Kudla 2013). Another factor that is associated with this is the cytoskeletal proteins (Steinhorst and Kudla 2013). It is believed that the enhanced Ca^{2+} causes depolymerization of F-actin, and hence, the growth ceases (Cárdenas et al. 2008). But the stretch-activated Ca^{2+} channels (SAC) that lead Ca^{2+} into the pollen cytoplasm are closed, and the cytosolic Ca^{2+} levels fall, which causes the reorganization of the cytoskeleton and the tube growth resumed again (Dutta and Robinson 2004; Cárdenas et al. 2008). Ca^{2+} also promotes fusion of vesicle carrying new cell wall materials at the expanding region of the tip (Battey et al. 1999). The proper movement of the vesicle is maintained by ROP1 GTPase and F-actin (Steinhorst and Kudla 2013). Ca^{2+} then causes F-actin disassembly so that the vesicle can fuse to the PM (Steinhorst and Kudla 2013). The vesicle fusion releases the cell wall material (mainly methyl-pectin) (Bosch and Hepler 2005). Once the methyl pectin is de-methoxylated, pectin cross links with Ca^{2+} to make the cell wall rigid enough not to burst during expansion (Hepler and Winship 2010). The clear zone

imports high Ca^{2+} concentration from the apoplast with the help of SAC channels, CNGCs, and GLRs (Dutta and Robinson 2004; Frietsch et al. 2007; Nakagawa et al. 2007; Michard et al. 2011). Compared to the apoplast, the information on the contribution of the internal stores for higher Ca^{2+} concentration in a clear zone in pollen cytoplasm is still elusive (Steinhorst and Kudla 2013). These Ca^{2+} influx channels could be targets of Ca^{2+} decoding machinery such as CaMs, CMLs, CDPKs, and CBL-CIPKs since these are expressed in the pollen tube (Rato et al. 2004; Pina et al. 2005; Yoon et al. 2006; Myers et al. 2009; Mahs et al. 2013; Steinhorst and Kudla 2013; Zhou et al. 2015a). One of the important Ca^{2+} decoders, CBL2/CBL3-CIPK12, is targeted into the vacuole (Steinhorst et al. 2015). It is speculated that this module may control the vacuolar dynamics as a means of pollen tube growth as most of the other processes (pollen development, cytoskeleton organization, and Ca^{2+} oscillations) are not affected on perturbing the CBL2/CBL3-CIPK12 complex (Steinhorst et al. 2015).

11.5.5 Ca^{2+} Signaling During Plant Symbiosis with Microorganisms

Plants need to interact with microorganisms as they can be a valuable source of nitrogen or other micronutrients. For this plants form a symbiosis with nitrogen-fixing bacteria or with arbuscular-mycorrhizal (AM) fungi (Dodd et al. 2010). Plants secrete flavonoids, which signal the bacteria to produce nod factors (NF) resulting in symbiosis signaling and Ca^{2+} oscillations (Oldroyd and Downie 2008; Oldroyd 2013; Kudla et al. 2018). The NOD FACTOR RECEPTORs (NFR5 and NFR1) and symbiosis receptor-like kinase (SYMRK) form a receptor complex to recognize the nod factors and initiate the Ca^{2+} signal in the cytosol, and this signal is taken inside the nucleus by a yet-unknown mechanism (Oldroyd and Downie 2008; Oldroyd 2013). There might also be another second messenger like mevalonate associated with this message transfer to the nucleus (Oldroyd 2013). The POLLUX and CASTOR K^+ channels of the nuclear envelope probably serve to uptake K^+ into the nuclear membrane to compensate for the release of Ca^{2+} into the nucleus, or they may activate a voltage-gated Ca^{2+} channel by changing the membrane potential (Singh and Parniske 2012). In the presence of NF, POLLUX (also known as does not make infection 1 (DMI1) in *Medicago tranculata*) interacts with CNGC15 to allow Ca^{2+} passage to the nucleus from the nuclear envelope (Charpentier et al. 2016). Ca^{2+} ATPase (MCA8) drives Ca^{2+} back into the nuclear envelope to maintain a balance in the nucleus (Charpentier et al. 2008; Capoen et al. 2011; Singh and Parniske 2012; Charpentier et al. 2016). This elevated Ca^{2+} is perceived by CaM, and it activates CCaMK (Oldroyd and Downie 2008). The CaM-CCaMK phosphorylates CYCLOPS transcription factor (Singh et al. 2014). This entire complex then mediates the transcription of nodule inception (*NIN*), required for arbuscular mycorrhization 1 (*RAM1*), and ERF, for nodulation 1 (*ERN*), genes by using the nodulation signaling pathway (NSP1 and NSP2) transcription factors in the nodulation process (Oldroyd 2013; Kudla et al. 2018). NIN has a role in nodule

organogenesis and bacterial infection thread formation (Singh et al. 2014), RAM1 acts in arbuscular development (Pimprikar et al. 2016), and ERN is required for bacterial infection (Cerri et al. 2017).

To initiate AM, plants secrete strigolactones that are perceived by the fungi, and reception of strigolactone promotes spore germination and hyphal branching of the fungi (Kretzschmar et al. 2012; Oldroyd 2013). Subsequently, production of hypothetical mycorrhizal factors (myc factors) and chitooligosaccharides that initiate Ca^{2+} spiking in the cell takes place (Gutjahr and Parniske 2013). Following this event, the fungal hyphae enter in the plant cell by formation of a pre-penetration apparatus, and this involves high frequency of Ca^{2+} oscillation (Sieberer et al. 2012; Gutjahr and Parniske 2013). The hyphae help the AM fungus to colonize the plant through their hyphal growth that ultimately results in the arbuscule formation, and during the entire process, Ca^{2+} oscillations are observed in the plant cell (Gutjahr and Parniske 2013; Oldroyd 2013). The SYMRK receptors are also hypothesized to be involved in mycorrhizal signaling, and the downstream mechanism that translates the signal into nuclear Ca^{2+} oscillations is largely unknown (Oldroyd 2013). However, the same CaM-CCaMK-CYCLOPS complex is believed to be involved in AM signaling (Oldroyd 2013). The transcription factors involved in this pathway are thought to be NSP2 and RAM1, and they drive the expression of RAM2 (Oldroyd 2013). RAM2 encodes glycerol-3-phosphate acyl transferase, which helps in the colonization of AM fungi by producing cutin monomers. This cutin is believed to allow the fungus to recognize plant surface and colonize it (Oldroyd 2013).

11.5.6 Role of Ca^{2+} in Plant Memory

While this topic is also discussed in a later chapter, here we will briefly emphasize the role of Ca^{2+} in plant memory (Fig. 11.5). The neurons in animals use the sophisticated Ca^{2+} signaling system to regulate brain rhythms, information processing, learning, and memory (Berridge 2014). The neuronal Ca^{2+} signaling is important for memory acquisition during both conscious and sleeping state (Berridge 2014). In fact, Ca^{2+} signaling is very important for the memory and learning functions of the mammalian brain (Berridge 2011). Ca^{2+} helps in both long-term potentiation (for memory formation) and long-term depolarization (for memory erasure) by modulating its concentration in the brain (Berridge 2011). Throughout the lifespan, a plant encounters both beneficial and detrimental stimuli. But does it have the capability to learn from these using the Ca^{2+} signaling system like animals? Can it memorize the experience and later recall it and act accordingly if faced with the same stimuli as seen in the case of animals?

The idea that plants are intelligent beings capable of complex behavior, memory, and learning had been proposed by plant biologists (Thellier et al. 1982; Trewavas 1999, 2003, 2005, 2009, 2012, 2016, 2017). In fact, Anthony Trewavas has opined that plant competition follows the "laws of game theory," a mathematical model used to understand economic trends and animal behavior (Trewavas 2016). It is thought that the cellular networks (like Ca^{2+} signaling pathways) operating in plant

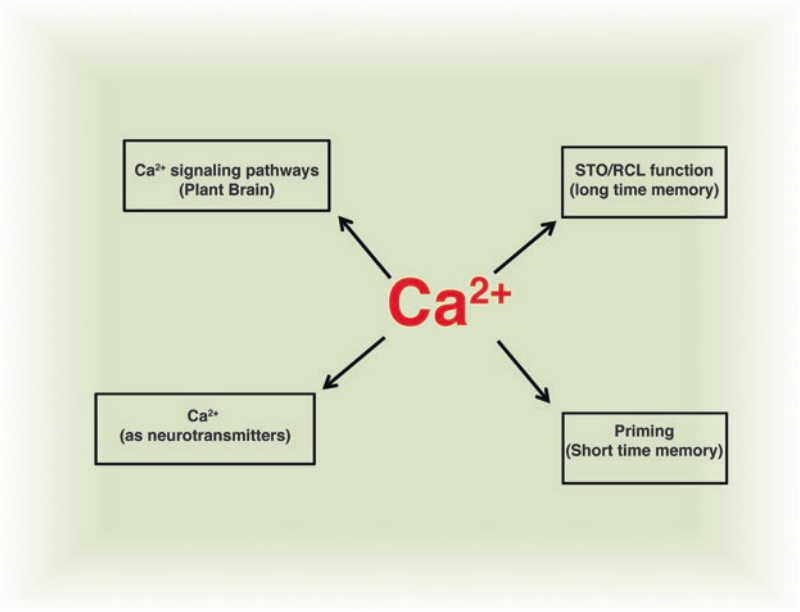

Fig. 11.5 Ca^{2+} is a central molecule in plant memory. The Ca^{2+} signaling pathways (and other signaling pathways) function as the plant memory network. The Ca^{2+} molecule also serves as the neurotransmitter in the plants. Ca^{2+} is hypothesized to play a significant role in both long-term and short-term memory formation in plants

cells act as the plant brain (van Loon 2016). The internal communication in plants is maintained primarily through low-molecular-weight compounds and Ca^{2+} (van Loon 2016). This system is probably analogous to the animal neurotransmitter system (van Loon 2016). The presence of AP in plants has already been discussed in an earlier section (Differences between plant and animal Ca^{2+} signaling). So all these facts indicate the presence of a brain-like processing system in plants, which makes a case for calling it intelligent. The next point that immediately implies itself is that an intelligent being with a well-developed neural system would be capable of learning and memorizing. There are many examples where plants have learnt from earlier exposure, stored the information, and modified their behavior accordingly (Thellier and Luttge 2013). A very elegant study demonstrated that plants can learn from previous exposure to a stimulus and change the Ca^{2+} signature in cytosol when the same stress is encountered again (Knight et al. 1998). The plant memory function has been divided into two forms: (a) plant can store (STO) the information and recall (RCL) it for later use [this is also known as STO/RCL] and (b) perception of stimuli changes the way the plant transduces subsequent stimuli (habituation/priming) (Trewavas 2003; Thellier and Luttge 2013). What probably differentiates these two forms of memory is that STO/RCL memory information can be stored for days, weeks, or months, but the priming stores memory only for minutes (Thellier and Luttge 2013). In case of priming, the Ca^{2+} signature generated from the first stimuli

orients the plants toward a response. If the same/new stimuli are perceived, through prior "priming" of Ca^{2+} signature, a plant can either (i) decrease the intensity of response if stimuli are innocuous or (ii) increase the intensity of response to save itself (Thellier and Luttge 2013). In case of STO/RCL, stimuli-generated Ca^{2+} signature results in the storage of information regarding the physical changes in the plant that were the final result of stimuli (Verdus et al. 2007). Where and how and in what form Ca^{2+} stores this memory is still not known, but it is hypothesized that generation of Ca^{2+} signature turns the STO function of plants "on" (Thellier and Luttge 2013). Similarly, another hypothesis forwarded by the same authors is that activation/deactivation of Ca^{2+}-dependent processes (they call it Ca^{2+} condensation/decondensation) is responsible for the functioning of the RCL function (Thellier and Luttge 2013). A study on STO/RCL memory's dependence on Ca^{2+} was reported by Verdus and colleagues (Verdus et al. 2007). Epidermal meristem production was delayed in flax seedlings subjected to stress stimuli due to the treatment of Ca^{2+} blockers (EGTA/lanthanum/ruthenium red) (Thellier and Luttge 2013). These blockers probably blocked the STO/RCL chain that was dependent on Ca^{2+}.

11.6 Conclusion and Future Perspective

Is Ca^{2+} signaling a plant defense response or growth response? Summarizing the two sets of physiological phenomenon presented in the chapter, we would say that Ca^{2+} signaling is a very important pathway for stimulus-response coupling. The knowledge on this particular pathway has been enriched because of the dedicated work done by the community; however, what we know as of now is just the tip of the iceberg. The information about the Ca^{2+} efflux machinery is very less, and one must think that only two members (CAX and ATPases) should not be enough to get rid of the excess Ca^{2+} instantaneously. Similarly, the substrates of the Ca^{2+} decoding component also present a challenge as to fully decipher a pathway. One has to find out all the components of this Ca^{2+}-triggered network. It is quite amazing how plants have used a comparatively less (than animals) diverse set of decoders to establish a very robust network. These proteins, in some cases, converge on a particular cellular protein. Why does plant have to run several redundant pathways to transfer information to a specific point? Is it the way plants keep the information chain functional in case of shutdown of certain pathways? Also, how complex is the nature of cross talk between Ca^{2+} binding proteins and other signaling pathways? How efficient are they in exchanging information among themselves? The role of Ca^{2+} and Ca^{2+} binding proteins in controlling a diverse array of biological processes, many of which are still unknown, must be looked into with greater detail. Lastly, the organelle Ca^{2+} signaling is probably the new path that must be investigated as it promises more information on the ever-increasing knowledge base for the Ca^{2+} family. Besides, it is time when we consider plants as intelligent beings that have the capacity to learn and choose. As in animals, where Ca^{2+} has already been investigated for its role in brain functions, the role of Ca^{2+} in similar functions in plants must also be undertaken to appreciate how these "intelligent" species are evolving and decipher the role of Ca^{2+} in these processes.

Acknowledgments We are thankful to the University of Delhi, University Grants Commission (UGC-SAP; DRSIII), Department of Biotechnology (DBT), and Department of Science and Technology (DST-PURSE grant) for research funding in our lab.

References

Albrecht V, Ritz O, Linder S, Harter K, Kudla J (2001) The NAF domain defines a novel protein-protein interaction module conserved in Ca^{2+} regulated kinases. EMBO J 20:1051–1063

Allen GJ, Sanders D (1994) Two voltage-gated calcium release channels coreside in the vacuolar membrane of broad bean guard cells. Plant Cell 6:685–694

Allen GJ, Muir SR, Sanders D (1995) Release of Ca^{2+} from individual plant vacuoles by both InsP3 and cyclic ADP-ribose. Science 268:735–737

Arpagaus S, Rawyler A, Braendle R (2002) Occurrence and characteristics of the mitochondrial permeability transition in plants. J Biol Chem 277:1780–1787

Axelsen KB, Palmgren MG (1998) Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol 46:84–101

Battey N, James N, Greenland A, Brownlee C (1999) Exocytosis and endocytosis. Plant Cell 11:643–660

Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KA, Sonnewald S, Sonnewald U, Kneitz S, Lachmann N, Mendel RR, Bittner F, Hetherington AM, Hedrich R (2013) The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Curr Biol 23:53–57

Bemm F, Becker D, Larisch C, Kreuzer I, Escalante-Perez M, Schulze WX, Ankenbrand M, Van de Weyer AL, Krol E, Al-Rasheid KA, Mithöfer A, Weber AP, Schultz J, Hedrich R (2016) Venus flytrap carnivorous lifestyle builds on herbivore defense strategies. Genome Res 26:812–825

Berridge MJ (2011) Calcium signalling and Alzheimer's disease. Neurochem Res 36:1149–1156

Berridge MJ (2014) Calcium regulation of neural rhythms memory and Alzheimer's disease. J Physiol 592:281–293

Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1:11–21

Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics homeostasis and remodelling. Nat Rev Mol Cell Biol 4:517–529

Blatt MR, Thiel G, Trentham DR (1990) Reversible inactivation of K^+ channels of Vicia stomatal guard cells following the photolysis of caged inositol 145-trisphosphate. Nature 346:766–769

Bosch M, Hepler PK (2005) Pectin methylesterases and pectin dynamics in pollen tubes. Plant Cell 17:3219–3226

Bouche N, Scharlat A, Snedden W, Bouchez D, Fromm H (2002) A novel family of calmodulin-binding transcription activators in multicellular organisms. J Biol Chem 277:21851–21861

Bouche N, Yellin A, Snedden WA, Fromm H (2005) Plant-specific calmodulin-binding proteins. Annu Rev Plant Biol 56:435–466

Boudsocq M, Sheen J (2013) CDPKs in immune and stress signaling. Trends Plant Sci 18:30–40

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464:418–422

Brini M, Carafoli E (2000) Calcium signalling: a historical account recent developments and future perspectives. Cell Mol Life Sci 57:354–370

Cai G, Cresti M (2009) Organelle motility in the pollen tube: a tale of 20 years. J Exp Bot 60:495–508

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, Hirsch S, Miwa H, Downie JA, Morris RJ, Ane JM, Oldroyd GE (2011) Nuclear membranes control symbiotic calcium signaling of legumes. Proc Natl Acad Sci U S A 108:14348–14353

Cárdenas L, Lovy-Wheeler A, Kunkel JG, Hepler PK (2008) Pollen tube growth oscillations and intracellular calcium levels are reversibly modulated by actin polymerization. Plant Physiol 146:1611–1621

Carraretto L, Formentin E, Teardo E, Checchetto V, Tomizioli M, Morosinotto T, Giacometti GM, Finazzi G, Szabo I (2013) A thylakoid-located two-pore K^+ channel controls photosynthetic light utilization in plants. Science 342:114–118

Cerri MR, Wang Q, Stolz P, Folgmann J, Frances L, Katzer K, Li X, Heckmann AB, Wang TL, Downie JA, Klingl A, de Carvalho-Niebel F, Xie F, Parniske M (2017) The ERN1 transcription factor gene is a target of the CCaMK/CYCLOPS complex and controls rhizobial infection in Lotus japonicus. New Phytol 215:323–337

Chae L, Pandey GK, Luan S, Cheong YH, Kim KN (2010) Protein kinases and phosphatases for stress signal transduction in plants. In: Pareek A, Sopory SK, Bohnert HJ (eds) Abiotic stress adaptation in plants. Springer, Dordrecht, pp 123–163

Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E, Parniske M (2008) Lotus japonicus castor and pollux are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. Plant Cell 20:3467–3479

Charpentier M, Sun J, Vaz Martins T, Radhakrishnan GV, Findlay K, Soumpourou E, Thouin J, Very AA, Sanders D, Morris RJ, Oldroyd GE (2016) Nuclear-localized cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. Science 352:1102–1105

Cheng NH, Hirschi KD (2003) Cloning and characterization of CXIP1 a novel PICOT domain-containing Arabidopsis protein that associates with CAX1. J Biol Chem 278:6503–6509

Cheng NH, Pittman JK, Zhu JK, Hirschi KD (2004a) The protein kinase SOS2 activates the Arabidopsis H^+/Ca^{2+} antiporter CAX1 to integrate calcium transport and salt tolerance. J Biol Chem 279:2922–2926

Cheng NH, Liu JZ, Nelson RS, Hirschi KD (2004b) Characterization of CXIP4 a novel Arabidopsis protein that activates the H^+/Ca^{2+} antiporter CAX1. FEBS Lett 559:99–106

Clapham DE (2007) Calcium signaling. Cell 131:1047–1058

Costa A, Drago I, Behera S, Zottini M, Pizzo P, Schroeder JI, Pozzan T, Lo Schiavo F (2010) H_2O_2 in plant peroxisomes: an in vivo analysis uncovers a Ca^{2+}-dependent scavenging system. Plant J 62:760–772

Davies JM (2014) Annexin-mediated calcium signalling in plants. Plants (Basel) 3:128–140

DeFalco TA, Bender KW, Snedden WA (2010) Breaking the code: Ca^{2+} sensors in plant signalling. Biochem J 425:27–40

DeFalco TA, Moeder W, Yoshioka K (2016) Opening the gates: insights into cyclic nucleotide-gated channel-mediated signaling. Trends Plant Sci 21:903–906

Derler I, Jardin I, Romanin C (2016) Molecular mechanisms of STIM/Orai communication. Am J Physiol Cell Physiol 310:C643–C662

Diaz M, Sanchez-Barrena MJ, Gonzalez-Rubio JM, Rodriguez L, Fernandez D, Antoni R, Yunta C, Belda-Palazon B, Gonzalez-Guzman M, Peirats-Llobet M, Menendez M, Boskovic J, Marquez JA, Rodriguez PL, Albert A (2016) Calcium-dependent oligomerization of CAR proteins at cell membrane modulates ABA signaling. Proc Natl Acad Sci U S A 113:E396–E405

Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant Biol 61:593–620

Drerup MM, Schlucking K, Hashimoto K, Manishankar P, Steinhorst L, Kuchitsu K, Kudla J (2013) The calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. Mol Plant 6:559–569

Drobak BK, Ferguson IB (1985) Release of Ca^{2+} from plant hypocotyl microsomes by inositol-145-trisphosphate. Biochem Biophys Res Commun 130:1241–1246

Du L, Yang T, Puthanveettil SV, Poovaiah BW (2011) Decoding of calcium signal through calmodulin: calmodulin-binding proteins in plants. In: Luan S (ed) Coding and decoding of calcium signals in plants. Springer, Berlin, pp 177–233

Dutta R, Robinson KR (2004) Identification and characterization of stretch-activated ion channels in pollen protoplasts. Plant Physiol 135:1398–1406

Edel KH, Kudla J (2016) Integration of calcium and ABA signaling. Curr Opin Plant Biol 33:83–91
Edel KH, Marchadier E, Brownlee C, Kudla J, Hetherington AM (2017) The evolution of calcium-based signalling in plants. Curr Biol 27:R667–r679
Emery L, Whelan S, Hirschi KD, Pittman JK (2012) Protein phylogenetic analysis of Ca^{2+}/cation antiporters and insights into their evolution in plants. Front Plant Sci 3:1
Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303:1831–1838
Frederickson Matika DE, Loake GJ (2014) Redox regulation in plant immune function. Antioxid Redox Signal 21:1373–1388
Frietsch S, Wang YF, Sladek C, Poulsen LR, Romanowsky SM, Schroeder JI, Harper JF (2007) A cyclic nucleotide-gated channel is essential for polarized tip growth of pollen. Proc Natl Acad Sci U S A 104:14531–14536
Gao C, Zhao Q, Jiang L (2015) Vacuoles protect plants from high magnesium stress. Proc Natl Acad Sci U S A 112:2931–2932
Geisler M, Axelsen KB, Harper JF, Palmgren MG (2000) Molecular aspects of higher plant P-type Ca^{2+}-ATPases. Biochim Biophys Acta 1465:52–78
Gifford JL, Walsh MP, Vogel HJ (2007) Structures and metal-ion-binding properties of the Ca^{2+}-binding helix-loop-helix EF-hand motifs. Biochem J 405:199–221
Gilroy S, Read ND, Trewavas AJ (1990) Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature 346:769–771
Guo H, Feng P, Chi W, Sun X, Xu X, Li Y, Ren D, Lu C, David Rochaix J, Leister D, Zhang L (2016a) Plastid-nucleus communication involves calcium-modulated MAPK signalling. Nat Commun 7:12173
Guo J, Zeng W, Chen Q, Lee C, Chen L, Yang Y, Cang C, Ren D, Jiang Y (2016b) Structure of the voltage-gated two-pore channel TPC1 from Arabidopsis thaliana. Nature 531:196–201
Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W (2009) Cyanobacterial photosystem II at 29-A resolution and the role of quinones lipids channels and chloride. Nat Struct Mol Biol 16:334–342
Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617
Hamilton DW, Hills A, Kohler B, Blatt MR (2000) Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc Natl Acad Sci U S A 97:4967–4972
Harmon AC, Gribskov M, Harper JF (2000) CDPKs – a kinase for every Ca^{2+} signal? Trends Plant Sci 5:154–159
Harper JF, Breton G, Harmon A (2004) Decoding Ca^{2+} signals through plant protein kinases. Annu Rev Plant Biol 55:263–288
Hashimoto K, Kudla J (2011) Calcium decoding mechanisms in plants. Biochimie 93:2054–2059
Hedrich R, Salvador-Recatala V, Dreyer I (2016) Electrical wiring and long-distance plant communication. Trends Plant Sci 21:376–387
Hepler PK, Wayne RO (1985) Calcium and plant development. Annu Rev Plant Physiol 36:397–439
Hepler PK, Winship LJ (2010) Calcium at the cell wall-cytoplast interface. J Integr Plant Biol 52:147–160
Himschoot E, Pleskot R, Van Damme D, Vanneste S (2017) The ins and outs of Ca^{2+} in plant endomembrane trafficking. Curr Opin Plant Biol 40:131–137
Hochmal AK, Schulze S, Trompelt K, Hippler M (2015) Calcium-dependent regulation of photosynthesis. Biochim Biophys Acta 1847:993–1003
Hochmal AK, Zinzius K, Charoenwattanasatien R, Gabelein P, Mutoh R, Tanaka H, Schulze S, Liu G, Scholz M, Nordhues A, Offenborn JN, Petroutsos D, Finazzi G, Fufezan C, Huang K, Kurisu G, Hippler M (2016) Calredoxin represents a novel type of calcium-dependent sensor-responder connected to redox regulation in the chloroplast. Nat Commun 7:11847
Hocking B, Conn SJ, Manohar M, Xu B, Athman A, Stancombe MA, Webb AR, Hirschi KD, Gilliham M (2017) Heterodimerization of Arabidopsis calcium/proton exchangers contributes to regulation of guard cell dynamics and plant defense responses. J Exp Bot 68:4171–4183

Huang L, Berkelman T, Franklin AE, Hoffman NE (1993) Characterization of a gene encoding a Ca^{2+}-ATPase-like protein in the plastid envelope. Proc Natl Acad Sci U S A 90:10066–10070

Jha SK, Sharma M, Pandey GK (2016) Role of cyclic nucleotide gated channels in stress management in plants. Curr Genomics 17:315–329

Johannes E, Brosnan JM, Sanders D (1992) Parallel pathways for intracellular Ca^{2+} release from the vacuole of higher plants. Plant J 2:97–102

Johnson CH, Knight MR, Kondo T, Masson P, Sedbrook J, Haley A, Trewavas A (1995) Circadian oscillations of cytosolic and chloroplastic free calcium in plants. Science 269:1863–1865

Johnson CH, Shingles R, Ettinger WF (2006) Regulation and role of calcium fluxes in the chloroplast. In: Wise RR, Hoober JK (eds) The structure and function of plastids. Springer, Berlin, pp 403–416

Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R (1999) Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. Proc Natl Acad Sci U S A 96:13807–13812

Kissoudis C, van de Wiel C, Visser RG, van der Linden G (2014) Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. Front Plant Sci 5:207

Knight H, Brandt S, Knight MR (1998) A history of stress alters drought calcium signalling pathways in Arabidopsis. Plant J 16:681–687

Konopka-Postupolska D, Clark G (2017) Annexins as overlooked regulators of membrane trafficking in plant. Cells Int J Mol Sci 18:pii: E863

Kretzschmar T, Kohlen W, Sasse J, Borghi L, Schlegel M, Bachelier JB, Reinhardt D, Bours R, Bouwmeester HJ, Martinoia E (2012) A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. Nature 483:341–344

Kudla J, Xu Q, Harter K, Gruissem W, Luan S (1999) Genes for calcineurin B-like proteins in Arabidopsis are differentially regulated by stress signals. Proc Natl Acad Sci U S A 96:4718–4723

Kudla J, Batistic O, Hashimoto K (2010) Calcium signals: the lead currency of plant information processing. Plant Cell 22:541–563

Kudla J, Becker D, Grill E, Hedrich R, Hippler M, Kummer U, Parniske M, Romeis T, Schumacher K (2018) Advances and current challenges in calcium signaling. New Phytol 218:414–431

Laohavisit A, Davies JM (2011) Annexins. New Phytol 189:40–53

Laohavisit A, Brown AT, Cicuta P, Davies JM (2010) Annexins: components of the calcium and reactive oxygen signaling network. Plant Physiol 152:1824–1829

Lecourieux D, Lamotte O, Bourque S, Wendehenne D, Mazars C, Ranjeva R, Pugin A (2005) Proteinaceous and oligosaccharidic elicitors induce different calcium signatures in the nucleus of tobacco cells. Cell Calcium 38:527–538

Lemtiri-Chlieh F, MacRobbie EA, Webb AA, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A 100:10091–10095

Li L, Kim BG, Cheong YH, Pandey GK, Luan S (2006) A Ca^{2+} signaling pathway regulates a K^+ channel for low-K response in Arabidopsis. Proc Natl Acad Sci U S A 103:12625–12630

Li T, Wu XY, Li H, Song JH, Liu JY (2016) A dual-function transcription factor AtYY1 is a novel negative regulator of the Arabidopsis ABA response. Netw Mol Plant 9:650–661

Lindzen E, Choi JH (1995) A carrot cDNA encoding an atypical protein kinase homologous to plant calcium-dependent protein kinases. Plant Mol Biol 28:785–797

Liu KH, Tsay YF (2003) Switching between the two action modes of the dual-affinity nitrate transporter. EMBO J 22:1005–1013

Logan DC, Knight MR (2003) Mitochondrial and cytosolic calcium dynamics are differentially regulated in plants. Plant Physiol 133:21–24

Loro G, Wagner S, Doccula FG, Behera S, Weinl S, Kudla J, Schwarzlander M, Costa A, Zottini M (2016) Chloroplast-specific in vivo Ca^{2+} imaging using yellow Cameleon fluorescent protein sensors reveals organelle-autonomous Ca^{2+} signatures in the stroma. Plant Physiol 171:2317–2330

Luan S (2009) The CBL-CIPK network in plant calcium signaling. Trends Plant Sci 14:37–42
Luo GZ, Wang HW, Huang J, Tian AG, Wang YJ, Zhang JS, Chen SY (2005) A putative plasma membrane cation/proton antiporter from soybean confers salt tolerance in Arabidopsis. Plant Mol Biol 59:809–820
Lyzenga WJ, Liu H, Schofield A, Muise-Hennessey A, Stone SL (2013) Arabidopsis CIPK26 interacts with KEG components of the ABA signalling network and is degraded by the ubiquitin-proteasome system. J Exp Bot 64:2779–2791
Ma W, Berkowitz GA (2007) The grateful dead: calcium and cell death in plant innate immunity. Cell Microbiol 9:2571–2585
Mahs A, Steinhorst L, Han JP, Shen LK, Wang Y, Kudla J (2013) The calcineurin B-like Ca^{2+} sensors CBL1 and CBL9 function in pollen germination and pollen tube growth in Arabidopsis. Mol Plant 6:1149–1162
Maierhofer T, Diekmann M, Offenborn JN, Lind C, Bauer H, Hashimoto K, Al-Rasheid KAS, Luan S, Kudla J, Geiger D, Hedrich R (2014) Site- and kinase-specific phosphorylation-mediated activation of SLAC1 a guard cell anion channel stimulated by abscisic acid. Sci Signal 7:ra86
Malho R, Trewavas AJ (1996) Localized apical increases of cytosolic free calcium control pollen tube orientation. Plant Cell 8:1935–1949
Marchadier E, Oates ME, Fang H, Donoghue PC, Hetherington AM, Gough J (2016) Evolution of the calcium-based intracellular signaling system. Genome Biol Evol 8:2118–2132
Maser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H, Talke IN, Amtmann A, Maathuis FJ, Sanders D, Harper JF, Tchieu J, Gribskov M, Persans MW, Salt DE, Kim SA, Guerinot ML (2001) Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol 126:1646–1667
McAinsh MR, Pittman JK (2009) Shaping the calcium signature. New Phytol 181:275–294
McAinsh MR, Webb A, Taylor JE, Hetherington AM (1995) Stimulus-induced oscillations in guard cell cytosolic free calcium. Plant Cell 7:1207–1219
McCormack E, Braam J (2003) Calmodulins and related potential calcium sensors of Arabidopsis. New Phytol 159:585–598
Mei H, Zhao J, Pittman JK, Lachmansingh J, Park S, Hirschi KD (2007) In planta regulation of the Arabidopsis Ca^{2+}/H^{+} antiporter CAX1. J Exp Bot 58:3419–3427
Michard E, Alves F, Feijo JA (2009) The role of ion fluxes in polarized cell growth and morphogenesis: the pollen tube as an experimental paradigm. Int J Dev Biol 53:1609–1622
Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliham M, Liu LH, Obermeyer G, Feijo JA (2011) Glutamate receptor-like genes form Ca^{2+} channels in pollen tubes and are regulated by pistil D-serine. Science 332:434–437
Miller DD, Callaham DA, Gross DJ, Hepler PK (1992) Free Ca^{2+} gradient in growing pollen tubes of Lillium. J Cell Sci 101:7–12
Mogami J, Fujita Y, Yoshida T, Tsukiori Y, Nakagami H, Nomura Y, Fujiwara T, Nishida S, Yanagisawa S, Ishida T, Takahashi F, Morimoto K, Kidokoro S, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2015) Two distinct families of protein kinases are required for plant growth under high external Mg^{2+} concentrations in Arabidopsis. Plant Physiol 167:1039–1057
Moreno I, Norambuena L, Maturana D, Toro M, Vergara C, Orellana A, Zurita-Silva A, Ordenes VR (2008) AtHMA1 is a thapsigargin-sensitive Ca^{2+}/heavy metal pump. J Biol Chem 283:9633–9641
Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI (2015) Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr Opin Plant Biol 28:154–162
Myers C, Romanowsky SM, Barron YD, Garg S, Azuse CL, Curran A, Davis RM, Hatton J, Harmon AC, Harper JF (2009) Calcium-dependent protein kinases regulate polarized tip growth in pollen tubes. Plant J 59:528–539
Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, Kishigami A, Sokabe M, Kojima I, Sato S, Kato T, Tabata S, Iida K, Terashima A, Nakano M, Ikeda M, Yamanaka T, Iida H (2007) Arabidopsis plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. Proc Natl Acad Sci U S A 104:3639–3644

Navazio L, Bewell MA, Siddiqua A, Dickinson GD, Galione A, Sanders D (2000) Calcium release from the endoplasmic reticulum of higher plants elicited by the NADP metabolite nicotinic acid adenine dinucleotide phosphate. Proc Natl Acad Sci U S A 97:8693–8698

Nomura H, Komori T, Kobori M, Nakahira Y, Shiina T (2008) Evidence for chloroplast control of external Ca^{2+}-induced cytosolic Ca^{2+} transients and stomatal closure. Plant J 53:988–998

Nomura H, Komori T, Uemura S, Kanda Y, Shimotani K, Nakai K, Furuichi T, Takebayashi K, Sugimoto T, Sano S, Suwastika IN, Fukusaki E, Yoshioka H, Nakahira Y, Shiina T (2012) Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nat Commun 3:926

Obermeyer G, Weisenseel MH (1991) Calcium channel blocker and calmodulin antagonists affect the gradient of free calcium ions in lily pollen tubes. Eur J Cell Biol 56:319–327

Oldroyd GE (2013) Speak friend and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Oldroyd GE, Downie JA (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546

Patil S, Takezawa D, Poovaiah AW (1995) Chimeric plant calcium/calmodulin-dependent protein kinase gene with a neural visinin-like calcium-binding domain. Proc Natl Acad Sci U S A 92:4897–4901

Pedersen CN, Axelsen KB, Harper JF, Palmgren MG (2012) Evolution of plant p-type ATPases. Front Plant Sci 3:31

Petroutsos D, Busch A, Janssen I, Trompelt K, Bergner SV, Weinl S, Holtkamp M, Karst U, Kudla J, Hippler M (2011) The chloroplast calcium sensor CAS is required for photoacclimation in Chlamydomonas reinhardtii. Plant Cell 23:2950–2963

Pierson ES, Miller DD, Callaham DA, Shipley AM, Rivers BA, Cresti M, Hepler PK (1994) Pollen tube growth is coupled to the extracellular calcium ion flux and the intracellular calcium gradient: effect of BAPTA-type buffers and hypertonic media. Plant Cell 6:1815–1828

Pimprikar P, Carbonnel S, Paries M, Katzer K, Klingl V, Bohmer MJ, Karl L, Floss DS, Harrison MJ, Parniske M, Gutjahr C (2016) A CCaMK-CYCLOPS-DELLA complex activates transcription of RAM1 to regulate Arbuscule branching. Curr Biol 26:987–998

Pina C, Pinto F, Feijo JA, Becker JD (2005) Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth division control and gene expression regulation. Plant Physiol 138:744–756

Pittman JK, Hirschi KD (2001) Regulation of CAX1 an Arabidopsis Ca^{2+}/H^{+} antiporter identification of an N-terminal autoinhibitory domain. Plant Physiol 127:1020–1029

Pittman JK, Hirschi KD (2016) CAX-ing a wide net: Cation/H^{+} transporters in metal remediation and abiotic stress signalling. Plant Biol 18:741–749

Pittman JK, Shigaki T, Cheng N-H, Hirschi KD (2002) Mechanism of N-terminal autoinhibition in the Arabidopsis Ca^{2+}/H^{+} antiporter CAX1. J Biol Chem 277:26452–26459

Plattner H, Verkhratsky A (2015) Evolution of calcium signalling. Cell Calcium 57:121–122

Pottosin II, Schonknecht G (2007) Vacuolar calcium channels. J Exp Bot 58:1559–1569

Pottosin II, Martinez-Estevez M, Dobrovinskaya OR, Muniz J (2005) Regulation of the slow vacuolar channel by luminal potassium: role of surface charge. J Membr Biol 205:103–111

Qi Z, Stephens NR, Spalding EP (2006) Calcium entry mediated by GLR33 an Arabidopsis glutamate receptor with a broad agonist profile. Plant Physiol 142:963–971

Qin Y, Yang Z (2011) Rapid tip growth: insights from pollen tubes. Semin Cell Dev Biol 22:816–824

Ragel P, Rodenas R, Garcia-Martin E, Andres Z, Villalta I, Nieves-Cordones M, Rivero RM, Martinez V, Pardo JM, Quintero FJ, Rubio F (2015) The CBL-interacting protein kinase CIPK23 regulates HAK5-mediated high-affinity K^{+} uptake in Arabidopsis roots. Plant Physiol 169:2863–2873

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends Plant Sci 15:395–401

Rathore KS, Cork RJ, Robinson KR (1991) A cytoplasmic gradient of Ca^{2+} is correlated with the growth of lily pollen tubes. Dev Biol 148:612–619

Rato C, Monteiro D, Hepler PK, Malho R (2004) Calmodulin activity and cAMP signalling modulate growth and apical secretion in pollen tubes. Plant J 38:887–897

Rodriguez L, Gonzalez-Guzman M, Diaz M, Rodrigues A, Izquierdo-Garcia AC, Peirats-Llobet M, Fernandez MA, Antoni R, Fernandez D, Marquez JA, Mulet JM, Albert A, Rodriguez PL (2014) C2-domain abscisic acid-related proteins mediate the interaction of PYR/PYL/RCAR abscisic acid receptors with the plasma membrane and regulate abscisic acid sensitivity in Arabidopsis. Plant Cell 26:4802–4820

Rosa M, Abraham-Juarez MJ, Lewis MW, Fonseca JP, Tian W, Ramirez V, Luan S, Pauly M, Hake S (2017) The maize MID-COMPLEMENTING ACTIVITY homolog CELL NUMBER REGULATOR13/NARROW ODD DWARF coordinates organ growth and tissue patterning plant. Cell 29:474–490

Sai J, Johnson CH (2002) Dark-stimulated calcium ion fluxes in the chloroplast stroma and cytosol. Plant Cell 14:1279–1291

Sanchez-Barrena MJ, Martinez-Ripoll M, Albert A (2013) Structural biology of a major signaling network that regulates plant abiotic stress: the CBL-CIPK mediated pathway. Int J Mol Sci 14:5734–5749

Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11:691–706

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. Plant Cell 14(Suppl):S401–S417

Sanyal SK, Pandey A, Pandey GK (2015) The CBL-CIPK signaling module in plants: a mechanistic perspective. Physiol Plant 155:89–108

Sanyal SK, Rao S, Mishra LK, Sharma M, Pandey GK (2016) Plant stress responses mediated by CBL-CIPK phosphorylation network. In: Lin C, Luan S (eds) The enzymes. Elsevier, Amsterdam, pp 31–64

Sanyal SK, Kanwar P, Yadav AK, Sharma C, Kumar A, Pandey GK (2017) Arabidopsis CBL interacting protein kinase 3 interacts with ABR1 an APETALA2 domain transcription factor to regulate ABA responses. Plant Sci 254:48–59

Schumaker KS, Sze H (1987) Inositol 145-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of oat roots. J Biol Chem 262:3944–3946

Seigneurin-Berny D, Gravot A, Auroy P, Mazard C, Kraut A, Finazzi G, Grunwald D, Rappaport F, Vavasseur A, Joyard J, Richaud P, Rolland N (2006) HMA1 a new Cu-ATPase of the chloroplast envelope is essential for growth under adverse light conditions. J Biol Chem 281:2882–2892

Sello S, Perotto J, Carraretto L, Szabo I, Vothknecht UC, Navazio L (2016) Dissecting stimulus-specific Ca^{2+} signals in amyloplasts and chloroplasts of Arabidopsis thaliana cell suspension cultures. J Exp Bot 67:3965–3974

Shi J, Kim KN, Ritz O, Albrecht V, Gupta R, Harter K, Luan S, Kudla J (1999) Novel protein kinases associated with calcineurin B-like calcium sensors in Arabidopsis. Plant Cell 11:2393–2405

Shigaki T, Rees I, Nakhleh L, Hirschi KD (2006) Identification of three distinct phylogenetic groups of CAX cation/proton antiporters. J Mol Evol 63:815–825

Sieberer BJ, Chabaud M, Fournier J, Timmers AC, Barker DG (2012) A switch in Ca^{2+} spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of Medicago truncatula. Plant J 69:822–830

Singh A, Pandey GK (2016) How phospholipase C regulates stress tolerance and development in plants? J Cell Signal 1:132

Singh S, Parniske M (2012) Activation of calcium- and calmodulin-dependent protein kinase (CCaMK) the central regulator of plant root endosymbiosis. Curr Opin Plant Biol 15:444–453

Singh S, Katzer K, Lambert J, Cerri M, Parniske M (2014) CYCLOPS a DNA-binding transcriptional activator orchestrates symbiotic root nodule development. Cell Host Microbe 15:139–152

Singh A, Bhatnagar N, Pandey A, Pandey GK (2015) Plant phospholipase C family: regulation and functional role in lipid signaling. Cell Calcium 58:139–146

Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu JK (2005) Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. Plant Cell 17:2384–2396

Spiegel S, Milstien S (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol 4:397–407
Stael S, Wurzinger B, Mair A, Mehlmer N, Vothknecht UC, Teige M (2012) Plant organellar calcium signalling: an emerging field. J Exp Bot 63:1525–1542
Steinhorst L, Kudla J (2013) Calcium – a central regulator of pollen germination and tube growth. Biochim Biophys Acta 1833:1573–1581
Steinhorst L, Mahs A, Ischebeck T, Zhang C, Zhang X, Arendt S, Schultke S, Heilmann I, Kudla J (2015) Vacuolar CBL-CIPK12 Ca^{2+}-sensor-kinase complexes are required for polarized pollen tube growth. Curr Biol 25:1475–1482
Straub T, Ludewig U, Neuhauser B (2017) The kinase CIPK23 inhibits ammonium transport in Arabidopsis thaliana. Plant Cell 29:409–422
Swarbreck SM, Colaco R, Davies JM (2013) Plant calcium-permeable channels. Plant Physiol 163:514–522
Takahashi Y, Ito T (2011) Structure and function of CDPK: a sensor responder of calcium. In: Luan S (ed) Coding and decoding of calcium signals in plants. Springer, Berlin, pp 129–146
Tang RJ, Luan S (2017) Regulation of calcium and magnesium homeostasis in plants: from transporters to signaling network. Curr Opin Plant Biol 39:97–105
Tang RJ, Zhao FG, Garcia VJ, Kleist TJ, Yang L, Zhang HX, Luan S (2015) Tonoplast CBL-CIPK calcium signaling network regulates magnesium homeostasis in Arabidopsis. Proc Natl Acad Sci U S A 112:3134–3139
Teardo E, Carraretto L, Wagner S, Formentin E, Behera S, De Bortoli S, Larosa V, Fuchs P, Lo Schiavo F, Raffaello A, Rizzuto R, Costa A, Schwarzlander M, Szabo I (2017) Physiological characterization of a plant mitochondrial calcium uniporter in vitro and in vivo. Plant Physiol 173:1355–1370
Thellier M, Luttge U (2013) Plant memory: a tentative model. Plant Biol 15:1–12
Thellier M, Desbiez MO, Champagnat P, Kergosien Y (1982) Do memory processes occur also in plants? Physiol Plant 56:281–284
Thion L, Mazars C, Nacry P, Bouchez D, Moreau M, Ranjeva R, Thuleau P (1998) Plasma membrane depolarization-activated calcium channels stimulated by microtubule-depolymerizing drugs in wild-type Arabidopsis thaliana protoplasts display constitutively large activities and a longer half-life in ton 2 mutant cells affected in the organization of cortical microtubules. Plant J 13:603–610
Tian Q, Zhang X, Yang A, Wang T, Zhang WH (2016) CIPK23 is involved in iron acquisition of Arabidopsis by affecting ferric chelate reductase activity. Plant Sci 246:70–79
Tiwari BS, Belenghi B, Levine A (2002) Oxidative stress increased respiration and generation of reactive oxygen species resulting in ATP depletion opening of mitochondrial permeability transition and programmed cell death. Plant Physiol 128:1271–1281
Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions abiotic stress and development. Curr Opin Plant Biol 8:397–403
Trewavas A (1999) Le calcium C'est la vie: calcium makes waves. Plant Physiol 120:1–6
Trewavas A (2003) Aspects of plant intelligence. Ann Bot 92:1–20
Trewavas A (2005) Green plants as intelligent organisms. Trends Plant Sci 10:413–419
Trewavas A (2009) What is plant behaviour? Plant Cell Environ 32:606–616
Trewavas AJ (2012) Plants are intelligent too. EMBO Rep 13:772–773
Trewavas A (2016) Intelligence cognition and language of green plants. Front Psychol 7:588
Trewavas A (2017) The foundations of plant intelligence. Interface Focus 7:20160098
Trewavas AJ, Malho R (1998) Ca^{2+} signalling in plant cells: the big network! Curr Opin Plant Biol 1:428–433
Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 A. Nature 473:55–60
van Loon LC (2016) The intelligent behavior of plants. Trends Plant Sci 21:286–294
Verdus MC, Le Sceller L, Norris V, Thellier M, Ripoll C (2007) Pharmacological evidence for calcium involvement in the long-term processing of abiotic stimuli in plants. Plant Signal Behav 2:212–220

Verret F, Wheeler G, Taylor AR, Farnham G, Brownlee C (2010) Calcium channels in photosynthetic eukaryotes: implications for evolution of calcium-based signalling. New Phytol 187:23–43

Virdi AS, Singh S, Singh P (2015) Abiotic stress responses in plants: roles of calmodulin-regulated proteins. Front Plant Sci 6:809

Virolainen E, Blokhina O, Fagerstedt K (2002) Ca^{2+}-induced high amplitude swelling and cytochrome c release from wheat (Triticum aestivum L) mitochondria under anoxic stress. Ann Bot 90:509–516

Wagner S, Behera S, De Bortoli S, Logan DC, Fuchs P, Carraretto L, Teardo E, Cendron L, Nietzel T, Fussl M, Doccula FG, Navazio L, Fricker MD, Van Aken O, Finkemeier I, Meyer AJ, Szabo I, Costa A, Schwarzlander M (2015) The EF-hand Ca^{2+} binding protein MICU choreographs mitochondrial Ca^{2+} dynamics in Arabidopsis. Plant Cell 27:3190–3212

Wagner S, De Bortoli S, Schwarzlander M, Szabo I (2016) Regulation of mitochondrial calcium in plants versus animals. J Exp Bot 67:3809–3829

Walter A, Mazars C, Maitrejean M, Hopke J, Ranjeva R, Boland W, Mithofer A (2007) Structural requirements of jasmonates and synthetic analogues as inducers of Ca^{2+} signals in the nucleus and the cytosol of plant cells. Angew Chem Int Ed Engl 46:4783–4785

Wang WH, Chen J, Liu TW, Han AD, Simon M, Dong XJ, He JX, Zheng HL (2014) Regulation of the calcium-sensing receptor in both stomatal movement and photosynthetic electron transport is crucial for water use efficiency and drought tolerance in Arabidopsis. J Exp Bot 65:223–234

Wang L, Yamano T, Takane S, Niikawa Y, Toyokawa C, Ozawa S, Tokutsu R, Takahashi Y, Minagawa J, Kanesaki Y, Yoshikawa H, Fukuzawa H (2016) Chloroplast-mediated regulation of CO2-concentrating mechanism by Ca^{2+}-binding protein CAS in the green alga Chlamydomonas reinhardtii. Proc Natl Acad Sci U S A 113:12586–12591

Wernimont AK, Amani M, Qiu W, Pizarro JC, Artz JD, Lin YH, Lew J, Hutchinson A, Hui R (2011) Structures of parasitic CDPK domains point to a common mechanism of activation. Proteins 79:803–820

Wheeler GL, Brownlee C (2008) Ca^{2+} signalling in plants and green algae – changing channels. Trends Plant Sci 13:506–514

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92:487–511

Xiong TC, Jauneau A, Ranjeva R, Mazars C (2004) Isolated plant nuclei as mechanical and thermal sensors involved in calcium signalling. Plant J 40:12–21

Xiong TC, Coursol S, Grat S, Ranjeva R, Mazars C (2008) Sphingolipid metabolites selectively elicit increases in nuclear calcium concentration in cell suspension cultures and in isolated nuclei of tobacco. Cell Calcium 43:29–37

Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, Wu WH (2006) A protein kinase interacting with two calcineurin B-like proteins regulates K^+ transporter AKT1 in Arabidopsis. Cell 125:1347–1360

Yamanaka T, Nakagawa Y, Mori K, Nakano M, Imamura T, Kataoka H, Terashima A, Iida K, Kojima I, Katagiri T, Shinozaki K, Iida H (2010) MCA1 and MCA2 that mediate Ca^{2+} uptake have distinct and overlapping roles in Arabidopsis. Plant Physiol 152:1284–1296

Yang T, Poovaiah BW (2002) Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. Proc Natl Acad Sci U S A 99:4097–4102

Yoon GM, Dowd PE, Gilroy S, McCubbin AG (2006) Calcium-dependent protein kinase isoforms in petunia have distinct functions in pollen tube growth including regulating polarity. Plant Cell 18:867–878

Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B, Johnson DM, Swift GB, He Y, Siedow JN, Pei ZM (2014) OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis. Nature 514:367–371

Zakharov SD, Ewy RG, Dilley RA (1993) Subunit III of the chloroplast ATP-synthase can form a Ca^{2+}-binding site on the lumenal side of the thylakoid membrane. FEBS Lett 336:95–99

Zeng H, Xu L, Singh A, Wang H, Du L, Poovaiah BW (2015) Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. Front Plant Sci 6:600

Zhao J, Shigaki T, Mei H, Guo YQ, Cheng NH, Hirschi KD (2009) Interaction between Arabidopsis Ca^{2+}/H^+ exchangers CAX1 and CAX3. J Biol Chem 284:4605–4615

Zhou L, Lan W, Chen B, Fang W, Luan S (2015a) A calcium sensor-regulated protein kinase calcineurin B-like protein-interacting protein kinase19 is required for pollen tube growth and polarity. Plant Physiol 167:1351–1360

Zhou X, Hao H, Zhang Y, Bai Y, Zhu W, Qin Y, Yuan F, Zhao F, Wang M, Hu J, Xu H, Guo A, Zhao H, Zhao Y, Cao C, Yang Y, Schumaker KS, Guo Y, Xie CG (2015b) SOS2-like protein kinase5 an SNF1-related protein kinase3-type protein kinase is important for abscisic acid responses in Arabidopsis through phosphorylation of abscisic acid-insensitive5. Plant Physiol 168:659–676

Sibaji Kumar Sanyal is pursuing his Ph.D. with Dr. Pandey on the functioning of CBL-CIPK module in ABA signal transduction in plants.

Swati Mahiwal is working in Dr. Pandey's lab to explore the interaction of Ca^{2+} signaling with oxidative stress in *Arabidopsis*.

Girdhar Kumar Pandey worked at ICGEB and obtained his Ph.D. from JNU, New Delhi (with the Editor), in the field of calcium (Ca^{2+}) signaling in plants. Subsequently, he pursued postdoctoral research on Ca^{2+}-CBL-CIPK, phosphatases, channels/transporters, and transcription factors involved in abiotic stresses at the Department of Plant and Microbial Biology, University of California at Berkeley. He is currently Professor at the Plant Molecular Biology Department, University of Delhi, South Campus. His group's research interest is to understand the mechanistic interplay of Ca^{2+}- mediated signaling networks in plants under mineral nutrient deficiency and abiotic stresses.

Nitric Oxide: A Tiny Decoder and Transmitter of Information

12

Jasmeet Kaur Abat and Renu Deswal

Abstract

Plants are immobile, yet they are considered sentient because of their capacity to sense and respond. Priming, cross-tolerance to stress, and trans-generational traits support their capacity to retain information. Plants respond to external as well as internal cues. Signaling mechanisms are intricate, and redox changes are the hallmark of these. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to these redox changes. Nitric oxide (NO) is one such gaseous RNS which mainly modifies protein functions by post-translational modifications (PTMs) of proteins. NO is considered a "do it all" molecule. It is produced in plants by oxidative and reductive pathways. Nitrosylation, i.e., addition of NO group to thiols in proteins, is a major protein modification. Several hundreds of nitrosylated proteins and NO-modified transcription factors are identified in plants. The spatial and temporal distribution of these nitrosylated targets suggests nitrosylation to be a global modification contributing to majority of cellular functions and pathways. Some of the nitrosylated proteins are functionally validated to show these as important redox hubs in cellular physiology.

Recently, the ERF VII transcription factor-dependent N-end rule proteolysis pathway has been implicated for NO perception. A NO perceptron concept may enrich and help in integrating NO signaling in different stress conditions. Some of the redox hubs may be vital targets for crop improvement and adaptation to stress in future. Many of the nitrosylated proteins are also modified by

J. K. Abat
Department of Botany, Gargi College, University of Delhi, New Delhi, India

Molecular Plant Physiology and Proteomics Laboratory, Department of Botany, University of Delhi, Delhi, India

R. Deswal (✉)
Molecular Plant Physiology and Proteomics Laboratory, Department of Botany, University of Delhi, Delhi, India

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_12

other NO modifications like nitration or a related redox modification called glutathionylation suggesting existence of PTM crosstalk, another level of regulation which needs to be deciphered in future.

Keywords
Nitric oxide · Nitrosylation · NO perception · Abiotic stress · Post-translational modification (PTM)-crosstalk · NO sensor · Reactive oxygen species (ROS) · Glutathionylation

12.1 Plants Are Sentient Like Animals

Unlike animals, plants are sessile, yet they are able to tolerate any stress and can perceive or feel the environment. Pioneer work by Sir Jagadish Chandra Bose, mentioned elsewhere in detail in later chapters also, suggested that plants are sentient and discovered that an electric death spasm occurs in plants when they die and that the actual moment of death in a plant could be accurately recorded. He said that "all around us, the plants are communicating, we just don't notice it." Plants not only communicate with each other, but communication also occurs within a plant. Different parts of the plant communicate with each other, exchanging information at cellular, physiological, and environmental level, and this has been discussed in detail in another chapter. For example, root growth is dependent on hormone auxin that is generated in the tips of shoots and transported to the growing roots, while shoot development is partially dependent on a signal that's generated in the roots.

Stress conditions faced by plants are broadly categorized as abiotic stress (including drought, heat, cold, salinity, and light) and biotic stress (arises mainly from bacteria, fungi, viruses, nematodes, and insects). Plants have adaptive mechanisms that allow them to survive in an ever-changing environment; they particularly show plasticity in organ formation after germination. A germinating seedling has an embryonic root and the cotyledons, while all other organs are formed post-embryonically; therefore, a plant's body architecture is determined by the conditions that the plant experiences, and its growth can be adjusted to suit those conditions.

Under stress conditions, plants adjust their physiology and development to assure survival. Plants developed sensitive and complex sensory mechanisms to integrate all dynamic and changing information, to survive in an ever-changing environment. Plants also have memory to remember the stress faced by them. For example, rabi crops like wheat remember that they have gone through winter before they start to flower. Also some stressed plants have sustained memory of environmental experiences and give rise to progeny that are more resistant to the same stress. Plant phenotypic responses to environmental stimulus can have either an immediate expression or even a transgenerational expression (Verhoeven and van Gurp 2012). Chemical priming using chemicals like NO, hydrogen peroxide (H_2O_2), hydrogen sulfide (H_2S), melatonin, and polyamines to provide abiotic and biotic stress tolerance supports the existence of "memory" in plants. It is observed that chemical priming leads to complex signaling as deciphered by proteomic, transcriptomic, and

metabolomic analysis (Savvides et al. 2016). "Priming" is being proposed a promising tool for crop improvement in future.

In addition to being sentient, another similarity between animals and plants that can be drawn is that plants also have immunity like animals. This aspect of plant responses to pathogens is discussed in a later chapter.

12.2 Plant Hormones and Reactive Nitrogen Species

Being sessile organisms, plants depend on complex signaling mechanisms to adjust their growth and metabolism with the constant changing environment. Plant hormones are key regulators in determining the ability of plants to adapt to changing environments and biotic challenges by regulating growth, development, and nutrient allocation (Peleg and Blumwald 2011; Wolters and Jurgens 2009). Manipulation of the endogenous phytohormone levels either by exogenous application or by using biotechnological tools can contribute to the adjustment of plant metabolism and development to various abiotic stress factors (Wani et al. 2016).

Mittler et al. (2012) reported that to counter the effects of, for example, heat stress, plants reprogram their transcriptome, proteome, and metabolome. Changes in temperature are sensed at the membrane level, and calcium channel(s) are activated. The inward flux of calcium activates signal transduction events including Ca^{2+} signaling, ROS signaling, and hormones leading to altered plant metabolism. Plants can perceive a signal and transduce it through the complex network or phloem which is also considered as "phytoneuron" in plants for transmission of signal (Calvo et al. 2017). A common factor among plant responses to abiotic and biotic stress is the overproduction of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydroxyl radicals, hydrogen peroxide (discussed in detail in another chapter), and reactive nitrogen species (RNS) that have signaling functions under normal conditions but have the potential to cause a number of deleterious events under a stressful environment (Ruelland and Zachowski 2010). Thus, the regulation of plant redox homeostasis is an important facet of stress tolerance (Vranová et al. 2002).

RNS are redox active molecules including nitric oxide (NO), peroxinitrite (ONOO), dinitrogen trioxide (N_2O_3) and dinitrogen tetraoxide (N_2O_4), S-nitrosoglutathione (GSNO), S-nitrosothiols (SNOs), and nitrogen dioxide (NO_2). RNS play a critical role in intracellular redox signaling and the activation of antioxidant defense mechanisms. RNS particularly NO is an important secondary messenger which plays a dominant role in transduction of the stress signal (Sahay and Gupta 2017). Reports suggest that all major classes of plant hormones such as auxins, gibberellins, cytokinins, abscisic acid, ethylene, salicylic acid, jasmonates, and brassinosteroids can influence the endogenous levels of NO and vice versa. NO may also affect biosynthesis, catabolism, transport, and perception of these phytohormones (Freschi 2013). It has been demonstrated that when plant cells are challenged by biotic stress (pathogens), NO is produced and there is expression of defense-related genes and the production of secondary metabolites leading to hypersensitive response (Bellin et al. 2013). The challenge is to understand how the information stored in the stress-induced increase in NO concentration helps to define the outcome of the response.

12.3 Nitric Oxide: A Skilled Molecule

Nitric oxide is a gaseous free radical with an unshared electron which can regulate a multitude of biological processes. The importance of NO as a redox-active reactive free radical in biological environment is well documented. NO acts as a signaling molecule that has direct and indirect regulatory roles in various functional processes in animal and plant systems. In animals, NO plays an important role as a mediator of vasodilation in blood vessels. It is induced by several factors, and once synthesized, it results in phosphorylation of several proteins that cause smooth muscle relaxation. In plants, NO is now recognized as a ubiquitous cell signaling molecule as a regulator of growth, development, immunity, stress tolerance, and environmental interactions. NO plays important regulatory roles in plants, including seed dormancy and germination, root development and hypocotyl elongation, floral transition, senescence, cell death, phytohormone signaling, and responses to abiotic and biotic stress conditions (Fig. 12.1). NO can act both as a promoter and as an inhibitor of cellular processes depending on its local concentration (Mur et al. 2013). NO plays important roles in diverse plant metabolic and physiological processes, along with phytohormones and secondary messengers, and due to this NO has gained special interest in research community in recent years.

12.3.1 Discovery of NO in Animals and Plants

Nitric oxide was first described in 1772 as "nitrous air" by Joseph Priestly. In 1984, Furchgott described it as an endothelium-derived relaxing factor (EDRF) which was unstable, acted via stimulation of the soluble guanylate cyclase (sGC), and was

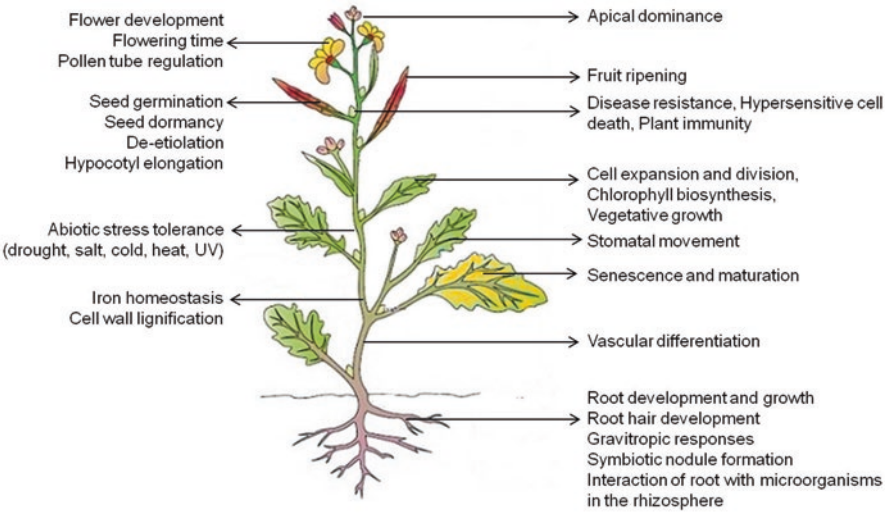

Fig. 12.1 Schematic representation of plant processes mediated by nitric oxide

inhibited by hemoglobin and methylene blue (Furchgott et al. 1984). In 1987, at a symposium, he proposed that the endogenous nitrovasodilator released by vascular endothelium that mediates the relaxation might be NO (Moncada et al. 1988). In vivo NO generation was reported in herbicide-treated *Glycine max* leaves and intact plants by Klepper in 1979. NO plays a key regulatory role in controlling many physiological functions in animals. Owing to its importance, NO was named as "molecule of the year" in 1992 by *Science* (SoRelle 1998); later, Robert F. Furchgott, Louis J Ignarro, and Farid Murrad (NO research pioneers) were given the noble prize in medicine for their discoveries proving NO as a signaling molecule in the cardiovascular system. In 1998, Durner et al. demonstrated that NO-related molecules increase levels of salicylic acid and pathogenesis-related protein (PR protein), indicating role of NO in plant immunity. They showed that "nitric oxide synthase" (NOS) protected tobacco plants from viral infection by triggering the induction of defense-related genes. Remarkably, NO does so by using the same signal transduction pathways that it uses in mammals. The enzymatic source of NO is well defined, mainly NOS contributing to the NO production, but in plants, a "NOS" homolog is still to be discovered. This is an interesting challenge waiting for a clear answer from the plant research community.

12.3.2 NO Synthesis in Plants

In animals, NO is reported to be produced by three NOS enzymes, which oxidize L-arginine to generate L-citrulline and NO (Mayer and Hemmens 1997; Wendehenne et al. 2001). Although pharmacological evidence using NOS inhibitors indicated the presence of NOS-like activity in plants yet in silico analysis of plant genomes, ortholog genes encoding NOS enzymes have not been identified and a similar biosynthesis mechanism in plants is still debatable (Talwar et al. 2012). Several possible routes for NO production have been proposed in plants (Astier et al. 2017). Genes encoding NOS-like enzymes were searched in algal genomes, and NOS-like sequences were identified in 15 of the 265 algal species analyzed. Though no gene or protein similar to NOS has not been reported in higher plants, recent studies have shown the existence of NOS-like enzymes in photosynthetic microalgae, *Ostreococcus tauri* (Weisslocker-Schaetzel et al. 2017), and cyanobacteria, *Synechococcus* PCC 7335 (Correa-Aragunde et al. 2018). Algal NOS showed similarity with animal NOS but lacks N-terminal Zn-binding domain, while cyanobacterial NOS have N-terminal globin domain but lack CaM-binding domain. Lack of NOS in higher plants led to the hypothesis that land plants might have evolved an efficient mechanism of NO production via nitrate assimilation and reduction processes by NR. Moreover, presence of NOS might not be necessary due to the availability of multiple routes of NO production in plants (Jeandroz et al. 2016). Therefore, production of NO is not confined to organisms containing NOS. Rather, nitrate reduction by bacteria, fungi, and plants is known to be an alternative source.

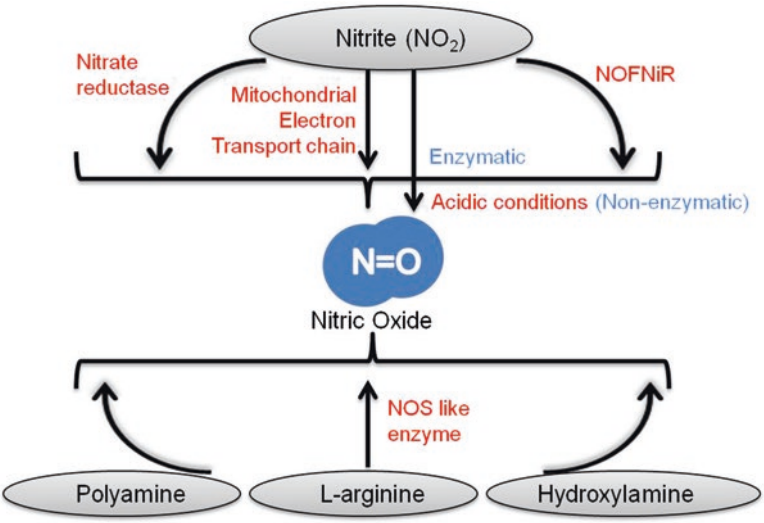

Fig. 12.2 Pathways of nitric oxide production in plants. Lower part of the diagram shows oxidative pathway of NO synthesis by NOS-like enzyme. The upper section shows another, the reductive pathway of NO production, which involves reduction of nitrite to NO via different nonenzymatic or enzymatic reactions majorly catalyzed by nitrate reductase (NR)

NO biosynthesis in plants includes both L-arginine and nitrite-dependent pathways (Fig. 12.2). L-arginine-dependent NO biosynthesis, the oxidative pathway of NO synthesis, relies on the NADPH-dependent oxidation of L-arginine via NO synthase (NOS)-like activity, while nitrite-dependent production of NO by the reductive pathway requires the formation of nitrite from nitrate via nitrate reductase (NR) activity and the subsequent reduction of nitrite into NO via NR itself or via the mitochondrial electron transport chain. Arabidopsis has two known NR genes, NIA1 and NIA2 (Campbell 1999). Comparative studies of individual and double mutants, nia1/nia2, showed a significant reduction in NO synthesis and different contribution to the synthesis of NO in different tissues (Modolo et al. 2006). Cytochrome P450, xanthine oxidase, or copper amine oxidase 1 have also been suggested as potential sources of NO production in plants. In *Chlamydomonas reinhardtii*, another NO-producing mechanism by NR was discovered. It was shown that NR interacts with the nitric oxide-forming nitrite reductase (NOFNiR) to produce NO from nitrite (Chamizo-Ampudia et al. 2016). Once produced, NO mediates its action via multiple signaling pathways.

12.4 NO Signaling: Transmitting the Information

Nitric oxide acts as a ubiquitous signal in plants, and NO signaling can be mediated via a cGMP-dependent or cGMP-independent pathway (Yu et al. 2014). The mechanism of cGMP-mediated signaling is initiated by the covalent bonding of

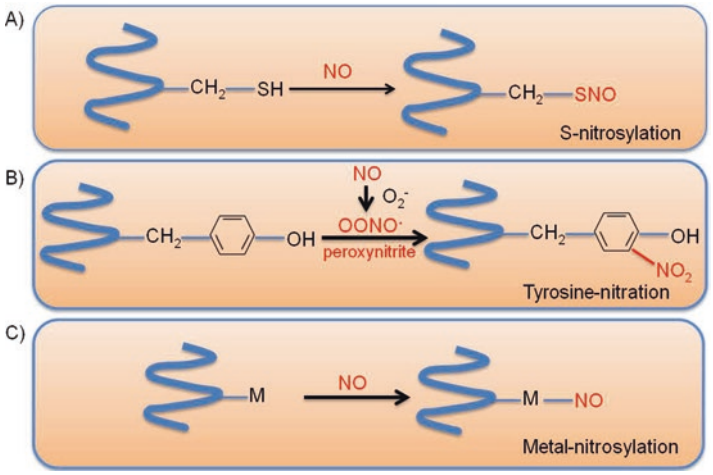

Fig. 12.3 Nitric oxide-mediated post-translational modifications (PTMs) of proteins. (**a**) S-nitrosylation of cysteine residues. (**b**) Tyrosine nitration. (**c**) Metal nitrosylation

NO with the heme group of guanylate cyclase, which enhances its enzymatic activity and affects the generation of cyclic GMP. The basal activity of the enzyme is increased up to 200 times on binding NO; however, the lifetime of the NO–heme complex is very short. In plant cells, similarly as in the case in animal cells, several signaling pathways coexist for NO-mediated signals, including, e.g., cyclic nucleotides, Ca^{2+} ions, protein kinases, as well as others. Introduction of animal NOS to tobacco leaves or treatment of tobacco cell suspension with an NO donor (S-nitrosoglutathione, GSNO) induced an increase in cGMP. Acting in a cGMP-independent manner, NO can interact with all cellular macromolecules including proteins (S-nitrosylation, tyrosine nitration, metal-nitrosylation) (Fig. 12.3), lipids (nitro-fatty acids), and nucleic acids. One of the main signal transduction mechanisms of NO is derived from its ability to reversibly bind cysteine (Cys) thiols to form post-translational, redox-sensitive S-nitrosothiol (SNO). S-nitrosylation can regulate protein activity, localization, structure, and protein–protein interaction (Spadaro et al. 2010). This redox modification is a central route for NO bioactivity, as it changes the cellular redox status. S-nitrosylation has been shown to modulate the enzyme activity, and several S-nitrosylated proteins have been identified in *Arabidopsis* (Lindermayr et al. 2005; Fares et al. 2011), *Brassica juncea* (Abat and Deswal 2009; Sehrawat et al. 2013), wheat (Gietler et al. 2016), *Kalanchoe pinnata* (Abat et al. 2008), pea (Ortega-Galisteo et al. 2012), potato (Kato et al. 2013), and citrus (Tanou et al. 2009). Proteome of *Arabidopsis* GSNOR knockout mutant atgsnor 1–3 was shown to contain 926 and 1195 S-nitrosylated proteins and peptides, respectively (Hu et al. 2015). S-nitrosylation can also regulate the activity of the target protein. In *B. juncea*, SNO modification of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) led to inhibition of its carboxylase activity (Abat and Deswal 2009), while fructose bisphosphate aldolase was reported to be positively regulated by S-nitrosylation (Sehrawat et al. 2013).

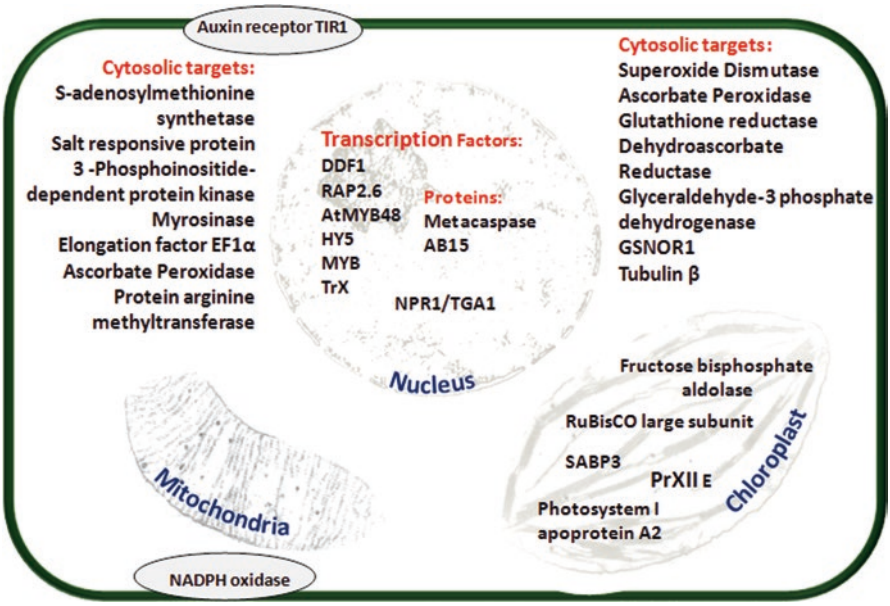

Fig. 12.4 Validated S-nitrosylated proteins. Till date hundreds of S-nitrosylated proteins have been identified in plants, but only a few have been functionally validated to confirm regulation of activity or localization by S-nitrosylation. The figure lists and provides subcellular localization of validated S-nitrosylated proteins in plant cell. GSNOR, S-nitrosoglutathione reductase; AB15, abscisic acid-responsive gene; PrxII E, peroxiredoxin II E; SABP3, salicylic acid-binding protein 3

In *Pisum sativum*, S-nitrosylation of cytosolic ascorbate peroxidase, which is involved in the regulation of cellular hydrogen peroxide (H_2O_2) content, promoted its enzyme activity (Begara-Morales et al. 2013). Also cold stress-mediated superoxide dismutase (SOD) activation by S-nitrosylation was reported in *B. juncea* (Sehrawat et al. 2013). Although many nitrosylated proteins are identified, the functional validation of only a few has been done. Figure 12.4 depicts the subcellular localization of some of the functionally validated S-nitrosylation proteins and NO-responsive transcription factors.

Recently, high-throughput transcriptome analysis was used to identify 673 transcription factors in *Arabidopsis* leaves which showed differential expression in response to S-nitrosocysteine (CySNO, NO donor). These transcription factors were shown to be involved in pathways like hormone signaling, protein degradation, development, and biotic and abiotic stress. Regulatory role of NO in plant growth and immunity was suggested by functional analysis of transcription factors *DDF1*, *RAP2.6*, and *AtMYB48* (Imran et al. 2018). Intricate crosstalk networks exist between NO and other signaling molecules like phytohormones, other second messengers, and key transcription factors. It has emerged that S-nitrosylation shows complex interplay with several other post-translational modifications, thereby expanding the large repertoire of cell signaling pathways it regulates (Skelly et al. 2016). Moreover, these observations suggest existence of PTM crosstalk which is

another level of regulating responses in plants. Recently Hu et al. (2017) demonstrated that S-nitrosylation selectively modulates enzymatic activity of arginine methyltransferase (PRMT5) protein, vital for abiotic stress tolerance. Protein methylation is an important modulator of signal transduction pathways, but methyltransferases themselves may also be controlled by S-nitrosylation, indicating presence of an intricate network of signaling regulators and super-regulators. GSNO (obtained from S-nitrosylation of glutathione and a stable reservoir of NO) was recently shown to work downstream of NO to mediate iron-deficiency signaling in *Arabidopsis*. On sensing iron deficiency, the plant sends a signal to the nucleus to activate the response via transcriptional reprogramming. Plant hormones and gaseous molecules, NO and carbon monoxide, were suggested to be involved in the signaling process (Yang et al. 2016; Kailasam et al. 2018). These proteins are redox hubs where crosstalk between metabolism and gene expression leads to integration of signals leading to appropriate responses.

12.4.1 Ethylene Responsive Factor (ERF) VII: A Putative NO Sensor

Sensing of a signal is a crucial step in signaling mechanism. NO sensing in plants is reported to be mediated by targeted degradation of plant-specific transcriptional regulators, group VII ethylene response factor (ERF) transcription factors via N-end rule pathway proteolysis (Gibbs et al. 2014). The group VII ERF transcription factors were identified as key regulators of many NO-mediated processes, and this pathway was suggested as a mechanism for NO perception in plants. ERF VII proteins have a redox-sensitive cysteine (cys), which can be recognized by proteolytic pathway called Arg-cys/N-end rule pathway (NERP) of protein degradation. This cys is destabilized by NO leading to its degradation and activation of the NERP pathway. Targeted proteolysis plays an important role in regulating various developmental and physiological processes by generating spatial gradient and varying the concentration of the signaling molecule. Ubiquitin-dependent proteolysis plays a major role in regulating the signaling by phytohormones such as auxins, gibberellins, and jasmonic acid (Graciet and Wellmer 2010). The N-end rule pathway is part of the ubiquitin–proteasome system in eukaryotes and has been shown to be involved in a multitude of cellular and developmental processes in animals also (Graciet and Wellmer 2010), again suggesting a commonality in sensory biology of plants and animals.

12.5 Perceptron: The Integrator of Information

All the above reports suggest that intricate crosstalk networks exist in plant cells. These networks intertwine most of the signaling molecules and are responsible for the overall plant responses to the environmental changes. Mostly, there is a shift from metabolism to defense signaling to enable the plant to be tolerant to particular stress condition at the expense of growth. Recently an analogy of perceptrons was proposed

in plant responses to the environment. Proteins and gene promoters were proposed to be the processing units like neurons which are linked through biochemical pathways and form information processing network of output which depends upon the combinations of inputs (Scheres and van der Putten 2017). In the future, a major challenge will be to understand how the information conveyed by the simple signaling molecules like NO with multiple functions is integrated during plant growth.

12.6 Future Directions

Future challenge would be to link phenotypes with the internal molecular changes which exist in plants and how these states can change and respond to the environment. With respect to NO signaling, redox changes initiated by the external and internal signals and the final response of these redox hubs would facilitate better regulation. Such studies would help in better understanding of biological significance of these redox switches and their contribution to the sensory physiology of plants. These advances may provide useful targets for crop improvement/adaptation to stress conditions. Moreover, a riddle which still is to be solved is whether a "NOS"-like enzymatic source of "NO" exists in plants.

Acknowledgments The nitric oxide signaling research work was funded by the Council of Scientific and Industrial Research (CSIR), University Grants Commission (UGC), and University of Delhi, Research and Development grant.

References

Abat JK, Deswal R (2009) Differential modulation of S-nitrosoproteome of *Brassica juncea* by low temperature: change in S-nitrosylation of Rubisco is responsible for the inactivation of its carboxylase activity. Proteomics 9:4368–4380

Abat JK, Mattoo AK, Deswal R (2008) S-nitrosylated proteins of a medicinal CAM plant *Kalanchoe pinnata*–ribulose-1, 5-bisphosphate carboxylase/oxygenase activity targeted for inhibition. FEBS J 275:2862–2872

Astier J, Gross I, Durner J (2017) Nitric oxide production in plants: an update. J Exp Bot 69:3401–3411

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Padilla MN, Carreras A, Corpas FJ, Barroso JB (2013) Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and S-nitrosylation. J Exp Bot 65:527–538

Bellin D, Asai S, Delledonne M, Yoshioka H (2013) Nitric oxide as a mediator for defense responses. Mol Plant-Microbe Interact 26:271–277

Calvo P, Sahi VP, Trewavas A (2017) Are plants sentient? Plant Cell Environ 40:2858–2869

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Annu Rev Plant Biol 50:277–303

Chamizo-Ampudia A, Sanz-Luque E, Llamas Á, Ocaña-Calahorro F, Mariscal V, Carreras A, Barroso JB, Galván A, Fernández E (2016) A dual system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO production in *Chlamydomonas*. Plant Cell Environ 39:2097–2107

Correa-Aragunde N, Foresi N, Castello FD, Lamattina L (2018) A singular nitric oxide synthase with a globin domain found in *Synechococcus* PCC 7335 mobilizes N from arginine to nitrate. Sci Rep 8:1–11

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP and cyclic ADP-ribose. Proc Natl Acad Sci U S A 95:10328–10333
Fares A, Rossignol M, Peltier JB (2011) Proteomics investigation of endogenous S-nitrosylation in Arabidopsis. Biochem Biophys Res Commun 416:331–336
Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:1–22
Furchgott RF, Cherry PD, Zawadzki JV, Jothianandan D (1984) Endothelial cells as mediators of vasodilation of arteries. J Cardiovasc Pharmacol 6:S336–S343
Gibbs DJ, Isa NM, Movahedi M, Lozano-Juste J, Mendiondo GM, Berckhan S, Marín-de la Rosa N, Conde JV, Correia CS, Pearce SP, Bassel GW (2014) Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors. Mol Cell 53:369–379
Gietler M, Nykiel M, Orzechowski S, Fettke J, Zagdanska B (2016) Proteomic analysis of S-nitrosylated and S-glutathionylated proteins in wheat seedlings with different dehydration tolerances. Plant Physiol Biochem 108:507–518
Graciet E, Wellmer F (2010) The plant N-end rule pathway: structure and functions. Trends Plant Sci 15:447–453
Hu J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J (2015) Site-specific nitrosoproteomic identification of endogenously S-nitrosylated proteins in Arabidopsis. Plant Physiol 167:1731–1746
Hu J, Yang H, Mu J, Lu T, Peng J, Deng X, Kong Z, Bao S, Cao X, Zuo J (2017) Nitric oxide regulates protein methylation during stress responses in plants. Mol Cell 67:702–710
Imran QM, Hussain A, Lee SU, Mun BG, Falak N, Loake GJ, Yun BW (2018) Transcriptome profile of NO-induced Arabidopsis transcription factor genes suggests their putative regulatory role in multiple biological processes. Sci Rep 8:1–14
Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK, Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase–like proteins in the plant kingdom. Sci Signal 9:1–9
Kailasam S, Wang Y, Lo JC, Chang HF, Yeh KC (2018) S-Nitrosoglutathione works downstream of nitric oxide to mediate iron-deficiency signaling in *Arabidopsis*. Plant J 94:157–168
Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of S-nitrosylated proteins in potato plant. Physiol Plant 148:371–386
Klepper L (1979) Nitric oxide (NO) and nitrogen dioxide (NO2) emissions from herbicide-treated soybean plants. Atmos Environ 13:537–542
Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of S-nitrosylated proteins in Arabidopsis. Plant Physiol 137:921–930
Mayer B, Hemmens B (1997) Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem Sci 22:477–481
Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? Trends Biochem Sci 37:118–125
Modolo LV, Augusto O, Almeida IMG, Pinto-Maglio CAF, Oliveira HC, Seligman K, Salgado I (2006) Decreased arginine and nitrite levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impair nitric oxide synthesis and the hypersensitive response to *Pseudomonas syringae*. Plant Sci 171:34–40
Moncada S, Palmer RM, Higgs EA (1988) The discovery of nitric oxide as the endogenous nitrovasodilator. Hypertension 12:365–372
Mur LA, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJ, Hebelstrup KH, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plants 5:1–17
Ortega-Galisteo AP, Rodríguez-Serrano M, Pazmiño DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. J Exp Bot 63:2089–2103
Peleg Z, Blumwald E (2011) Hormone balance and abiotic stress tolerance in crop plants. Curr Opin Plant Biol 14:290–295
Ruelland E, Zachowski A (2010) How plants sense temperature. Environ Exp Bot 69:225–232

Sahay S, Gupta M (2017) An update on nitric oxide and its benign role in plant responses under metal stress. Nitric Oxide 67:39–52
Savvides A, Ali S, Tester M, Fotopoulos V (2016) Chemical priming of plants against multiple abiotic stresses: Mission possible? Trends Plant Sci 21:329–340
Scheres B, van der Putten WH (2017) The plant perceptron connects environment to development. Nature 543:337–345
Sehrawat A, Abat JK, Deswal R (2013) RuBisCO depletion improved proteome coverage of cold responsive S-nitrosylated targets in *Brassica juncea*. Front Plant Sci 4:1–14
Skelly MJ, Frungillo L, Spoel SH (2016) Transcriptional regulation by complex interplay between post-translational modifications. Curr Opin Plant Biol 33:126–132
SoRelle R (1998) Nobel Prize awarded to scientists for nitric oxide discoveries. Circulation 98:2365–2366
Spadaro D, Yun BW, Spoel SH, Chu C, Wang YQ, Loake GJ (2010) The redox switch: dynamic regulation of protein function by cysteine modifications. Physiol Plant 138:360–371
Talwar PS, Gupta R, Maurya AK, Deswal R (2012) *Brassica juncea* nitric oxide synthase like activity is stimulated by PKC activators and calcium suggesting modulation by PKC-like kinase. Plant Physiol Biochem 60:157–164
Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804
Verhoeven KJ, van Gurp TP (2012) Transgenerational effects of stress exposure on offspring phenotypes in apomictic dandelion. PLoS One 7:1–8
Vranová E, Inzé D, Van Breusegem F (2002) Signal transduction during oxidative stress. J Exp Bot 53:1227–1236
Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176
Weisslocker-Schaetzel M, André F, Touazi N, Foresi N, Lembrouk M, Dorlet P, Frelet-Barrand A, Lamattina L, Santolini J (2017) The NOS-like protein from the microalgae *Ostreococcus tauri* is a genuine and ultrafast NO-producing enzyme. Plant Sci 265:100–111
Wendehenne D, Pugin A, Klessig DF, Durner J (2001) Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends Plant Sci 6:177–183
Wolters H, Jurgens G (2009) Survival of the flexible: hormonal growth control and adaptation in plant development. Nat Rev Genet 10:305–317
Yang L, Ji J, Wang H, Harris-Shultz KR, Abd_Allah EF, Luo Y, Guan Y, Hu X (2016) Carbon monoxide interacts with auxin and nitric oxide to cope with iron deficiency in Arabidopsis. Front Plant Sci 7:1–15
Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

Jasmeet Kaur Abat did her Ph.D. with Prof. Renu Deswal. She is an Assistant Professor in Gargi College, University of Delhi. She initiated Nitric oxide research and contributed significantly by analyzing nitrosylation and its effect on cold stress signaling.

Renu Deswal did her Ph.D. with the Editor from JNU on "Purification, characterization and regulation of Glyoxalase I from *Brassica juncea*." Currently, she is a Professor in Department of Botany, University of Delhi, India. Her area of expertise is functional genomics. Majorly, proteomics tools are being used to understand abiotic stress signaling (cold/freezing stress). Nitric oxide signaling and antifreeze proteins along with nano-biotechnology are other areas of her interest. She is a visiting faculty to the University of Western Ontario, Canada, is a recipient of Biotechnology fellowship from the Government of Germany, and was also provided Department of Biotechnology, GOI, Overseas Fellowship.

A Tale of Sugars and Hormones: Perception and Responses

13

Muhammed Jamsheer K, Sunita Jindal, Mohan Sharma, Manvi Sharma, Dhriti Singh, Archna Tiwari, Harshita B. Saksena, Bhuwaneshwar Mishra, Sunita Kushwah, Zeeshan Z. Banday, and Ashverya Laxmi

Abstract

The survival of organisms is dependent on the perception of various external and internal cues and modulating growth according to the available conditions. This is achieved through highly coordinated and interconnected signalling pathways which are highly complex in eukaryotic systems. In order to circumvent the sessile nature, plants are evolved to have enhanced plasticity and robust environmental sensing mechanisms. Sugars produced by the plants are perceived by a dedicated set of receptors which leads to the modulation of the specific signalling pathway to ultimately fine-tune plant growth and defence responses according to the sugar and

Equal first authors: Muhammed Jamsheer K and Sunita Jindal
Equal contributing authors: Mohan Sharma, Manvi Sharma, Dhriti Singh, Archna Tiwari, Harshita B. Saksena, Bhuwaneshwar Mishra, Sunita Kushwah and Zeeshan Z. Banday

Muhammed Jamsheer K
National Institute of Plant Genome Research, New Delhi, India

Amity Food & Agriculture Foundation, Amity University, Noida, Uttar Pradesh, India

S. Jindal · M. Sharma · M. Sharma · D. Singh · A. Tiwari · H. B. Saksena
B. Mishra · A. Laxmi (✉)
National Institute of Plant Genome Research, New Delhi, India
e-mail: ashverya_laxmi@nipgr.ac.in

S. Kushwah
National Institute of Plant Genome Research, New Delhi, India

Umeå Plant Science Centre, Umeå, Sweden

Z. Z. Banday
National Institute of Plant Genome Research, New Delhi, India

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, USA

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_13

energy availability. Different phytohormone signalling pathways which originated at different facets of plant evolution play a pivotal role in controlling the growth, development and defence strategies. Research in the past two decades uncovered the extent of interaction of sugar and phytohormone signalling pathways in controlling and fine-tuning various plant growth and stress responses. The following chapter concisely summarizes the molecular and physiological interaction of different sugar signalling pathways with hormone signalling pathways which is ultimately important in the regulation of plant development and stress responses.

Keywords
Energy signalling · Hexokinase 1 · Phytohormones · Regulators of G-protein signalling · Signalling crosstalk · SNF-related protein kinase 1 · Sugar signalling · Target of rapamycin (TOR)

13.1 Introduction

The sustenance of life on earth is heavily dependent on the production of sugars by plants. Sugars produced by green plants reach to heterotrophic organisms through the food chain. Sugar and energy status regulate almost all processes of life from growth, reproduction, defence and ageing (Rolland et al. 2001). Organisms need to sense the fluctuations in the sugar availability to rearrange growth to improve their adaptability. Consistent with this, eukaryotes share many highly conserved signalling pathways across the lineage to optimize growth according to the energy availability. Although sugar is transported majorly as sucrose (Suc) from source to sink, glucose (Glc) emerged as the most potent signalling sugar even in plants (Rolland et al. 2001; Ramon et al. 2008).

Sugars are important structural constituents of the plant cell. Being autotrophic, plants produce sugars by photosynthesis. The source-to-sink transport of sugars, consumption for the energy production (i.e. respiration) and its utilization for the biosynthesis of other macromolecules is a tightly coordinated process (Rolland et al. 2006; Ramon et al. 2008). Owing to its importance in all aspects of plant life, sugar status was found to regulate seed germination, seedling development and root and shoot development, flowering and senescence (Ramon et al. 2008). Further, sugar status works as a key signal which controls different developmental transitions in plant lifecycle such as heterotrophic to photoautotrophic transition at the early seedling stage, juvenile-to-adult phase transition at the late seedling stage and adult-to-reproductive transition which leads to flowering (Seo et al. 2011; Wahl et al. 2013; Yang et al. 2013; Yu et al. 2013). Apart from all these, sugar status is also an important determinant in regulating the defence strategies of the plant against both biotic and abiotic factors (Ramon et al. 2008; Bolouri Moghaddam and Van Den Ende 2012). Thus, the sugar status and the coordination of sugar signalling are key regulators of plant productivity.

Phytohormones are key signalling molecules that can quickly alter the plant responses towards internal and external cues. Hormones such as auxin work as morphogen, and its maximal or minimal accumulation in particular tissue types drives organogenesis in plants (Finet and Jaillais 2012). However, signalling or biosynthesis of hormones which are majorly involved in stress responses (such as abscisic acid, jasmonates and salicylic acid) is rapidly enhanced in response to external threats (McSteen and Zhao 2008; Santner and Estelle 2009; Wang et al. 2015). Meticulous research uncovered the details of the perception and signalling mechanisms of all major phytohormones, and their role in controlling different aspects of plant growth and responses is well identified (Santner and Estelle 2009). Most of the plant hormone pathways originated in algae and bryophytes or during the origin of angiosperms which further developed into well-integrated pathways including receptors for perception and downstream signalling effectors. Further, many repressors also originated and coevolved with the hormone signalling pathways to fine-tune the responses (Wang et al. 2015). Thus a coordinated network of perception and signalling mechanism with communication between different hormone pathways controls plant growth and stress responses.

The communication of hormonal signalling with central nutrient pathway such as sugar signalling has emerged from numerous studies, and it is now well appreciated that sugar and hormonal pathways are intimately involved in controlling various plant responses towards endogenous and exogenous cues. The succeeding sections briefly discuss the different sugar perception pathways and how they coordinate with various hormonal pathways to regulate growth and survival strategies of plants. It is noteworthy that although the function of individual sugar-sensing pathways is explored in great detail, how these different pathways interact at the molecular level to optimize the growth is yet to be explored.

13.2 Sugar Sensing and Signalling Mechanisms in Plants

Physiological studies identified that although Glc promotes growth in general, very high concentrations (such as 6%) cause developmental arrest of the *Arabidopsis* seedlings (Jang et al. 1997; Ramon et al. 2008). This phenotype was later exploited to identify the mutants defective in Glc signalling (Ramon et al. 2008). Studies using this strategy revealed two distinct glucose-sensing mechanisms in plants. Forward genetic screens identified that hexokinase 1 (HXK1), the very first enzyme of glycolytic pathway, works as a glucose sensor independent of the catalytic activity (Moore et al. 2003). The REGULATOR OF G-PROTEIN SIGNALLING 1 (RGS1), an atypical component of G-protein signalling, was also identified as a glucose sensor in plants (Chen and Jones 2004). A highly conserved energy-sensing pathway also exists in eukaryotes where two antagonistic serine/threonine kinases, SNF1-RELATED PROTEIN KINASE 1 (SnRK1) and TARGET OF RAPAMYCIN (TOR), regulate the growth according to the energy availability (Broeckx et al. 2016; Dobrenel et al. 2016).

13.2.1 Hexokinase 1-Dependent Glucose Signalling

The isolation of *glucose-insensitive 2* (*gin2*) mutant which is impaired in Glc sensing without affecting the catalytic activity of HXK1 identified that the Glc sensing and the catalytic activity are undertaken by different modules of the protein (Moore et al. 2003). In the abundant light conditions, *gin2* plants show smaller leaves, petiole and root system and reduced number of flowers and siliques indicating that HXK1-dependent signalling is involved in the Glc-dependent acceleration of growth in favourable growth conditions. Glc influences the expression of a wide variety of genes, and the HXK1-dependent pathway is majorly implicated in the regulation of Glc-dependent gene expression (Ramon et al. 2008). The Glc regulation of expression of genes involved in photosynthesis, nitrate assimilation, aliphatic glucosinolate biosynthesis, RNA turnover and starvation and stress response was found to be dependent on this pathway (Jang et al. 1997; Moore et al. 2003; Lin et al. 2011; Miao et al. 2013; Kunz et al. 2015). Although predominantly cytosolic, HXK1 is also found in the nucleus where it forms a complex with the 19S regulatory particle of proteasome subunit (RPT5B) and vacuolar H+-ATPase B1 (VHA-B1). This complex binds to the promoters of the Glc-regulated genes suggesting their role in transcriptional regulation. In agreement with their role in Glc signalling, mutants of RPT5B and VHA-B1 show phenotypes similar to *gin2* (Cho et al. 2006). Apart from the regulation of gene expression, HXK1-dependent Glc signalling pathway is also involved in many other Glc-regulated processes such as sugar-mediated stomatal closure and Glc-dependent degradation of ETHYLENE-INSENSITIVE 3 (EIN3), a major transcription factor in the ethylene signalling (Yanagisawa et al. 2003; Kelly et al. 2013). Functional analysis in tobacco and rice suggests that the role of HXK1 as a Glc sensor is conserved across the plant lineage (Cho et al. 2009; Kim et al. 2013).

13.2.2 Regulator of G-Protein Signalling 1-Dependent Glucose Signalling

The plasma membrane-bound RGS1 which is a hybrid protein formed by the fusion of G-protein-coupled receptor (GPCR) and a C-terminal RGS box was also identified as an independent Glc sensor in *Arabidopsis* (Chen et al. 2003; Chen and Jones 2004). Glc causes a quick and transient enhancement of the interaction of RGS1 with G-PROTEIN ALPHA SUBUNIT 1 (GPA1) (Johnston et al. 2007). Glc also promotes concentration-dependent endocytosis of RGS1 which is mediated through the phosphorylation of RGS1 by WITH NO LYSINE KINASEs (WNKs) (Urano et al. 2012; Fu et al. 2014). This phosphorylation-dependent endocytosis of RGS1 accelerates the downstream G-protein signalling through GPA1. GPA1 regulate many diverse aspects of plant growth and development including cell division, elongation, organ development and hormone response (Urano et al. 2013). GPA1 also interacts with a chloroplastic protein, THYLAKOID FORMATION1 (THF1), which was also rapidly degraded by Glc (Huang et al. 2006).

The Glc-dependent expression of approximately 30 genes is perturbed in the mutant of RGS1 indicating that the RGS-dependent pathway is involved in the

regulation of a subset of Glc-regulated genes (Grigston et al. 2008). The altered expression of Glc-regulated genes was also observed in the mutants of other components of the RGS1 pathway described above indicating the involvement of whole signalling pathway in Glc response (Grigston et al. 2008; Urano et al. 2012). At the physiological level, the RGS1-dependent signalling components are essential for the sugar-mediated mitigation of salt stress (Colaneri et al. 2014). Analysis of the dependence of Glc-regulated gene expression on both HXK1 and RGS1 pathway identified a nuanced interaction where both synergistic and antagonistic interaction of both pathways is observed (Huang et al. 2015). The Glc-induced glucosinolate production is synergistically regulated by both pathways indicating the possible interaction between both Glc signalling pathways (Miao et al. 2013). However, more molecular studies are needed to dissect this interaction. Recently, a WD40-repeat protein, RGS1-HXK1 INTERACTING PROTEIN 1 (RHP1), was found to be interacting with both RGS1 and HXK1 (Huang et al. 2015). RHP1 is proposed to be a scaffolding protein of HXK1 and RGS1, and it might be important in mediating the molecular interaction between these pathways.

13.2.3 Energy-Sensing and Signalling Pathway Exists in Plants Too

Cellular respiration is directly related to the sugar availability to the organism. Although the source of sugar is different in green plants and heterotrophic organisms, they share a common energy-sensing pathway which is conserved in all eukaryotes (Roustan et al. 2016). In response to energy and nutrient abundance, the TOR pathway is activated which promotes all growth processes (Dobrenel et al. 2016). Depending on the components of the TOR complex, two types of complexes exist in mammals. The mTOR Complex 1 (mTORC1) contains mTOR, the REGULATORY-ASSOCIATED PROTEIN OF mTOR (RAPTOR) and mammalian LETHAL WITH SEC13 PROTEIN 8 (mLST8), and this complex is majorly responsible for the energy-dependent promotion of protein synthesis (Ma and Blenis 2009). The mTOR Complex 2 (mTORC2) also possesses mTOR and mLST8. Apart from these components, it also contains RAPAMYCIN-INSENSITIVE COMPANION OF MTOR (RICTOR) and mammalian STRESS-ACTIVATED PROTEIN KINASE INTERACTING PROTEIN 1 (mSIN1). mTORC2 is a major regulator of actin cytoskeleton organization (Sarbassov et al. 2004). Plants possess the homologues of mTORC1, and similar to its role in mammals, this complex regulates the energy-dependent protein synthesis (Deprost et al. 2007; Ren et al. 2012; Dobrenel et al. 2016). The plant TOR kinase is also implicated in the regulation of general transcription and E2 FACTOR (E2F)-mediated transcription during cell cycle progression (Ren et al. 2011, 2012; Xiong et al. 2013; Kim et al. 2014). Phenotypic analysis of the mutants and overexpression of *TOR*, *RAPTOR* and *LST8* and downstream components identified that this pathway is essential for diverse processes of plant growth including embryo development, photoautotrophic transition, root and shoot growth, root hair and silique development, etc. (Menand et al. 2002; Deprost et al. 2007; Ren et al. 2011, 2012; Moreau et al. 2012; Caldana et al. 2013; Schepetilnikov et al. 2013; Xiong et al. 2013). The interaction

between TOR and various phytohormone pathways is recently emerging indicating that the TOR pathway in plants underwent significant evolutionary changes to rewire the pathway according to the lifestyle of plants (Schepetilnikov et al. 2013, 2017; Zhang et al. 2016; Song et al. 2017; Xiong et al. 2017).

SnRK1 directs adaptive responses of plants in response to energy deficit (Baena-González et al. 2007; Broeckx et al. 2016). The homologues of SnRK1 are known as AMP-ACTIVATED PROTEIN KINASE/SUCROSE NON-FERMENTING (AMPK/SNF1) in mammals and yeast, respectively. This conserved eukaryotic energy gauge originated in a common eukaryotic ancestor (Roustan et al. 2016). Although there are slight differences in the activation mechanisms, their role as the master regulator of growth during energy starvation is highly conserved across different eukaryotic lineages (Broeckx et al. 2016; Roustan et al. 2016). It is an obligate heterotrimeric serine/threonine kinase complex composed of α kinase and β and γ regulatory subunits (Broeckx et al. 2016). In plants, a hybrid βγ subunit evolved by the fusion of specific domains from β and γ subunits works as the canonical γ subunit (Ramon et al. 2013). Depending on the tissue types, different isoenzyme complexes contribute to the formation of the heterotrimeric enzyme (Emanuelle et al. 2015). During energy deficit, through a series of phosphorylation events, SnRK1 attenuates the energy-consuming processes and promotes energy-producing processes including photosynthesis (Baena-González et al. 2007). SnRK1 works as a central hub complex and interacts and directs the activity of various proteins which include other kinases, transcription factors, enzymes, etc. (Broeckx et al. 2016). In response to energy starvation, AMPK/SnRK1 inhibits TOR activity by phosphorylating RAPTOR which results in its dissociation from the TOR complex (Gwinn et al. 2008; Nukarinen et al. 2016). Through this direct phosphorylation and many intermediate regulatory proteins, AMPK/SnRK1 negatively regulates energy-dependent protein synthesis (Ma and Blenis 2009; Nukarinen et al. 2016). Phenotypic analysis of mutant and overexpression of SnRK1 subunits identified that this pathway is essential in the regulation of seedling growth, flowering time, reproductive development and senescence in plants (Baena-González et al. 2007; Gao et al. 2016). SnRK1 is also found to be important in the mitigation of various abiotic stresses such as submergence, salt, osmotic, oxidative and drought stress (Cho et al. 2012, 2016; Chen et al. 2017; Soto-Burgos and Bassham 2017).

The available evidences indicate that antagonistic interaction of TOR and SnRK1 optimizes plant growth according to energy availability. This antagonism is also observed in the regulation of autophagy where SnRK1 works as a promoter of autophagy, while TOR inhibits it (Liu and Bassham 2010; Chen et al. 2017; Pu et al. 2017; Soto-Burgos and Bassham 2017). Pathogens utilize the TOR pathway to colonize on plants, while SnRK1 pathway predominantly restricts pathogen attack (Schepetilnikov et al. 2011; Hulsmans et al. 2016; Meteignier et al. 2017; De Vleesschauwer et al. 2018). Although the antagonistic interaction of TOR and SnRK1 is evident at the downstream level, how the activity of these kinases is regulated at the molecular level in response to energy remains a mystery. Recently, a novel class of zinc finger proteins named FCS-like Zinc finger proteins has been identified which interact with both SnRK1 and TOR complex and participate in the arms race between these kinases in plants (Jamsheer and Laxmi 2014, 2015;

Nietzsche et al. 2014, 2016; Jamsheer et al. 2018). Further, elucidation of the interaction between SnRK1-TOR signalling with HXK1- and RGS1-dependent Glc sensors will provide a more comprehensive picture of the intricate network of sugar signalling in plants.

13.2.4 Other Sugar Signalling Pathways

The disaccharide trehalose and its sugar-phosphate trehalose-6-phosphate (T6P) are already implicated in the regulation of many growth and stress responses in plants (Tsai and Gazzarrini 2014). Compared to Glc and sucrose, these compounds are present in the plants in very low amounts; however, T6P inhibits SnRK1 activity in micromolar concentrations indicating that the trehalose signalling network functionally interacts with the SnRK1 signalling pathway in plants (Zhang et al. 2009; Broeckx et al. 2016). An independent fructose-sensing mechanism is also proposed in plants where a FRUCTOSE-1,6-BISPHOSPHATASE (FBP) is implicated as a pivotal regulator of this signalling pathway (Cho and Yoo 2011). Although the existence of a sucrose sensing pathway is yet to be established, some molecular and physiological responses in plants were found to be sucrose-specific (Tognetti et al. 2013). A remarkable example is the conserved Upstream Open Reading Frame (uORF)-mediated translation repression of S1-group bZIP transcription factors by sucrose (Peviani et al. 2016). The uORFs are small ORFs upstream of the main ORF which inhibit the translation of the main ORF through ribosome stalling (von Arnim et al. 2014). Sucrose causes the repression of the translation of S1-group bZIP transcription factors through the upstream uORFs (Rook et al. 1998; Wiese et al. 2004; Rahmani et al. 2009). Intriguingly, Glc and fructose were ineffective in inducing this response indicating that plants may also possess a distinct sucrose sensing and signalling pathway (Rook et al. 1998).

13.3 Interaction of Sugar and Phytohormone Signalling Pathways

13.3.1 Sugars and Auxin

Auxin regulates many developmental processes in plants. It is generated in the shoot apical region and is transported basipetally. However, both acropetal and basipetal auxin transport occur in root. This auxin transport is facilitated by a number of plasma membrane-bound auxin transport proteins such as AUXIN RESISTANT 1 (AUX1) which facilitates auxin influx into the cell, whereas PIN-FORMED PROTEIN (PIN) and ATP-BINDING CASSETTE B/P-GLYCOPROTEIN (ABCB/PGP) export auxin out of the cell. Auxin response is regulated by the auxin co-receptors of the TRANSPORT INHIBITOR RESPONSE 1-AUXIN SIGNALLING F-BOX (TIR1-AFB), Aux/IAA family of auxin signalling repressors and transcription factors of the AUXIN RESPONSE FACTORs (ARFs) family. In the absence or low auxin levels, Aux/IAA proteins heterodimerize with ARFs and, therefore, repress the expression of auxin-responsive genes. Presence of auxin facilitates the proteolysis of these Aux/IAA

repressors through SKP1/CULLIN1/F-BOX (SCF) TIR1/AFB-type 3 ubiquitin ligase complex and releases ARFs for the induction of auxin-responsive genes.

In the past decade, the first link between the interaction of sugar and auxin signalling emerged from the study of Glc-insensitive mutant *gin2* which showed resistance to exogenous auxin (Moore et al. 2003). In another report, the role of turanose-insensitive mutant (*tin*) in maintaining auxin homeostasis by increasing auxin biosynthesis and repressing its conjugation in root quiescent centre has been shown (Gonzali et al. 2005). *HOOKLESS 1* (*HLS1*) regulates apical hook formation in dark grown seedlings (Lehman et al. 1996). Lack of functional *HLS1* resulted in hypersensitivity to exogenous sucrose and negatively regulated auxin-induced *AUXIN UPREGULATED 3* (*AUR3*) expression (Ohto et al. 2006). There are reports which suggested the role of heterotrimeric G-proteins in auxin- and sugar-mediated lateral root development (Mudgil et al. 2009). Several reports also suggested the role of auxin and sugar signalling in regulating root system architecture and shoot development (Mishra et al. 2009; Kircher and Schopfer 2012; Mudgil et al. 2009, 2016). Under iron deficiency, sucrose increases nitric oxide production and, therefore, facilitates iron uptake by roots through changing auxin signalling (Lin et al. 2016). Sugar-induced expression of *WOX7* negatively regulates lateral root initiation by directly repressing cell cycle gene *CYCD6.1*. In contrast, auxin represses *WOX7* expression in regulating lateral root development suggesting a fine tuning between auxin and sugar signalling in regulating lateral root initiation (Kong et al. 2016). *ABSCISIC ACID INSENSITIVE 5* (*ABI5*) regulates meristem size by reducing PIN1 accumulation through Glc (Yuan et al. 2014). Photosynthesis-generated sugars regulate auxin biosynthesis through PIF proteins (Sairanen et al. 2012). Among various sugars, Glc is an emerging player in controlling root and shoot directional responses (Mishra et al. 2009; Singh et al. 2014a, b; Gupta et al. 2015a, b). Application of exogenous Glc enhanced the root gravitropic response of auxin signalling and transport mutants. This suggests the involvement of Glc in modulating root directional response through alteration in auxin signalling (Mishra et al. 2009; Singh et al. 2014a, b). Whole-genome microarray study by Mishra et al. (2009) showed that 62% of auxin-affected genes were also regulated by Glc suggesting convergence between Glc and auxin signalling pathways. Lately, an atypical bHLH protein, *REGULATED BY SUGAR AND SHADE1* (*RSS1*), has been shown to regulate hypocotyl length elongation response by integrating Glc, light and auxin signalling (Singh et al. 2017).

Since SnRK1 and TOR kinases are directly implicated in energy-sensing processes, the functional connection between these kinases and auxin signalling has been demonstrated in several studies. The *snf1a snf1b* double mutants in *Physcomitrella* displayed hypersensitivity to auxin (Thelander et al. 2004). In response to low energy, bZIP class of transcription factors such as bZIP1, 11 and 53 was found to be involved in SnRK1-dependent metabolic reprogramming (Baena-González et al. 2007). Sucrose negatively regulates bZIP11 translation, whereas auxin-TOR-mediated signalling positively regulates bZIP translation through promotion of polysomal loading at bZIP11 mRNA (Schepetilnikov et al. 2017). Weiste et al. (2017) showed that bZIP11 negatively regulates root meristem by activation of *SHORT HYPOCOTYL 2* (*IAA/SHY2*) expression which decreases the expression of

auxin transporter PINs. Auxin promotes TOR kinase activity via activation of a small GTPase Rho-related protein 2 (ROP2) that leads to translation reinitiation of uORF-containing mRNA through RIBOSOMAL PROTEIN S6 KINASE 1 (S6K1) phosphorylation of elF3h (Schepetilnikov et al. 2013). Inactivation of TOR either by ATP competitive TOR Kinase inhibitor Torin-1 or through RNAi suppression abolished the auxin-TOR-dependent transcription re-initiation which led to defect in root gravitropism, suggesting that auxin-activated TOR signalling is vital for plant development (Schepetilnikov et al. 2013). Auxin controls cell cycle reactivation through binding of Lateral Organ Boundary (LBD) protein at *E2Fa* gene promoter to activate transcription (Berckmans et al. 2011). In the root apexes, Glc-mediated energy signalling is required to activate TOR kinase. On the contrary, both Glc and light stimuli are requisite to activate TOR kinase in shoot apexes. However, external auxin application could replace light signal for activation of TOR in shoot apexes in promotion of true leaf development. This suggests that low to high ratio of auxin in shoot and root apexes might be responsible for distinctive light requirement in shoot and root apexes. Light-auxin signal transduces via ROP2 to activate TOR kinase which, in turn, triggers transcription factors E2Fa and E2Fb for activation of cell cycle genes in shoot apexes (Li et al. 2017). Importantly, constitutive photomorphogenesis 1 (COP1) acts upstream to ROP2 in regulating auxin-ROP2-TOR signal in response to light (Cai et al. 2017). A molecular model linking sugar and auxin signalling pathways is shown in Fig. 13.1.

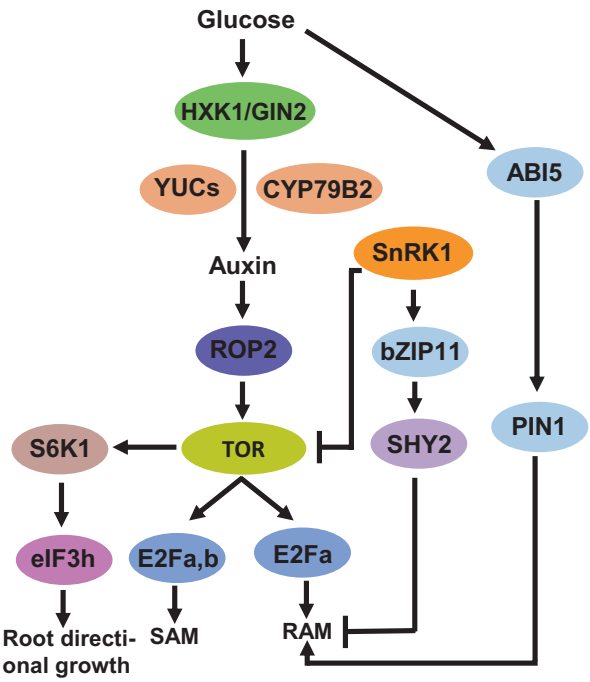

Fig. 13.1 Molecular crosstalk between sugar and auxin signalling pathways to regulate meristem activity and root directional growth

13.3.2 Sugars and Cytokinin

Cytokinins (CKs) are crucial in regulating various important developmental processes and responses of plants such as embryogenesis, seed development, organogenesis, vascular patterning, senescence and stress resilience (Kieber 2002). CK perception and signalling in plants are mediated by a multistep phosphorelay system which is a complex two-component signalling which has been described in detail in Chap. 10.

It is already well known that auxin and CK interact with each other extensively and their dynamics regulate various vital plant growth and developmental processes (Schaller et al. 2015). In literature, there are several reports which suggest a strong interaction between sugar (Glc) and CKs. Sugars and CKs are fundamental molecules in plants and modulate various similar processes. They can act both synergistically (Riou-Khamlichi et al. 2000; Hartig and Beck 2006; Kushwah et al. 2011) and antagonistically (Moore et al. 2003; Franco-Zorrilla et al. 2005) or independently (Aki et al. 2007). Most of the aspects of CK homeostasis from CK biosynthesis to degradation are influenced by Glc. Glc affects expression of CK-regulated genes both transcriptionally and nontranscriptionally. To find out Glc-CK interaction, Kushwah and Laxmi (2014) have done whole-genome transcript profiling with physiological analysis to identify the extent of overlap at gene expression level between these two components. In the study, they found that 76% of CK-regulated genes were transcriptionally affected by Glc at whole-genome level, out of which most of the co-regulated genes were agonistically regulated (approx. 89%). Various CK metabolism and signalling genes were regulated by Glc. CK and Glc commonly regulate a number of gene families involved in various developmental and stress processes. Kushwah et al. (2011) reported that CK-induced root directional growth response is increased with Glc application in the medium and increasing concentrations of Glc could also affect primary root length, gravitropic curvature of the roots, lateral root numbers and root hair (Mishra et al. 2009). Hypocotyl elongation of dark grown seedlings of *Arabidopsis* is regulated by both Glc and CK. Glc and CK act antagonistically at low Glc concentration but work synergistically at higher Glc concentrations for hypocotyl length regulation. Root growth in light, hypocotyl length in dark, chlorophyll and anthocyanin content, all these parameters could be regulated by both Glc and CK (Kushwah and Laxmi 2014). Zwack and Rashotte (2013) showed that CK regulated changes in sink/source-sugar relationships, which led to delayed senescence in plants.

Sugar and CK synergistically regulate cyclin D3 (CycD3) expression (Riou-Khamlichi et al. 2000; Hartig and Beck 2006), early seedling development (Németh et al. 1998; Salchert et al. 1998; Kushwah et al. 2011) and anthocyanin accumulation (Das et al. 2012). Sucrose hypersensitivity and CK resistance was observed in *cytokinin resistant 1* (*cnr1*) mutant (Laxmi et al. 2006). The *gin-2* mutant displayed delayed leaf senescence and hypersensitivity towards CKs for shoot regeneration (Moore et al. 2003), putting forward a strong interaction between CK and sugar. Taken together, these studies suggest that sugars and CKs extensively interact during plant growth and developmental processes, and these interactions can be both direct and indirect, and involve cell-specific and long-distance interactions.

13.3.3 Sugars and Ethylene

Ethylene is the chemically simplest plant hormone that controls various vital plant processes such as seed germination, root hair formation, flower senescence, abscission and fruit ripening (Johnson and Ecker 1998). The ethylene perception by the plant is mediated by a family of receptors which include ETHYLENE RESPONSE factors (ETR1 and ETR2), ETHYLENE RESPONSE SENSOR factors (ERS1 and ERS2) and ETHYLENE INSENSITIVE 4 (EIN4) in *Arabidopsis*, and out of these, the *etr1*, *etr2* and *ein4* have been identified as dominant ethylene-insensitive mutants (Hall et al. 2007). In the absence of ethylene, CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) is activated by the free receptors and negatively regulates ethylene signalling by phosphorylating EIN2 (Kieber et al. 1993; Ju et al. 2012). The *CTR1* loss-of-function mutant *ctr1* exhibits constitutive expression of ethylene signalling pathway genes (Kieber et al. 1993). The *ein2* null mutants are completely ethylene unresponsive throughout the plant development (Alonso et al. 1999).

In wild-type *Arabidopsis*, a high Glc concentration blocks the post-germination seedling development. However, the Glc-insensitive mutants such as *gin1*, *gin4*, *gin5*, *gin6*, etc. have impaired Glc-induced developmental arrest, and they germinate and develop on higher doses of Glc indiscriminately to a normal sugar dose (Arenas-Huertero et al. 2000). The *gin1-1* seeds germinate faster, and the plant has darker green rosettes (Zhou et al. 1998). These symptoms are phenocopied by constitutive ethylene biosynthesis and signalling mutants *eto1* and *ctr1*, respectively, and also by ACC treatment of wild-type plants (Zhou et al. 1998). The ethylene overproducer mutants *eto1* and *eto3* have elevated ethylene biosynthesis owing to different posttranscriptional regulation of ACS (Woeste et al. 1999). Among the other Glc-insensitive mutants, *gin4* also phenocopies the *ctr1* mutant suggesting that *gin* mutants are allelic to the *ctr1*. The ethylene-insensitive mutant *etr1-1* however, shows an opposite Glc response as compared to the *gin1* mutant suggesting the antagonistic crosstalk between Glc and ethylene (Zhou et al. 1998). Ethylene has an inhibitory but reversible effect on photosynthesis (Kays and Pallas 1980) which could be the result of ethylene-induced senescence and thus breakdown of the photosynthetic machinery. However, *Arabidopsis* and tobacco ethylene-insensitive genotypes are deficient in Rubisco content and photosynthetic capacity probably because of their delayed senescence (Tholen et al. 2004, 2008). The functional links between ethylene and cellular energy sensors TOR and SnRK1 have begun to emerge in recent years (Fig. 13.2). In a study by Dong et al. (2015), application of AZD, an active-site TOR inhibitor, upregulated the expression of genes encoding the ETHYLENE RESPONSE FACTOR (ERF), ETHYLENE RESPONSE ELEMENT-BINDING FACTOR (ERE-BF) and ethylene biosynthetic enzymes indicating the antagonistic interaction of TOR and ethylene. Further, it is reported that ethylene-inducible hypocotyl growth is suppressed by PSII deficiency-inducible SnRK1α1 in *Arabidopsis*. The SnRK1directly interacts, phosphorylates and destabilizes EIN3, the key transcription factor in ethylene signalling (Kim et al. 2017).

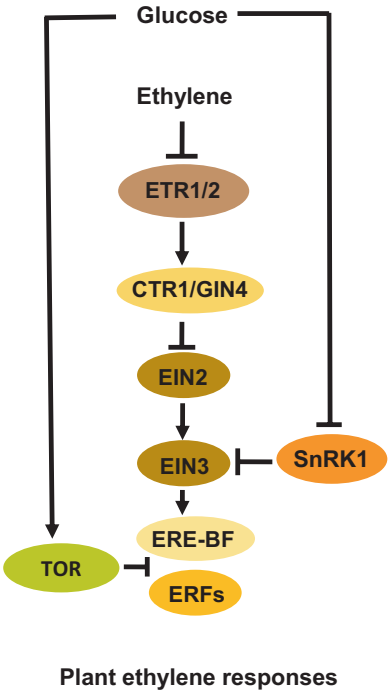

Fig. 13.2 Regulation of the ethylene signalling pathway by sugar signalling components

13.3.4 Sugars and Abscisic Acid

Plants mitigate abiotic stresses such as cold, drought, salt and wounding through an adaptive process that chiefly includes ABA which is biosynthesized from β-carotene in several enzymatic steps. A minimal amount of ABA is always present in the plant, but its level is elicited during stress challenges that entail triggering ABA biosynthetic pathway genes (Tuteja 2007). These genes correspond to zeaxanthin epoxidase (*ZEP*; *ABA1* in *Arabidopsis*), 9-cis-epoxycarotenoid dioxygenase (*NECED*), xanthoxin dehydrogenase (*ABSCISIC ACID DEFICIENT2/GLUCOSE INSENSITIVE1*) (Cheng et al. 2002) and ABA-aldehyde oxidase (*AAO*). The early ABA signal transduction employs PYR/RCAR-PP2C-SnRK2 module where PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) are ABA-binding receptors; PP2Cs are phosphatases that negatively regulate early ABA signalling pathway (Finkelstein 2013).

Several sugar signalling mutants like *gin1*, *gin5*, *gin6*, etc. have lower levels of endogenous ABA as evidenced by several biochemical and physiological studies. The *gin1* mutant is defective in the biosynthetic gene *ABA DEFICIENT 2* (*ABA2)* (Cheng et al. 2002) which attributes to the lower endogenous ABA levels in *gin1* plants. Consistent with this, many ABA-related mutants such as *aba1*, *aba2* and *aba3* display *gin*-like phenotype. Mutations in sugar signalling-related and ABA biosynthesis and signalling genes are mapped closely and display allelism (Cheng et al. 2002). Glc activates *ABA2* transcript at 2% concentration in wild-type *Arabidopsis* but not in the ABA-deficient *aba2*/*gin1* mutants establishing that activation of ABA biosynthesis

synergistically requires Glc and ABA (Cheng et al. 2002). Among other sugar mutants, *gin6*, *isi3*, *sis5* and *sun6* exhibit allelism to ABA-induced transcription factor ABSCISIC ACID INSENSITIVE 4 (ABI4) mutant *abi4* (Arenas-Huertero et al. 2000). ABI4 directly binds to the ABI4 binding motif of many target genes and activates or represses their expression. One of such ABI4-inducible transcription factors is ANAC060 which is encoded by a quantitative trait locus (QTL) responsible for sugar sensitivity in *Arabidopsis*. It is present in long and short versions because of differential mRNA splicing caused by a single-nucleotide polymorphism (SNP). The shorter version of the ANAC060 lacks the transmembrane domain (TMD) leading to its nuclear retention. It is involved in a negative feedback loop to regulate sugar-ABA signalling. ABI4 induces ANAC060 expression, but its nuclear retention leads to inhibition of Glc-induced ABA accumulation and ABI4 expression thereby leading to reduced sugar responsiveness (Li et al. 2014a). ABA signalling pathway also interacts with the TOR-SnRK pathway in order to integrate stress and growth. *Arabidopsis* seedlings overexpressing SnRK1α1 exhibit ABA-hypersensitivity which is further enhanced upon Glc addition to the media. The *35S:SnRK1.1-3* × *gin1-1* seedlings however show Glc-insensitive phenotype same as *gin1-1* mutant on 6% Glc suggesting that SnRK1.1-induced ABA-hypersensitivity response requires ABA and that ABA2/GIN1 and SnRK1α1are epistatic (Jossier et al. 2009). SnRK1 and SnRK2, which are implicated in metabolic and stress signalling, respectively, are dephosphorylated by PP2Cs. PP2Cs directly interact with the catalytic subunit of SnRK1 in the absence of ABA and cause its dephosphorylation and thus deactivation. The *pp2c* knockout mutant displays SnRK1α1 overexpression-like characteristics (Rodrigues et al. 2013). Similarly, the SnRK2 dephosphorylation by PP2Cs represses its downstream signalling and ABA responses. In the presence of ABA, the ABA-bound PYR1/RCAR interacts with PP2C which sets SnRK2 free and allows phosphorylation of downstream protein targets (Finkelstein 2013). Conversely, TOR inhibition by AZD leads to expression remodeling of 19 ABA signalling pathway genes, out of which, 18 are upregulated (Dong et al. 2015). Consistent with this, *Arabidopsis raptor1b*, a mutant of TOR interactor RAPTOR1B, accumulates significantly higher amount of ABA in seeds as compared to wild type, and the *raptor1b* seedlings are hypersensitive to even extremely low amounts of ABA leading to germination deterioration (Salem et al. 2017). To balance plant growth and stress responses, a mutual regulation process is employed wherein the stress response is kept under check during unstressed conditions, whereas upon stress perception, the growth is minimized. In order to achieve this, the growth promontory TOR phosphorylates the ABA receptor PYL preventing its activation during stress-free conditions, while stress- and ABA-induced SnRK2 phosphorylates RAPTOR to suppress the TOR function in *Arabidopsis* (Wang et al. 2018). Figure 13.3 depicts the interaction between glucose and ABA signalling pathways.

ABA and ethylene share a subset of functions with opposite effects to antagonistically fine tune the plant processes like seed germination and early seedling establishment (Zhou et al. 1998). The enhanced response to ABA3 mutant (*era3*) is allelic to *ein2* which overaccumulates ABA and also some ethylene-response mutants show alterations in ABA sensitivity (Ghassemian et al. 2000). The ABA-deficient mutants *Arabidopsis aba2* and tomato *flacca* and *notabilis* are overproducers of ethylene (Ghassemian et al. 2000). In *Arabidopsis*, ABA antagonizes ethylene by

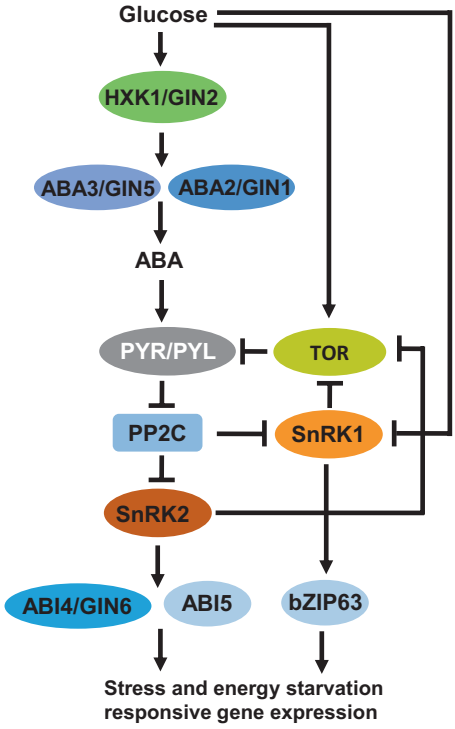

Fig. 13.3 Molecular crosstalk between ABA and sugar signalling pathways to balance growth and stress responses

transcriptional repression of ACS4 and 8 through ABI4 (Dong et al. 2016). Thus, a close interplay of ethylene and ABA signalling controls plant growth, development and stress mitigation.

13.3.5 Sugars and Gibberellins

Chemically, gibberellins (GAs) are the group of cyclic diterpenoid carboxylic acids that are essential for different developmental processes such as germination, enzyme induction, leaf expansion, stem elongation, trichome development and flowering. In higher plants, GAs are synthesized by the action of terpene synthases (TPSs), cytochrome P450 monooxygenases (P450s) and 2-oxoglutarate-dependent dioxygenases (2ODDs), localized in plastids, the endomembrane system and the cytosol, respectively. A soluble, nuclear-localized GIBBERELLIN INSENSITIVE DWARF1 (GID1) protein has been identified as GA receptor in rice (Ueguchi-Tanaka et al. 2005). Other components of GA signalling are DELLA protein and an F-box protein SLEEPY1 (SLY1). DELLAs are plant-specific GRAS family transcription regulators which inhibit plant growth by triggering transcriptional reprogramming of genes involved in cell division, expansion and differentiation. However, canonical DNA-binding domain is absent in DELLA proteins. Several genetic and biochemical studies showed that DELLA proteins regulate molecular and developmental processes through direct interaction with diverse classes of regulators such as PHYTOCHROME

INTERACTING FACTORs (PIFs) (de Lucas et al. 2008; Feng et al. 2008), JASMONATE ZIM-DOMAIN (JAZ) (Hou et al. 2010), JASMONATE INSENSITIVE1 (JIN1/MYC2) (Hong et al. 2012; Wild et al. 2012), BRASSINAZOLE RESISTANT1 (BZR1) (Bai et al. 2012) and chromatin remodeling enzyme PICKLE (PKL) (Zhang et al. 2014; Park et al. 2017). Binding of GA with GID1 facilitates the interaction of GID1 with DELLA protein and targets them for degradation by proteasome. Interaction of DELLA proteins with GA-GID1 causes a conformation changes in GRAS domain of DELLA protein which enhances its recognition by F-box proteins SLY1/GID2 of SCF complex (Hirano et al. 2010). Subsequently, the $SCF^{SLY1/GID2}$ complex promotes ubiquitylation of DELLA, which leads to the degradation by the 26S proteasome. The degradation of DELLA proteins releases the inhibitory effect consequently allowing GA regulated growth and development to resume.

GA and sugar interaction during seed germination and anthocyanin accumulation has been well studied. GA antagonistically interacts with Glc in regulating seed germination (Yuan and Wysocka-Diller 2006). GA has been shown to have a positive effect on seed germination by inducing the expression of enzyme involved in reserve food mobilization. Glc affects the GA-mediated α-amylase expression in barley embryos which leads to the mobilization of the reserve food (Perata et al. 1997). During seed germination, stored starch is degraded by the action of α-amylase into sugars to provide energy and materials for embryo growth. When sugar supply exceeds the demand of the sink cells, α-amylase expression is repressed via a process involving sugar sensing. Several studies show that sugar and GA regulate gene expression by sharing the same *cis*-regulatory element (Morita et al. 1998; Chen et al. 2002). Pyrimidine box and GARE motif are required for sucrose-dependent repression of the gene, while these elements are also involved in GA responsiveness (Washio 2003; Gubler and Jacobsen 1992). Furthermore, GA induced the expression of transcription factor MYBGA in endosperms which interacts with GARE element of α-amylase promoters and inhibits the sugar-dependent feedback repression of α-amylase genes in endosperms (Chen et al. 2006). A gibberellic acid-stimulated *Arabidopsis* (GASA) family protein, *AtGASA6*, functionally integrates the GA, sugar and ABA signalling in seed germination. ABA and Glc downregulated while GA upregulated the expression of *AtGASA6* in germinating seeds. The *AtGASA6*-overexpressing seeds germinated faster, whereas mutant seeds exhibited delayed seed germination on Glc, paclobutrazol (gibberellin biosynthesis inhibitor) and ABA (Zhong et al. 2015). These results suggest that *AtGASA6* is involved in Glc-GA signalling as a nodal point in regulating seed germination. Another report by Fennell et al. (2012) showed that a rare sugar D-allose inhibits the GA-mediated seed germination and early seedling development by inhibiting the expression of scaffold protein Receptor for Activated C Kinase 1A (RACK1A) in *Arabidopsis*. Exogenous application of paclobutrazol on sugar beet (*Beta vulgaris*) petioles inhibited the expression of neutral and vacuolar invertase genes, suggesting a role of GA in sugar metabolism. In addition, sugar, GA and light regulated the expression of *Rosa hybrida vacuolar invertase 1* gene (*RhVI1*) which in turn controls buds to grow out (Rabot et al. 2012, 2014). GA is also known to regulate expression pattern of the sugar transporter genes (Murcia et al. 2017). Interestingly a study by Kanno et al. (2016) showed that *Arabidopsis* sugar transport proteins AtSWEET13 and

AtSWEET14 were also involved in cellular GA uptake when expressed in yeast and *Xenopus* oocytes. Consistently, *sweet13 sweet14* double mutant shows a reduced GA transport as well as displays altered responses to GA during seed germination and seedling stages. The mutants of negative regulators of GA-signalling *rgl2* and *spy* were resistant to Glc-induced delay in seed germination (Yuan and Wysocka-Diller 2006) suggesting that sugar signalling may be involved in repression of GA signalling. Another novel finding in sugar-GA signalling interaction came from the study of Li et al. (2014b) suggesting that sucrose, but not Glc, stabilized the DELLA protein which in turn activates MYB75 expression and enhanced anthocyanin biosynthesis. All these studies together suggest that sugar and GA interact with each other at molecular and physiological level to regulate a number of common responses.

13.3.6 Sugars and Brassinosteroids

Brassinosteroids (BRs) are major growth-promoting hormones. They are a class of polyhyroxylated sterol derivatives and were discovered in pollen extract due to their growth promotional ability (Mitchell et al. 1970). Genetic, molecular and proteomic approaches have led to the discovery of major BR signalling pathway components and thousands of target genes (Clouse 2011; Sun et al. 2010; Wang et al. 2011; Zhu et al. 2013). BR binds to a receptor kinase BRASSINOSTEROID-INSENSITIVE 1 (BRI1) and its homologues BRI1 LIKE 1 and 3 (BRL1 and BRL3) (She et al. 2013) which functions in association with its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE/SOMATIC EMBRYOGENESIS RECEPTOR KINASE (BAK1/SERK3) (Li and Chory 1997; Li et al. 2002; Nam and Li 2002). Binding of BR to BRI1 induces partial BRI1 kinase activity resulting in its dissociation from BRI1 KINASE INHIBITOR1 (BKI1) and association with BAK1 leading to complete activation of the BRI1. BRI1 then phosphorylates BR SIGNALLING KINASES (BSKs) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1) to promote their binding to and phosphorylation of BRI1-SUPPRESSOR 1 (BSU1) phosphatase. Activated BSU1 dephosphorylates and thereby inactivates the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) which phosphorylate and deactivate two major transcription factors of BR signalling BRASSINAZOLE RESISTANT1 (BZR1) and BRI1-EMS1-SUPPRESSOR1/BRASSINAZOLE RESISTANT2 (BES1/BZR2). BR also causes proteasomal degradation of BIN2 through an F-box protein, KIB1. BIN2 inactivation relieves BZR1 and BES1 transcription factors and PP2A dephosphorylates them. Unphosphorylated BZR1 and BES1 regulate the expression of thousands of genes comprising 20% of the *Arabidopsis* genome (Guo et al. 2013a). These target genes have been shown to be involved in a large array of responses such as plant growth, stress responses and other signalling pathways such as light and almost all hormonal pathways (Saini et al. 2015; Guo et al. 2013a).

Early evidences of sugar and BR signalling came from the correlation between sugar levels and the expression level of BR-related genes. Szekeres et al. (1996) showed that high levels of sugar cause repression of an important brassinolide biosynthesis gene, CONSTITUTIVE PHOTOMORPHOGENIC DWARF (CPD). BR has also been reported to modulate tissue-specific source/sink regulation. They observed

that localized BR-dependent growth response of hypocotyl elongation zone of tomato seedlings coincides with specific induction of *Lin6* mRNA. This induction happens exclusively in corresponding tissues resulting in elevated uptake of sucrose via hexose monomers (Goetz et al. 2000). The *brassinosteroid, light, sugar (bls1)* mutant is hypersensitive to metabolizable sugars. This hypersensitivity can be rescued by application of exogenous BR, suggesting that sugar and BR might be interacting with each other (Laxmi et al. 2004). Metabolic study of tomato d^x mutant containing a defective gene of the brassinosteroid biosynthetic sterol reductase *DIMINUTO1* revealed that they have reduced levels of starch and various sugars, thus suggesting a link between BR biosynthesis and sugar level in plants (Lisso et al. 2006). Transgenic rice plants overexpressing C-22 hydroxylases that control BR levels produced more and heavier seeds with enhanced assimilation of Glc to starch in the seeds (Wu et al. 2008). Vicentini et al. (2009) showed a putative involvement of a LRR-RLK ScBAK1 in cellular signalling cascade mediated by high levels of sugars in bundle sheath cells of sugarcane leaves. A recent study reported that BR-regulated gene *EXORDIUM-LIKE 1* (*EXL1*) expression is also regulated by carbon and energy status; sugar starvation and anoxia induce its expression. The mutant *exl1* showed reduced survival under extended night/anoxia stress thus suggesting its important role in plant adaptation to carbon- and energy-limiting growth condition (Schröder et al. 2011). Plants contain many β-amylase-like proteins (BAMs), which are usually associated with starch breakdown and possess BZR1-type DNA-binding domains. In *Arabidopsis*, two BZR1-BAM proteins inversely regulate many BR-responsive genes. They might be involved in regulation of plant growth and development through BR and metabolic signal crosstalk (Reinhold et al. 2011).

Recently, there are reports suggesting a comprehensive crosstalk between Glc and BR signalling (Fig. 13.4). Glc and sucrose have been reported to antagonize the BR-mediated negative regulation of shoot gravitropism (Vandenbussche et al. 2011). Additionally, Gupta et al. (2012) showed that Glc antagonizes BR-induced randomization of hypocotyl growth of etiolated *Arabidopsis* seedlings. They also showed that Glc

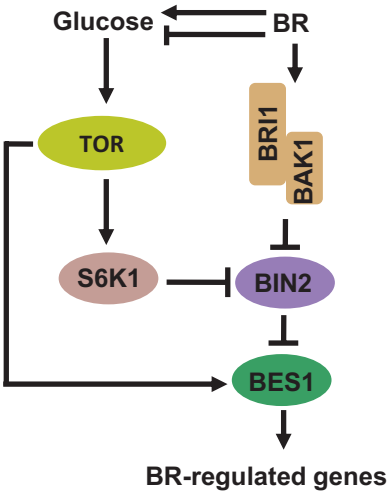

Fig. 13.4 Molecular crosstalk between sugar and brassinosteriod signalling pathways to regulate growth responses

inhibits BR-regulated gene expression and antagonizes BR-induced microtubule changes and cell-patterning across the hypocotyl, whereas BR enhances Glc-mediated root growth deviation from vertical suggesting a synergistic relationship between them in regulation of root direction growth. Glc treatment has been shown to enhance the BRI1 endocytosis in early endosomes leading to its increased accumulation. This may result in enhanced BR signalling and thus more deviation from vertical (Singh et al. 2014a, b). Glc and BR also interact with each other during lateral root production/emergence. BR works downstream to Glc in regulation of lateral root emergence as well as lateral root density (Gupta et al. 2015b). Sugar has been shown to induce hypocotyl elongation of *Arabidopsis* seedlings in dark with the help of BR which induces BZR1 transcription and promotes the stability of the BZR1 protein. Evidences suggest that BR may act downstream to HXK1 in regulation of Glc-mediated hypocotyl elongation in dark (Zhang and He 2015). There are recent reports indicating interconnections between energy signalling and BR signalling pathways. TOR plays a key role in sugar-induced hypocotyl elongation in dark through activating BR pathway. Additionally, TOR signalling promotes the accumulation of BZR1. Similarly, under starvation conditions, TOR is inactivated which leads to BZR1 degradation through autophagy. Thus, TOR and BR signalling balance the growth with carbon availability and energy status (Zhang et al. 2016). HDA6, a histone deacetylase, can deacetylate BIN2 that inhibits its kinase activity, but Glc has been shown to enhance BIN2 acetylation (Hao et al. 2016). TOR has been shown to play a pivotal role in regulating the transition from heterotrophic to photoautotrophic growth in *Arabidopsis* via S6K2 and BIN2. In this signalling cascade, S6K2 works directly downstream to TOR and phosphorylate BIN2 which possibly downregulates BIN2 activity (Xiong et al. 2017).

Shoot and root gravitropism as well as lateral roots play a vital role in plant adaption in different environmental conditions. Hypocotyl growth direction and shoot gravitropism are important for seedling growth in soil. Root growth direction and lateral root density play key roles in root system architecture and therefore are important for anchorage and water and nutrient uptake. Root growth direction also helps the plant to escape various adverse conditions such as water shortage, heat, nutrient limitation and pathogen. Root growth direction and lateral root density help plants to optimize their water and nutrient uptake under different environmental conditions such as drought and salt stress. All these reports suggest that Glc and BR interact with each other to modulate these parameters and thereby enhance the plant plasticity and adaptability. Further they play a key role in optimizing growth according to energy status of the cell, thus promoting the plant fitness.

13.3.7 Sugars and Jasmonic Acid

Jasmonic acid (JA) and its derivatives, collectively called jasmonates (JAs), are oxylipin compounds involved in a plethora of plant growth and developmental processes as well as biotic and abiotic challenges (Wasternack and Hause 2013). In recent times, remarkable progress has been made to understand JA signalling. The bioactive ligand jasmonoyl-isoleucine (JA-Ile) (Fonseca et al. 2009b) is perceived by F-box protein CORONATINE-INSENSITIVE1 (COI1) (Feys et al. 1994;

Fonseca et al. 2009a, b; Sheard et al. 2010) and JASMONATE-ZIM domain (JAZ) repressor proteins leading to the proteasomal degradation of the latter (Chini et al. 2007; Thines et al. 2007; Yan et al. 2007). This frees MYC2 and its homologs from repression which then binds to G-box element present in jasmonate responsive genes leading to downstream signal transduction (Fernández-Calvo et al. 2011).

The interaction between sugars and JAs has only recently begun to be understood. Initial accounts suggest MeJA and sugars to be synergistic regulators of vegetative storage protein (VSP) expression in *Glycine max*. When either of the inducers is limiting, VSP mRNA accumulation is inhibited (Mason et al. 1992). Anthocyanins are antioxidant molecules that protect plants from reactive oxygen species (ROS) and are also a rich source of abundant nutrition. Various reports have revealed a synergistic effect of JA and sucrose on anthocyanin accumulation. In *Arabidopsis*, JA enhanced the sucrose-induction of expression of genes involved in anthocyanin biosynthesis including *PRODUCTION OF ANTHOCYANIN PIGMENT 1* and *2* (*PAP1* and *PAP2*) (Loreti et al. 2008). The results also indicated the role of COI1 in sucrose-dependent signalling to modulate anthocyanin production, as there was no induction of anthocyanin biosynthetic genes in *coi1* mutant as compared to WT when treated with either sucrose or combination of JA and sucrose. This suggests the convergence of two signalling pathways to govern the response. Recent reports have demonstrated the involvement of SnRK1 in sucrose-induced anthocyanin accumulation (Liu et al. 2017; Baena-González et al. 2007). Liu and co-workers have formulated a molecular mechanism showing that *MdSnRK1.1* phosphorylates and destabilizes *MdJAZ18* protein, thus releasing MdbHLH3 TFs to promote anthocyanin biosynthesis (Liu et al. 2017).

JAs have been shown to have a positive effect on aliphatic and indolic glucosinolates synthesis in various plant species by activating various TFs such as MYBs and biosynthetic genes involved in glucosinolate biosynthesis. Sugar-induced glucosinolate accumulation has been accounted previously in *Arabidopsis* and broccoli sprouts. However, little research has been focussed on JA-sugar interplay in regulating glucosinolate accumulation. A finding by Guo et al. (2013b) suggests a synergism between the two in inducing glucosinolate accumulation. Genetic analyses have revealed the role of JAR1, COI1 and MYC2 in positively regulating the induction of glucosinolates by JA and Glc. Moreover, glucosinolate accumulation was reduced in Glc signalling mutants *rgs1-2* and *abi5-7* in the presence of Glc and JA treatments.

The functional connection between JA and cellular energy sensor TOR has recently begun to be understood. Global transcriptome analysis of cotton seedlings treated with TOR inhibitor AZD8055 has identified many key JA biosynthetic and signalling genes that are differentially expressed suggesting a potential crosstalk between TOR and JA signalling (Song et al. 2017). Also, TOR inhibited cotton seedlings showed enhanced endogenous JA levels. *Arabidopsis* synthesis and perception mutants including *jar1*, *coi1-2* and *myc2-2* were shown to be insensitive to AZD treatment, whereas *jaz10* and COI1ox showed growth-retarding effects of TOR inhibition (Song et al. 2017). All these observations suggest the negative influence of TOR on JA signalling. Another finding uncovers TOR as a negative regulator of plant immunity and antagonizes plant defences by interfering with JA and SA (De Vleesschauwer et al. 2018). Rice suspension cells infected with virulent Xoo cultures when treated with rapamycin showed increased resistance to MeJA treatment and a strong upregulation

of JA marker genes *JiPR10* and *JaMYB*, thus suggesting TOR acting as a negative regulator of plant defence (De Vleesschauwer et al. 2018). In summary, there are quite a few developmental processes controlled by either antagonistic or synergistic action of JA and Glc. Further molecular and physiological works are required to dissect out the broad interaction between these two signalling molecules.

13.3.8 Sugars and Salicylic Acid

Salicylic acid (SA) is a phenolic phytohormone (Raskin 1992) which is biosynthesized by two discrete pathways. The first pathway comprises of PHENYLALANINE AMMONIA LYASE (PAL) which catalyses the conversion of phenylalanine into trans-cinnamic acid (Vlot et al. 2009; Janda and Ruelland 2015). The second pathway is localized to the chloroplasts and involves the enzyme ISOCHORISMATE SYNTHASE (ICS) which converts chorismate into isochorismate (Janda and Ruelland 2015). NONEXPRESSOR OF PATHOGENESIS-RELATED 1 (NPR1) is one of the integral components of SA signalling (Cao et al. 1994) and regulates the expression of most SA-dependent genes (Wang et al. 2005; Janda and Ruelland 2015). NPR1 is localized in the cytosol in the form of an oligomer; however, increase in SA level leads to the monomerization of the complex. NPR1 monomers translocate to the nucleus (Vlot et al. 2009; Janda and Ruelland 2015) and bind to TGA transcription factors followed by their direct binding to the promoter of pathogenesis-related (PR) genes and thus activate their expression (Jakoby et al. 2002; Janda and Ruelland 2015). SA is majorly involved in plant-pathogen interaction. However, it has a widespread role in various physiological functions ranging from seed germination to senescence (Rivas-San Vicente and Plasencia 2011) and also in several abiotic stresses (Horváth et al. 2007; Rivas-San Vicente and Plasencia 2011). SA activates the biosynthesis of various enzymes involved in metabolic pathways such as the glyoxylate cycle, the pentose phosphate pathway, glycolysis and gluconeogenesis suggesting that SA promotes the mobilization of resources and rescues from the metabolically inactive state to the active state (Rajjou et al. 2006; Rivas-San Vicente and Plasencia 2011). SA has a role in regulating photosynthesis by modulating the activity of enzymes such as RuBisCO (ribulose-1,5 bisphosphate carboxylase/oxygenase) and carbonic anhydrase (Pancheva and Popova 1998; Slaymaker et al. 2002). Treatment with SA in banana resulted in decreased levels of invertase and reducing sugar content, while it had an opposite effect on non-reducing sugar content, thereby delaying fruit ripening (Srivastava and Dwivedi 2000; Asghari and Aghdam 2010). To address the effect of SA on sugar metabolism, Dong et al. (2011) treated cucumber seedlings with SA and reported that activity of sucrose phosphate synthase (SPS), a key enzyme in sucrose synthesis, was upregulated by SA treatment in cucumber leaves. It also resulted in accumulation of higher percentage of soluble sugars and improved water uptake capacity and tolerance to salinity stress caused by NaCl (Dong et al. 2011). Poór et al. (2011) similarly proved that exogenous application of SA could decrease the activity of HXK leading to increased Glc and fructose content in leaf and increased sucrose content in the root of tomato plants, thereby minimizing the effect of salt stress through osmotic adjustment (Dong et al. 2011). SA is also involved in senescence regulation (Rivas-San Vicente and

Plasencia 2011). A rapid decline in photosynthesis serves as a key signal for induction of senescence (Jiang et al. 1993; Smart 1994; Bleecker and Patterson 1997; Quirino et al. 2000). Studies show that higher sugar levels downregulate the expression of photosynthesis-associated genes (Jang et al. 1997; Dai et al. 1999; Quirino et al. 2000). Thus, it can be hypothesized that sugars and SA might work synergistically in controlling senescence via repression of photosynthesis machinery. Studies in transgenic tomato (Dai et al. 1999; Swartzberg et al. 2011) and *Arabidopsis* (Kelly et al. 2012) plants revealed that overexpression of HXK1 led to early senescence, while the HXK1 mutant *gin2-1* responded poorly to glucose treatment (Pourtau et al. 2006; Wingler 2018) and showed delayed senescence (Moore et al. 2003). Evidence of direct crosstalk between sugars and SA exists in the mammalian system wherein salicylate activates AMPK which regulates cell growth and metabolism (Hawley et al. 2012). However, recently, it has been reported by Crozet et al. (2016) that in plants, SA had no effect on SnRK1-dependent gene expression in transient systems. Microarray analysis revealed that SA treatments induced the systemic acquired resistance (SAR) marker genes, but could not induce SnRK1 marker genes (Crozet et al. 2016). Therefore, it can be concluded that interconnections between sugar and SA signalling in controlling plant growth and development still need further exploration.

Salicylic acid (SA) affects photosynthesis and thus regulates sugar biosynthesis (Uzunova and Popova 2000; Pancheva and Popova 1998). SA influences photosynthesis in a dose-dependent manner (Pancheva et al. 1996) and changes leaf ultrastructure, increasing chloroplast volume (Uzunova and Popova 2000). This altered photosynthetic activity, owing to the SA treatment, is due to its effects on the thylakoid membranes and the reactions catalysed therein. In *Arabidopsis*, the SA signalling pathway contributes towards optimal photosynthetic activity by regulating acclimation to light, culminating into altered sugar biosynthesis (Mateo et al. 2006). Soluble sugars are highly sensitive to environmental stresses. This sensitivity greatly affects the distribution of sugars in plants. Sugars not only are the carbon source for energy but also play crucial regulatory functions regulating growth, development and defence responses in plants. The production and distribution of sugars to various tissues, to meet energy demands, are highly regulated.

The role of sugars and SA interaction has largely been implicated in plant immune responses. Glc activates the expression of several PR genes, many of which are strongly induced by SA. The presence of HXK1 is required for the induction of some of these genes. Sensing hexose levels has been shown to be important for mediating the repression of photosynthetic genes and expression of defence genes in plants (Herbers et al. 1996). RGS1 has also been reported to be involved in defence responses through stimulation of ROS generation (Xiao et al. 2000). Moreover, sucrose functions as a signalling molecule in plant defence (Wind et al. 2010) and regulates the expression of anthocyanin biosynthesis genes. Trehalose has also been shown to induce partial resistance against powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat by the activation of phenylalanine ammonia-lyase (PAL). PAL catalyses the critical first step in the biosynthesis of SA. *Arabidopsis siz1* displays altered responses to exogenous sugar supplementation. The *siz1* mutant accumulates higher levels of SA which interferes with sugar-dependent responses and signalling events. The signalling effect of sugars has also been shown to be independent of SA by using the bacterial SA-degrading

enzyme coded by *NahG* (Castro et al. 2016). In most compatible host-microbe interactions, pathogeneses rely on the supply of sugars synthesized by the colonized host tissue. It has been found in rice that phloem-localized sucrose transporter SWEET can be reprogrammed by bacterial effectors to establish compatibility. *sweet11/sweet12* double mutants exhibit increased resistance towards the fungal pathogen *Colletotrichum higginsianum* (Ch). During the course of Ch infection, the soluble sugar turnover increases in the sweet11/sweet12 mutants, and accumulation of free hexoses and sucrose also increases significantly in these double mutant leaves (Gebauer et al. 2017). Interestingly, the amount of total SA and the expression of SA-related genes were high in *sweet11/sweet12* plants, suggesting a possible sugar-mediated priming of SA signalling (Gebauer et al. 2017). Disease profiling of SA-deficient *sweet11/sweet12/sid2* triple mutants revealed that the increased tolerance observed in *sweet11/sweet12* mutants was dependent on the SA pathway (Gebauer et al. 2017). Since SWEET genes efflux sucrose into phloem (Chen et al. 2012), the defective phloem loading of sucrose in sweet mutants can influence SA priming and disease outcomes.

How do microbial pathogens reprogramme the host carbohydrate metabolism is not fully understood. However, pathogens are known to affect sugar synthesis to mediate pathogenesis. TOR acts as a molecular switch to regulate cellular immunity and interferes with SA signalling thereby regulating disease response in plants (De Vleesschauwer et al. 2018). An antagonistic relationship between TOR and SA reinforces the hypothesis that the trade-off between growth and defence is due to the differential activation of hormone signalling pathways rather than due to competition for the available resources (Eichmann and Schäfer 2015; Kliebenstein 2016). Additionally, an increasing number of studies suggest a key role of SnRK1-mediated signalling in plant interactions with pathogens (Hulsmans et al. 2016). The regulation of plant-pathogen interactions by SnRK1 is diverse and includes the regulation of primary carbohydrate metabolism. SnRK1 and its downstream processes are often targeted during stress tolerance (Hulsmans et al. 2016).

The differential accumulation of free sugars in pathogen infected tissues of maize plants causes the downregulation of the photosynthetic apparatus in these infected leaves (Doehlemann et al. 2008). During host immune response, microbe-/pathogen-associated molecular patterns (MAMPs/PAMPs) are recognized by the plasma membrane (PM) resident pattern recognition receptors to initiate pattern-triggered immunity (PTI). This is discussed in detail in Chap. 21. It has been suggested that sugars can act as PAMPs or DAMPs and activate PTI. The first identified sugar elicitors were β-glucans produced from *Phytophthora megasperma* pv. *Sojae* (Ayers et al. 1976). Thereafter, a large number of studies have demonstrated the roles of oligosaccharides in eliciting defence responses in plants (Shibuya and Minami 2001; Inui et al. 1997; Klarzynski et al. 2000; Ferrari 2013; Denoux et al. 2008). Sugars have a well-known role in innate immunity in plants and activate various defence genes. Genetic analyses have showed extensive interactions between sugar and hormone signalling in plants. SA signalling defective mutants such as *cpr5-1* and *sid2* have impaired photosynthetic activity (Mateo et al. 2006; Abreu and Munné-Bosch 2009). SA also controls sugar metabolism by regulating mitochondrial electron transport and oxidative phosphorylation in plants (Xie and Chen 1999; Norman et al. 2004). Sugars are influenced by stresses and hormone signalling and act in concert to

coordinate responses to environmental stresses (Rolland et al. 2006). Sugars have also been shown to have antioxidant roles and function as key components of the cellular redox network (Keunen et al. 2013; Bolouri-Moghaddam et al. 2010). SA levels, on the other hand, are also required for redox homeostasis (Mateo et al. 2006). Sugars serve as signals for the regulation of defence genes (Ehness et al. 1997; Roitsch et al. 2003; Bolton 2009) often mimicking the role of SA. The key roles of sugars in plant immunity have led to the coinage of "sweet immunity" or "sugar-enhanced defense" for the sugar-mediated immune responses (Bolouri Moghaddam and Van Den Ende 2013; Sonnewald et al. 2012) which is further reinforced by genetic interaction between SA and sugar signalling.

13.4 Sugars and Strigolactones

Strigolactones (SLs) are recently discovered plant hormones produced in roots and were initially recognized as germination stimulants of root parasitic plants such as *Striga*, *Orobanche* and *Phelipanche* (Cook et al. 1966). However, subsequent studies demonstrated that SLs also stimulated hyphal branching as well as root colonization of the symbiotic arbuscular mycorrhizal fungi and also act as long-distance signalling molecules to inhibit shoot branching (Akiyama et al. 2005; Besserer et al. 2006; Gomez-Roldan et al. 2008; Umehara et al. 2008; Kohlen et al. 2012; Xie et al. 2010). SLs also regulate primary root growth, lateral root formation, adventitious root formation, root hair development, seed germination, photomorphogenesis, stress response, nodulation and protonema branching (Czarnecki et al. 2014). Chemically, SLs are terpenoid lactones containing a butenolide group which is connected to tricyclic lactone via an enol ether bridge. Mutant study and biochemical analysis showed that SLs are synthesized from carotenoids by consecutive oxidation and oxidative cleavage (Sorefan et al. 2003; Booker et al. 2004, 2005; Snowden et al. 2005; Beveridge and Kyozuka 2010).

The SL signalling pathway shows a remarkably high similarity to auxin, JA and GA signalling pathways in which the key regulatory step is ubiquitin-mediated protein degradation of negative regulators. A subunit of the SCF ubiquitin E3 ligase complex, Leu-rich F-box protein, MORE AXILLARY GROWTH 2 (MAX2), play vital roles in SL signal perception and transduction by determining the repressor proteins (such as D53/SMXLs and BES1) for subsequent ubiquitination and degradation through 26S proteasome-mediated pathway. (Stirnberg et al. 2002, 2007; Ishikawa et al. 2005; Johnson et al. 2006; Arite et al. 2009; Nelson et al. 2011; Nakamura et al. 2013; Jiang et al. 2013; Wang et al. 2013; Zhou et al. 2013; Soundappan et al. 2015; Liang et al. 2016). Using genetic approaches it was found that α-/β-fold hydrolase DWARF 14 (D14)/DECREASED APICAL DOMINANCE2 (DAD2)/HTD2 is also involved in SL signal perception and transduction; however, mechanism of SL reception by the enzyme is still not well understood. In rice, GA signalling repressor protein DELLA also interacts with D14 in an SL-dependent manner, but the biological significant of this interaction is still unknown (Nakamura et al. 2013).

The role of SL and sugars is well established in regulating shoot branching. SL negatively regulates while sugars promote the shoot branching. Further studies

showed that negative regulators of the shoot branching gene *BRANCHED1* (*BRC1*) act as an integrator of sugar and SL signalling pathways in controlling axillary bud outgrowth. In pea, *PsBRC1* transcript levels are upregulated by SLs, while CKs and sucrose downregulated it during axillary bud extension (Braun et al. 2012; Mason et al. 2014). All these results together suggested an antagonistic interaction between sugar and SL in shoot branching. A detailed study by Li et al. (2016) at seedling stage suggested that SLs work with sugar signalling to regulate early seedling development. SL biosynthesis mutant *max1* and signalling mutant *max2* show less sensitivity than wild type in terms of sugar-induced growth repression, and SL was found to work synergistically with Glc in repressing seedling establishment. Genome-wide transcriptome profiling showed that sugar and SL together regulate genes which are involved in stress responses and root hair development (Li et al. 2016). In conclusion, SL and sugars interact either antagonistically or synergistically to regulate morphological or developmental process; however, more molecular and physiological works are required to dissect out crosstalk between SL and sugar signalling.

13.5 Conclusions

In nature, sugars are not as such available to the plant for uptake unlike other nutrients and solely produced by photosynthesis in source tissues and transported to sink tissues primarily as sucrose via phloem. Plant responses to sugars will be of great concern in the future, as atmospheric CO_2 concentration will continue to rise due to urbanization, deforestation and industrial revolution. Elevated environmental CO_2 causes increased photosynthesis which leads to more production of carbohydrates and thus greater allocation of them to sink tissues where they affect growth and development. Elevated environmental CO_2 concentration has positive effect on growth, biomass and yield, whereas it has negative effect on nutrient quality of crop plants. There are plethora of reports in past decade which show that any increase in endogenous sugar level either by elevated environmental CO_2 concentration or by exogenous supplementation of sugars in growth medium affect seedling architecture, plant growth, nutrient acquisition and hormone crosstalk. Sugars crosstalk with the hormone regulatory network involved in growth and development at the levels of biosynthesis, degradation, transport, signalling and gene expression. Plants have evolved as masters in the suppression and stimulation of growth as they modify their shape throughout life to adjust to their environment. Molecular pathways governing these growth processes must be tightly coordinated to produce organized development. However, the complete knowledge of these interaction networks is still notably poor and one of the big questions in plant biology. Therefore, the detailed understanding of molecular pathways governed by sugars either as a metabolite or as a signalling molecule and/or in association with other signalling pathways will become increasingly important and also a prerequisite due to a large overlap of candidate genes and phenotypes shared by these signals. Uncovering the entry point of sugars either alone or in association with other signals in developmental program will be certainly beneficial for targeted engineering of plants and in order to develop new varieties that can better withstand today's varied climate conditions. The study of sugar hormone crosstalk will also add a piece of information in understanding the complicated puzzle of plant growth and development.

Acknowledgements The research in AL laboratory is supported by Project Grants from the Department of Biotechnology, Government of India, and a Core Grant from the National Institute of Plant Genome Research. MJK, SJ, MS, MS, DS, AT, HBS, BM, SK and ZZB duly acknowledge research fellowships from the Department of Biotechnology, Government of India; Department of Science and Technology, Government of India; Council of Scientific and Industrial Research, Government of India; and University Grants Commission, Government of India. The authors acknowledge DBT-eLibrary Consortium (DeLCON) for providing access to e-resources.

References

Abreu ME, Munné-Bosch S (2009) Salicylic acid deficiency in NahG transgenic lines and sid2 mutants increases seed yield in the annual plant Arabidopsis thaliana. J Exp Bot 60:1261–1271

Aki T, Konishi M, Kikuchi T, Fujimori T, Yoneyama T, Yanagisawa S (2007) Distinct modulations of the hexokinase1-mediated glucose response and hexokinase1-independent processes by HYS1/CPR5 in Arabidopsis. J Exp Bot 58:3239–3248

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science 284:2148–2152

Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, León P (2000) Analysis of Arabidopsis glucose insensitive mutants, gin5 and gin6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev 14:2085–2096

Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J (2009) d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiol 50:1416–1424

Asghari M, Aghdam MS (2010) Impact of salicylic acid on post-harvest physiology of horticultural crops. Trends Food Sci Technol 21:502–509

Ayers AR, Ebel J, Valent B, Albersheim P (1976) Host-pathogen interactions: X. fractionation and biological activity of an elicitor isolated from the mycelial walls of Phytophthora megasperma var. sojae. Plant Physiol 57:760–765

Baena-González E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signalling. Nature 448:938–942

Bai MY, Shang JX, Oh E, Fan M, Bai Y, Zentella R, Sun TP, Wang ZY (2012) Brassinosteroid, gibberellin and phytochrome impingeon a common transcription module in Arabidopsis. Nat Cell Biol 14:810–817

Berckmans B, Vassileva V, Schmid SPC, Maes S, Parizot B, Naramoto S, Magyar Z, Kamei CLA, Koncz C, Bogre L et al (2011) Auxin-dependent cell cycle reactivation through transcriptional regulation of Arabidopsis E2Fa by lateral organ boundary proteins. Plant Cell 23:3671–3683

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:e226

Beveridge CA, Kyozuka J (2010) New genes in the strigolactone-related shoot branching pathway. Curr Opin Plant Biol 13:34–39

Bleecker AB, Patterson SE (1997) Last exit: senescence, abscission, and meristem arrest in Arabidopsis. Plant Cell 9:1169–1179

Bolouri Moghaddam MR, Van Den Ende W (2012) Sugars and plant innate immunity. J Exp Bot 63(11):3989–3998

Bolouri Moghaddam MR, Van Den Ende W (2013) Sweet immunity in the plant circadian regulatory network. J Exp Bot 64:1439–1449

Bolouri-Moghaddam MR, Le Roy K, Xiang L, Rolland F, Van Den Ende W (2010) Sugar signalling and antioxidant network connections in plant cells. FEBS J 277:2022–2037

Bolton MD (2009) Primary metabolism and plant defense-fuel for the fire. Mol Plant-Microbe Interact 22:487–497

Booker J, Auldridge M, Wills S, McCarty D, Klee H, Leyser O (2004) MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Curr Biol 14:1232–1238

Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O (2005) MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Dev Cell 8:443–449

Braun N, de Saint GA, Pillot JP, Boutet-Mercey S, Dalmais M, Antoniadi I, Li X, Maia-Grondard A, Le Signor C, Bouteiller N, Luo D, Bendahmane A, Turnbull C, Rameau C (2012) The pea TCP transcription factor PsBRC1 acts downstream of Strigolactones to control shoot branching. Plant Physiol 158:225–238

Broeckx T, Hulsmans S, Rolland F (2016) The plant energy sensor: evolutionary conservation and divergence of SnRK1 structure, regulation, and function. J Exp Bot 67:6215–6252

Cai W, Li X, Liu Y, Wang Y, Zhou Y, Xu T, Xiong Y (2017) COP1 integrates light signals to ROP2 for cell cycle activation. Plant Signal Behav 12:e1363946

Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P (2013) Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in Arabidopsis thaliana. Plant J 73:897–909

Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583–1592

Castro PH, Verde N, Tavares RM, Bejarano ER, Azevedo H (2016) Sugar signaling regulation by Arabidopsis SIZ1-driven sumoylation is independent of salicylic acid. Plant Signal Behav 13:e1179417

Chen J-G, Jones AM (2004) AtRGS1 function in Arabidopsis thaliana. Methods Enzymol 389:338–350

Chen PW, Lu CA, Yu TS, Tseng TH, Wang CS, Yu SM (2002) Rice alpha-amylase transcriptional enhancers direct multiple mode regulation of promoters in transgenic rice. J Biol Chem 277:6113641–61113649

Chen J-G, Willard FS, Huang J, Liang J, Chasse SA, Jones AM, Siderovski DP (2003) A seven-transmembrane RGS protein that modulates plant cell proliferation. Science 301:1728–1731

Chen PW, Chiang CM, Tseng TH, Yu SM (2006) Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of alpha-amylase genes. Plant Cell 18:2326–2340

Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335:207–211

Chen L, Su Z-Z, Huang L, Xia F-N, Qi H, Xie L-J, Xiao S, Chen Q-F (2017) The AMP-activated protein kinase KIN10 is involved in the regulation of autophagy in Arabidopsis. Front Plant Sci 8:1201

Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T (2002) A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14:2723–2743

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, Micol JL (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448:666–671

Cho Y-H, Yoo S-D (2011) Signaling role of fructose mediated by FINS1/FBP in Arabidopsis thaliana. PLoS Genet 7:e1001263

Cho Y-H, Yoo S-D, Sheen J (2006) Regulatory functions of nuclear Hexokinase1 complex in glucose signaling. Cell 127:579–589

Cho JI, Ryoo N, Eom JS, Lee DW, Kim HB, Jeong SW, Lee YH, Kwon YK, Cho MH, Bhoo SH, Hahn TR (2009) Role of the rice hexokinases OsHXK5 and OsHXK6 as glucose sensors. Plant Physiol 149:745–759

Cho Y-H, Hong J-W, Kim E-C, Yoo S-D (2012) Regulatory functions of SnRK1 in stress-responsive gene expression and in plant growth and development. Plant Physiol 158:1955–1964

Cho H-Y, Wen T-N, Wang Y-T, Shih M-C (2016) Quantitative phosphoproteomics of protein kinase SnRK1 regulated protein phosphorylation in Arabidopsis under submergence. J Exp Bot 67:2745–2760

Clouse SD (2011) Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. Plant Cell 23:1219–1230
Colaneri AC, Tunc-Ozdemir M, Huang J, Jones AM (2014) Growth attenuation under saline stress is mediated by the heterotrimeric G protein complex. BMC Plant Biol 14:129
Cook CE, Whichard LP, Turner B, Wall ME, Egley GH (1966) Germination of witchweed (Striga lutea Lour.): isolation and properties of a potent stimulant. Science 154:1189–1190
Crozet P, Margalha L, Butowt R, Fernandes N, Elias CA, Orosa B, Tomanov K, Teige M, Bachmair A, Sadanandom A, González EB (2016) SUMOylation represses SnRK1 signaling in Arabidopsis. Plant J 85:120–133
Czarnecki O, Yang J, Wang X, Wang S, Muchero W, Tuskan GA, Chen JG (2014) Characterization of MORE AXILLARY GROWTH genes in Populus. PLoS One 9:e102757
Dai N, Schaffer A, Petreikov M, Shahak Y, Giller Y, Ratner K, Levine A, Granota D (1999) Overexpression of Arabidopsis hexokinase in tomato plants inhibits growth, reduces photosynthesis, and induces rapid senescence. Plant Cell 11:1253–1266
Das PK, Shin DH, Choi S-B, Park Y-I (2012) Sugar-hormone cross-talk in anthocyanin biosynthesis. Mol Cells 34:501–507
de Lucas M, Davière JM, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E, Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. Nature 451:480–486
De Vleesschauwer D, Filipe O, Hoffman G, Seifi HS, Haeck A, Canlas P, Van Bockhaven J, De Waele E, Demeestere K, Ronald P, Hofte M (2018) Target of rapamycin signaling orchestrates growth-defense trade-offs in plants. New Phytol 217:305–319
Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel FM, Dewdney J (2008) Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. Mol Plant 1:423–445
Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolaï M, Bedu M, Robaglia C, Meyer C (2007) The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. EMBO Rep 8:864–870
Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016) TOR signaling and nutrient sensing. Annu Rev Plant Biol 67:261–285
Doehlemann G, Wahl R, Horst RJ, Voll LM, Usadel B, Poree F, Stitt M, Pons-Kühnemann J, Sonnewald U, Kahmann R, Kämper J (2008) Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph Ustilago maydis. Plant J 56:181–195
Dong CJ, Wang XL, Shang QM (2011) Salicylic acid regulates sugar metabolism that confers tolerance to salinity stress in cucumber seedlings. Sci Hortic 129:629–636
Dong P, Xiong F, Que Y, Wang K, Yu L, Li Z, Ren M (2015) Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in Arabidopsis. Front Plant Sci 6:677
Dong Z, Yu Y, Li S, Wang J, Tang S, Huang R (2016) Abscisic acid antagonizes ethylene production through the ABI4-mediated transcriptional repression of ACS4 and ACS8 in Arabidopsis. Mol Plant 9:126–135
Ehness R, Ecker M, Godt DE, Roitsch T (1997) Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. Plant Cell 9:1825–1841
Eichmann R, Schäfer P (2015) Growth versus immunity – a redirection of the cell cycle? Curr Opin Plant Biol 26:106–112
Emanuelle S, Hossain MI, Moller IE, Pedersen HL, van de Meene AML, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WGT et al (2015) SnRK1 from Arabidopsis thaliana is an atypical AMPK. Plant J 82:183–192
Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, Schäfer E, Fu X, Fan LM, Deng XW (2008) Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 451:475–480
Fennell H, Olawin A, Mizanur R, Ken I, Chen JG, Ullah H (2012) Arabidopsis scaffold protein RACK1A modulates rare sugar D-allose regulated gibberellin signaling. Plant Signal Behav 7:1771–1780

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J (2011) The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell 23:701–715

Ferrari S (2013) Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. Front Plant Sci 4:49

Feys BJF, Benedetti CE, Penfold CN, Turner JG (1994) Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell 6:751–759

Finet C, Jaillais Y (2012) AUXOLOGY: when auxin meets plant evo-devo. Dev Biol 369:19–31

Finkelstein R (2013) Abscisic acid synthesis and response. Arabidopsis Book 11:e0166

Fonseca S, Chico JM, Solano R (2009a) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol 12:539–547

Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R (2009b) (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol 5:344–350

Franco-Zorrilla JM, Martín AC, Leyva A, Paz-Ares J (2005) Interaction between phosphate-starvation, sugar, and cytokinin signaling in Arabidopsis and the roles of cytokinin receptors CRE1/AHK4 and AHK3. Plant Physiol 138:847–857

Fu Y, Lim S, Urano D, Tunc-Ozdemir M, Phan NG, Elston TC, Jones AM (2014) Reciprocal encoding of signal intensity and duration in a glucose-sensing circuit. Cell 156:1084–1095

Gao X-Q, Liu CZ, Li DD, Zhao TT, Li F, Jia XN, Zhao X-Y, Zhang XS (2016) The Arabidopsis KINβγ subunit of the SnRK1 complex regulates pollen hydration on the stigma by mediating the level of reactive oxygen species in pollen. PLoS Genet 12:e1006228

Gebauer P, Korn M, Engelsdorf T, Sonnewald U, Koch C, Voll LM (2017) Sugar accumulation in leaves of Arabidopsis sweet11/sweet12 double mutants enhances priming of the salicylic acid-mediated defense response. Front Plant Sci 8:1378

Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 12:1117–1126

Goetz M, Godt DE, Roitsch T (2000) Tissue-specific induction of the mRNA for an extracellular invertase isoenzyme of tomato by brassinosteroids suggests a role for steroid hormones in assimilate partitioning. Plant J 22:515–522

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194

Gonzali S, Novi G, Loreti E, Paolicchi F, Poggi A, Alpi A, Perata P (2005) A turanose-insensitive mutant suggests a role for WOX5 in auxin homeostasis in Arabidopsis thaliana. Plant J 44:633–645

Grigston JC, Osuna D, Scheible W-R, Liu C, Stitt M, Jones AM (2008) D-glucose sensing by a plasma membrane regulator of G signaling protein, at RGS1. FEBS Lett 582:3577–3584

Gubler F, Jacobsen JV (1992) Gibberellin-responsive elements in the promoter of a barley high-pI alpha-amylase gene. Plant Cell 4:1435–1441

Guo H, Li L, Aluru M, Aluru S, Yin Y (2013a) Mechanisms and networks for brassinosteroid regulated gene expression. Curr Opin Plant Biol 16:545–553

Guo R, Shen W, Qian H, Zhang M, Liu L, Wang Q (2013b) Jasmonic acid and glucose synergistically modulate the accumulation of glucosinolates in Arabidopsis thaliana. J Exp Bot 64:5707–5719

Gupta A, Singh M, Jones AM, Laxmi A (2012) Hypocotyl directional growth in Arabidopsis: a complex trait. Plant Physiol 159:1463–1476

Gupta A, Singh M, Laxmi A (2015a) Multiple interactions between glucose and brassinosteroid signal transduction pathways in arabidopsis are uncovered by whole-genome transcriptional profiling. Plant Physiol 168:1091–1105

Gupta A, Singh M, Laxmi A (2015b) Interaction between glucose and brassinosteroid during regulation of lateral root development in Arabidopsis thaliana. Plant Physiol 168:307–320

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 30:214–226

Hall BP, Shakeel SN, Schaller GE (2007) Ethylene receptors: ethylene perception and signal transduction. J Plant Growth Regul 26:118–130
Hao Y, Wang H, Qiao S, Leng L, Wang X (2016) Histone deacetylase HDA6 enhances brassinosteroid signaling by inhibiting the BIN2 kinase. Proc Natl Acad Sci U S A 113:10418–10423
Hartig K, Beck E (2006) Crosstalk between auxin, cytokinins, and sugars in the plant cell cycle. Plant Biol 8:389–396
Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard J, Kemp BE, Sakamoto K, Steinberg GR, Hardie DG (2012) The ancient drug salicylate directly activates AMP-activated protein kinase. Science 336:918–922
Herbers K, Meuwly P, Frommer W, Metraux J, Sonnewald U (1996) Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. Plant Cell 8:793–803
Hirano K, Asano K, Tsuji H, Kawamura M, Mori H, Kitano H, Ueguchi-Tanaka M, Matsuoka M (2010) Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. Plant Cell 22:2680–2696
Hong GJ, Xue XY, Mao YB, Wang LJ, Chen XY (2012) Arabidopsis MYC2 interacts with DELLA proteins in regulating sesquiterpene synthase gene expression. Plant Cell 24:2635–2648
Horváth E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid signaling. J Plant Growth Regul 26:290–300
Hou X, Lee LYC, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell 19:884–894
Huang J, Taylor JP, Chen J-G, Uhrig JF, Schnell DJ, Nakagawa T, Korth KL, Jones AM (2006) The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. Plant Cell 18:1226–1238
Huang J-P, Tunc-Ozdemir M, Chang Y, Jones AM (2015) Cooperative control between AtRGS1 and AtHXK1 in a WD40-repeat protein pathway in Arabidopsis thaliana. Front Plant Sci 6:851
Hulsmans S, Rodriguez M, De Coninck B, Rolland F (2016) The SnRK1 energy sensor in plant biotic interactions. Trends Plant Sci 21:648–661
Inui H, Yamaguchi Y, Hirano S (1997) Elicitor actions of N-acetylchitooligosaccharides and laminarioligosaccharides for chitinase and l-phenylalanine ammonia-lyase induction in rice suspension culture. Biosci Biotechnol Biochem 61:975–978
Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J (2005) Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiol 46:79–86
Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci 7:106–111
Jamsheer KM, Laxmi A (2014) DUF581 is plant specific FCS-like zinc finger involved in protein-protein interaction. PLoS One 9:e99074
Jamsheer KM, Laxmi A (2015) Expression of Arabidopsis FCS-like zinc finger genes is differentially regulated by sugars, cellular energy level, and abiotic stress. Front Plant Sci 6:1–12
Jamsheer KM, Sharma M, Singh D, Mannully CT, Jindal S, Shukla BN, Laxmi A (2018) FCS-like zinc finger 6 and 10 repress SnRK1 signalling in Arabidopsis. Plant J 94:232–245
Janda M, Ruelland E (2015) Magical mystery tour: salicylic acid signalling. Environ Exp Bot 114:117–128
Jang JC, León P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell 9:5–19
Jiang CZ, Rodermel SR, Shibles RM (1993) Photosynthesis, rubisco activity and amount, and their regulation by transcription in senescing soybean leaves. Plant Physiol 101:105–112
Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y, Yi W, Zhao L, Ma H, He Y, Wu Z, Melcher K, Qian Q, Xu HE, Wang Y, Li J (2013) DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature 504:401–405
Johnson PR, Ecker JR (1998) The ethylene gas signal transduction pathway: a molecular perspective. Annu Rev Genet 32:227–254
Johnson X, Brcich T, Dun EA, Goussot M, Haurogné K, Beveridge CA, Rameau C (2006) Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. Plant Physiol 142:1014–1026

Johnston CA, Taylor JP, Gao Y, Kimple AJ, Grigston JC, Chen J-G, Siderovski DP, Jones AM, Willard FS (2007) GTPase acceleration as the rate-limiting step in Arabidopsis G protein-coupled sugar signaling. Proc Natl Acad Sci 104:17317–17322

Jossier M, Bouly J-P, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame Hardie D, Thomas M (2009) SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. Plant J 59:316–328

Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, Garrett WM, Kessenbrock M, Groth G, Tucker ML, Cooper B, Kieber JJ, Chang C (2012) CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc Natl Acad Sci 109:19486–19491

Kanno Y, Oikawa T, Chiba Y, Ishimaru Y, Shimizu T, Sano N, Koshiba T, Kamiya Y, Ueda M, Seo M (2016) AtSWEET13 and AtSWEET14 regulate gibberellin-mediated physiological processes. Nat Commun 7:13245

Kays SJ, Pallas JE (1980) Inhibition of photosynthesis by ethylene. Nature 285:51–52

Kelly G, David-Schwartz R, Sade N, Moshelion M, Levi A, Alchanatis V, Granot D (2012) The pitfalls of transgenic selection and new roles of *AtHXK1*: a high level of *AtHXK1* expression uncouples hexokinase1-dependent sugar signaling from exogenous sugar. Plant Physiol 159:47–51

Kelly G, Moshelion M, David-Schwartz R, Halperin O, Wallach R, Attia Z, Belausov E, Granot D (2013) Hexokinase mediates stomatal closure. Plant J 75:977–988

Keunen E, Peshev D, Vangronsveld J, Van Den Ende W, Cuypers A (2013) Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant Cell Environ 36:1242–1255

Kieber JJ (2002) Cytokinins. Arabidopsis Book 1:e0063

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell 72:427–441

Kim Y-M, Heinzel N, Giese J-O, Koeber J, Melzer M, Rutten T, Von Wirén N, Sonnewald U, Hajirezaei M-R (2013) A dual role of tobacco hexokinase 1 in primary metabolism and sugar sensing. Plant Cell Environ 36:1311–1327

Kim Y-K, Kim S, Shin Y, Hur Y-S, Kim W-Y, Lee M-S, Cheon C-I, Verma DPS (2014) Ribosomal protein S6, a target of rapamycin, is involved in the regulation of rRNA genes by possible epigenetic changes in Arabidopsis. J Biol Chem 289:3901–3912

Kim G-D, Cho Y-H, Yoo S-D (2017) Phytohormone ethylene-responsive Arabidopsis organ growth under light is in the fine regulation of photosystem II deficiency-inducible AKIN10 expression. Sci Rep 7:2767

Kircher S, Schopfer P (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in Arabidopsis. Proc Natl Acad Sci U S A 109:11217–11221

Klarzynski O, Plesse B, Joubert J-M, Yvin J-C, Kopp M, Kloareg B, Fritig B (2000) Linear β-1,3 glucans are elicitors of defense responses in tobacco. Plant Physiol 124:1027–1038

Kliebenstein DJ (2016) False idolatry of the mythical growth versus immunity tradeoff in molecular systems plant pathology. Physiol Mol Plant Pathol 95:55–59

Kohlen W, Charnikhova T, Lammers M, Pollina T, Tóth P, Haider I, Pozo MJ, de Maagd RA, Ruyter-Spira C, Bouwmeester HJ, López-Ráez JA (2012) The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. New Phytol 196:535–547

Kong D, Hao Y, Cui H (2016) The WUSCHEL related homeobox protein WOX7 regulates the sugar response of lateral root development in Arabidopsis thaliana. Mol Plant 9:261–270

Kunz S, Gardeström P, Pesquet E, Kleczkowski LA (2015) Hexokinase 1 is required for glucose-induced repression of bZIP63, At5g22920, and BT2 in Arabidopsis. Front Plant Sci 6:525

Kushwah S, Laxmi A (2014) The interaction between glucose and cytokinin signal transduction pathway in Arabidopsis thaliana. Plant Cell Environ 37:235–253

Kushwah S, Jones AM, Laxmi A (2011) Cytokinin interplay with ethylene, auxin, and glucose signaling controls Arabidopsis seedling root directional growth. Plant Physiol 156:1851–1866

Laxmi A, Paul LK, Peters JL, Khurana JP (2004) Arabidopsis constitutive photomorphogenic mutant, bls1, displays altered brassinosteroid response and sugar sensitivity. Plant Mol Biol 56:185–201

Laxmi A, Paul LK, Raychaudhuri A, Peters JL, Khurana JP (2006) Arabidopsis cytokinin-resistant mutant, cnr1, displays altered auxin responses and sugar sensitivity. Plant Mol Biol 62:409–425

Lehman A, Black R, Ecker JR (1996) HOOKLESS1 an ethylene response gene, is required for differential cell elongation in the arabidopsis hypocotyl. Cell 85:183–194

Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinsteroid signal transduction. Cell 90:929–938

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an Arabidopsis LRR receptor like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 110:213–222

Li P, Zhou H, Shi X, Yu B, Zhou Y, Chen S, Wang Y, Peng Y, Meyer RC, Smeekens SC, Teng S (2014a) The ABI4-induced Arabidopsis ANAC060 transcription factor attenuates ABA signaling and renders seedlings sugar insensitive when present in the nucleus. PLoS Genet 10:1–10

Li Y, Van den Ende W, Rolland F (2014b) Sucrose induction of anthocyanin biosynthesis is mediated by DELLA. Mol Plant 7:570–572

Li S, Chen L, Li Y, Yao R, Wang F, Yang M, Gu M, Nan F, Xie D, Yan J (2016) Effect of GR24 stereoisomers on plant development in Arabidopsis. Mol Plant 9:1432–1435

Li X, Cai W, Liu Y, Li H, Fu L, Liu Z, Xu L, Liu H, Xu T, Xiong Y (2017) Differential TOR activation and cell proliferation in Arabidopsis root and shoot apexes. Proc Natl Acad Sci U S A 114:2765–2770

Liang Y, Ward S, Li P, Bennett T, Leyser O (2016) SMAX1-LIKE7 signals from the nucleus to regulate shoot development in Arabidopsis via partially EAR motif-independent mechanisms. Plant Cell 28:1581–1601

Lin P-C, Pomeranz MC, Jikumaru Y, Kang SG, Hah C, Fujioka S, Kamiya Y, Jang J-C (2011) The Arabidopsis tandem zinc finger protein AtTZF1 affects ABA- and GA-mediated growth, stress and gene expression responses. Plant J 65:253–268

Lin XY, Ye YQ, Fan SK, Jin CW, Zheng SJ (2016) Increased sucrose accumulation regulates iron-deficiency responses by promoting auxin signaling in Arabidopsis plants. Plant Physiol 170:907–920

Lisso J, Altmann T, Müssig C (2006) Metabolic changes in fruits of the tomato dx mutant. Phytochemistry 67:2232–2238

Liu Y, Bassham DC (2010) TOR is a negative regulator of autophagy in Arabidopsis thaliana. PLoS One 5:e11883

Liu XJ, An XH, Liu X, Hu DG, Wang XF, You CX, Hao YJ (2017) MdSnRK1.1 interacts with MdJAZ18 to regulate sucrose-induced anthocyanin and proanthocyanidin accumulation in apple. J Exp Bot 68:2977–2990

Loreti E, Povero G, Novi G, Solfanelli C, Alpi A, Perata P (2008) Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. New Phytol 179:1004–1016

Ma XM, Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10:307–318

Mason HS, DeWald DB, Creelman RA, Mullet JE (1992) Coregulation of soybean vegetative storage protein gene expression by methyl jasmonate and soluble sugars. Plant Physiol 98:859–867

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. Proc Natl Acad Sci U S A 111:6092–6097

Mateo A, Funck D, Mühlenbock P, Kular B, Mullineaux PM, Karpinski S (2006) Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J Exp Bot 57(8):1795–1807

McSteen P, Zhao Y (2008) Plant hormones and signaling: common themes and new developments. Dev Cell 14:467–473

Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proc Natl Acad Sci U S A 99:6422–6427

Meteignier L-V, El Oirdi M, Cohen M, Barff T, Matteau D, Lucier J-F, Rodrigue S, Jacques P-E, Yoshioka K, Moffett P (2017) Translatome analysis of an NB-LRR immune response identifies important contributors to plant immunity in Arabidopsis. J Exp Bot 68:2333–2344

Miao H, Wei J, Zhao Y, Yan H, Sun B, Huang J, Wang Q (2013) Glucose signalling positively regulates aliphatic glucosinolate biosynthesis. J Exp Bot 64:1097–1109

Mishra BS, Singh M, Aggrawal P, Laxmi A (2009) Glucose and auxin signaling interaction in controlling arabidopsis thaliana seedlings root growth and development. PLoS One 4:e4502.

Mitchell JW, Mandava N, Worley JF, Plimmer JR, Smith MV (1970) Brassins – a new family of plant hormones from rape pollen. Nature 225:1065–1066

Moore B, Zhou L, Rolland F, Hall Q, Cheng W-H, Liu Y-X, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300:332–336

Moreau M, Azzopardi M, Clement G, Dobrenel T, Marchive C, Renne C, Martin-Magniette M-L, Taconnat L, Renou J-P, Robaglia C, Meyer C (2012) Mutations in the Arabidopsis homolog of LST8/G L, a partner of the target of rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long days. Plant Cell 24:463–481

Morita A, Umemura T, Kuroyanagi M, Futsuhara Y, Perata P, Yamaguchi J (1998) Functional dissection of a sugar-repressed alpha-amylase gene (RAmy1 A) promoter in rice embryos. FEBS Lett 423:6181–6185

Mudgil Y, Uhrig JF, Zhou J, Temple B, Jiang K, Jones AM (2009) Arabidopsis N-MYC DOWNREGULATED-LIKE1, a positive regulator of auxin transport in a G protein-mediated pathway. Plant Cell 21:3591–3609

Mudgil Y, Karve A, Teixeira PJPL, Jiang K, Tunc-Ozdemir M, Jones AM (2016) Photosynthate regulation of the root system architecture mediated by the heterotrimeric G protein complex in Arabidopsis. Front Plant Sci 7:1–13

Murcia G, Pontin M, Piccoli P (2017) Role of ABA and Gibberellin A3 on gene expression pattern of sugar transporters and invertases in Vitis vinifera cv. Malbec during berry ripening. Plant Growth Regul 84(2):275–283

Nakamura H, Xue YL, Miyakawa T, Hou F, Qin HM, Fukui K, Shi X, Ito E, Ito S, Park SH, Miyauchi Y, Asano A, Totsuka N, Ueda T, Tanokura M, Asami T (2013) Molecular mechanism of strigolactone perception by DWARF14. Nat Commun 4:2613

Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110:203–212

Nelson DC, Scaffidi A, Dun EA, Waters MT, Flematti GR, Dixon KW, Beveridge CA, Ghisalberti EL, Smith SM (2011) F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in Arabidopsis thaliana. Proc Natl Acad Sci U S A 108:8897–8902

Németh K, Salchert K, Putnoky P, Bhalerao R, Koncz-Kálmán Z, Stankovic-Stangeland B, Bakó L, Mathur J, Okrész L, Stabel S, Geigenberger P, Stitt M, Rédei GP, Schell J, Koncz C (1998) Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. Genes Dev 12:3059–3073

Nietzsche M, Schießl I, Börnke F (2014) The complex becomes more complex: protein-protein interactions of SnRK1 with DUF581 family proteins provide a framework for cell- and stimulus type-specific SnRK1 signaling in plants. Front Plant Sci 5:54

Nietzsche M, Landgraf R, Tohge T, Börnke F (2016) A protein–protein interaction network linking the energy-sensor kinase SnRK1 to multiple signaling pathways in Arabidopsis thaliana. Curr Plant Biol 5:36–44

Norman C, Howell KA, Millar AH, Whelan JM, Day DA (2004) Salicylic acid is an uncoupler and inhibitor of mitochondrial electron transport. Plant Physiol 134:492–501

Nukarinen E, Nägele T, Pedrotti L, Wurzinger B, Mair A, Landgraf R, Börnke F, Hanson J, Teige M, Baena-Gonzalez E, Dröge-Laser W, Weckwerth W (2016) Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation. Sci Rep 6:31697

Ohto MA, Hayashi S, Sawa S, Hashimoto-Ohta A, Nakamura K (2006) Involvement of HLS1 in sugar and auxin signaling in Arabidopsis leaves. Plant Cell Physiol 47:1603–1611

Pancheva TV, Popova LP (1998) Effect of salicylic acid on the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase in barley leaves. J Plant Physiol 152:381–386
Pancheva TV, Popova LP, Uzunova AN (1996) Effects of salicylic acid on growth and photosynthesis in barley plants. J Plant Physiol 149:57–63
Park J, Oh DH, Dassanayake M, Nguyen KT, Ogas J, Choi G, Sun TP (2017) Gibberellin signaling requires chromatin remodeler PICKLE to promote vegetative growth and phase transitions. Plant Physiol 173:1463–1474
Perata P, Matsukura C, Vernieri P, Yamaguchi J (1997) Sugar repression of a gibberellin-dependent signaling pathway in barley embryos. Plant Cell 9:612197–612208
Peviani A, Lastdrager J, Hanson J, Snel B (2016) The phylogeny of C/S1 bZIP transcription factors reveals a shared algal ancestry and the pre-angiosperm translational regulation of S1 transcripts. Sci Rep 6:30444
Poór P, Gémes K, Horváth F, Szepesi Á, Simon ML, Tari I (2011) Salicylic acid treatment via the rooting medium interferes with stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. Plant Biol 13:105–114
Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A (2006) Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in Arabidopsis. Planta 224:556–568
Pu Y, Luo X, Bassham DC (2017) TOR-dependent and -independent pathways regulate autophagy in Arabidopsis thaliana. Front Plant Sci 8:1204
Quirino BF, Noh Y-S, Himelblau E, Amasino RM (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5:278–282
Rabot A, Henry C, Ben Baaziz K, Mortreau E, Azri W, Lothier J, Hamama L, Boummaza R, Leduc N, Pelleschi-Travier S, Gourrierec JL, Sakr S (2012) Insight into the role of sugars in bud burst under light in the rose. Plant Cell Physiol 53:1068–1082
Rabot A, Portemer V, Péron T, Mortreau E, Leduc N, Hamama L, Coutos-Thévenot P, Atanassova R, Sakr S, Gourrierec JL (2014) Interplay of sugar, light and gibberelling in expression of Rosa hybrida vacuolar invertase 1 regulation. Plant Cell Physiol 55:1734–1748
Rahmani F, Hummel M, Schuurmans J, Wiese-Klinkenberg A, Smeekens S, Hanson J (2009) Sucrose control of translation mediated by an upstream open reading frame-encoded peptide. Plant Physiol 150:1356–1367
Rajjou L, Belghazi M, Huguet R, Robin C, Moreau A, Job C, Job D (2006) Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms. Plant Physiol 141:910–923
Ramon M, Rolland F, Sheen J (2008) Sugar sensing and signaling. Arabidopsis Book 6:e0117
Ramon M, Ruelens P, Li Y, Sheen J, Geuten K, Rolland F (2013) The hybrid four-CBS-domain KINβγ subunit functions as the canonical γ subunit of the plant energy sensor SnRK1. Plant J 75:11–25
Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43:439–463
Reinhold H, Soyk S, Simková K, Hostettler C, Marafino J, Mainiero S, Vaughan CK, Monroe JD, Zeeman SC (2011) β-amylase-like proteins function as transcription factors in Arabidopsis, controlling shoot growth and development. Plant Cell 23:1391–1403
Ren M, Qiu S, Venglat P, Xiang D, Feng L, Selvaraj G, Datla R (2011) Target of rapamycin regulates development and ribosomal RNA expression through kinase domain in Arabidopsis. Plant Physiol 155:1367–1382
Ren M, Venglat P, Qiu S, Feng L, Cao Y, Wang E, Xiang D, Wang J, Alexander D, Chalivendra S, Logan D, Mattoo A, Selvaraj G, Datla R (2012) Target of rapamycin signaling regulates metabolism, growth, and life span in Arabidopsis. Plant Cell 24:4850–4874
Riou-Khamlichi C, Menges M, Healy JM, Murray JA (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. Mol Cell Biol 20:4513–4521
Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 62:3321–3338
Rodrigues A, Adamo M, Crozet P, Margalha L, Confraria A, Martinho C, Elias A, Rabissi A, Lumbreras V, González-Guzmán M, Antoni R (2013) ABI1 and PP2CA phosphatases are negative regulators of Snf1-related protein kinase1 signaling in Arabidopsis. Plant Cell 25:3871–3884

Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK (2003) Extracellular invertase: key metabolic enzyme and PR protein. J Exp Bot 54:513–524
Rolland F, Winderickx J, Thevelein JM (2001) Glucose-sensing mechanisms in eukaryotic cells. Trends Biochem Sci 26:310–317
Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709
Rook F, Gerrits N, Kortstee A, van Kampen M, Borrias M, Weisbeek P, Smeekens S (1998) Sucrose-specific signalling represses translation of the Arabidopsis ATB2 bZIP transcription factor gene. Plant J 15:253–263
Roustan V, Jain A, Teige M, Ebersberger I, Weckwerth W (2016) An evolutionary perspective of AMPK–TOR signaling in the three domains of life. J Exp Bot 67:3897–3907
Saini S, Sharma I, Pati PK (2015) Versatile roles of brassinosteroid in plants in the context of its homoeostasis, signaling and crosstalks. Front Plant Sci 6:950
Sairanen I, Novak O, Pencik A, Ikeda Y, Jones B, Sandberg G, Ljung K (2012) Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. Plant Cell 24:4907–4916
Salchert K, Bhalerao R, Koncz-Kalman Z, Koncz C (1998) Control of cell elongation and stress responses by steroid hormones and carbon catabolic repression in plants. Philos Trans R Soc Lond B Biol Sci 353:1517–1520
Salem MA, Li Y, Wiszniewski A, Giavalisco P (2017) Regulatory-associated protein of TOR (RAPTOR) alters the hormonal and metabolic composition of Arabidopsis seeds, controlling seed morphology, viability and germination potential. Plant J 92:525–545
Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signalling. Nature 459:1071–1078
Sarbassov DD, Ali SM, Kim D-H, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) RICTOR, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14:1296–1302
Schaller GE, Bishopp A, Kieber JJ (2015) The yin-yang of hormones: cytokinin and auxin interactions in plant development. Plant Cell 27:44–63
Schepetilnikov M, Kobayashi K, Geldreich A, Caranta C, Robaglia C, Keller M, Ryabova LA (2011) Viral factor TAV recruits TOR/S6K1 signalling to activate reinitiation after long ORF translation. EMBO J 30:1343–1356
Schepetilnikov M, Dimitrova M, Mancera-Martínez E, Geldreich A, Keller M, Ryabova LA (2013) TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. EMBO J 32:1087–1102
Schepetilnikov M, Makarian J, Srour O, Geldreich A, Yang Z, Chicher J, Hammann P, Ryabova LA (2017) GTPase ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin. EMBO J 36:886–903
Schröder F, Lisso J, Müssig C (2011) EXORDIUM-LIKE1 promotes growth during low carbon availability in Arabidopsis. Plant Physiol 156:1620–1630
Seo PJ, Ryu J, Kang SK, Park C-M (2011) Modulation of sugar metabolism by an INDETERMINATE DOMAIN transcription factor contributes to photoperiodic flowering in Arabidopsis. Plant J 65:418–429
She J, Han Z, Zhou B, Chai J (2013) Structural basis for differential recognition of brassinolide by its receptors. Protein Cell 4:475–482
Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu FF, Sharon M, Browse J, He SY, Rizo J, Howe GA, Zheng N (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405
Shibuya N, Minami E (2001) Oligosaccharide signalling for defence responses in plant. Physiol Mol Plant Pathol 59:223–233
Singh M, Gupta A, Laxmi A (2014a) Glucose and phytohormone interplay in controlling root directional growth in Arabidopsis. Plant Signal Behav 9:e29219
Singh M, Gupta A, Laxmi A (2014b) Glucose control of root growth direction in Arabidopsis thaliana. J Exp Bot 65:2981–2993
Singh M, Gupta A, Singh D, Khurana JP, Laxmi A (2017) Arabidopsis RSS1 mediates cross-talk between glucose and light signaling during hypocotyl elongation growth. Sci Rep 7:16101

Slaymaker DH, Navarre DA, Clark D, del Pozo O, Martin GB, Klessig DF (2002) The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proc Natl Acad Sci U S A 99:11640–11645

Smart CM (1994) Gene expression during leaf senescence. New Phytol 126:419–448

Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ (2005) The decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. Plant Cell 17:746–759

Song Y, Zhao G, Zhang X, Li L, Xiong F, Zhuo F, Zhang C, Yang Z, Datla R, Ren M, Li F (2017) The crosstalk between target of rapamycin (TOR) and jasmonic acid (JA) signaling existing in Arabidopsis and cotton. Sci Rep 7:45830

Sonnewald S, Priller JPR, Schuster J, Glickmann E, Hajirezaei MR, Siebig S, Mudgett MB, Sonnewald U (2012) Regulation of cell wall-bound invertase in pepper leaves by Xanthomonas campestris pv. vesicatoria type three effectors. PLoS One 7:e51763

Sorefan K, Booker J, Haurogné K, Goussot M, Bainbridge K, Foo E, Chatfield S, Ward S, Beveridge C, Rameau C, Leyser O (2003) MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. Genes Dev 17:1469–1474

Soto-Burgos J, Bassham DC (2017) SnRK1 activates autophagy via the TOR signaling pathway in Arabidopsis thaliana. PLoS One 12:e0182591

Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson DC (2015) SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in Arabidopsis. Plant Cell 27:3143–3159

Srivastava MK, Dwivedi UN (2000) Delayed ripening of banana fruit by salicylic acid. Plant Sci 158:87–96

Stirnberg P, van de Sande K, Leyser HMO (2002) MAX1 and MAX2 control shoot lateral branching in Arabidopsis. Development 129:1131–1141

Stirnberg P, Furner IJ, Leyser HMO (2007) MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. Plant J 50:80–94

Sun Y, Fan XY, Cao DM, Tang W, He K, Zhu JY, He JX, Bai MY, Zhu S, Oh E, Patil S, Kim TW, Ji H, Wong WH, Rhee SY, Wang ZY (2010) Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell 19:765–777

Swartzberg D, Hanael R, Granot D (2011) Relationship between hexokinase and cytokinin in the regulation of leaf senescence and seed germination. Plant Biol 13:439–444

Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei GP, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell 85:171–182

Thelander M, Olsson T, Ronne H (2004) Snf1-related protein kinase 1 is needed for growth in a normal day-night light cycle. EMBO J 23:1900–1910

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature 448:661–665

Tholen D, Voesenek LACJ, Poorter H (2004) Ethylene insensitivity does not increase leaf area or relative growth rate in Arabidopsis, Nicotiana tabacum, and Petunia x hybrida. Plant Physiol 134:1803–1812

Tholen D, Pons TL, Voesenek LA, Poorter H (2008) The role of ethylene perception in the control of photosynthesis. Plant Signal Behav 3:108–109

Tognetti JA, Pontis HG, Martínez-Noël GMA (2013) Sucrose signaling in plants: a world yet to be explored. Plant Signal Behav 8:e23316

Tsai AY-L, Gazzarrini S (2014) Trehalose-6-phosphate and SnRK1 kinases in plant development and signaling: the emerging picture. Front Plant Sci 5:119

Tuteja N (2007) Abscisic acid and abiotic stress signaling. Plant Signal Behav 2:135–138

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, Matsuoka M (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437:693–698

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195–200

Urano D, Phan N, Jones JC, Yang J, Huang J, Grigston J, Philip Taylor J, Jones AM (2012) Endocytosis of the seven-transmembrane RGS1 protein activates G-protein-coupled signalling in Arabidopsis. Nat Cell Biol 14:1079–1088

Urano D, Chen J-G, Botella JR, Jones AM (2013) Heterotrimeric G protein signalling in the plant kingdom. Open Biol 3:120186

Uzunova AN, Popova LP (2000) Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. Photosynthetica 38:243–250

Vandenbussche F, Suslov D, De Grauwe L, Leroux O, Vissenberg K, Van der Straeten D (2011) The role of brassinosteroids in shoot gravitropism. Plant Physiol 156:1331–1336

Vicentini R, Felix Jde M, Dornelas MC, Menossi M (2009) Characterization of a sugarcane (Saccharum spp.) gene homolog to the brassinosteroid insensitive1-associated receptor kinase 1 that is associated to sugar content. Plant Cell Rep 28:481–491

Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177–206

von Arnim AG, Jia Q, Vaughn JN (2014) Regulation of plant translation by upstream open reading frames. Plant Sci 214:1–12

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M (2013) Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science 339:704–707

Wang D, Weaver ND, Kesarwani M, Dong X (2005) Induction of protein secretory pathway is required for systemic acquired resistance. Science 308:1036–1040

Wang H, Yang C, Zhang C, Wang N, Lu D, Wang J, Zhang S, Wang ZX, Ma H, Wang H (2011) Dual role of BKI1 and 14-3-3s in brassinosteroid signaling to link receptor with transcription factors. Dev Cell 21:825–834

Wang Y, Sun S, Zhu W, Jia K, Yang H, Wang X (2013) Strigolactone/MAX2-induced degradation of brassinosteroid transcriptional effector BES1 regulates shoot branching. Dev Cell 27:681–688

Wang C, Liu Y, Li S-S, Han G-Z (2015) Insights into the origin and evolution of the plant hormone signaling machinery. Plant Physiol 167:872–886

Wang P, Zhao Y, Li Z, Hsu C-C, Liu X, Fu L, Hou Y-J, Du Y, Xie S, Zhang C, Gao J, Cao M, Huang X, Zhu Y, Tang K, Wang X, Tao WA, Xiong Y, Zhu JK (2018) Reciprocal regulation of the TOR kinase and ABA receptor balances plant growth and stress response. Mol Cell 69:100–112

Washio K (2003) Functional dissections between GAMYB and Dof transcription factors suggest a role for protein-protein associations in the gibberellin-mediated expression of the RAmy1A gene in the rice aleurone. Plant Physiol 133:850–863

Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann Bot 111:1021–1058

Weiste C, Pedrotti L, Selvanayagam J, Muralidhara P, Fröschel C, Novák O, Ljung K, Hanson J, Dröge-Laser W (2017) The Arabidopsis bZIP11 transcription factor links low-energy signalling to auxin-mediated control of primary root growth. PLoS Genet 13:e1006607

Wiese A, Elzinga N, Wobbes B, Smeekens S (2004) A conserved upstream open reading frame mediates sucrose-induced repression of translation. Plant Cell 16:1717–1729

Wild M, Davière JM, Cheminant S, Regnault T, Baumberger N, Heintz D, Baltz R, Genschik P, Achard P (2012) The Arabidopsis DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell 24:3307–3319

Wind J, Smeekens S, Hanson J (2010) Sucrose: metabolite and signaling molecule. Phytochemistry 71:1610–1614

Wingler A (2018) Transitioning to the next phase: the role of sugar signaling throughout the plant life cycle. Plant Physiol 176:1075–1084

Woeste KE, Ye C, Kieber JJ (1999) Two Arabidopsis mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. Plant Physiol 119:521–530

Wu CY, Trieu A, Radhakrishnan P, Kwok SF, Harris S, Zhang K, Wang J, Wan J, Zhai H, Takatsuto S, Matsumoto S, Fujioka S, Feldmann KA, Pennell RI (2008) Brassinosteroids regulate grain filling in rice. Plant Cell 20:2130–2145

Xiao W, Sheen J, Jang JC (2000) The role of hexokinase in plant sugar signal transduction and growth and development. Plant Mol Biol 44:451–461

Xie Z, Chen Z (1999) Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. Plant Physiol 120:217–226

Xie X, Yoneyama K, Yoneyama K (2010) The strigolactone story. Annu Rev Phytopathol 48:93–117

Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen J (2013) Glucose-TOR signalling reprograms the transcriptome and activates meristems. Nature 496:181–186

Xiong F, Zhang R, Meng Z, Deng K, Que Y, Zhuo F, Feng L, Guo S, Datla R, Ren M (2017) Brassinosteriod insensitive 2 (BIN2) acts as a downstream effector of the target of rapamycin (TOR) signaling pathway to regulate photoautotrophic growth in Arabidopsis. New Phytol 213:233–249

Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, Dubugnon L, Farmer EE (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell 19:2470–2483

Yanagisawa S, Yoo S-D, Sheen J (2003) Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature 425:521–525

Yang L, Xu M, Koo Y, He J, Poethig RS (2013) Sugar promotes vegetative phase change in Arabidopsis thaliana by repressing the expression of MIR156A and MIR156C. Elife 2:e00260

Yu S, Cao L, Zhou C-M, Zhang T-Q, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang J-W (2013) Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. Elife 2:e00269

Yuan K, Wysocka-Diller J (2006) Phytohormone signalling pathways interact with sugars during seed germination and seedling development. J Exp Bot 57:613359–613367

Yuan TT, Xu HH, Zhang KX, Guo TT, Lu YT (2014) Glucose inhibits root meristem growth via ABA INSENSITIVE 5, which represses PIN1 accumulation and auxin activity in Arabidopsis. Plant Cell Environ 37:1338–1350

Zhang Y, He J (2015) Sugar-induced plant growth is dependent on brassinosteroids. Plant Signal Behav 10:e1082700

Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ (2009) Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. Plant Physiol 149:1860–1871

Zhang D, Jing Y, Jiang Z, Lin R (2014) The chromatin-remodeling factor PICKLE integrates brassinosteroid and gibberellin signaling during skotomorphogenic growth in Arabidopsis. Plant Cell 26:2472–2485

Zhang Z, Zhu J-Y, Roh J, Marchive C, Kim S-K, Meyer C, Sun Y, Wang W, Wang Z-Y (2016) TOR signaling promotes accumulation of BZR1 to balance growth with carbon availability in Arabidopsis. Curr Biol 26:1854–1860

Zhong C, Xu H, Ye S, Wang S, Li L, Zhang S, Wang X (2015) Gibberellic acid-stimulated Arabidopsis6 serves as an integrator of gibberellin, abscisic acid, and glucose signaling during seed germination in Arabidopsis. Plant Physiol 169:2288–2303

Zhou L, J-C J, Jones TL, Sheen J (1998) Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc Natl Acad Sci U S A 95:10294–10299

Zhou F, Lin Q, Zhu L, Ren Y, Zhou K, Shabek N, Wu F, Mao H, Dong W, Gan L, Ma W, Gao H, Chen J, Yang C, Wang D, Tan J, Zhang X, Guo X, Wang J, Jiang L, Liu X, Chen W, Chu J, Yan C, Ueno K, Ito S, Asami T, Cheng Z, Wang J, Lei C, Zhai H, Wu C, Wang H, Zheng N, Wan J (2013) D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signaling. Nature 504:406–410

Zhu JY, Sae-Seaw J, Wang ZY (2013) Brassinosteroid signalling. Development 140:1615–1620

Zwack PJ, Rashotte AM (2013) Cytokinin inhibition of leaf senescence. Plant Signal Behav 8:e24737

Muhammed Jamsheer K did his Ph.D. with Dr. Ashverya Laxmi at NIPGR, and currently he is working as an Assistant Professor/DST-INSPIRE Faculty Fellow at the Amity Food and Agriculture Foundation, Amity University, Uttar Pradesh, Noida.

Sunita Jindal is Ph.D. from CSIR-CIMAP Lucknow, mentored by Dr. Vikrant Gupta. She is working as a Postdoctoral Research Associate in the lab of Dr. Ashverya Laxmi.

Mohan Sharma is pursuing Ph.D. from NIPGR under Dr. Ashverya Laxmi at NIPGR currently being mentored by Dr. Ashverya Laxmi.

Manvi Sharma is pursuing Ph.D. from NIPGR under Dr. Ashverya Laxmi at NIPGR currently being mentored by Dr. Ashverya Laxmi.

Dhriti Singh is pursuing Ph.D. from NIPGR under Dr. Ashverya Laxmi at NIPGR currently being mentored by Dr. Ashverya Laxmi.

Archna Tiwari is pursuing Ph.D. from NIPGR under Dr. Ashverya Laxmi at NIPGR currently being mentored by Dr. Ashverya Laxmi.

Harshita B. Saksena is pursuing Ph.D. from NIPGR under Dr. Ashverya Laxmi at NIPGR currently being mentored by Dr. Ashverya Laxmi.

Bhuwaneshwar Mishra did his Ph.D. with Dr. Ashverya Laxmi and pursued his postdoctoral studies from UDSC.

Sunita Kushwah who was a former Ph.D. student under Dr. Ashverya Laxmi's mentorship, is currently a Postdoctoral Fellow at Plant Cell Wall Biology group at Umeå Plant Science Centre, Umeå, Sweden.

Zeeshan Z. Banday is currently a Postdoctoral Fellow at the Department of Molecular Genetics and Cell Biology, The University of Chicago. He earlier worked as a Research Associate with Dr. Ashverya Laxmi at NIPGR and was mentored by Prof. Ashish K. Nandi at JNU, New Delhi, during his Ph.D.

Ashverya Laxmi obtained her Ph.D. in Plant Molecular Biology in the year 2002 from UDSC, South Campus, with Professor Jitendra P. Khurana wherein she worked on to understand the nature of crosstalk between light and hormone signal transduction pathways. She did her first Postdoctoral Fellowship with Dr. J.C. Jang, Ohio State University, Ohio, USA, wherein she deciphered molecular mechanism of sugar signal transduction pathway in *Arabidopsis*. She did her second Postdoctoral Research Fellowship at Samuel Robert Noble Foundation, Ardmore, Oklahoma, USA, with Dr. Rujin Chen wherein she further pursued her interest to characterize role of light in controlling auxin transport and its eventual effect on plant root growth and development. She returned back to India in the year 2006 and has since been working as a Staff Scientist at National Institute of Plant Genome Research (NIPGR), New Delhi. From then on she has been in contact with the Editor who is the Chair of the Scientific and Advisory committee of NIPGR. Her group has extensively characterized glucose-hormone signaling interactions in controlling plant growth and development. She also has several significant leads in understanding role of glucose/energy signaling in regulating abiotic stress responses in plants.

ROS Signaling and Its Role in Plants

14

Mrinalini Manna, V. Mohan M. Achary,
and Malireddy K. Reddy

Abstract

Reactive oxygen species (ROS) are the unavoidable byproducts of aerobic metabolism. They are the necessary evils for every living organism whose lives are dependent on atmospheric oxygen in one form or another. While excess level of ROS is toxic for the plants and causes oxidative stress, an optimum basal level of ROS is required to be maintained in the cells as it is indispensable for plant's proper growth and development. Various latest studies have discovered that ROS signaling is essential for carrying out various biological activities such as cellular proliferation, differentiation, physiological cell death, cell-to-cell communication, stress acclimation, pathogen defense, and so on. Judicious manipulation of key regulators of ROS signaling can bring about improved adaptation of the plants to the recent climate changes happening across the globe.

Keywords

Adaptation · Cell signaling · Oxidative stress · Reactive oxygen species homeostasis · Stress response

14.1 Introduction

Reactive oxygen species (ROS) are partially reduced (e.g., O_2^-, H_2O_2, OH^-) or exited (e.g., $^1O_2$) forms of atmospheric molecular oxygen (O_2) (Halliwell and Gutteridge 2007). They appeared on earth since the evolution of aerobic organisms about 2.4–3.8 billion years ago and have remained a part of biological activities of cells since then (Wood et al. 2003; Halliwell and Gutteridge 2007; Anbar 2008;

M. Manna · V. M. M. Achary · M. K. Reddy (✉)
International Centre for Genetic Engineering and Biotechnology, New Delhi, India
e-mail: reddy@icgeb.res.in

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_14

Mittler et al. 2011; Miller 2012; Boyd et al. 2014; Mittler 2016). The chemistry of O_2 is the reason behind formation of ROS in the cells. Despite containing an even number of electrons, O_2 has two unpaired electrons with the same spin in its molecular orbital. The O_2 is an oxidizing agent whereby it attracts a pair of electrons from an electropositive molecule to pair with its two unpaired electrons. For this oxidation step to happen, the electron donor should donate two electrons having the same spin quantum number but opposite in direction in comparison to the two unpaired electrons of O_2. However, most of the electrons present in atomic or molecular orbitals of various chemical reactants have anti-parallel spin posing a constraint on O_2-mediated oxidation. In order to avoid this spin restriction, O_2 molecule reacts with paramagnetic elements such as iron (Fe) and copper (Cu), which possess unpaired electrons. The O_2 oxidizes Fe^{3+} into Fe^{2+}, and upon accepting its one electron, O_2 gets reduced into superoxide radical (O_2^-), a very reactive ROS with a half-life of about 1–4 μs. In aqueous solution, O_2^- reacts with H^+ to form either hydrogen peroxide (H_2O_2) or hydroxyl radical (OH^-) which is far more reactive than the former. While H_2O_2 has a half-life of about 1 ms, OH^- has a very short life span with half-life of just 1 ns. The reaction of H_2O_2 with Fe^{2+} (called Fenton reaction) also results in the formation of OH^- radical in the cells (Tripathy and Oelmüller 2012; Mittler 2016). Singlet oxygen ($^1O_2$) is another kind of ROS produced in the cells. The ground state of quite nonreactive O_2 is called triplet state ($^3O_2$) in which its unpaired electrons have the same spin (↑↑) in the molecular orbital. When $^3O_2$ absorbs enough energy, spin of one of the electrons is reversed resulting in the formation of $^1O_2$ which has a half-life of about 1–4 μs. The $^1O_2$ radicals also form when O_2^- radicals interact with OH^- radicals. Thus, ROS production inside a cell is imminent wherever there is presence of O_2, and due to this, ROS production is considered as a byproduct of aerobic metabolism (i.e., photosynthesis, respiration, and photorespiration). Various kinds of ROS are being depicted in Fig. 14.1a. Being very reactive, ROS damages various cellular components. For instance, O_2^- reacts with Fe-S proteins; OH^- radical damages nucleic acids, proteins, and lipids; H_2O_2 denatures proteins by attacking their Cys and Met residues, and it also causes damage to heme-containing proteins and DNA; and $^1O_2$ oxidizes lipids, proteins (having Trp, His, Tyr, Met, and Cys residues), and G-residues of DNA. These damages are collectively termed as *oxidative stress* (Mittler 2016). To overcome the ROS-mediated cellular toxicity, the organisms on earth have invented various kinds of antioxidative enzymes and antioxidants (Mittler et al. 2004) (Fig. 14.1b). The presence of antioxidative enzyme superoxide dismutase (SOD) in all kingdoms of life and its evolution before the separation lineage of Eubacteria and Archaea (Miller 2012) suggest that ROS scavenging system had always been an integral part of cells to counter the harmful effects of ROS. The ROS scavenging system plays a crucial role in keeping the ROS level at a safer nontoxic level in the cells, and when ROS is present in excess, ROS-mediated cellular signaling (called *ROS signaling*) occurs which is essential for the organism's adaptation during the oxidative stress (Mittler 2016). A fine balance exists between aerobic metabolism-mediated ROS production, diffusion, reactivity, signaling, ROS scavenging, and ROS perception in various cellular compartments (*ROS signaling*) (Mittler 2016). Different environmental stimuli (such as scorching sunlight, gusty wind, salt stress, water logging, dehydration,

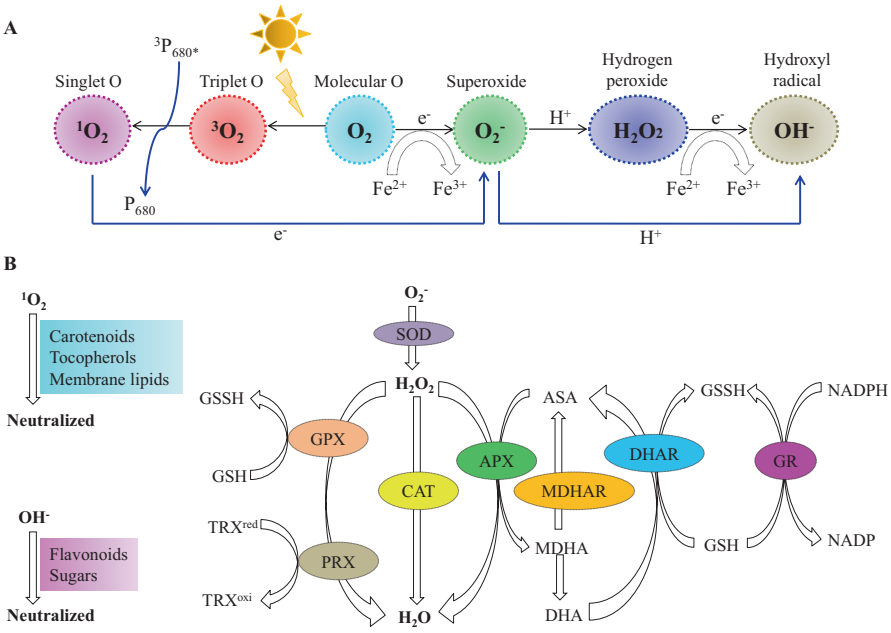

Fig. 14.1 ROS production and their scavenging inside the cell. (**a**) Schematic diagram showing method of production of various types of ROS from molecular oxygen. (**b**) Different enzymatic and nonenzymatic methods of ROS scavenging in a cell

insect attack, pathogen invasion, weed infestation, etc.) result in different types of ROS networking which in turn determines the response of the organism to a particular stimulus (Fig. 14.2). This paper attempts to describe various aspects of ROS signaling in plants.

14.2 Source of ROS in Plants

ROS are generated in cellular compartments where production and/or consumption of O_2 occurs or redox potential is high or flow of electrons is intense. Chloroplasts, mitochondria, and peroxisomes having the abovementioned properties are the major sites of ROS production in plants. The ROS production can also happen at any other cellular compartments which contain proteins or molecules with high redox potential for donating electrons to O_2. For example, membrane-bound NADPH oxidases and cell wall-localized amine oxidases produce ROS (Tripathy and Oelmüller 2012; Mittler 2016).

In C_3 plants, when photosynthesis exceeds respiration and intense sunlight, heat and water stress lead to stomatal closure, and O_2 concentration inside chloroplasts increases leading to photorespiration. During photorespiration, ribulose bisphosphate carboxylase/oxygenase (RuBisCO) enzyme having higher affinity toward O_2 reacts with it to form glycolate, which is transported to peroxisome, and glycolate

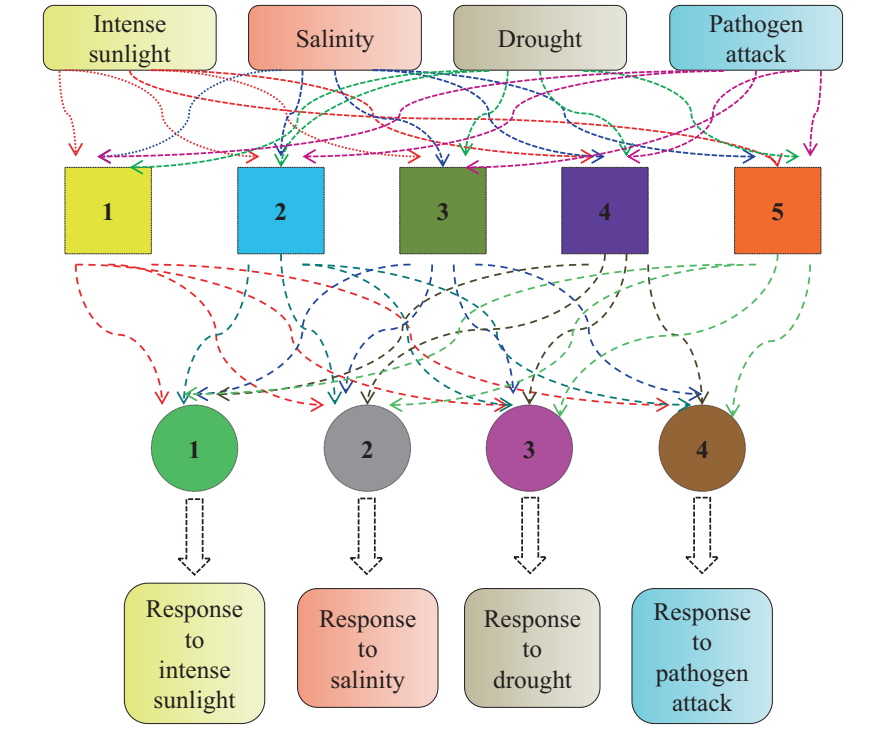

Fig. 14.2 Schematic representation showing how differential activation of ROS signal receptors takes place and how their differential interaction with various downstream signaling molecules occurs to bring about overall stress adaptation response when plants experience environmental perturbations

oxidase enzyme present there oxidizes glycolate to produce H_2O_2 (Tripathy and Oelmüller 2012). Chloroplasts are also the sites of $^1O_2$ production. Chlorophyll pigment is the major light-absorbing component of the light-harvesting complexes (LHCs) present in both the photosystems (PS) in green plants. Upon absorption of light energy, chlorophyll molecules reach a short-lived excitation state leading to establishment of an electrochemical potential via charge separation. The energy generated due to the formation of electrochemical gradient is dissipated to downstream molecules involved in light reaction of photosynthesis. However, if energy transfer is inefficient, it leads to the formation of triplet-state chlorophyll which reacts with $^3O_2$ to produce the extremely reactive $^1O_2$ species. Carotenoids present in LHC quench $^1O_2$ (Frank et al. 1999). In the presence of excess light, when light absorption by the leaves exceeds consumption of light energy by photosynthesis, the plastoquinone A and plastoquinone B in the electron transport chain are over-reduced causing inadequate charge separation between P_{680} and pheophytin.

This situation favors formation of triplet state of reaction center chlorophyll P_{680} (or P_{680}) leading to the formation of $^1O_2$ (Foote et al. 1984; Barber and Andersson 1992; Aro et al. 1993; Ohad et al. 1994).

Chlorophyll biosynthesis intermediates present in the thylakoids such as protochlorophyllide, protoporphyrin IX, 5-aminolevulinic acid, etc. produce $^1O_2$ in plants and are the reasons for oxidative cellular damages (Rebeiz et al. 1984, 1991, 1998; Tripathy and Chakraborty 1991; Chakraborty and Tripathy 1992; Mock and Grimm 1997; Shalygo et al. 1998; Lermontova and Grimm 2006; Tripathy et al. 2007; Jung et al. 2008). The ROS scavenging carotenoids being spatially far from chlorophyll biosynthesis intermediates are incapable of efficiently scavenging ROS (Havaux et al. 2007; Mozzo et al. 2008). There is hardly any overproduction of chlorophyll biosynthetic intermediates for their synthesis is highly regulated. However, when plants are exposed to high light intensity and many other oxidative stresses, these chlorophyll biosynthesis intermediates are capable of producing $^1O_2$ leading to oxidative damage of the cells (Chakraborty and Tripathy 1992).

Mitochondria are the other sites of ROS production in plant cells where a single electron from the electron transport chain is transferred to O_2 causing production of O_2^- and other species of ROS (Purvis 1997). However, unlike animal cells, mitochondria of plant cells are not major sites of ROS production (Maxwell et al. 1999). The mitochondrial alternative oxidase (AOX) catalyzes ROS oxidation in an O_2-dependent manner (Purvis 1997). When *Arabidopsis* and catalase mutant tobacco cells were treated with H_2O_2, the AOX level was found to increase in the mitochondria which indicated that recognition of over-accumulation of ROS in plant cells causes induction of mitochondrial AOX enzymes to scavenge the toxic ROS (Sweetlove et al. 2008).

The NADPH-dependent oxidases present in the plasma membrane of plant cells contain a multimeric flavocytochrome that are capable of forming an electron transport chain, and acceptance of an electron by a molecule of O_2 results in the formation of O_2^- (Allan and Fluhr 1997). Additionally, pH-dependent amine oxidases, oxalate oxidase, cell wall peroxidase, polyamine oxidase, and apoplastic peroxidases also contribute to ROS production in the apoplast of plant cells (Hu et al. 2003; Walters 2003). The Respiratory Burst Oxidase Homologues (RBOHs) present in the cytoplasm oxidize NADPH and transfer the electron to O_2, thereby producing O_2^- which is then converted to H_2O_2 (Tripathy and Oelmüller 2012).

14.3 Mechanism of ROS Homeostasis in Different Cellular Compartments

We have discussed above that ROS are generated in chloroplasts, mitochondria, peroxisomes, and apoplast in response to highly reducing atmosphere of the cells or by means of enzymatic actions. Excess of ROS is detrimental for the cells, and hence, a healthy cell maintains the amount of ROS production both spatially and temporally by scavenging the excess of ROS produced. In this section, the detoxification mechanisms of excess ROS by various enzymatic and nonenzymatic means are being discussed.

Chloroplasts are the major sites of $^1O_2$ production (Fischer et al. 2013). No enzyme has evolved to directly detoxify $^1O_2$, and it is scavenged by carotenoids, tocopherols, and membrane lipids (Krieger-Liszkay and Trebst 2006; Ramel et al. 2012; Farmer and Mueller 2013). The half-life of $^1O_2$ is about 1 μs, and it spontaneously dismutates to H_2O_2 in the stromal side of the thylakoid membrane where it is removed enzymatically by the superoxide dismutases (SODs) belonging to three different classes such as iron-SOD, copper-SOD, and zinc-SOD. Stromal ascorbate peroxidases (APXs), glutathione peroxidase-like enzymes (GPXLs), and peroxiredoxins (Prxs) also detoxify chloroplastic H_2O_2 (Asada 2006). Further, water-water cycle, where flow of electron occurs from O_2^- to H_2O_2 to water, is also involved in scavenging ROS detoxification in the chloroplast (Awad et al. 2015).

Mitochondrial ROS are mainly scavenged by alternative oxidases (AOXs) (Giraud et al. 2008). Plant mitochondria are also seen to divert electron flow by bypassing complexes III and IV of electron transport chain (ETC) to avoid ROS production (Huang et al. 2016).

Photorespiration inside peroxisome results in H_2O_2 production. However, rise in H_2O_2 concentration is prevented by mainly activity of catalases present in this organelle (Queval et al. 2007). Catalases can effectively scavenge peroxisomal H_2O_2 and keep its concentration below 10 μM (Foyer and Noctor 2016). Ascorbate-glutathione cycle also helps in controlling the ROS levels in peroxisome (Del Rio and Lopez-Huertas 2016).

Apoplasts are the major sites of O_2^- production, and it is converted to H_2O_2 either spontaneously or by the activity of apoplastic superoxide dismutases (SODs) (Cheng et al. 2009).

14.4 ROS Sensing and Signaling

The common feature of all kinds of abiotic and biotic stresses in plants is ROS overproduction. This implies that ROS have evolved to play a vital role in sensing various environmental cues and relaying those signals to nuclei for gene expression so that plants adapt to such stresses. A fine balance between ROS generation and their scavenge helps in generation of innumerable types of ROS signatures inside the cell resulting in differential gene expression in response to different types of stresses. These ROS signatures also interact with other signaling events of the cells such as Ca^{2+} signaling to bring about variation in the overall response.

The ROS signaling essentially involves the ability of ROS to react with various metabolites and proteins present in the cells. Initial ROS sensing involves ROS-mediated oxidation of sensory proteins (via posttranslational modifications (PTMs)) and metabolites. The primary targets for H_2O_2-mediated oxidative PTMs of ROS-sensitive proteins are the sulfur (S) atoms present in cysteine and methionine residues of sensory proteins. The reaction of H_2O_2 with S atoms of cysteine leads to the formation of cysteine sulfenic acid (-SOH) group which reacts with either glutathione (GSH) or other thiol groups resulting in the formation of S-glutathionylation (-SSG) group or inter−/intra-molecular disulfide (-S-S-) bonds, respectively (Roos and Messens 2011; Waszczak et al. 2014). Deglutathionylation

and reduction of disulfide bonds are catalyzed by glutathione peroxidases (GPXs) and thioredoxin peroxidases (TRXs) (Meyer et al. 2012). The oxidized sensory proteins further oxidize effector proteins, forming a redox relay. So far, the sole example of such redox relay in plants is GLUTATHIONE PEROXIDASE-LIKE3 (GPXL3)–ABA-INSENSITIVE2 (ABI2) H_2O_2-sensing system, which has been speculated to regulate stomatal closure (Miao et al. 2006). *In planta* studies have revealed that GPXL3 upon oxidation interacted with and oxidized ABI2 that led to inhibition of AB12 activity (Miao et al. 2006). However, recent studies revealed that GPXL3 localizes to the ER membrane (Attacha et al. 2017) and ABI2 to cytoplasm, thereby making their interaction highly unlikely. Thus, the actual method of H_2O_2-mediated redox relay involving GPXL3 remains to be elucidated. Analogous redox relay mechanisms in yeast found that the thiol peroxidase GPX3 oxidized the transcription factor YAP1 which finally resulted in its nuclear import and transcriptional activity (Delaunay et al. 2002).

Apart from cysteine thiols, methionine residues are also subjected to H_2O_2-mediated PTM leading to the formation of methionine sulfoxide ($-(S=O)-CH_3$) and its subsequent irreversible oxidation to methionine sulfone ($-(SO_2)-CH_3$). Methionine sulfoxide is reduced by a large group of methionine sulfoxide reductases which utilize TRX as the electron donor (Tarrago et al. 2009). When *Arabidopsis* plants were exposed to photorespiratory stress, approximately 400 proteins were found to be oxidized at their methionine residues (Jacques et al. 2015). However, methionine oxidation-mediated signaling events have not been extensively elucidated yet. Methionine oxidation generally leads to inactivation of protein function (Jacques et al. 2015; Lee et al. 2014); however, recent data from bacteriological studies reveal that the opposite effect is also a possibility (Drazic et al. 2013).

Chloroplastic ROS signaling reveals that a correlation exists between H_2O_2 formation in the chloroplast and alteration in its metabolite production and gene expression in the nucleus (Chan et al. 2016b; de Souza et al. 2017; Leister 2017). However, since H_2O_2 has several sites of origin, a question arises as to how the nucleus specifically recognizes chloroplastic H_2O_2 level. Recent reports indicate that chloroplastic ROS signature is effectively relayed to the nucleus by signaling events involving (a) direct stromule-mediated delivery of ROS and posttranslationally modified proteins to the nucleus; (b) fast regulation of nuclear H_2O_2 concentration by a population of companion chloroplasts localized around the nucleus; and (c) signaling via accumulation of chloroplast metabolites, their oxidative derivatives, or both (Waszczak et al. 2018). Stromules are the dynamic plastid projections through which plastids and nuclei maintain a direct contact between them (Erickson et al. 2017; Hanson and Sattarzadeh 2013). The stromules were seen to be formed in response to treatment of plants with ROS-generating chemicals and during photosynthesis, when there is formation of ROS and electrons (Brunkard et al. 2015). Further, in case of chloroplasts present near the nuclei, the chloroplastic ROS are directly transferred to nuclei in a stromule-independent manner (Exposito-Rodriguez et al. 2017; Caplan et al. 2015). When ROS accumulate inside the chloroplasts, the 3′-phosphoadenosine 5′-phosphate (PAP) phosphatase SAL1 undergoes redox- or H_2O_2-dependent oxidative inactivation which further leads to PAP accumulation

(Chan et al. 2016a). PAP is suggested to act as a secondary messenger involved in relaying ROS levels in chloroplasts to the nuclei (Estavillo et al. 2011). Further, PAP is also involved in relaying ROS-generated signal from mitochondria to nuclei (Waszczak et al. 2018).

Cell-to-cell transfer of ROS signaling phenomena involves activation of NADPH oxidases that produce O_2^- anions on the apoplastic side of the plasma membrane. Further, the O_2^-ions are dismutated to H_2O_2 and diffuse to the neighboring cells. Apoplastic H_2O_2 activates plasma membrane-bound Ca^{2+} channels which ultimately lead to an increase in the concentration of Ca^{2+} in the cytoplasm resulting from an influx from the apoplast and other intracellular Ca^{2+} stores. The increased Ca^{2+} levels either directly activate RBOHD by binding to its EF-hands (which are the Ca^{2+}-binding motifs) or indirectly activate multiple CDPKs, which in turn lead to ROS accumulation within the apoplast of neighboring cells. These processes are thought to be associated with signaling events triggered by apoplastic or cytoplasmic ROS sensors. Such a sequence of events allows the systemic spread of information in the form of a self-propelling wave (Waszczak et al. 2018). Figure 14.3 highlights the mechanism of ROS perception, signaling, and their relay for bringing about various physiological changes in the plants (Fig. 14.3).

14.5 ROS Signaling in Various Cellular Compartments

ROS, being the indispensible component of aerobic life, has evolved to be an important signaling molecule for aiding cell-to-cell communication and transduction of environmental cues inside the cells. This signaling network is essential for plant's adaptation and survival under changing environmental conditions. However, the ROS-mediated signaling network has still not been completely discovered due to the immense complexity of ROS perception by various cellular receptor molecules, interaction of ROS signaling pathways with other signaling networks such as hormonal signaling and Ca^{2+} signaling pathways, and multiplicity of ROS targets. The following section describes the different aspects of ROS signaling pathways in various plant organelles.

14.5.1 Chloroplasts

The $^1O_2$ produced inside the chloroplast is highly reactive, and hence, it reacts with various chloroplastic molecules to form the secondary signaling compounds unless quenched rapidly. The β-cyclocitral, a β-carotene derivative produced by $^1O_2$-mediated oxidation of the carotenoids, is one such secondary signaling compound. It is a reactive electrophile species (RES) which causes changes in gene expression by chemically interacting with proteins and nucleic acids. Though higher production of RES is responsible for apoptosis, low RES levels may contribute to the expression of cell survival genes and lead to plant's survival during stress (Laloi and Havaux 2015). The fluorescent (flu) mutant of *Arabidopsis* accumulates a chlorophyll precursor, protochlorophyllide, in dark, and during dark-to-light transition,

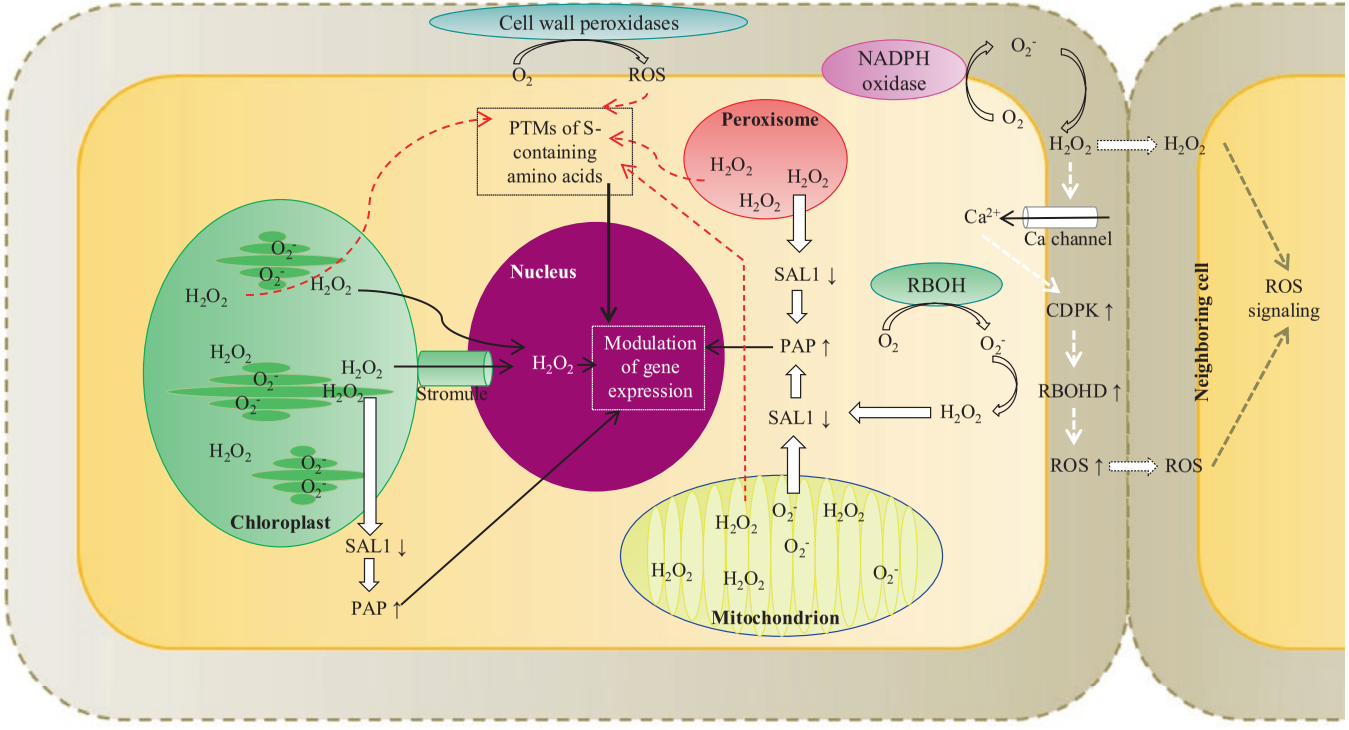

Fig. 14.3 Schematic diagram showing ROS signaling events inside and cell and cell-to-cell transfer of ROS signal. Abbreviations: PAP, 3′-phosphoadenosine 5′-phosphate; SAL, a chloroplastic PAP phosphatase; PTM, posttranslational modification; CDPK, Ca^{2+}-dependent protein kinase; RBOH, respiratory burst oxidase homolog

the precursor causes overproduction of $^1O_2$, thereby triggering apoptosis which ultimately manifests as lesions on the leaves (Camp et al. 2003). Two chloroplast proteins, EXECUTER1 (EX1) and EXECUTER2 (EX2), have been found to be involved in $^1O_2$-dependent chloroplast retrograde signaling (Lee et al. 2007). The expression of REDOX-RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1), belonging to the AP2/ERF transcription factor family, is found to be induced in response to $^1O_2$ various biotic and abiotic stresses (Foyer et al. 2014). The $^1O_2$-mediated signaling is also responsible for the activation of CALCIUM-SENSING RECEPTOR (CAS), a chloroplast-localized protein which is responsible for inducing salicylic acid accumulation and hypersensitive cell death in response to biotic stress. CAS protein is also induced by pathogen-associated molecular pattern (PAMP) during biotic stress and is involved in expression of defense-related genes in the plants (Dodd et al. 2010). Further, increased level of $^1O_2$ accumulation inside the chloroplasts of flu mutants induces jasmonic and salicylic acid production and expression of PATHOGENESIS-RELATED PROTEIN 1 (PR1) and PR5 genes (Ochsenbein et al. 2006). Another *Arabidopsis* mutant chlorina1 (ch1), deficient in chlorophyll b, showed elevated levels of $^1O_2$ under oxidative stress (Triantaphylidès et al. 2008).

The chloroplastic H_2O_2 is also involved in ROS signaling and expression of various genes encoding transcription factors, secondary signaling molecules, mitochondrial retrograde signaling molecules, and the biosynthesis of defense compounds (Sewelam et al. 2014). H_2O_2 is responsible for the activation of one of the MAPKKKs in *Arabidopsis*, ANP1, and oxidative signal-inducible 1 (OXI1) kinase, both of which lead to the activation of MPK3- and MPK6-dependent signaling cascade during transition of the plants from low to high light (Kovtun et al. 2000). The phosphorylation of MPK6 causes expression of several transcription factors from the APETALA2/ETHYLENE RESPONSE FACTOR family, such as ERF6 and ERF104 (Vogel et al. 2014). Additionally, the MEKK1–MKK1/MKK2–MPK4 cascade regulates ROS homeostasis and programmed cell death in plants (Pitzschke et al. 2009). *Arabidopsis* MPK4 has been found to be activated by both biotic and abiotic stresses (Droillard et al. 2004; Zhang et al. 2012). MPK4 is responsible for negatively regulating immune defenses in a salicylic acid-dependent manner and positively regulating photosynthesis, ROS metabolism, and growth at the same time (Gawroński et al. 2014).

A chloroplast membrane-bound plant homeodomain transcription factor with transmembrane domains (PTM) is found to be involved in chloroplast-to-nucleus retrograde signal transduction. During stress, PTM is proteolytically cleaved, and it gathers inside the nucleus, where it activates another transcription factor, ABA INSENSITIVE 4 (ABI4), which is responsible for the downregulation of many nucleus-encoded photosynthesis genes (Sun et al. 2011).

14.5.2 Mitochondria

Inside the mitochondria, H_2O_2 is mainly involved in ROS signaling. This is due to the high activity of MnSOD which dismutates O^{2-} into H_2O_2. Additionally, mitochondrial H_2O_2 has much a longer lifespan than O^{2-} and can easily pass through the

mitochondrial membranes and activate downstream signaling pathways that induce the expression of genes in response to a broad range of biotic and abiotic stresses (De Clercq et al. 2013). During H_2O_2-mediated signaling, it oxidizes the thiol group of various proteins such as AOX1 and some tricarboxylic acid (TCA)-cycle enzymes (Yoshida and Hisabori 2014; Yoshida et al. 2013; Daloso et al. 2015). In *Arabidopsis*, the inactivation of twin-Cys proteins, At12Cys-1 and At12Cys-2 proteins, results in enhanced tolerance to drought and light stresses and increased plant antioxidant capacity, thus highlighting the fact that both these genes negatively regulate stress homeostasis in the plants (Wang et al. 2016).

Mitochondria transmit their redox status to the nucleus through a signaling process called mitochondrial retrograde regulation (MRR) for modifying gene expression (Ng et al. 2014). Various transcription factors from the NAC and WRKY families and cyclin-dependent kinases (CDKs) take part in MRR (Ng et al. 2014). It has been found that overexpression of two ARABIDOPSIS NAC DOMAIN-CONTAINING PROTEINS, ANAC013 and ANAC017, increases tolerance against oxidative stresses (Ng et al. 2013). It has also been revealed that both ANAC013 and ANAC017 reside in the endoplasmic reticulum under normal conditions, and when plants witness stress, they travel to the nucleus to take part in transcriptional regulation of mitochondrial proteins (Ng et al. 2013).

Mitochondria-derived ROS cross talk with plant hormones such as ABA, SA, and auxins. For instance, mutation in a gene encoding mitochondrial inner membrane-bound protease AtFtsH4 leads to increased H_2O_2 levels and auxin oxidation, causing excessive axillary branching and dwarf phenotype (Zhang et al. 2014). Treatment of plant mitochondria with salicylic acid results in inhibition of respiration and ROS overproduction indicating involvement of salicylic acid in ROS production in plant mitochondria (Nie et al. 2015).

Study of *Arabidopsis* Mosaic Death 1 (MOD1) mutant has identified integration of chloroplastic and mitochondrial ROS signaling for bringing about programmed cell death in the plant (Wu et al. 2015).

14.5.3 Peroxisomes

Peroxisomes are the sites of photorespiration that leads to H_2O_2 production and the ROS takes part in signaling. Mutation of *Arabidopsis* LSD1 protein leads to reduced stomatal conductance and reduced peroxisomal catalase activity leading to higher H_2O_2 production and associated programmed cell death (Mühlenbock et al. 2008; Mateo et al. 2004). In *Arabidopsis*, catalase-deficient plants, i.e., CATALASE 2 mutant (cat2), serve as model systems to study signal transduction by the accumulation of peroxisomal H_2O_2 (Queval et al. 2012; Vanderauwera et al. 2005). Such plants show altered gene expression where various genes encoding enzymes engaged in protein refolding, repair, and degradation are seen to be induced suggesting the fact that H_2O_2 production in peroxisomes enhances plant's stress acclimation and tolerance responses (Sewelam et al. 2014). A gene encoding UDP-glucosyl transferase (UGT74E2) is strongly induced in cat2 plants, and it is involved in auxin homeostasis and increased tolerance to salt and drought stresses (Tognetti et al. 2010).

Other plant hormones such as salicylic acid and abscisic acid cross talk with peroxisome-derived H_2O_2 for the induction of programmed cell death (Kaurilind et al. 2015). Salicylic acid is responsible for cat2-driven programmed cell death. In the cat2/sid2 (SALICYLIC ACID INDUCTION DEFICIENT 2) double mutant, deficient in both CAT2 and ICS1 (required for SA biosynthesis), necrotic lesion formation triggered by peroxisomal H_2O_2 was suppressed which indicates that both the proteins are responsible for programmed cell death in *Arabidopsis*. Similarly, cat2/abi1 (ABA INSENSITIVE 1) mutants exhibited fewer necrotic lesions and increased plant survival suggesting involvement of ABA in peroxisomal H_2O_2-triggered programmed cell death (Chaouch et al. 2010). It has been found that increase in Ca^{2+} concentration inside peroxisome causes enhanced catalase activity and, thus, there is cross talk between Ca^{2+} and ROS signaling in peroxisome (Costa et al. 2010).

14.5.4 Apoplast

In apoplast, H_2O_2, derived from RBOH-produced O^{2-}, diffuses freely across the plasma membrane to the cytoplasm via the aquaporins (Bienert et al. 2007). Since ROS cause lipid peroxidation, membrane lipids are thought to transfer the ROS signals themselves. ROS have been found to regulate ion fluxes through the membrane by opening the ion channels (Garcia-Mata et al. 2010). As many apoplastic proteins are relatively Cys rich in their extracellular domain, they might be able to sense redox changes. Recently, a secreted Cys-rich protein has been shown to be proteolytically cleaved by METACASPASE 9 (MC9), for induction of ROS-dependent programmed cell death (Wrzaczek et al. 2015).

The Cys-rich receptor-like kinases (CRKs), which are a subfamily of receptor-like kinases (RLKs), have been suggested as ROS sensors and redox signal transmitters during abiotic stresses like ozone, UV radiation, and salinity stress conditions (Bourdais et al. 2015). They are thought to be involved in transmitting extracellular ROS signaling in order to activate intracellular MAPK cascades (Burdiak et al. 2015; Vainonen and Kangasjärvi 2015). Treating plants with ozone induces apoplastic ROS production which further activates MPK3 and MPK6 in *Arabidopsis* (Ahlfors et al. 2004). Further, *Arabidopsis* MPK2, MPK4, and MPK7 are also induced by the oxidative burst (Desikan et al. 2001; Ortiz-Masia et al. 2007). In *Arabidopsis*, MKK1 induces CAT1 expression by triggering H_2O_2 production in response to drought and salt stress (Xing et al. 2007). Many transcription factors have been recognized to act downstream of MAPKs in ROS responses, namely, MYB DOMAIN PROTEIN 44 (MYB44) (Persak and Pitzschke 2014), HEAT STRESS TRANSCRIPTION FACTOR A-4A (HSFA4A) (Pérez-Salamó et al. 2014), and ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 6 (ERF6) (Wang et al. 2013).

Mutation studies have revealed cross talks existing between apoplastic ROS signaling and hormone signaling in plants. It has been found that salicylic acid and ethylene are positive regulators of apoplastic ROS-induced programmed cell death, while jasmonic acid is a negative regulator (Tamaoki 2008).

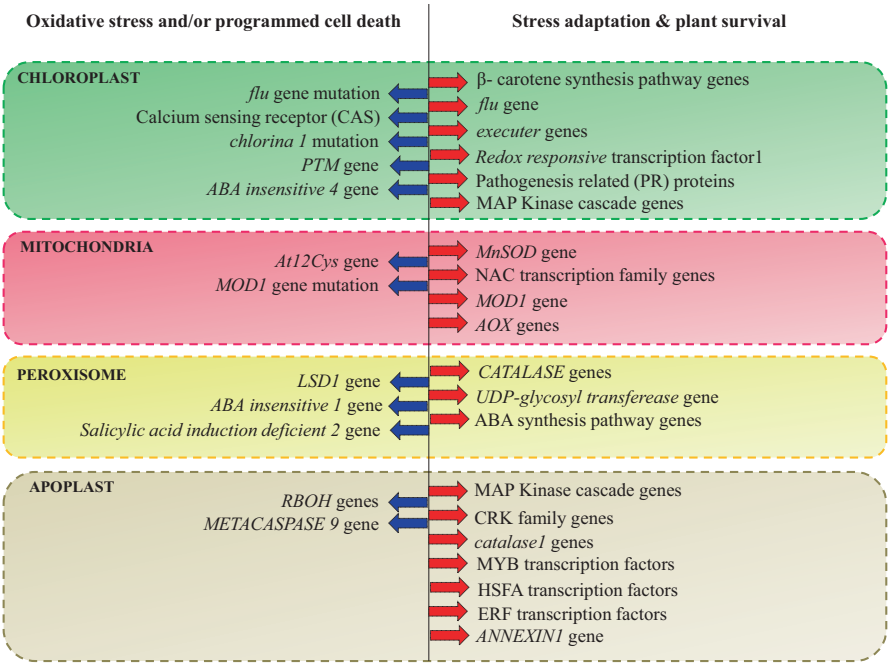

Fig. 14.4 Various genes and transcription factors involved in ROS signaling in different plant organelles for bringing about programmed cell death or stress adaption and plant survival. The description of the genes can be found in the text

Apoplastic polyamines have been shown to activate Ca^{2+}-ATPases located in the plasma membrane which are involved in the removal of Ca^{2+} from the cell (Pottosin et al. 2014). When the cytoplasmic Ca^{2+} concentration increases, multiple Ca^{2+}-dependent protein kinases such as CPK3, 4, 5, 6, 11, 21, and 23 are activated (Waszczak et al. 2015). In *Arabidopsis*, the ANNEXIN1 protein (ANN1) has been found to mediate ROS-dependent Ca^{2+} fluxes in the roots by binding to lipid membranes and stimulating the Ca^{2+} influx. It has been found that ann1 mutants are hypersensitive to drought (Konopka-Postupolska et al. 2009). The roles of various organelle genes and transcription factors in carrying out ROS signaling-mediated cell survival and death are being schematically shown in Fig. 14.4.

14.6 Role of ROS Signaling in Plants

The aerobic organisms have a highly conserved ROS signaling network controlling a wide array of biological processes constituting growth, development, and responses to abiotic and biotic stresses (Mittler et al. 2011). Though overproduction of ROS is harmful, nontoxic levels of ROS are essential for perception of various environmental stimuli and stress conditions and relay of those informations to the nuclei required for gene expression and plant's adaptation. In *Arabidopsis*, RBOHs,

the key enzymes involved in ROS production, affect various physiological processes in plants such as stomatal closure, growth of root hair, pollen tube development, and acclimation to different abiotic and biotic stresses (Torres and Dangl 2005; McInnis et al. 2006; Monshausen et al. 2007; Jammes et al. 2009; Miller et al. 2009; Nishimura and Dangl 2010; Suzuki et al. 2011).

ROS signaling is vital for plant's acclimatization to various biotic and abiotic stresses. Being sessile, plants have developed very sophisticated ROS signaling network to adapt to the changing climate. Among various enzymes involved in ROS production, RBOHs are known to play the most widespread role. The plant RBOHs have a cytosolic N-terminal region consisting of two Ca^{2+}-binding EF-hand motifs and phosphorylation target sites which are essential for their activity (Kobayashi et al. 2007; Oda et al. 2010; Kimura et al. 2012; Drerup et al. 2013). RBOHs generate O_2^- radicals in the apoplast region which is dismutated to H_2O_2 spontaneously or enzymatically by the action of SOD (Lin et al. 2009). H_2O_2 is membrane permeable, and it facilitates long-distance ROS signaling for modulating various metabolic processes of plants (Sagi et al. 2004; Xia et al. 2009). Several studies indicate that Ca^{2+} binding and phosphorylation events stimulate the ROS-producing activity of RBOHD and RBOHF in *Arabidopsis* (Ogasawara et al. 2008; Kimura et al. 2012). In this regard, increase in the level of cytosolic Ca^{2+} is essential for the activation of RBOHD (Ogasawara et al. 2008). Recent studies revealed that PLDα1 and its lipid derivative phosphatidic acid are essential for abscisic acid (ABA)-induced production of ROS in guard cells via the activity of RBOHD and RBOHF which further facilitate stomatal closure (Zhang et al. 2009a, b). OPEN STOMATA 1 (OST1) protein was found to phosphorylate RBOHF during ABA-dependent stomatal closure (Sirichandra et al. 2009). A recent study revealed that calcium-dependent protein kinase 5 (CPK5) phosphorylates RBOHD during pathogen defense (Dubiella et al. 2013). In rice, OsRac1 protein was found to be upregulated during pathogen attack, and it activated OsRBOHB by directly interacting with EF-motifs of N-terminal region in a Ca^{2+}-dependent manner (Wong et al. 2007; Oda et al. 2008). Two Ca^{2+}-dependent protein kinases, StCDPK4 and StCDPK5, were found to activate StRBOHB in *Solanum tuberosum* (potato) (Kobayashi et al. 2007). In *Capsicum annuum* (pepper), receptor-like protein kinase 1 (CaRLK1) was found to get induced during pathogen infection and exogenous application of H_2O_2 (Yi et al. 2010). The ROS signaling is also involved in priming the plants to tolerate various abiotic stresses. The stress hormone abscisic acid (ABA) which regulates various biological functions is also shown to interact with ROS (Kwak et al. 2003; Sagi et al. 2004; Ma et al. 2012; Drerup et al. 2013). Treatment of plants with ABA and SA was shown to increase H_2O_2 production which further induced tolerance to salt, high light, heat, and oxidative stress (Xia et al. 2009; Suzuki et al. 2013). ROS signaling is found to control cell death in plants. Jasmonic acid (JA) is thought to play a key role in the regulation of cell death by interacting with H_2O_2 and salicylic acid (SA) signaling during insect attack and wounding (Pasqualini et al. 2003; Zhou et al. 2009; Lin et al. 2011). Local application of high light is found to induce tolerance of plants to pathogen infection and oxidative stress (Rossel et al. 2007; Muhlenbock et al. 2008; Karpinski et al. 2013). ROS signaling-mediated regulation of growth and development of plants has also been studied. RBOHC has been shown to localize in the root

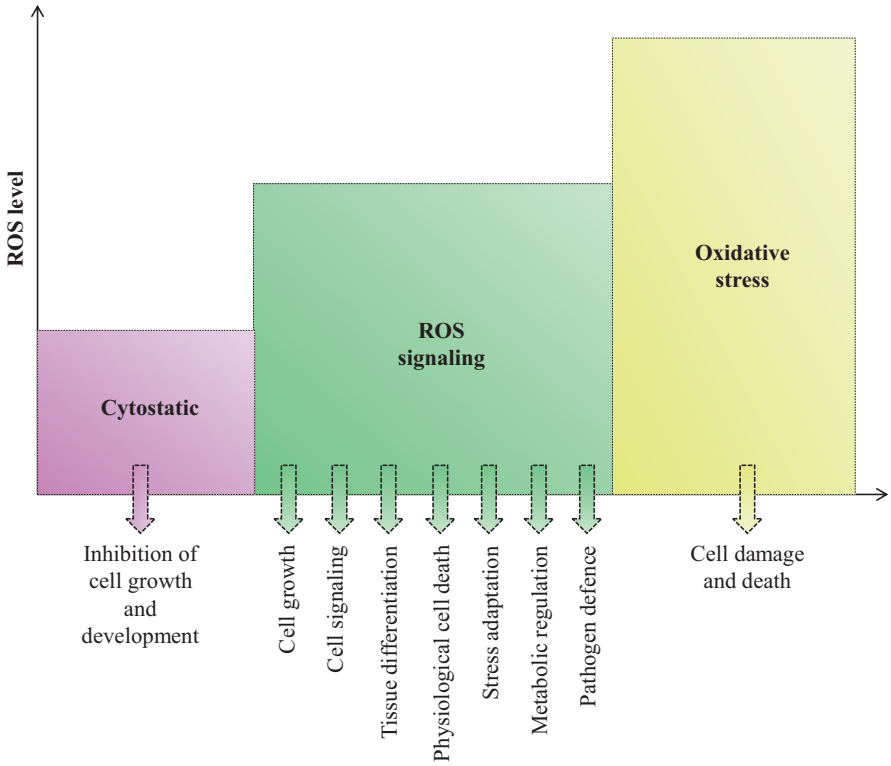

Fig. 14.5 Schematic diagram showing how ROS levels modulate various physiological processes inside the plant cells. An optimum basal level of ROS is essential for ROS signaling to occur, and this brings about beneficial biological processes for plant's growth, development, and stress acclimation. On the other hand, sub-basal ROS level is cytostatic, and excess ROS production causes oxidative stress, cellular damage, and cell death

tips, and ROS production by its activity triggers influx of extracellular Ca^{2+} required for root elongation (Foreman et al. 2003; Takeda et al. 2008). During proper growth of pollen tube, two RBOH isoforms, RBOHH and RBOHJ, were seen to play a crucial role (Boisson-Dernier et al. 2013; Kaya et al. 2014; Lassig et al. 2014). Further, RBOHD has been found to be involved in cellular lignification in plants (Denness et al. 2011). Thus, coordinated function of various signal networks involving ROS signaling is essential for proper growth, development, and stress tolerance in plants (Fig. 14.5).

14.7 Manipulation of ROS Signaling Pathway for Plant Stress Adaptation

Abiotic stresses have capacity to decrease a plant's potential yield by more than 80%, and hence, management of various oxidative stresses such as drought, heat, waterlogging, salinity, cold, and intense sunlight is very essential to realize increased

agricultural production. Manipulations like overexpression and downregulation of various proteins, enzymes, and transcription factors involved in ROS signaling pathway have been shown to render stress tolerance in crop plants, and these are being briefly described in the following section.

Extensive studies have been done in rice to enhance its abiotic stress tolerance by manipulating the ROS signaling genes. Overproduction of *OsMn-SOD1* (manganese-superoxide dismutase) led to less O_2^- production in mitochondria under oxidative stresses (Li et al. 2013), and overproduction of *OsAPX1* and *OsAPX2* (ascorbate peroxidase) led to increased abiotic stress resistance in rice (Sato et al. 2011). When *OsTRXh1* (h-type thioredoxin) was overexpressed, there was less H_2O_2 production under salt stresses and reduced expression of salt-responsive genes, which led to a salt-sensitive phenotype in rice (Zhang et al. 2011). Overproduction of *OsCPK4* (calcium-dependent protein kinase) resulted in enhanced tolerance to salt and drought stresses by reducing levels of membrane lipid peroxidation under stress conditions (Campo et al. 2014). When *OsCPK12* was overproduced in rice, it enhanced the plant's salt tolerance by downregulating ROS-producing NADPH oxidase gene (*OsRBOH1*) and upregulating two ROS scavenging enzymes (*OsAPx2* and *OsAPx8*) (Asano et al. 2012). Mutants of *OsPP18* (protein phosphatase) gene have been found to be sensitive to drought and oxidative stresses due to reduced activity of ROS scavenging enzymes (You et al. 2014). Mutants of *OsDST* (a C2H2 zinc finger-containing salt and drought tolerance gene) transcription factor were found to be salt tolerant as in these plants, there was increased accumulation of H_2O_2 in the guard cells which led to stomatal closure under salt and drought stresses (Huang et al. 2009). Overexpression of *OsTZF1* (a CCCH-tandem zinc finger protein) transcription factor was found to confer tolerance to oxidative stresses by negatively regulating leaf senescence and enhancing expression of redox homeostasis genes (Jan et al. 2013). *OsSUB1A* (a ERF class of transcription factor named submergence tolerance) gene was found to be responsible for positively affecting submergence tolerance in rice by decreasing accumulation of ROS in aerial tissues during submergence and enhancing production of ROS scavenging enzymes (Fukao et al. 2011). Overexpression of *OsNAC3* (NAM (no apical meristem)/ATAF (*Arabidopsis* transcription activation factor)/CUC (cup-shaped cotyledon) transcription factors) transcription factor led to enhanced tolerance to heat and drought stresses in rice (Fang et al. 2015). Increased expression of *OsSKIPa* (Ski-interacting protein) in rice led to drought stress tolerance by enhancement of ROS scavenging ability of the transgenic plants (Hou et al. 2009). When *OsSRO1c* (similar to RCD (radical-induced cell death) 1) gene was overexpressed in rice, it caused increased accumulation of H_2O_2 in guard cells, which, in turn, decreased stomatal aperture and reduced water loss (You et al. 2013). Overproduction of *OsDSM2* (drought-sensitive mutant) gene in rice led to increase in xanthophyll content and non-photochemical quenching activity and enhanced expression of ABA-responsive genes resulting in improved tolerance to drought and oxidative stresses (Du et al. 2010). Downregulation of *OsABA8ox3* (abscisic acid 8′-hydroxylase) has been found to enhance SOD and CAT activities and reduce malondialdehyde (MDA) level during dehydration treatment in rice (Nguyen et al. 2015). *OsANN1* (annexin

protein) gene was found to be responsible for abiotic stress tolerance by decreasing ROS accumulation (Qiao et al. 2015). Upon overproduction of *OsSUV3* (a NTP-dependent RNA/DNA helicase) in rice, plants showed lesser lipid peroxidation and H_2O_2 production resulting in plants becoming tolerant to high salinity (Tuteja et al. 2013). Overproduction of *OsOAT* (ornithine δ-aminotransferase) in rice enhanced ornithine δ-aminotransferase activity and increased proline content of plants resulting in oxidative, drought, and osmotic stress tolerance (You et al. 2012).

Similar studies in other crops involving manipulation of ROS signaling genes have also been shown to enhance their stress tolerance. For instance, overproduction of cotton gene *GhMKK1* (a mitogen-activated protein kinase kinase) in tobacco improved its salt and drought stresses by enhancement of its ROS scavenging capacity (Lu et al. 2013). Overexpression of *Stylosanthes guianensis* gene *SgNCED1* (9-cis-epoxycarotenoid dioxygenase) in tobacco enhanced ABA level and tolerance to drought and salt stresses (Zhang et al. 2009a, b), and overproduction of rice gene *OsACA6* (a type IIB Ca^{2+} ATPase) in tobacco conferred salinity, drought, and cadmium tolerance in tobacco (Huda et al. 2013; Shukla et al. 2014). Overexpression of *TaCIPK29* (calcineurin B-like protein-interacting protein kinase) in tobacco resulted in increased salt tolerance by maintenance of high K^+/Na^+ ratios and Ca^{2+} levels and increase in activity of ROS scavenging enzymes (Deng et al. 2013). When *Poncirus trifoliate* gene *PtADC* (arginine decarboxylase) was overproduced in tobacco and tomato, it enhanced their endogenous polyamine which provides protective roles to negatively charged proteins, DNA, and RNA and reduced ROS accumulation conferred tolerance to drought (Jang et al. 2012). Overproduction of finger millet gene *EcNAC1* in tobacco led to increased ROS scavenging and tolerance to various oxidative stresses (Ramegowda et al. 2012). *TaASR1* (ABA-, stress-, and ripening-induced transcription factor) gene of wheat was found to be involved in drought and osmotic stress tolerance (Hu et al. 2013). Further, overexpression of wheat gene *TaSRO1* (similar to RCD (radical-induced cell death) 1) gene in wheat and *Arabidopsis* was found to cause ROS accumulation (Liu et al. 2014), and overproduction of wheat gene *TaOPR1* (2-oxo-phytodienoic acid reductase) in wheat and *Arabidopsis* resulted in increased salt stress tolerance by regulation of ROS and ABA signaling pathways in the plants (Dong et al. 2013). Overproduction of apple gene *MdSOS2L1* (a CIPK protein kinase) conferred salt tolerance in tomato and apple by enhancing production of ROS scavenging enzymes and antioxidant metabolites such as procyanidin and malate (Hu et al. 2015), and overexpression of soybean gene *GmWRKY27* (amino acids WRKY domain-containing proteins) in soybean enhanced its salt and drought tolerance (Wang et al. 2015).

Apart from the transgenic approaches which involve overproduction or down-regulation of ROS signaling genes, mild treatment of plants with various oxidative stress conditions enhances plant's capacities to tolerate enhanced abiotic stress conditions for longer duration. For example, when maize plants were made to witness drought (the condition was achieved by withholding water for 7 days), the stress-adapted plants were able to withstand 5 °C for 5 days (Irigoyen et al. 1996). When rice plants were subjected to 1–1000 μM H_2O_2 or sodium nitroprusside treatment for 2 days, these plants were able to tolerate 100 mM NaCl treatments for 8 days

(Uchida et al. 2002). Spraying of 1 mM salicylic acid over tomato plants enhanced their salt tolerance, and the stress-adapted plants could withstand 100 mM salt stress for 14 days (He and Zhu 2008). Cinnamic acid (50 μM) treatment of cucumber plants for 2 days made them cold (8 ° C) tolerant for 1 day (Li et al. 2011). When wheat plants were subjected to 1–120 mM H_2O_2 treatment for 8 h, the plants became salt (150 mM) tolerant for 15 days (Wahid et al. 2007). These examples indicate that optimum ROS production is an adaptive response employed by plants for withstanding various oxidative stresses.

14.8 Conclusions

Ubiquitously located in all aerobic organisms, ROS are the necessary evils for the cells. Earlier, they were considered unwanted as their cellular toxicity was the only known phenomenon. However, subsequent studies revealed their role in various biological processes of the cell including cellular growth, differentiation, stress acclimation, pathogen defense, physiological cell death, etc. Now, it has been proved beyond doubt that plants are required to maintain an optimum basal level of ROS for their proper growth and development. Sub-basal ROS level is cytostatic, and excess ROS causes oxidative stress-mediated cellular damages and death in the plants. These observations have opened up a new arena for studying regulatory processes involved in ROS signaling. As controlled production of ROS is critical for plant's growth and stress adaption, it implies that ROS signaling is a highly coordinated phenomenon and various levels of cross talk exist within ROS signaling and various other cellular signaling events also cross talk among themselves to bring about overall effect in response to a particular environmental stimulus. However, the detailed information of how plants perceive cellular levels of ROS, how the downstream ROS signaling events take place, and how their interactions occur is mostly unknown till date. Further studies are essential to identify the global picture of ROS signaling and their role in stress acclimation in plants. This will enable better manipulation of ROS signaling for the development of plants adaptable to the harsh climatic changes.

References

Ahlfors R, Macioszek V, Rudd J, Brosché M, Schlichting R, Scheel D, Kangasjärvi J (2004) Stress hormone-independent activation and nuclear translocation of mitogen-activated protein kinases in Arabidopsis thaliana during ozone exposure. Plant J Cell Mol Biol 40:512–522

Allan AC, Fluhr R (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. Plant Cell 9:1559–1572

Anbar AD (2008) Elements and evolution. Science 322:1481–1483

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143:113–134

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Asano T, Hayashi N, Kobayashi M, Aoki N, Miyao A, Mitsuhara I, Ichikawa H, Komatsu S, Hirochika H, Kikuchi S, Ohsugi R (2012) A rice calcium-dependent protein kinase OsCPK12 oppositely modulates salt stress tolerance and blast disease resistance. Plant J 69:26–36

Attacha S, Solbach D, Bela K, Moseler A, Wagner S, Schwarzländer M, Aller I, Müller SJ, Meyer AJ (2017) Glutathione peroxidase-like enzymes cover five distinct cell compartments and membrane surfaces in Arabidopsis thaliana. Plant Cell Environ 40:1281–1295

Awad J, Stotz HU, Fekete A, Krischke M, Engert C, Havaux M, Berger S, Mueller MJ (2015) 2-cysteine peroxiredoxins and thylakoid ascorbate peroxidase create a water-water cycle that is essential to protect the photosynthetic apparatus under high light stress conditions. Plant Physiol 167:1592–1603

Barber J, Andersson B (1992) (1992) too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci 17:61–66

Bienert GP, Møller ALB, Kristiansen KA, Schulz A, Møller IM, Schjoerring JK, Jahn TP (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282:1183–1192

Boisson-Dernier A, Lituiev DS, Nestorova A, Franck CM, Thirugnanarajah S, Grossniklaus U (2013) ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases. PLoS Biol 11:e1001719

Bourdais G, Burdiak P, Gauthier A, Nitsch L, Salojärvi J, Rayapuram C, Idänheimo N, Hunter K, Kimura S, Merilo E, Vaattovaara A, Oracz K, Kaufholdt D, Pallon A, Anggoro DT, Glów D, Lowe J, Zhou J, Mohammadi O, Puukko T, Albert A, Lang H, Ernst D, Kollist H, Brosché M, Durner J, Borst JW, Collinge DB, Karpiński S, Lyngkjær MF, Robatzek S, Wrzaczek M, Kangasjärvi J (2015) Large-scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative stress. PLoS Genet 11:e1005373

Boyd ES, Thomas KM, Dai Y, Boyd JM, Outten FW (2014) Interplay between oxygen and Fe–S cluster biogenesis: insights from the Suf pathway. Biochemistry 53:5834–5847

Brunkard JO, Runkel AM, Zambryski PC (2015) Chloroplasts extend stromules independently and in response to internal redox signals. Proc Natl Acad Sci U S A 112:10044–10049

Burdiak P, Rusaczonek A, Witoń D, Głów D, Karpiński S (2015) Cysteine-rich receptorlike kinase CRK5 as a regulator of growth, development, and ultraviolet radiation responses in *Arabidopsis thaliana*. J Exp Bot 66:3325–3337

Camp RGLO d, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Göbel C, Feussner I, Nater M, Apel K (2003) Rapid induction of distinct stress responses after the release of singlet oxygen in *Arabidopsis*. Plant Cell 15:2320–2332

Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M, SanSegundo B (2014) Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiol 165:688–704

Caplan JL, Kumar AS, Park E, Padmanabhan MS, Hoban K, Modla S, Czymmek K, Dinesh-Kumar SP (2015) Chloroplast stromules function during innate immunity. Dev Cell 34:45–57

Chakraborty N, Tripathy BC (1992) 5-aminolevulinic acid induced photodynamic reactions in thylakoid membranes of cucumber (*Cucumis sativus* L.) cotyledons. J Plant Biochem Biotechnol 1:65–68

Chan KX, Mabbitt PD, Phua SY, Mueller JW, Nisar N, Gigolashvili T, Stroeher E, Grassl J, Arlt W, Estavillo GM, Jackson CJ, Pogson BJ (2016a) Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. Proc Natl Acad Sci U S A 113:4567–4576

Chan KX, Phua SY, Crisp P, McQuinn R, Pogson BJ (2016b) Learning the languages of the chloroplast: retrograde signaling and beyond. Annu Rev Plant Biol 67:25–53

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois- Meurinne M, Van Breusegem F, Saindrenan P, Noctor G (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. Plant Physiol 153:1692–1705

Cheng F, Blackburn K, Lin Y, Goshe MB, Williamson JD (2009) Absolute protein quantification by LC/MSE for global analysis of salicylic acid-induced plant protein secretion responses. J Proteome Res 8:82–93

Costa A, Drago I, Behera S, Zottini M, Pizzo P, Schroeder JI, Pozzan T, Schiavo FL (2010) H_2O_2 in plant peroxisomes: an in vivo analysis uncovers a Ca^{2+}- dependent scavenging system. Plant J Cell Mol Biol 62:760–772

Daloso DM, Müller K, Obata T, Florian A, Tohge T, Bottcher A, Riondet C, Bariat L, Carrari F, Nunes-Nesi A, Buchanan BB, Reichheld JP, Araújo WL, Fernie AR (2015) Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. Proc Natl Acad Sci U S A 112:E1392–E1400

De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, MMurcha MW, Law SR, Inzé A, Ng S, Ivanova A, Rombaut D, van de Cotte B, Jaspers P, Van de Peer Y, Kangasjärvi J, Whelan J, Van Breusegem F (2013) The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in Arabidopsis. Plant Cell 25:3472–3490

de Souza A, Wang J-Z, Dehesh K (2017) Retrograde signals: integrators of interorganellar communication and orchestrators of plant development. Annu Rev Plant Biol 68:85–108

Del Rio LA, Lopez-Huertas E (2016) ROS generation in peroxisomes and its role in cell signaling. Plant Cell Physiol 57:1364–1376

Delaunay A, Pflieger D, Barrault M-B, Vinh J, Toledano MB (2002) A thiol peroxidase is an H2O2 receptor and redox-transducer in gene activation. Cell 111:471–481

Deng X, Hu W, Wei S, Zhou S, Zhang F, Han J, Chen L, Li Y, Feng J, Fang B, Luo Q, Li S, Liu Y, Yang G, He G (2013) TaCIPK29, a CBL-interacting protein kinase gene from wheat, confers salt stress tolerance in transgenic tobacco. PLoS One 8:e69881

Denness L, McKenna JF, Segonzac C, Wormit A, Madhou P, Bennett M, Mansfield J, Zipfel C, Hamann T (2011) Cell wall damage-induced lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process in *Arabidopsis*. Plant Physiol 156:1364–1374

Desikan R, Hancock JT, Ichimura K, Shinozaki K, Neill SJ (2001) Harpin induces activation of the *Arabidopsis* mitogen-activated protein kinases AtMPK4 and AtMPK6. Plant Physiol 126:1579–1587

Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant Biol 61:593–620

Dong W, Wang M, Xu F, Quan T, Peng K, Xiao L, Xia G (2013) Wheat oxophytodienoate reductase gene TaOPR1 confers salinity tolerance via enhancement of abscisic acid signaling and reactive oxygen species scavenging. PlantPhysiol 161:1217–1228

Drazic A, MiuraH PJ, Le Y, Bach NC, Kriehuber T, Winter J (2013) Methionine oxidation activates a transcription factor in response to oxidative stress. Proc Natl Acad Sci U S A 110:9493–9498

Drerup MM, Schlucking K, Hashimoto K, Manishankar P, Steinhorst L, Kuchitsu K, Kudla J (2013) The calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. Mol Plant 6:559–569

Droillard MJ, Boudsocq M, Barbier-Brygoo H, Laurière C (2004) Involvement of MPK4 in osmotic stress response pathways in cell suspensions and plantlets of Arabidopsis thaliana: activation by hypoosmolarity and negative role in hyperosmolarity tolerance. FEBS Lett 574:42–48

Du H, Wang N, Cui F, Li X, Xiao J, Xiong L (2010) Characterization of the beta-carotene hydroxylase gene DSM2 conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice. Plant Physiol 154:1304–1318

Dubiella U, Seybold H, Durian G, Komander E, Lassig R, Witte CP, Schulze WX, Romeis T (2013) Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. Proc Natl Acad Sci U S A 110:8744–8749

Erickson JL, Kantek M, Schattat MH (2017) Plastid-nucleus distance alters the behavior of stromules. Front Plant Sci 8:1135

Estavillo GM, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, Carrie C, Giraud E, Whelan J, David P, Javot H, Brearley C, Hell R, Marin E, Pogson BJ (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. Plant Cell 23:3992–4012

Exposito-Rodriguez M, Laissue PP, Yvon-Durocher G, Smirnoff N, Mullineaux PM (2017) Photosynthesis-dependent H_2O_2 transfer from chloroplasts to nuclei provides a high-light signaling mechanism. Nat Commun 8:49

Fang Y, Liao K, Du H, Xu Y, Song H, Li X (2015) A stress- responsive NAC transcription factor SNAC3 confers heat and drought tolerance through modulation of reactive oxygen species in rice. J Exp Bot 66:6803

Farmer EE, Mueller MJ (2013) ROS-mediated lipid peroxidation and RES-activated signaling. Annu Rev Plant Biol 64:429–450

Fischer BB, Hideg E, Krieger-Liszkay A (2013) Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxid Redox Signal 18:2145–2162

Foote CS, Shook FC, Abakerli RB (1984) Characterization of singlet oxygen. Methods Enzymol 105:36–47

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446

Foyer CH, Noctor G (2016) Stress-triggered redox signalling: What's in prospect? Plant Cell Environ 39:951–964

Foyer CH, Karpinska B, Krupinska K (2014) The functions of WHIRLY1 and REDOXRESPONSIVE TRANSCRIPTION FACTOR 1 in cross tolerance responses in plants: a hypothesis. Philos Trans R Soc Lond Ser B Biol Sci 369:20130226

Frank HA, Young AJ, Britton G, Cogdell RJ (1999) The photochemistry of carotenoids. In: Advances in photosynthesis [and respiration], vol 8. Kluwer Academic Publishers. (now Springer, Dordrecht

Fukao T, Yeung E, Bailey-Serres J (2011) The submergence tolerance regulator SUB1A mediates cross talk between submergence and drought tolerance in rice. Plant Cell 23:412–427

Garcia-Mata C, Wang J, Gajdanowicz P, Gonzalez W, Hills A, Donald N, Riedelsberger J, Amtmann A, Dreyer I, Blatt MR (2010) A minimal cysteine motif required to activate the SKOR K+ channel of Arabidopsis by the reactive oxygen species H_2O_2. J Biol Chem 285:29286–29294

Gawroński P, Witoń D, Vashutina K, Bederska M, Betliński B, Rusaczonek A, Karpiński S (2014) Mitogen-activated protein kinase 4 is a salicylic acid-independent regulator of growth but not of photosynthesis in *Arabidopsis*. Mol Plant 7:1151–1166

Giraud E, Ho LHM, Clifton R, Carroll A, Estavillo G, Tan YF, Howell KA, Ivanova A, Pogson BJ, Millar AH, Whelan J (2008) The absence of ALTERNATIVE OXIDASE1a in Arabidopsis results in acute sensitivity to combined light and drought stress. Plant Physiol 147:595–610

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 5th edn. Oxford university Press, Oxford

Hanson MR, Sattarzadeh A (2013) Trafficking of proteins through plastid stromules. Plant Cell 25:2774–2782

Havaux M, Dall'osto L, Bassi R (2007) Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in *Arabidopsis* leaves and functions independent of binding to PSII antennae. Plant Physiol 145:1506–1520

He Y, Zhu ZJ (2008) Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicon esculentum*. Biol Plant 52:792–795

Hou X, Xie K, Yao J, Qi Z, Xiong L (2009) A homolog of human ski- interacting protein in rice positively regulates cell viability and stress tolerance. Proc Natl Acad Sci U S A 106:6410–6415

Hu X, Bidney DL, Yalpani N, Duvick JP, Crasta O, Folkerts O, Lu G (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol 133:170–181

Hu W, Huang C, Deng X, Zhou S, Chen L, Li Y, Wang C, Ma Z, Yuan Q, Wang Y, Cai R, Liang X, Yang G, He G (2013) TaASR1, a transcription factor gene in wheat, confers drought stress tolerance in transgenic tobacco. Plant Cell Environ 36:1449–1464

Hu DG, Ma QJ, Sun CH, Sun MH, You CX, Hao YJ (2015) Overexpression of MdSOS2L1, a CIPK protein kinase, increases the antioxidant metabolites to enhance salt tolerance in apple and tomato. Physiol Plant 156:201–214

Huang XY, Chao DY, Gao JP, Zhu MZ, Shi M, Lin HX (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev 23:1805–1817

Huang S, Van Aken O, Schwarzl¨ander M, Belt K, Millar AH (2016) The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. Plant Physiol 171:1551–1559

Huda KM, Banu MS, Garg B, Tula S, Tuteja R, Tuteja N (2013) OsACA6, a P-type IIB Ca^{2+} ATPase promotes salinity and drought stress tolerance in tobacco by ROS scavenging and enhancing the expression of stress-responsive genes. Plant J 76:997–1015

Irigoyen JJ, Perez de Juan J, Sanchez-Diaz M (1996) Drought enhances chilling tolerance in a chilling sensitive maize (Zea mays) variety. New Phytol 134:53–59

Jacques S, Ghesqui'ere B, De Bock PJ, Demol H, Wahni K, Willems P, Messens J, Van Breusegem F, Gevaert K (2015) Protein methionine sulfoxide dynamics in *Arabidopsis thaliana* under oxidative stress. Mol Cell Proteomics 14:1217–1229

Jammes F, Song C, Shin D, Munemasa S, Takeda K, Gu D, Cho D, Lee S, Giordo R, Sritubtim S, Leonhardt N, Ellis BE, Murata Y, Kwak JM (2009) MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. Proc Natl Acad Sci U S A 106:20520–20525

Jan A, Maruyama K, Todaka D, Kidokoro S, Abo M, Yoshimura E, Shinozaki K, Nakashima K, Yamaguchi-Shinozaki K (2013) OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. Plant Physiol 161:1202–1216

Jang SJ, Wi SJ, Choi YJ, An G, Park KY (2012) Increased polyamine biosynthesis enhances stress tolerance by preventing the accumulation of reactive oxygen species: T-DNA mutational analysis of *Oryza sativa* lysine decarboxylase-likeprotein1. MolCells 34:251–262

Jung S, Lee HJ, Lee Y, Kang K, Kim YS, Grimm B, Back K (2008) Toxic tetrapyrrole accumulation in protoporphyrinogen IX oxidase-overexpressing transgenic rice plants. Plant Mol Biol 67:535–546

Karpinski S, Szechynska-Hebda M, Wituszynska W, Burdiak P (2013) Light acclimation, retrograde signalling, cell death and immune defences in plants. Plant Cell Environ 36:736–744

Kaurilind E, Xu E, Brosché M (2015) A genetic framework for H2O2 induced cell death in *Arabidopsis thaliana*. BMC Genomics 16:837

Kaya H, Nakajima R, Iwano M, Kanaoka MM, Kimura S, Takeda S, Kawarazaki T, Senzaki E, Hamamura Y, Higashiyama T, Takayama S, Abe M, Kuchitsu K (2014) Ca^{2+}-activated reactive oxygen species production by *Arabidopsis* RbohH and RbohJ is essential for proper pollen tube tip growth. Plant Cell 26:1069–1080

Kimura S, Kaya H, Kawarazaki T, Hiraoka G, Senzaki E, Michikawa M, Kuchitsu K (2012) Protein phosphorylation is a prerequisite for the Ca^{2+}-dependent activation of *Arabidopsis* NADPH oxidases and may function as a trigger for the positive feedback regulation of Ca^{2+} and reactive oxygen species. Biochim Biophys Acta 1823:398–405

Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H (2007) Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19:1065–1080

Konopka-Postupolska D, Clark G, Goch G, Debski J, Floras K, Cantero A, Fijolek B, Roux S, Hennig J (2009) The role of annexin 1 in drought stress in *Arabidopsis*. Plant Physiol 150:1394–1410

Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci U S A 97:2940–2945

Krieger-Liszkay A, Trebst A (2006) Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction Centre. J Exp Bot 57:1677–1684

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J 22:2623–2633

Laloi C, Havaux M (2015) Key players of singlet oxygen-induced cell death in plants. Front Plant Sci 6:39

Lassig R, Gutermuth T, Bey TD, Konrad KR, Romeis T (2014) Pollen tube NAD(P)H oxidases act as a speed control to dampen growth rate oscillations during polarized cell growth. Plant J 78:94–106

Lee KP, Kim C, Landgraf F, Apel K (2007) EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 104:10270–10275

Lee SH, Li CW, Koh KW, Chuang HY, Chen YR, Lin CS, Chan MT (2014) MSRB7reverses oxidation of GSTF2/3 to confer tolerance of Arabidopsis thaliana to oxidative stress. J Exp Bot 65:5049–5062

Leister D (2017) Piecing the puzzle together: the central role of ROS and redox hubs in chloroplast retrograde signaling. Antioxid Redox Signal 30:1206–1219.

Lermontova I, Grimm B (2006) Reduced activity of plastid protoporphyrinogen oxidase causes attenuated photodynamic damage during high-light compared to low-light exposure. Plant J 48:499–510

Li Q, Yu B, Gao Y, Dai AH, Bai JG (2011) Cinnamic acid pretreatment mitigates chilling stress of cucumber leaves through altering antioxidant enzyme activity. J Plant Physiol 168:927–934

Li CR, Liang DD, Li J, Duan YB, Li H, Yang YC, Qin RY, Li L, Wei PC, Yang JB (2013) Unravelling mitochondrial retrograde regulation in the abiotic stress sinduction of rice ALTERNATIVE OXIDASE1 genes. Plant Cell Environ 36:775–788

Lin F, Ding HD, Wang JX, Zhang H, Zhang AY, Zhang Y, Tan MP, Dong W, Jiang MY (2009) Positive feedback regulation of maize NADPH oxidase by mitogen-activated protein kinase cascade in abscisic acid signalling. J Exp Bot 60:3221–3238

Lin CC, Jih PJ, Lin HH, Lin JS, Chang LL, Shen YH, Jeng ST (2011) Nitric oxide activates superoxide dismutase and ascorbate peroxidase to repress the cell death induced by wounding. Plant Mol Biol 77:235–249

Liu S, Wang M, Wei T, Meng C, Xia G (2014) A wheat SIMILAR TO RCD-ONE gene enhances seedling growth and abiotic stress resistance by modulating redox homeostasis and maintaining genomic integrity. Plant Cell 26:164–180

Lu W, Chu X, Li Y, Wang C, Guo X (2013) Cotton GhMKK1 induces the tolerance of salt and drought stress, and mediates defence responses to pathogen infection in transgenic *Nicotiana benthamiana*. PLoS One 8:e68503

Ma L, Zhang H, Sun L, Jiao Y, Zhang G, Miao C, Hao F (2012) NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na^+/K^+ homeostasis in *Arabidopsis* under salt stress. J Exp Bot 63:305–317

Mateo A, Mühlenbock P, Rustérucci C, Chang CCC, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S (2004) LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. Plant Physiol 136:2818–2830

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci U S A 96:8271–8276

McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytol 172:221–228

Meyer Y, Belin C, Delorme-Hinoux V, Reichheld J-P, Riondet C (2012) Thioredoxin and glutaredoxin systems in plants: molecular mechanisms, crosstalks, and functional significance. Antioxid Redox Signal 17:1124–1160

Miao Y, Lv D, Wang P, Wang X-C, Chen J, Miao C, Song CP (2006) An *Arabidopsis* glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell 18:2749–2766

Miller AF (2012) Superoxide dismutases: ancient enzymes and new insights. FEBS Lett 586:585–595

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R (2009) The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Sci Signal 2:ra45

Mittler R (2016) ROS are good! Trends Plant Sci 22:11–19

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F (2011) ROS signaling: the new wave? Trends Plant Sci 16:300–309

Mock HP, Grimm B (1997) Reduction of uroporphyrinogen decarboxylase by antisense RNA expression affects activities of other enzymes involved in tetrapyrrole biosynthesis and leads to light dependent necrosis. Plant Physiol 113:1101–1112

Monshausen GB, Bibikova TN, Messerli MA, Shi C, Gilroy S (2007) Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. Proc Natl Acad Sci U S A 104:20996–21001

Mozzo M, Dall'Osto L, Hienerwadel R, Bassi R, Croce R (2008) Photoprotection in the antenna complexes of photosystem II: role of individual xanthophylls in chlorophyll triplet quenching. J Biol Chem 283:6184–6192

Mühlenbock P, Szechynska-Hebda M, Plaszczyca M, Baudo M, Mateo A, Mullineaux PM, Parker JE, Karpinska B, Karpinski S (2008) Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in *Arabidopsis*. Plant Cell 20:2339–2356

Muhlenbock P, Szechynska-Hebda M, Plaszczyca M, Baudo M, Mullineaux PM, Parker JE, Karpinska B, Karpinski S (2008) Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. Plant Cell 20:2339–2356

Ng S, Ivanova A, Duncan O, Law SR, Van Aken O, De Clercq I, Wang Y, Carrie C, Xu L, Kmiec B, Walker H, Van Breusegem F, Whelan J, Giraud E (2013) A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in *Arabidopsis*. Plant Cell 25:3450–3471

Ng S, De Clercq I, Van Aken O, Law SR, Ivanova A, Willems P, Giraud E, Van Breusegem F, Whelan J (2014) Anterograde and retrograde regulation of nuclear genes encoding mitochondrial proteins during growth, development, and stress. Mol Plant 7:1075–1093

Nguyen HT, Cai S, Jiang G, Ye N, Chu Z, Xu X, Zhang J, Zhu G (2015) A key ABA catabolic gene, OsABA8ox3, is involved in drought stress resistance in rice. PLoS One 10:e0116646

Nie S, Yue H, Zhou J, Xing D (2015) Mitochondrial-derived reactive oxygen species play a vital role in the salicylic acid signaling pathway in *Arabidopsis thaliana*. PLoS One 10:e0119853

Nishimura MT, Dangl JL (2010) *Arabidopsis* and the plant immune system. Plant J 61:1053–1066

Ochsenbein C, Przybyla D, Danon A, Landgraf F, Göbel C, Imboden A, Feussner I, Apel K (2006) The role of EDS1 (enhanced disease susceptibility) during singlet oxygen-mediated stress responses of *Arabidopsis*. Plant J Cell Mol Biol 47:445–456

Oda T, Hashimoto H, Kuwabara N, Hayashi K, Kojima C, Kawasaki T, Shimamoto K, Sato M, Shimizu T (2008) Crystallographic characterization of the N-terminal domain of a plant NADPH oxidase. Acta Crystallogr Sect F Struct Biol Cryst Commun 64:867–869

Oda T, Hashimoto H, Kuwabara N, Akashi S, Hayashi K, Kojima C, Wong HL, Kawasaki T, Shimamoto K, Sato M, Shimizu T (2010) Structure of the N-terminal regulatory domain of a plant NADPH oxidase and its functional implications. J Biol Chem 285:1435–1445

Ogasawara Y, Kaya H, Hiraoka G, Yumoto F, Kimura S, Kadota Y, Hishinuma H, Senzaki E, Yamagoe S, Nagata K, Nara M, Suzuki K, Tanokura M, Kuchitsu K (2008) Synergistic activation of the *Arabidopsis* NADPH oxidase AtrbohD by Ca^{2+} and phosphorylation. J Biol Chem 283:8885–8892

Ohad I, Keren N, Zer H, Gong H, Mor TS (1994) Light induced degradation of the photosystem II reaction center D1 protein in vivo: an integrative approach. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis: from molecular mechanisms to the field. BIOS Scientific Publishers, Oxford, pp 161–177

Ortiz-Masia D, Perez-Amador MA, Carbonell J, Marcote MJ (2007) Diverse stress signals activate the C1 subgroup MAP kinases of *Arabidopsis*. FEBS Lett 581:1834–1840

Pasqualini S, Piccioni C, Reale L, Ederli L, Della Torre G, Ferranti F (2003) Ozone-induced cell death in tobacco cultivar bel W3 plants. The role of programmed cell death in lesion formation. Plant Physiol 133:1122–1134

Pérez-Salamó I, Papdi C, Rigó G, Zsigmond L, Vilela B, Lumbreras V, Nagy I, Horváth B, Domoki M, Darula Z, Medzihradszky K, Bögre L, Koncz C, Szabados L (2014) The heat shock factor A4A confers salt tolerance and is regulated by oxidative stress and the mitogen-activated protein kinases MPK3 and MPK6. Plant Physiol 165:319–334

Persak H, Pitzschke A (2014) Dominant repression by Arabidopsis transcription factor MYB44 causes oxidative damage and hypersensitivity to abiotic stress. Int J Mol Sci 15:2517–2537

Pitzschke A, Djamei A, Bitton B, Hirt H (2009) A major role of the MEKK1–MKK1/2–MPK4 pathway in ROS signaling. Mol Plant 2:120–137

Pottosin I, Velarde-Buendía AM, Bose J, Zepeda-Jazo I, Shabala S, Dobrovinskaya O (2014) Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. J Exp Bot 65:1271–1283

Purvis AC (1997) Role of the alternative oxidase in limiting superoxide production by plant mitochondria. Physiol Plant 100:165–170

Qiao B, Zhang Q, Liu D, Wang H, Yin J, Wang R, He M, Cui M, Shang Z, Wang D, Zhu Z (2015) A calcium-binding protein, rice annexin OsANN1, enhances heat stress tolerance by modulating the production of H_2O_2. J Exp Bot 66:5853–5866

Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gaki'ere B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G (2007) Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. Plant J 52:640–657

Queval G, Neukermans J, Vanderauwera S, Van Breusegem F, Noctor G (2012) Day length is a key regulator of transcriptomic responses to both CO_2 and H_2O_2 in *Arabidopsis*. Plant Cell Environ 35:374–387

Ramegowda V, Senthil-Kumar M, Nataraja KN, Reddy MK, Mysore KS, Udayakumar M (2012) Expression of a finger millet transcription factor, EcNAC1, in tobacco confers abiotic stress-tolerance. PLoS One 7:e40397

Ramel F, Birtic S, Cuin'e S, Triantaphylid'es C, Ravanat JL, Havaux M (2012) Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol 158:1267–1278

Rebeiz CA, Montazer-Zouhoor A, Hopen HJ, Wu SM (1984) Photodynamic herbicides: 1. Concept and phenomenology. Enzym Microb Technol 6:390–401

Rebeiz CA, Nandihalli UB, Reddy KN (1991) In: Baker NR, Percival MP (eds) Topics in Photosynthesis – volume 10, herbicides. Elsevier, Amsterdam, pp 173–208

Rebeiz CA, Montazer-Zouhoor A, Mayasich JM, Tripathy BC, Wu SM, Rebeiz CC (1998) Photodynamic herbicides. Recent development and molecular basis of selectivity. CRC Crit Rev Plant Sci 6:385–436

Roos G, Messens J (2011) Protein sulfenic acid formation: from cellular damage to redox regulation. Free Radic Biol Med 51:314–326

Rossel JB, Wilson PB, Hussain D, Woo NS, Gordon MJ, Mewett OP, Howell KA, Whelan J, Kazan K, Pogson BJ (2007) Systemic and intracellular responses to photooxidative stress in Arabidopsis. Plant Cell 19:4091–4110

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. Plant Cell 16:616–628

Sato Y, Masuta Y, Saito K, Murayama S, Ozawa K (2011) Enhanced chilling tolerance at the booting stage in rice by transgenic overexpression of the ascorbate peroxidase gene, OsAPXa. Plant Cell Rep 30:399–406

Sewelam N, Jaspert N, Van Der Kelen K, Tognetti V, Schmitz J, Frerigmann H, Stahl E, Zeier J, Van Breusegem F, Maurino VG (2014) Spatial H_2O_2 signaling specificity: H_2O_2 from chloroplasts and peroxisomes modulates the plant transcriptome differentially. Mol Plant 7:1191–1210

Shalygo NV, Mock HP, Averina NG, Grimm B (1998) Photodynamic action of uroporphyrin and protochlorophyllide in greening barley leaves treated with cesium chloride. J Photochem Photobiol 42:151–158

Shukla D, Huda KM, Banu MS, Gill SS, Tuteja R, Tuteja N (2014) OsACA6, a P-type 2B Ca^{2+} ATPase functions in cadmium stress tolerance in tobacco by reducing the oxidative stress load. Planta 240:809–824

Sirichandra C, Gu D, Hu HC, Davanture M, Lee S, Djaoui M, Valot B, Zivy M, Leung J, Merlot S, Kwak JM (2009) Phosphorylation of the Arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. FEBS Lett 583:2982–2986

Sun X, Feng P, Xu X, Guo H, Ma J, Chi W, Lin R, Lu C, Zhang L (2011) A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. Nat Commun 2:477

Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R (2011) Respiratory burst oxidases: the engines of ROS signaling. Curr Opin Plant Biol 14:691–699

Suzuki N, Miller G, Salazar C, Mondal HA, Shulaev E, Cortes DF, Shuman JL, Luo X, Shah J, Schlauch K, Shulaev V, Mittler R (2013) Temporal–spatial interaction between ROS and ABA controls rapid systemic acclimation in plants. Plant Cell 25:3553–3569

Sweetlove LJ, Fell D, Fernie AR (2008) Getting to grips with the plant metabolic network. Biochem J 409:27–41

Takeda S, Gapper C, Kaya H, Bell E, Kuchitsu K, Dolan L (2008) Local positive feedback regulation determines cell shape in root hair cells. Science 319:1241–1244

Tamaoki M (2008) The role of phytohormone signaling in ozone-induced cell death in plants. Plant Signal Behav 3:166–174

Tarrago L, Laugier E, Rey P (2009) Protein-repairing methionine sulfoxide reductases in photosynthetic organisms: gene organization, reduction mechanisms, and physiological roles. Mol Plant 2:202–217

Tognetti VB, Van Aken O, Morreel K, Vandenbroucke K, van de Cotte B, De Clercq I, Chiwocha S, Fenske R, Prinsen E, Boerjan W, Genty B, Stubbs KA, Inzé D, Van Breusegem F (2010) Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase UGT74E2 modulates arabidopsis architecture and water stress tolerance[W]. Plant Cell 22:2660–2679

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8:397–403

Triantaphylidès C, Krischke M, Hoeberichts FA, Ksas B, Gresser G, Havaux M, Van Breusegem F, Mueller MJ (2008) Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. Plant Physiol 148:960–968

Tripathy BC, Chakraborty N (1991) 5-Aminolevulinic acid induced photodynamic damage of the photosynthetic electron transport chain of cucumber (*Cucumis sativus* L.) cotyledons. Plant Physiol 96:761–767

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. Plant Signal Behav 7:1621–1633

Tripathy BC, Mohapatra A, Gupta I (2007) Impairment of the photosynthetic apparatus by oxidative stress induced by photosensitization reaction of protoporphyrin IX. Biochim Biophys Acta 1767:860–868

Tuteja N, Sahoo RK, Garg B, Tuteja R (2013) OsSUV3 dual helicase functions in salinity stress tolerance by maintaining photosynthesis and antioxidant machinery in rice (*Oryza sativa* L.cv. IR64). Plant J 76:115–127

Uchida A, Andre T, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci 163:515–523

Vainonen JP, Kangasjärvi K (2015) Plant signalling in acute ozone exposure. Plant Cell Environ 38:240–252

Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inzé D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol 139:806–821

Vogel MO, Moore M, König K, Pecher P, Alsharafa K, Lee J, Dietz KJ (2014) Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in *Arabidopsis*. Plant Cell 26:1151–1165

Wahid A, Perveen M, Gelani S, Basra SMA (2007) Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. J Plant Physiol 164:283–294

Walters DR (2003) Polyamines and plant disease. Phytochemistry 64:97–107

Wang P, Du Y, Zhao X, Miao CP, Song Y (2013) The MPK6-ERF6-ROS-responsive cis-acting Element7/GCC box complex modulates oxidative gene transcription and the oxidative response in *Arabidopsis*. Plant Physiol 161:1392–1408

Wang F, Chen HW, Li QT, Wei W, Li W, Zhang WK, Ma B, Bi YD, Lai YC, Liu XL, Man WQ, Zhang JS, Chen SY (2015) GmWRKY27 interacts with GmMYB174 to reduce expression of GmNAC29 for stress tolerance in soybean plants. Plant J 83:224–236

Wang Y, Lyu W, Berkowitz O, Radomiljac JD, Law SR, Murcha MW, Carrie C, Teixeira PF, Kmiec B, Duncan O, Van Aken O, Narsai R, Glaser E, Huang S, Roessner U, Millar AH, Whelan J (2016) Inactivation of mitochondrial complex I induces the expression of a twin cysteine protein that targets and affects cytosolic, chloroplastidic and mitochondrial function. Mol Plant 9:696–710

Waszczak C, Akter S, Eeckhout D, Persiau G, Wahni K, Bodra N, Van Molle I, De Smet B, Vertommen D, Gevaert K, De Jaeger G, Van Montagu M, Messens J, Van Breusegem F (2014) Sulfenome mining in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 111:11545–11550

Waszczak C, Akter S, Jacques S, Huang J, Messens J, Van Breusegem F (2015) Oxidative post-translational modifications of cysteine residues in plant signal transduction. J Exp Bot 66:2923–2934

Waszczak C, Carmody M, Kangasjärvi J (2018) Reactive oxygen species in plant signaling. Annu Rev Plant Biol 69:209–236

Wong HL, Pinontoan R, Hayashi K, Tabata R, Yaeno T, Hasegawa K, Kojima C, Yoshioka H, Iba K, Kawasaki T, Shimamoto K (2007) Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension. Plant Cell 19:4022–4034

Wood ZA, Poole LB, Karplus PA (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. Science 300:650–653

Wrzaczek M, Vainonen JP, Stael S, Tsiatsiani L, Help-Rinta-Rahko H, Gauthier A, Kaufholdt D, Bollhöner B, Lamminmäki A, Staes A, Gevaert K, Tuominen H, Van Breusegem F, Helariutta Y, Kangasjärvi J (2015) GRIM REAPER peptide binds to receptor kinase PRK5 to trigger cell death in *Arabidopsis*. EMBO J 34:55–66

Wu J, Sun Y, Zhao Y, Zhang J, Luo L, Li M, Wang J, Yu H, Liu G, Yang L, Xiong G, Zhou JM, Zuo J, Wang Y, Li J (2015) Deficient plastidic fatty acid synthesis triggers cell death by modulating mitochondrial reactive oxygen species. Cell Res 25:621–633

Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K, Asami T, Chen Z, Yu JQ (2009) Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. Plant Physiol 150:801–814

Xing Y, Jia W, Zhang J (2007) AtMEK1 mediates stress-induced gene expression of CAT1 catalase by triggering H2O2 production in *Arabidopsis*. J Exp Bot 58:2969–2981

Yi SY, Lee DJ, Yeom SI, Yoon J, Kim YH, Kwon SY, Choi D (2010) A novel pepper (*Capsicum annuum*) receptor-like kinase functions as a negative regulator of plant cell death via accumulation of superoxide anions. New Phytol 185:701–715

Yoshida K, Hisabori T (2014) Mitochondrial isocitrate dehydrogenase is inactivated upon oxidation and reactivated by thioredoxin-dependent reduction in *Arabidopsis*. Front Environ Sci 2:38

Yoshida K, Noguchi K, Motohashi K, Hisabori T (2013) Systematic exploration of thioredoxin target proteins in plant mitochondria. Plant Cell Physiol 54:875–892

You J, Hu H, Xiong L (2012) An ornithine delta-amino transferase gene OsOAT confers drought and oxidative stress tolerance in rice. Plant Sci 197:59–69

You J, Zong W, Li X, Ning J, Hu H, Xiao J, Xiong L (2013) The SNAC1- targeted gene OsSRO1c modulates stomatal closure and oxidative stress tolerance by regulating hydrogen peroxide in rice. J Exp Bot 64:569–583

You J, Zong W, Hu H, Li X, Xiao J, Xiong L (2014) A STRESS- RESPONSIVE NAC1-regulated protein phosphatase gene rice protein phosphatase18 modulates drought and oxidative stress

tolerance through abscisic acid-independent reactive oxygen species scavenging in rice. Plant Physiol 166:2100–2114

Zhang Y, Tan J, Guo Z, Lu S, He S, Shu W, Zhou B (2009a) Increased abscisic acid levels in transgenic tobacco over-expressing 9cis-epoxycarotenoid dioxygenase influence H_2O_2 and NO production and antioxidant defences. Plant Cell Environ 32:509–519

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009b) Phospholipase dalpha1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. Plant Cell 21:2357–2377

Zhang CJ, Zhao BC, Ge WN, Zhang YF, Song Y, Sun DY, Guo Y (2011) An apoplastic h-type thioredoxin is involved in the stress response through regulation of the apoplastic reactive oxygen species in rice. Plant Physiol 157:1884–1899

Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y (2012) Disruption of PAMP-induced MAP kinase cascade by a Pseudomonas syringae effector activates plant immunity mediated by the NB-LRR protein SUMM2. Cell Host Microbe 11:253–263

Zhang S, Zhang D, Yang C (2014) AtFtsH4 perturbs the mitochondrial respiratory chain complexes and auxin homeostasis in *Arabidopsis*. Plant Signal Behav 9:e29709

Zhou G, Qi J, Ren N, Cheng J, Erb M, Mao B, Lou Y (2009) Silencing OsHI-LOX makes rice more susceptible to chewing herbivores, but enhances resistance to a phloem feeder. Plant J 60:638–648

Mrinalini Manna obtained her Ph.D. from ICGEB in the area of phosphite-mediated weed control and improvement of phosphorus use efficiency in rice. Currently, she holds a research position at NIPGR India where she is involved with the development of transgenics in various crop plants including rice, tomato, and mustard. Presently, her research interests involve analysis of metabolome and transcriptome changes in rice in response to phosphite treatment.

V. Mohan M. Achary obtained his doctoral degree in plant science under Prof. B. B. Panda from Berhampur University, India, on the mechanism of plant adaptation and genome protection. He is presently working at ICGEB as Research Associate. His current research work is focused on crop improvement by employment of various biotechnological approaches. He is involved with the development of transgenic rice plants resistant to broad-spectrum and nonselective herbicides such as glyphosate and sulfonylureas for post- and preemergent applications including targeted genome editing using CRISPR-Cas9 technology for improving micronutrient bioavailability, yield, plant architecture, disease resistance, and stress tolerance in economically important crop plants.

Malireddy K. Reddy obtained his doctoral degree under Prof N. C. Subrahmanyam from the University of Hyderabad, Telangana, India, on molecular plant genetics. Later he joined as Research Scientist at ICGEB to study the mechanism of chloroplast DNA replication in Plant Molecular Biology group which was headed by the Editor. He had his postdoctoral training in Prof K. K. Tewari Lab at University of California, Irvine, USA. He visited Prof Ralf Oelmuller's lab at University of Jena, Germany, under INSA-DFG Fellowship and visited Prof Andrew Paterson's lab at University of Georgia, USA, under the DBT Overseas Fellowship. He is currently a Group Leader of the crop improvement group at ICGEB. In past, his research focused on understanding plant adaptation to environmental stress-induced oxidative damage. His current research interests are toward development of herbicide-tolerant crops for chemical-based effective weed management in agriculture and also exploring the targeted genome editing using CRISPR/Cas technology to create traits of agricultural value.

Extracellular ATP Signaling in Animals and Plants: Comparison and Contrast

15

Stanley J. Roux and Greg Clark

Abstract

Although the key role of extracellular nucleotides as signaling agents in animals and plants is not often discussed in text books, it is a major topic in the primary literature, with typically over 400 papers published on this topic every year for the past two decades. For research in animal cells, this literature became quite extensive following the discovery, over three decades ago, of multiple purinergic receptors for extracellular nucleotides such as extracellular ATP (eATP) in mammals and other vertebrates. On the other hand, research on eATP signaling in plant cells is relatively more recent and limited, but it has begun to expand significantly after the discovery of an eATP receptor in *Arabidopsis* in 2014. Although the structural characteristics of the purinergic receptors in animals and plants differ significantly, the signaling steps that follow the activation of these receptors are similar in plants and animals, both having an increase in $[Ca^{2+}]_{cyt}$ within seconds as one of the earliest steps, and both leading to increased levels of reactive oxygen species within minutes as a critical intermediate in the signaling pathway. New downstream molecular and physiological responses to receptor activation by extracellular nucleotides are being discovered every year, and this chapter will discuss underlying similarities and distinct differences in these responses in plants and animals. In both animals and plants, the main enzyme limiting the [eATP] is a nucleoside triphosphate-diphosphohydrolase (NTPDase), more often referred to in the plant literature as apyrase. These enzymes have features that have been conserved throughout evolution, from primitive algae through to humans. This fact, plus the observation that physiologically significant levels of ATP can be found in the open ocean, suggest that eATP signaling is an ancient method of regulating cellular responses.

S. J. Roux (✉) · G. Clark
Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA
e-mail: sroux@austin.utexas.edu

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_15

Keywords
Apyrase · Calcium signaling · Extracellular ATP · Purinoceptor · Wound response

15.1 Introduction

In both animals and plants, a variety of different stimuli induce the release of ATP from intracellular stores into the extracellular matrix (ECM). These include wounding, touch stimuli, membrane expansion, and pathogen attack (Burnstock and Verkhratsky 2009). In both animals and plants, once the extracellular ATP [eATP] concentration rises above a low threshold (typically above 1 μM), it can bind to and activate plasma membrane-localized receptors and induce signaling changes (Khakh and Burnstock 2009). Increased $[Ca^{2+}]_{cyt}$ is typically one of the earliest signaling steps, and increased levels of ROS and mitogen-activated protein kinase (MAPK) activation are often later steps (Clark and Roux 2011). In both animals (Guan et al. 2007) and plants (Peiter 2016; Dindas et al. 2018), the release of ATP from cells in one tissue locale can induce the production of Ca^{2+} waves that propagate to and induce signaling in distant cells, and ecto-phosphatases are the enzymes most often used to terminate the eATP signal, with conserved ecto-NTPDases (ecto-apyrases) playing the main role in this activity (Yegutkin 2014). Critical features of both the primary and 3-D crystal structures of ecto-NTPDase are conserved across the animal and plant kingdoms of life (Summers et al. 2017). Among the downstream physiological responses to eATP and NTPDase activity, the early signaling steps of increased $[Ca^{2+}]_{cyt}$, increased levels of reactive oxygen species, and enhanced MAPK activities, and later responses, such as induced defense activities against pathogens, and enhanced immune responses, have been especially well documented in both animals and plants (Clark et al. 2014).

Although there are remarkable similarities in the start and progression of eATP signaling in animals and plants, there are also major differences. Most vertebrates have more than a dozen different purinergic receptors that are activated by extracellular NTP and NDP nucleotides (Jacobson and Müller 2016), whereas thus far, only one receptor has been extensively characterized in plants (Choi et al. 2014). Vertebrate receptors fall into two major classes, P2X, which are ion channel-linked receptors, and P2Y, which are G-protein-linked receptors, whereas the one receptor known in plants, DORN1, is a receptor kinase, structurally unlike either P2X or P2Y receptors.

Earlier reviews on eATP signaling typically focused on responses in either animals or plants, and they only cursorily commented on the similarities and differences between responses in animals and plants. This chapter will discuss these similarities and differences more in depth, and, due to space limitations, it will emphasize more on recent discoveries made since 2014.

15.2 Mechanisms of ATP Release

15.2.1 Wounding: Cell Membrane Breakdown

The cytoplasmic [ATP] is typically in the high μM or low mM range in animals (Imamura et al. 2009) and plants (Gout et al. 1992), although this would vary depending on the metabolic state of cells (Surin et al. 2014). In contrast, the resting [ATP] in the ECM of unstimulated cells is typically near 5 nM or below (Helenius et al. 2012). Given this steep [ATP] gradient across the plasma membrane, the most obvious event that would lead to a release of ATP to the ECM would be a wound, or any other change that would cause the cell membrane to breakdown. Instantaneously, the level of [eATP] after a membrane break would rise close to that of [ATP] of the cytoplasm, but then, this level would drop rapidly as extracellular phosphatases degraded the eATP. When the [eATP] was measured at the wound site of *Arabidopsis* leaves within 3 min after they were punctured, the level had already dropped to 40 μM (Song et al. 2006), but even this level would be far above the threshold needed to activate the P2K1 (DORN1) nucleotide receptor (Choi et al. 2014). As discussed more in Sect. 15.4, the damage-induced release of ATP initiates a signaling pathway that leads to repair and defense responses to the injury that are remarkably similar in animals and plants, including increased ROS and MAPK cascades (Hernández-Oñate and Herrera-Estrella 2015).

There is also a release of ATP from cells undergoing cell death either by necrosis or apoptosis, and this signal is among the common molecular regulators that participate in these two forms of cell death (Schulze-Lohoff et al. 1998). In both plants (Feng et al. 2015a) and animals (Chekeni et al. 2010), the eATP signal induces cellular changes that can advance the death process. In animals, the channel that releases ATP from apoptotic cells has been identified as pannexin 1, and its activation is initiated by caspase cleavage of its C-terminal autoinhibitory domain (Sandilos et al. 2012). As yet, no close homologue of pannexin has been reported in plants.

15.2.2 Touch Stimuli, Membrane Stretching, and Mechanosensitive Channels

In both animals (Nakamura and Stritmatter 1996; Lazarowski et al. 2003) and plants (Jeter et al. 2004; Weerasinghe et al. 2009), touch and other mechanical stimuli induce the release of ATP from cells by stretching membranes and activating channels that allow the diffusion of nucleotides from the cytoplasm into the ECM. Membrane stretching or deformation can also occur by reversible cell swelling and shrinkage induced either by osmotic shock or by physiologically induced ion uptake or release, such as in plant stomata (Clark et al. 2011), and these stimuli also can activate ATP-release channels (Wu et al. 2017). A number of plasma membrane (PM)-localized channels have pores large enough to release nucleotides, such as ATP. In particular, Piezo1 channels, which function in mechanoreception in

several different cell types (Volkers et al. 2015), can help mediate the mechanotransductive release of ATP from red blood cells (Cinar et al. 2015). Pannexin 1 also functions as a channel for ATP release from mechanically stimulated cells (Chiu et al. 2017). Thus far, homologues of neither Piezo1 nor pannexin have been found in plants. However, plants do have small conductance mechanosensitive channels (MscS), and these channels permit the passive transport of any charged molecule smaller than 1 K molecular mass, including ATP (Peyronnet et al. 2014). As yet, a role for MscS channels in ATP release from touch- or mechanically stimulated plant cells has not been genetically demonstrated, so the molecular mechanism for channel-mediated release of ATP from plant cells has not yet been discovered.

15.2.3 Secretory Vesicles

Remarkably, ATP can accumulate in secretory vesicles to concentrations even higher than that in the cytoplasm (Estevez-Herrera et al. 2016). For example, the concentration of ATP in secretory granules from chromaffin cells reaches up to 150 mM, almost 100 times higher than that typically found in the cytoplasm (Winkler and Westhead 1980). In animals, the nucleotide transporter VNUT is the main protein responsible for this accumulation of ATP in secretory vesicles (Sawada et al. 2008). When these vesicles fuse with the plasma membrane, their contents are typically released into the ECM, so this process is another means by which eATP levels increase. Interestingly, the vesicular release of ATP from neurons (Moriyama and Nomura 2018) and neutrophils (Harada et al. 2018) requires the mediation of VNUT. The significant relevance of vesicular ATP release in animal cells has recently been reviewed (Moriyama et al. 2017).

In plants, the secretion of vesicles delivering wall material is critically needed both for polarized cell growth (Bibeau et al. 2018) and for reversible swelling and shrinking of cells, such as guard cells (Shope and Mott 2006). In both cases, ATP release into the ECM accompanies these changes in cell size (Wu et al. 2007; Clark et al. 2011). Increased [eATP] is also found outside expanding cells in the elongation zone of primary roots (Roux et al. 2008). High levels of eATP can inhibit plant cell growth (Wu et al. 2007; Clark et al. 2010a, b), and in *Arabidopsis*, increased [eATP] is typically accompanied by increased expression of ecto-NTPDases (ecto-apyrases) (Wu et al. 2007), which have the lowest Km among all the eATP-hydrolyzing enzymes in eukaryotes (Knowles 2011). This increased expression is apparently important for maintaining optimal levels of eATP for continued growth (Roux and Steinebrunner 2007), because suppression of ecto-NTPDase expression or activity results in concurrent increases in [eATP] and growth suppression (Wu et al. 2007; Lim et al. 2014). The issues of which members of the *Arabidopsis* apyrase family are ecto-NTPDases and which can hydrolyze ATP are discussed in Sect. 15.4.

15.2.4 Active Transport and Facilitated Diffusion

Whereas the passive movement of ATP from the cytoplasm into the ECM through damaged membranes or mechanotransductive channels and its release via secretory vesicles are the main modes of moving ATP out of cells, there are also energy-dependent mechanisms that use ATP-dependent carriers. Schwiebert (1999) reviewed the evidence that ATP-binding cassette proteins (ABC) could be one of these carriers in animal cells, but inhibitors used to implicate these transporters in mediating ATP release also inhibit VNUT (Kato et al. 2013). More recently, Verkhratsky and Burnstock (2014) noted that whether active ATP transport contributes significantly to purinergic signaling in vertebrates remains unclear.

In plants, Thomas et al. (2000) found that when a gene encoding an *Arabidopsis* ABC glycoprotein (*AtPGP1*, or *MDR1*, *or AtABCB1*) was expressed in yeast, it promoted ATP release into the culture medium, and when it was overexpressed in *Arabidopsis*, it increased ATP accumulation on leaf surfaces. Another study found that ABC transport inhibitors suppressed elicitor-induced ATP release in *Salvia* hairy roots (Wu et al. 2011). Those results supported the conclusion that active transport is another mode of ATP release in plants. As in animals, it is not yet clear what, if any, role active ATP transport plays in eATP signaling in plants. However, because AtABCB1 promotes auxin transport (Noh et al. 2001) and ecto-apyrase expression promotes ABC transport activity (Thomas et al. 2000), the role of ecto-apyrases in maintaining a steep ATP gradient between the inside (mM) and outside (μM) of the plasma membrane may help explain the results of Liu et al. (2012), who found that the overexpression of AtAPY1 could promote auxin transport.

The transmembrane steep ATP gradient could allow the facilitated diffusion of ATP from the cytoplasm into the ECM, and Rieder and Neuhaus (2011) have identified a plasma membrane-localized transporter, PM-ANT1, that promotes the export of ATP during pollen maturation. That this transport of ATP has functional significance was demonstrated by studies that showed suppression of PM-ANT1 transcript levels resulting in reduced self-pollination and seed yield (Rieder and Neuhaus 2011).

15.2.5 ATP-Induced ATP Release and ATP Wave Propagation

In both plants (Peiter 2016; Dindas et al. 2018) and animals (Guan et al. 2007), signaling induced in specific cells can be propagated to distant cells by waves of Ca^{2+}. In animals, ATP release is induced by calcium signals (Boudreault and Grygorczyk 2004), and, since an early response of cells to eATP is an increase in $[Ca^{2+}]_{cyt}$, eATP can induce ATP release, and a propagated wave of ATP release in distant cells can result. In retinal astrocytes and Müller cells, the outward propagation of the wave of ATP release from the site of stimulus has a faster velocity (41 μm/s) than the propagation of Ca^{2+} waves (28 μm/s). In developing cochlea, the propagation of Ca^{2+} signals between cells is critically dependent on ATP-induced ATP release (Ceriani et al. 2016).

In plants, there is as yet no report of ATP-induced ATP release, and the propagation of Ca^{2+} waves is more closely linked to the propagation of reactive oxygen species (ROS) (Gilroy et al. 2016; Peiter 2016). However, eATP does induce calcium oscillations in root cells (Tanaka et al. 2010). Because these oscillations were damped by brefeldin, which inhibits vesicle trafficking, the authors concluded that, to the extent the oscillations were due to ATP released by cells, that release would be via vesicle secretion (Tanaka et al. 2010).

15.3 Receptor Structures and Functions

Because of their critical role in human physiology, the most studied purinergic receptors are those in vertebrates. There are multiple outstanding reviews of these receptors (e.g., Puchalowicz et al. 2014; Verkhratsky and Burnstock 2014; Jacobson and Müller 2016), so here we will simply summarize some of the main similarities and differences between these receptors and the one so far identified in plants.

The two main types of purinergic receptors in vertebrates are P2Xs, which are a family of eATP-gated cation channels, and P2Ys, which are members of the A class of G-protein-coupled receptors (GPCRs). In humans, there are seven subtypes of P2X receptors and eight members of the P2Y family. There are crystal structures available for both P2X (Minato et al. 2016) and P2Y (Zhang et al. 2014) receptors, and these have provided an advanced understanding of how the receptors bind nucleotides and how the binding changes their structures. The activation of both types of receptors by eATP rapidly leads to an increase in $[Ca^{2+}]_{cyt}$, but by different mechanisms. When P2X is activated, its cation channel opens to allow Ca^{2+} to enter cells, which raises the $[Ca^{2+}]_{cyt}$. The activation of P2Y receptors leads to an increase in cytoplasmic IP3, which then opens intracellular channels, resulting in an increased $[Ca^{2+}]_{cyt}$.

As noted in the Introduction, thus far only one receptor for extracellular nucleotides has been identified in plants, and that is P2K1 (initially named DORN1), which, unlike either P2X or P2Y receptors, is a lectin-receptor Ser/Thr kinase (Choi et al. 2014). Although structurally different from the animal receptors, P2K1 activation by ATP rapidly leads to an increase in $[Ca^{2+}]_{cyt}$. How P2K1 activation is linked to increased $[Ca^{2+}]_{cyt}$ is still being investigated, but presumably the link is indirect, since P2K1 is not itself an ATP-gated cation channel. In both animals and plants, the rapid Ca^{2+} signal generated by receptor activation leads to similar downstream signaling changes, as discussed in Sect. 15.4.

Given that there are multiple receptors for eATP in most vertebrates and multiple receptors for most plant hormones, it is unlikely that plants have only one purinergic receptor. Already there are some plant responses to eATP identified that persist in mutants null for P2K1, and this points to the likelihood that these responses are mediated by a nucleotide receptor different from P2K1.

15.4 eATP-Induced Responses

15.4.1 Early Signaling Steps

As previously discussed, the first detectable signaling step after eATP activation of the receptor is a rapid increase in $[Ca^{2+}]_{cyt}$ in both animal and plant cells. P2X receptors are ligand-gated ion channels and thus directly mediate Ca^{2+} influx, whereas P2Y receptors are G-protein linked and their activation results in intracellular release of Ca^{2+} from the endoplasmic reticulum. Thus, the kinetics and characteristics of the cytosolic Ca^{2+} changes differ depending on the type of purinoceptor which is activated. In plant cells, extracellular nucleotide-induced increases in $[Ca^{2+}]_{cyt}$ have also shown different kinetics and characteristics, but the source of these differences needs to be further characterized. For example, eATP and eADP induced an increase in $[Ca^{2+}{}_{cyt}]$ with different kinetics—the response to eATP treatment occurred in approximately 30 s, while the response to eADP treatment was more rapid, only taking 2 s (Demidchik et al. 2009, 2011).

Whereas in animal cells, the mechanisms for eATP-induced changes in $[Ca^{2+}]_{cyt}$ are well documented, it is not yet certain how activation of the plant P2K1 receptor (DORN1) leads to increased $[Ca^{2+}]_{cyt}$ in plant cells (Roux 2014). The cytoplasmic kinase domain of P2K1 is required for activation of the receptor to induce the change in $[Ca^{2+}]_{cyt}$ (Choi et al. 2014). Although there are numerous examples in the literature connecting receptor kinase activity to calcium signaling in plant cells, so far this connection for P2K1 is yet to be determined.

In *Arabidopsis*, an annexin, AnnAt1, was suggested as a possible candidate for the eATP-induced-calcium influx by Shang et al. (2009). Other studies have also linked this annexin to the function of facilitating calcium influx (Clark et al. 2012). Interestingly, AnnAt1 is subject to phosphorylation (Konopka-Postupolska et al. 2011), so if Ca^{2+} transport activity of AnnAt1 was regulated by phosphorylation, and it was a substrate for activated P2K1, this could be a mechanism for P2K1-induced changes in $[Ca^{2+}]_{cyt}$.

Recently, Zhu et al. (2017) reported a root avoidance response to high ATP. They found that this response was P2K1-independent, and that in this growth response, the eATP-induced increase in $[Ca^{2+}]_{cyt}$ was dependent on a heterotrimeric G-protein. Loss-of-function Gα mutants did not respond to eATP, while the gain-of-function Gα mutants were more responsive. Because the root avoidance response still occurred in mutants null for P2K1 (*dorn1–1, dorn1–3*), the results of this study also suggest the existence of another plant eATP receptor.

Another downstream signaling step of eATP receptor activation, found in both animal and plant cells, is an increase in reactive oxygen species (ROS). There is abundant evidence that eATP leads to an increase in ROS via activation of NADPH oxidase in animal cells (Bilbao et al. 2007; Katz et al. 2008; Roberts et al. 2017). In skeletal muscle cells, ROS production induced by eATP appears to be mediated by protein kinase C activation of NADPH oxidase (Díaz-Vegas et al. 2015). In *Arabidopsis* leaves, eATP-activated DORN1 induces the phosphorylation of the NADPH oxidase, respiratory burst oxidase homologue D (RBOHD), which leads to

increased ROS levels, then to stomatal closure, and increased resistance to attack by the bacterial pathogen *Pseudomonas syringae* (Chen et al. 2017). This provides a direct link between the kinase activity of DORN1 and the production of the second messenger, ROS, apparently without the need for an intermediate step of increased $[Ca^{2+}]_{cyt}$. In plants, RBOHD-dependent ROS and Ca^{2+} act as intercellular messages that can be propagated to distant cells as waves used for systemic immune signaling (Gilroy et al. 2016).

In both animals and plants, the eATP-induced ROS signal often leads the activation of MAPKs (Song et al. 2006; Buzzi et al. 2009). In plants the MAPK signaling pathway as well as RBOH-mediated Ca^{2+} signaling are regulated by calcium-dependent protein kinases in immune responses (Kobayashi et al. 2007; Xie et al. 2014), whereas in animals, MAPK and calcium signaling pathways can cross talk via protein kinase C (Tsao et al. 2013).

Nitric oxide (NO) is another eATP-induced intracellular messenger found in common between animal and plant cells. In animal cells multiple studies indicate that eATP induces an increase in NO in diverse tissues by activating nitric oxide synthase in a calcium-dependent manner (Lowe et al. 2013; Zimmermann 2016; Ulker 2018). A connection between eATP and NO was even found in the cell swelling response of the single-celled amoeba, *Dictyostelium* (Sivaramakrishnan and Fountain 2015). In plant cells, eATP also induces increased levels of NO. This increase is phosphatidic acid-dependent and occurs via nitrate reductase activity (Clark et al. 2010a; Sueldo et al. 2010; Salmi et al. 2013). However, the role of nitric oxide synthase in NO signaling in plants is still being investigated (Santolini et al. 2017). In both animal and plant cells, nitric oxide can promote Ca^{2+} influx (Jeandroz et al. 2013; Tang et al. 2015), further highlighting the interaction between eATP-generated second messengers.

15.4.2 Defense/Immune Responses to Pathogen Attack

Among the main responses mediated by eATP in both animal and plant cells are defense responses. In fact, much of the current research on purinergic signaling in animals is aimed at developing pharmacological approaches to treating diseases and disorders which affect human health (Burnstock 2017; Stokes et al. 2017). As discussed previously the most obvious mechanism for release of ATP from plant cells is via wounding, and this release of ATP acts as a damage-associated molecular pattern (DAMP) signal (Cao et al. 2014). With the discovery of P2K1 as the receptor for this eATP defense signal, the plant signaling response to injury and immune response to disease have become better understood. Plant immune responses are complex and involve cross talk between eATP and hormones that result in signaling changes in Ca^{2+} and ROS levels (Seybold et al. 2014).

A recent study demonstrated that in *Arabidopsis*, there is cross talk between P2K1-mediated eATP defense signaling and the plant defense hormone, jasmonate, in the response to attack by the necrotrophic fungus, *Botrytis cinerea* (Tripathi et al. 2018). This interaction between eATP and jasmonate involved the second

messengers Ca^{2+}, ROS, and NO. There is also cross talk between eATP and another plant defense hormone, salicylic acid, in mediating programmed cell death (Feng et al. 2015b).

Because the [eATP] can be limited by ecto-apyrase activity, it is not surprising that several studies have also shown a key role for ecto-apyrases in defense responses to fungal pathogens. An ecto-apyrase found in the cell walls of pea epicotyls functions in a protein complex with copper amine oxidase, an enzyme that is involved in extracellular H_2O_2 production in the defense response to fungal attack by *Mycosphaerella pinodes* (Toyoda et al. 2012). This apyrase appears to be the target of elicitor and suppressor molecules secreted by the fungus. Ectopic expression of a *Medicago* ecto-apyrase in *Nicotiana benthamiana* reduced the size of necrotic lesions induced by a virulent fungus (Toyoda et al. 2014). Correspondingly, treatment with ecto-apyrase inhibitors can block the ability of diverse pathogenic fungi to efflux fungicides, making fungicide treatments more effective against these pathogens (Tripathy et al. 2016).

In animal cell immune responses, eATP acts in a pro-inflammatory manner, whereas adenosine, which is produced as a breakdown product of eATP, acts mainly as an anti-inflammatory (Faas et al. 2017). In the alga *Dasycladus vermicularis*, treatment with adenosine blocks eATP- and wound-induced NO production (Torres et al. 2008), and in *Arabidopsis*, the treatment of root hairs and cotton fibers with adenosine blocks eATP-mediated changes in growth (Clark et al. 2010a, b). More recently, it was shown that accumulation of apoplastic adenosine in plant leaves makes them more susceptible to fungal attack (Daumann et al. 2015). Thus adenosine appears to act in an antagonistic fashion to some plant responses to eATP, including defense responses, but the mechanism for this antagonism is unknown.

eATP and ecto-apyrases are also implicated in responses to a variety of abiotic stresses including cold, salt, and drought (Deng et al. 2015; Zhao et al. 2016; Veerappa et al. 2018). For example, cold and salt stress result in membrane disruption and increased release of ATP (Deng et al. 2015; Zhao et al. 2016). High [eATP], in turn, can inhibit vesicular trafficking and membrane repair, and lead to cell death (Sun et al. 2012). By increasing the hydrolysis of eATP, the enhanced expression of ecto-apyrase can protect cells against excessive eATP accumulation and thus promote vesicular trafficking and membrane repair. This mechanism was proposed to help explain how the ectopic expression of APYRASE2 could promote sustained growth in cold-stressed *Arabidopsis* (Deng et al. 2015). Thus far, there is less of a parallel for eATP involvement in stress responses in animal cells, although purinergic signaling has been implicated in ageing (Burnstock and Dale 2015).

15.4.3 Growth Responses

In animal cells, eATP affects growth mainly by regulating cell division. In recent years, much research in this field has been focused on eATP effects on growth of cancer cells (Di Virgilio and Adinolfi 2017). In contrast, eATP regulates growth in plant cells, primarily via regulation of cell expansion. Thus far, eATP and

ecto-apyrases appear to regulate growth in every cell or tissue type tested, including roots, hypocotyls, and leaves, as well as in single cells such as pollen tubes, cotton fibers, and root hairs (Clark and Roux 2011; Clark et al. 2014).

In root hairs, there is a biphasic growth response to ATPγS with low levels promoting growth while high levels inhibiting growth (Clark et al. 2010a). Correspondingly, treatment with apyrase inhibitors or anti-AtAPY1 antibodies also inhibits growth because, as expected, these treatments cause an increase in the [eATP] (Clark et al. 2010b; Lim et al. 2014). One explanation for these growth changes would be the effects of apyrase expression or [eATP] on auxin transport (Tang et al. 2003; Liu et al. 2012), because inhibition of auxin transport can inhibit growth even in single root hairs (Velasquez et al. 2016). Typically, growth is inhibited when plants are responding to biotic stress, so the high [eATP] that results when plants suffer from insect or microbe attacks may also play a role in mediating this feature of defense responses.

Treatment with eATP/eADP or altered expression of AtAPY1 affects the root skewing growth response in *Arabidopsis* (Haruta and Sussman 2012; Yang et al. 2015). Both the root skewing growth response and the eATP-induced Ca^{2+} influx are reduced in the loss-of-function mutants for the H^+-ATPase (AHA2) (Haruta and Sussman 2012). This result indicates that the plasma membrane proton motive force plays an important role in eATP growth responses at alkaline pH.

15.5 Mechanisms of Decreasing or Terminating the eATP Signal

15.5.1 Ecto-NTPDases (Ecto-Apyrases)

A general rule of signaling pathways is that cells must have a mechanism for turning off whatever chemical signal initiates the pathway. In the case of eATP, the main enzyme typically used by plants and animals to turn off the cellular responses initiated by eATP is ecto-NTPDase, the enzyme with the lowest Km for ATP (Knowles 2011). In general, this enzyme removes the terminal phosphate from NTPs and NDPs, but not NMPs; however, different versions of the enzyme have different affinities for NTPs and NDPs (Zimmermann et al. 2012; Yegutkin 2014). The ecto-NTPDase family of enzymes is highly conserved (Clark et al. 2014), with its members having four or five "apyrase conserved regions," that is, domains with very similar primary sequences.

Not all NTPDases are "ecto," but in vertebrates, NTPDases 1, 2, 3, and 8 all function on the plasma membrane with their active site facing out into the ECM, where they play a key role in limiting the [eATP] (Yegutkin 2014). In plants, ecto-NTPDases have been identified from potato (Riewe et al. 2008), pea (Thomas et al. 2000; Shibata et al. 2002), soybean (Day et al. 2000), and *Arabidopsis* (Wu et al. 2007; Lim et al. 2014), although, as discussed later, which among the seven NTPDases in *Arabidopsis* is "ecto" and has triphosphatase activity is not yet settled (Massalski et al. 2015). Furthermore, in peas, the same apyrase, that is, "ecto," also

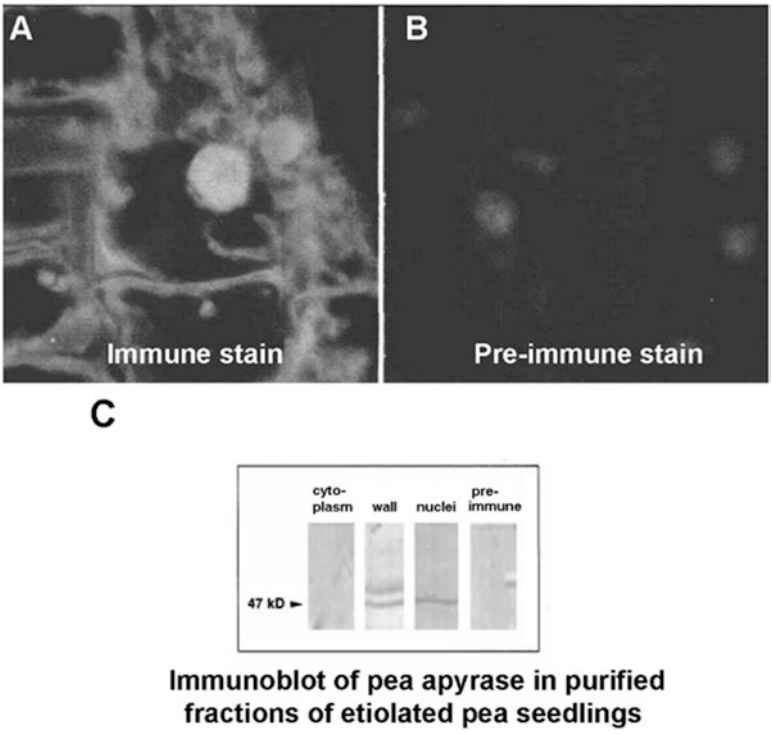

Fig. 15.1 As judged by immunocytochemistry (**a**, **b**) and by immunoblots (**c**), the pea apyrase (psNTP9/PsAPY1) is localized both in nuclei and in the wall of pea seedlings. These results are taken from Tong et al. (1993) (**a**, **b**), and from Thomas et al. (1999) (**c**), and were independently confirmed by Shibata et al. (2002)

localizes to the nucleus (Fig. 15.1), and, likely, to other subcellular locales (Tong et al. 1993; Thomas et al. 1999: Shibata et al. 2002). There are crystal structures available for both animal (Zebisch et al. 2012) and plant (Summers et al. 2017) NTPDases, and these show remarkable similarity and may share a common catalytic mechanism.

Purification and enzymatic characterization of plant NTPDases was originally done on psNTP9, extracted from pea nuclei (Chen et al. 1987), and later was carried out on the two almost identical NTPDases from *Arabidopsis*, AtAPY1 and AtAPY2, that were heterologously expressed in *Escherichia coli* (Steinebrunner et al. 2000). Both reports found that the purified NTPDases hydrolyzed ATP better than ADP. More recently, however, Massalski et al. (2015) reported that AtAPY1 has no ATPase activity, and, instead, it can only hydrolyze nucleoside diphosphates. The two versions of AtAPY1 assayed by Massalski et al. were both modified: one purified from *Arabidopsis* was tagged with GFP, and the other purified from the human embryonic kidney cell expression system (HEK293) was missing its N-terminal transmembrane domain (i.e., included only residues 67–470). These differences

could help explain the discrepancy between the findings of Steinebrunner et al. (2000) and Massalski et al. (2015) relative to the substrate preferences of AtAPY1, because the AtAPY1 purified by Steinebrunner et al. was full-length, and was not modified with GFP. The heterologously expressed AtAPY1 purified from *E. coli* did have a His-tag (Steinebrunner et al. 2000), so to test whether this altered its NTPDase activity, a native NTPDase sample that was purified from *Arabidopsis* to >90% purity without any tag, and that was recognized by an antibody directed to a unique peptide region of AtAPY1 and was assayed, and this sample had higher ATPase activity than ADPase activity (G. Weeraratne and S.J. Roux, unpublished).

As regards which (if any) *Arabidopsis* NTPDase is an ecto-NTPDase, there is both immunological and genetic evidence that AtAPY1 helps to regulate the [eATP] (Wu et al. 2007; Lim et al. 2014). However, two localization studies using fluorescently tagged versions of AtAPY1 and AtAPY2 found that both were localized primarily in Golgi, and neither study observed any fluorescent signal for AtAPY1 or AtAPY2 associated with the cell periphery (i.e., plasma membrane or wall) (Chiu et al. 2012; Schiller et al. 2012). As proposed by Clark and Roux (2014), these results could be reconciled with the immunological and genetic evidence if AtAPY1 and AtAPY2 regulated the [eATP] within the lumen of the Golgi, which could be the ultimate source of secreted ATP. Alternatively, AtAPY1 could reside primarily in the Golgi but move from the Golgi to the plasma membrane only under certain conditions or in certain tissues not observed in the fluorescent localization studies. Studies of fluorescently tagged versions of the other five members of the *Arabidopsis* NTPDase family (AtAPY3, 4, 5, 6, 7) indicated none of them were associated with the plasma membrane or wall (Yang 2011; Yang et al. 2013; Chiu et al. 2015). For now, although available evidence indicates that among the seven NTPDases in *Arabidopsis*, only AtAPY1 and AtAPY2 can regulate the [eATP], final definitive evidence as to which, if any, of them is an ecto-NTPDase will require additional studies.

15.5.2 Genetic Control of Ecto-NTPDase Activity

The best approach to defining the function of an enzyme is to observe the consequences of either knocking out or overexpressing the gene that encodes it. In animals there are a number of studies that documented the effects of knocking out an NTPDase on different tissue functions in mice. Here we briefly summarize three of these. Enjyoji et al. (1999) found that mutant mice in which there was a targeted disruption of NTPDase 1 (CD39) had prolonged bleeding times indicating they had disordered hemostasis and defective thromboregulation. Although blood flow to the ear is important for cochlear function, mice null for NTPDase 1 had normal brainstem responses to noise over a range of test frequencies, and did not differ from wild-type mice in their response to acoustic trauma, so the authors concluded that this knockout did not alter cochlear function (Vlajkovic et al. 2009). More recently, Vandenbeuch et al. (2013) found that knocking out NTPDase2 in mice resulted in increased [eATP] in tongue tissue, which desensitized the taste receptors on nerve

fibers there. They warned that pharmaceutical agents that target NTPDases could disrupt taste function as an unintended consequence.

In plants most of the knockout studies and overexpression studies on NTPDases have been focused on AtAPY1 and AtAPY2, because of the major role these enzymes play in growth control. These two NTPDases, which are 87% identical in their primary structure, complement each other's function in part. That is, single knockouts of either one show only minor phenotypic differences from wild-type plants, whereas the knockout of both is male lethal; i.e., pollen null for both genes cannot germinate (Steinebrunner et al. 2003). An RNAi approach has been used to study growth effects in mutants null for one of the genes and knocked down for the other, and such plants are dwarf and have major changes in gene expression that partially explain their defective growth (Lim et al. 2014). In contrast, mutants overexpressing either AtAPY1 or AtAPY2 have an enhanced growth phenotype, which is explained in part by their enhanced transport of the growth hormone auxin (Liu et al. 2012). These discoveries predicted the possibility that ectopic expression of an NTPDase in a crop plant could have beneficial effects on the growth and seed yield of that plant, and initial results suggest that this prediction is true (Veerappa et al. 2018).

Whereas a main focus of this chapter has been on eATP signaling and on the role of ecto-NTPDases in regulating the [eATP], it should not be concluded that the effects of overexpressing AtAPY1 or AtAPY2 noted earlier are necessarily due only to the "ecto" function of these enzymes. Recent studies have found that AtAPY1 and AtAPY2 can be purified from nuclei of *Arabidopsis* seedlings, and that these purified nuclei give a strong and specific nuclear immunofluorescent stain (G. Weeraratne and S.J. Roux, unpublished). These findings raise the possibility that these *Arabidopsis* enzymes, like the pea NTPDase (Tong et al. 1993), may function in both the ECM and in the nucleus. Regulating nuclear [NTP] would impact diverse nuclear functions, ranging from transcription and chromatin remodeling (Wright et al. 2016) to nuclear splicing (Ali and Reddy 2006). Comparable immunocytochemical and biochemical studies documenting nuclear NTPDases in animals have not yet been published.

15.5.3 Other Ectonucleotidases That Control [eATP]

Even though ecto-NTPDases are the most important enzymes in limiting the [eATP], in animals, there is ample documentation that there are ectonucleotidases other than NTPDases that help control the [eATP] (Yegutkin 2008). The three main families of these enzymes are ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP), alkaline phosphatases, and the ecto-5′-nucleotidase (E5′Nt/CD73). There is genetic evidence that at least some of these enzymes could be critically involved in controlling responses to eATP. For example, as discussed later, eATP can serve as a danger signal that induces a proinflammatory response in animals. Because CD73 plays a key role in converting proinflammatory ATP into immunosuppressive adenosine,

mice deficient in CD73 expression have a stronger inflammatory response to disease infections than wild-type mice (Alam et al. 2014).

In plants, the role of ectonucleotidases in regulating [eATP] has been less explored. There are definitely extracellular NPP enzymes in plants, and they play important roles in enabling plants to adapt to stress, just as ecto-NTPDases do, but why they help plants tolerate stress is not yet understood (Gutierrez-Luna et al. 2018). It would be surprising if NPPs and other extracellular phosphatases did not participate at some level in the mechanisms for controlling the [eATP], but as yet, there is not enough information to evaluate what that level is.

15.6 Conclusions

Although the diversity and structural characteristics of the purinergic receptors in animals and plants differ significantly (Hou and Cao 2016), the signaling steps that follow the activation of these receptors are remarkably similar in plants and animals (Fig. 15.2). In both, receptor activation results rapidly in increased $[Ca^{2+}]_{cyt}$ and increased levels of reactive oxygen species and nitric oxide (Clark et al. 2014; Zimmermann 2016). In both, the main enzyme limiting the [eATP] is an ecto-nucleoside triphosphate-diphosphohydrolase (NTPDase), referred to, here and in

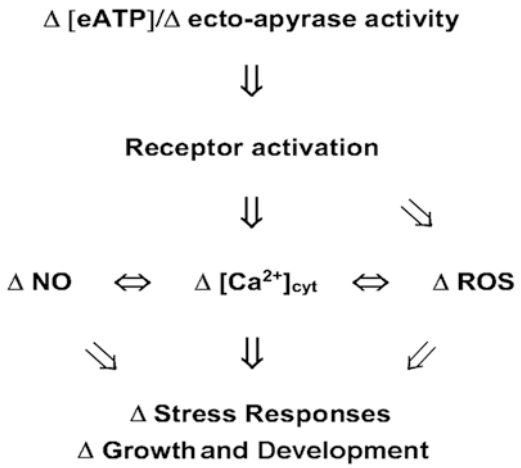

Fig. 15.2 Common features of extracellular nucleotide signaling in animals and plants, here illustrated by eATP initiation. Receptor activation depends on the [eATP], which can be increased by one or more mechanisms of ATP release from the cytoplasm or (less commonly) by synthesis in the ECM and decreased mainly by ecto-apyrase enzymes (ecto-NTPDases). The most rapid cellular change induced by receptor activation is typically an increase in $[Ca^{2+}]_{cyt}$, although in plants this increase may be mediated by an upstream activation of ROS production via the phosphorylation of RBOHD by the PK21 receptor kinase. Increased $[Ca^{2+}]_{cyt}$ can rapidly induce higher levels of NO and ROS, both of which can induce increased $[Ca^{2+}]_{cyt}$, as indicated by the bidirectional arrows. The main downstream effects of these early amplifiers of the eATP signal in animals and plants are adaptive stress responses and/or changes in growth and development

most of the plant literature, as ecto-apyrase. The conserved features of these enzymes, the documented regulatory functions of P2X-like receptor in primitive algae (Fountain et al. 2008), and the observation that physiologically significant levels of ATP can be found in the open ocean (Azam and Hodson 1977) suggest that eATP signaling is an ancient method of regulating cellular responses.

References

Alam MS, Kuo JL, Ernst PB, Derr-Castillo V, Pereira M, Gaines D, Costales M, Bigley E, Williams K (2014) Ecto-5′-nucleotidase (CD73) regulates host inflammatory responses and exacerbates murine salmonellosis. Sci Rep 4:4486

Ali GS, Reddy ASN (2006) ATP, phosphorylation and transcription regulate the mobility of plant splicing factors. J Cell Sci 119:3527–3538

Azam F, Hodson RE (1977) Dissolved ATP in the sea and its utilization by marine bacteria. Nature 267:696–698

Bibeau JP, Kingsley JL, Furt F, Tüzel E, Vidali L (2018) F-actin mediated focusing of vesicles at the cell tip is essential for polarized growth. Plant Physiol 176:352–363

Bilbao PS, Boland R, de Boland AR, Santillán G (2007) ATP modulation of mitogen activated protein kinases and intracellular Ca^{2+} in breast cancer (MCF-7) cells. Arch Biochem Biophys 466:15–23

Boudreault F, Grygorczyk R (2004) Cell swelling induced ATP release is tightly dependent on intracellular calcium elevations. J Physiol 561:499–513

Burnstock G (2017) Purinergic signalling: therapeutic developments. Front Pharmacol 8:661

Burnstock G, Dale N (2015) Purinergic signalling during development and ageing. Purinergic Signal 11:277–305

Burnstock G, Verkhratsky A (2009) Evolutionary origins of the purinergic signaling system. Acta Physiol 195:415–447

Buzzi N, Bilbao PS, Boland R, de Boland AR (2009) Extracellular ATP activates MAP kinase cascades through a P2Y purinergic receptor in the human intestinal Caco-2 cell line. Biochim Biophys Acta 1790:1651–1659

Cao Y, Tanaka K, Nguyen CT, Stacey G (2014) Extracellular ATP is a central signaling molecule in plant stress responses. Curr Opin Plant Biol 20:82–87

Ceriani F, Pozzan T, Mammano F (2016) Critical role of ATP-induced ATP release for Ca^{2+} signaling in nonsensory cell networks of the developing cochlea. Proc Natl Acad Sci U S A 113:E7194–E7201

Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, Armstrong AJ (2010) Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 467:863–867

Chen YR, Datta N, Roux SJ (1987) Purification and partial characterization of a calmodulin-stimulated nucleoside triphosphatase from pea nuclei. J Biol Chem 262:10689–10694

Chen D, Cao Y, Li H, Kim D, Ahsan N, Thelen J, Stacey G (2017) Extracellular ATP elicits DORN1-mediated RBOHD phosphorylation to regulate stomatal aperture. Nat Commun 8:2265

Chiu T-Y, Christiansen K, Moreno I, Lao J, Loqué D, Orellana A, Heazlewood J, Clark G, Roux S (2012) AtAPY1 and AtAPY2 function as endomembrane nucleoside diphosphatases in *Arabidopsis thaliana*. Plant Cell Physiol 53:1913–1925

Chiu T-Y, Lao J, Manalansan B, Loqué D, Roux SJ, Heazlewood JL (2015) Biochemical characterization of Arabidopsis APYRASE family reveals their roles in regulating endomembrane NDP/NMP homeostasis. Biochem J 472:43–54

Chiu Y-H, Schappe MS, Desai BN, Bayliss DA (2017) Revisiting multimodal activation and channel properties of Pannexin 1. J Gen Physiol 150:19–39

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee SY, Stacey G (2014) Identification of a plant receptor for extracellular ATP. Science 343:290–294

Cinar E, Zhou S, DeCourcey J, Want Y, Waugh RE, Wan J (2015) Piezo1 regulates mechanotransductive release of ATP from human RBCs. Proc Natl Acad Sci U S A 112:11783–11788

Clark G, Roux SJ (2011) Apyrases, extracellular ATP and the regulation of growth. Curr Opin Plant Biol 14:700–706

Clark G, Wu M, Wat N, Onyirimba J, Pham T, Herz N, Ogoti J, Gomez D, Canales AA, Aranda G, Blizard M, Nyberg T, Terry A, Torres J, Wu J, Roux SJ (2010a) Both the stimulation and inhibition of root hair growth induced by extracellular nucleotides in Arabidopsis are mediated by nitric oxide and reactive oxygen species. Plant Mol Biol 74:423–435

Clark G, Torres J, Finlayson S, Guan XY, Handley C, Lee J, Kays JE, Chen ZJ, Roux SJ (2010b) Apyrase (Nucleoside Triphosphate-Diphosphohydrolase) and extracellular nucleotides regulate cotton fiber elongation in cultured ovules. Plant Physiol 152:1073–1083

Clark G, Fraley D, Steinebrunner I, Cervantes A, Onyirimba J, Liu A, Torres T, Tang W, Kim J, Roux SJ (2011) Extracellular nucleotides and apyrases regulate stomatal aperture in *Arabidopsis*. Plant Physiol 156:1740–1753

Clark G, Morgan RO, Fernandez P, Roux SJ (2012) Evolutionary adaptation of plant annexins has diversified their molecular structures, interactions and functional roles. New Phytol 196:695–712

Clark G, Morgan RO, Fernandez M-P, Salmi ML, Roux SJ (2014) Breakthroughs spotlighting roles for extracellular nucleotides and apyrases in stress responses and growth and development. Plant Sci 225:107–116

Daumann M, Fischer M, Niopek-Witz S, Girke C, Möhlmann T (2015) Apoplastic nucleoside accumulation in Arabidopsis leads to reduced photosynthetic performance and increased susceptibility against *Botrytis cinerea*. Front Plant Sci 6:1158

Day RB, McAlvin CB, Loh JT, Denny RL, Wood TC, Young ND, Stacey G (2000) Differential expression of two soybean apyrases, one of which is an early nodulin. Mol Plant-Microbe Interact 13:1053–1070

Demidchik V, Shang ZL, Shin R, Thompson E, Rubio L, Laohavisit A, Mortimer JC, Chivasa S, Slabas AR, Glover BJ, Schachtman DP, Shabala SN, Davies JM (2009) Plant extracellular ATP signalling by plasma membrane NADPH oxidase and Ca^{2+} channels. Plant J 58:903–913

Demidchik V, Shang Z, Shin R, Colaco R, Laohavisit A, Shabala S, Davies JM (2011) Receptor-like activity evoked by extracellular ADP in Arabidopsis root epidermal plasma membrane. Plant Physiol 156:1375–1385

Deng S, Sun J, Zhao R, Ding M, Zhang Y, Sun Y, Wang W, Tan Y, Liu D, Ma X, Hou P, Wang M, Lu C, Shen X, Chen S (2015) *Populus euphratica* APYRASE2 enhances cold tolerance by modulating vesicular trafficking and extracellular ATP in Arabidopsis plants. Plant Physiol 169:530–548

Di Virgilio F, Adinolfi E (2017) Extracellular purines, purinergic receptors and tumor growth. Oncogene 36:293–303

Díaz-Vegas A, Campos CA, Contreras-Ferrat A, Casas M, Buvinic S, Jaimovich E, Espinosa A (2015) ROS production via P2Y1-PKC-NOX2 is triggered by extracellular ATP after electrical stimulation of skeletal muscle cells. PLoS One 10:e0129882

Dindas J, Scherzer S, Roelfsema MRG, von Meyer K, Muller HM, Al-Rasheid KAS, Palme K, Dietrich P, Becker D, Bennett MJ, Hedrich R (2018) AUX1-mediated root hair auxin influx governs $SCF^{TIR1/AFB}$-type Ca^{2+} signaling. Nat Commun 9:1174

Enjyoji K, Sevigny J, Lin Y, Frenette PS, Christie PD, JSA E, Imai M, Edelberg JM, Rayburn H, Lech M, Beeler DL, Csizmadia E, Wagner DD, Robson SC, Rosenberg RD (1999) Targeted disruption of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nat Med 5:1010–1017

Estevez-Herrera J, Dominguez N, Pardo MR, Gonzalez-Santan A, Westhead EW, Borges R, Machado JD (2016) ATP: the crucial component of secretory vesicles. Proc Natl Acad Sci U S A 113:E4098–E4106

Faas MM, Sáez U, de Vos P (2017) Extracellular ATP and adenosine: the Yin and Yang in immune responses? Mol Asp Med 55:9–19

Feng H, Guan D, Bai J, Sun K, Jia L (2015a) Extracellular ATP: a potential regulator of cell death. Mol Plant Pathol 16:633–639

Feng H, Guan D, Sun K, Fang Y, Zhao Y, Jia Y (2015b) Extracellular ATP is involved in the salicylic acid-induced cell death in suspension-cultured tobacco cells. Plant Prod Sci 18:154–160

Fountain SJ, Cao LS, Young MT, North RA (2008) Permeation properties of a P2X receptor in the green algae *Ostreococcus tauri*. J Biol Chem 283:15122–15126

Gilroy S, Białasek M, Suzuki N, Górecka M, Devireddy AR, Karpinski S, Mittler R (2016) ROS, calcium, and electric signals: key mediators of rapid systemic signaling in plants. Plant Physiol 171:1606–1615

Gout E, Bligny R, Douce R (1992) Regulation of intracellular pH values in higher plant cells. J Biol Chem 267:13903–13909

Guan C-B, Xu H-T, Jin M, Yuan XB, Poo MM (2007) Long-range Ca^{2+} signaling from growth cone to soma mediates reversal of neuronal migration induced by Slit-2. Cell 129:385–395

Gutierrez-Luna FM, Hernandez-Dominguez EE, Valencia-Turcotte LG, Rodriguez-Sotres R (2018) Review: "pyrophosphate and pyrophosphatases in plants, their involvement in stress responses and their possible relationship to secondary metabolism". Plant Sci 267:11–19

Harada Y, Kato Y, Miyaji T, Omote H, Moriyama Y, Hiasa M (2018) Vesicular nucleotide transporter mediates ATP release and migration in neutrophils. J Biol Chem 293:3770–3779

Haruta M, Sussman MR (2012) The effect of a genetically reduced plasma membrane proton motive force on vegetative growth of Arabidopsis. Plant Physiol 158:1158–1171

Helenius M, Jalkanen S, Yegutkin GG (2012) Enzyme-coupled assays for simultaneous detection of nanomolar ATP, ADP, AMP, adenosine, inosine and pyrophosphate concentrations in extracellular fluids. Biochim Biophys Acta 1823:1967–1975

Hernández-Oñate MA, Herrera-Estrella A (2015) Damage response involves mechanisms conserved across plants animals and fungi. Curr Genet 61:359–372

Hou ZR, Cao J (2016) Comparative study of the P2X gene family in animals and plants. Purinergic Signal 12:269–281

Imamura H, Nhat KPH, Togawab H, Saitoc K, Iinob R, Kato-Yamadad Y, Nagaia T, Noji H (2009) Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. Proc Natl Acad Sci U S A 106:15651–15656

Jacobson KA, Müller CE (2016) Medicinal chemistry of adenosine, P2Y and P2X receptors. Neuropharmacology 104:31–49

Jeandroz S, Lamotte O, Astier J, Rasul S, Trapet P, Besson-Bard A, Bourque S, Nicolas-Frances V, Ma W, Berkowitz GA, Wendehenne D (2013) There's more to the picture than meets the eye: nitric oxide cross talk with Ca^{2+} signaling. Plant Physiol 163:459–470

Jeter C, Tang W, Henaff E, Butterfield T, Roux SJ (2004) Evidence of a novel cell signaling role for extracellular adenosine triphosphates and diphosphates in Arabidopsis. Plant Cell 16:2652–2664

Kato Y, Omote H, Miyaji T (2013) Inhibitors of ATP release inhibit vesicular nucleotide transporter. Biol Pharm Bull 36:1688–1691

Katz S, Boland R, Santillán G (2008) Purinergic (ATP) signaling stimulates JNK1 but not JNK2 MAPK in osteoblast-like cells: contribution of intracellular Ca^{2+} release, stress activated and L-voltage-dependent calcium influx, PKC and Src kinases. Arch Biochem Biophys 477:244–252

Khakh BS, Burnstock GB (2009) The double life of ATP. Sci Am 301:84–92

Knowles I (2011) The GDA1_CD39 superfamily: NTPDases with diverse functions. Purinergic Signal 7:21–45

Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H (2007) Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19:1065–1080

Konopka-Postupolska D, Clark G, Hofmann A (2011) Structure, function and membrane interactions of plant annexins: an update. Plant Sci 181:230–241

Lazarowski ER, Boucher RC, Harden TK (2003) Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. Mol Pharmacol 64:785–795

Lim MH, Wu J, Yao JC, Gallardo IF, Dugger JW, Webb LJ, Huang J, Salmi ML, Song J, Clark G, Roux SJ (2014) Apyrase suppression raises extracellular ATP levels and induces gene expression and cell wall changes characteristic of stress responses. Plant Physiol 164:2054–2067

Liu X, Wu J, Clark G, Lundy S, Lim M, Arnold D, Chan J, Tang W, Muday G, Gardner G, Roux SJ (2012) Role for apyrases in polar auxin transport in Arabidopsis. Plant Physiol 160:1985–1995

Lowe M, Park SJ, Nurse CA, Campanucci VA (2013) Purinergic stimulation of carotid body efferent glossopharyngeal neurons increases intracellular Ca^{2+} and nitric oxide production. Exp Physiol 98:1199–1212

Massalski C, Bloch J, Zebisch M, Steinebrunner I (2015) The biochemical properties of the Arabidopsis ecto-nucleoside triphosphate diphosphohydrolase AtAPY1 contradict a direct role in purinergic signaling. PLoS One 10:e0115832

Minato Y, Suzuki S, Hara T, Kofuku Y, Kasuya G, Fujiwara Y, Igarashi S, Suzuki E, Nureki O, Hattori M, Ueda T, Shimada I (2016) Conductance of P2X4 purinergic receptor is determined by conformational equilibrium in the transmembrane region. Proc Natl Acad Sci U S A 113:4741–4746

Moriyama Y, Nomura M (2018) Clodronate: a vesicular ATP release blocker. Trends Pharma Sci 39:13–23

Moriyama Y, Hiasa M, Sakamoto S, Omote H, Nomura M (2017) Vesicular nucleotide transporter (VNUT): appearance of an actress on the stage of purinergic signaling. Purinergic Signal 13:387–404

Nakamura F, Stritmatter SM (1996) P2Y1 purinergic receptors in sensory neurons: contribution to touch-induced impulse generation. Proc Natl Acad Sci U S A 93:10465–10470

Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. Plant Cell 13:2441–2454

Peiter E (2016) The ever-closer union of signals: propagating waves of calcium and ROS are inextricably linked. Plant Physiol 172:3–4

Peyronnet R, Tran D, Girault T, Frachisse J-M (2014) Mechanosensitive channels: feeling tension in a world under pressure. Front Plant Sci 5:1–14

Puchalowicz TM, Baranowska-Bosiacka I, Chlubek D, Dziedziejko V (2014) P2X and P2Y receptors-role in the pathophysiology of the nervous system. Int J Mol Sci 15:23672–23704

Rieder B, Neuhaus HE (2011) Identification of an Arabidopsis plasma membrane-located ATP transporter important for anther development. Plant Cell 23:1932–1944

Riewe D, Grosman L, Fernie AR, Wucke C, Geigenberger P (2008) The potato-specific apyrase is apoplastically localized and has influence on gene expression, growth, and development. Plant Physiol 147:1092–1109

Roberts JS, Atanasova KR, Lee J, Diamond G, Deguzman J, Hee Choi C, Yilmaz Ö (2017) Opportunistic pathogen *Porphyromonas gingivalis* modulates danger signal ATP-mediated antibacterial NOX2 pathways in primary epithelial cells. Front Cell Infect Microbiol 7:291

Roux SJ (2014) A start point for extracellular nucleotide signaling. Mol Plant 7:937–938

Roux SJ, Steinebrunner I (2007) Extracellular ATP: an unexpected role as a signaler in plants. Trends Plant Sci 12:522–527

Roux S, Wu J, Henaff E, Torres J, Clark G (2008) Regions of growth are regions of highest release of ATP and highest expression of ectonucleotidases AtAPY1 and AtAPY2 in *Arabidopsis*. Purinergic Signal 4:S112

Salmi ML, Clark G, Roux S (2013) Current status and proposed roles for nitric oxide as a key mediator of the effects of extracellular nucleotides on plant growth. Front Plant Sci 4:427

Sandilos JK, Chiu Y-H, Chekeni FB, Armstrong AJ AJ, Walk SF SF, Kodi S, Ravichandran KS, Douglas A, Bayliss DA (2012) Pannexin 1, an ATP release channel, is activated by caspase cleavage of its pore-associated C-terminal autoinhibitory region. J Biol Chem 287:11303–11131

Santolini J, Andre F, Jeandroz S, Wendehenne D (2017) Nitric oxide synthase in plants: where do we stand? Nitric Oxide Biol Chem 63:30–38

Sawada K, Echigo N, Juge N, Miyaji T, Otsuka M, Omote H, Yamamoto A, Moriyama Y (2008) Identification of a vesicular nucleotide transporter. Proc Natl Acad Sci U S A 105:5683–5686

Schiller M, Massalski C, Kurth T, Steiebrunner I (2012) The Arabidopsis apyrase AtAPY1 is localized in the Golgi instead of the extracellular space. BMC Plant Biol 12:123

Schulze-Lohoff E, Hugo C, Rost S, Arnold S, Gruber A, Brune B, Sterzel RB (1998) Extracellular ATP causes apoptosis and necrosis of cultured mesangial cells via P2Z/P2X(7) receptors. Am J Physiol Renal Physiol 275:F962–F971

Schwiebert EM (1999) ABC transporter-facilitated ATP conductive transport. Am J Physiol 276:C1–C8

Seybold H, Trempel F, Ranf S, Scheel D, Romeis T, Lee J (2014) Ca^{2+} signalling in plant immune response: from pattern recognition receptors to Ca^{2+} decoding mechanisms. New Phytol 204:782–790

Shang Z, Laohavisit A, Davies JM (2009) Extracellular ATP activates an Arabidopsis plasma membrane Ca^{2+}-permeable conductance. Plant Signal Behav 4:989–991

Shibata K, Abe S, Yoneda M, Davies E (2002) Sub-cellular distribution and isotypes of a 49-kDa apyrase from *Pisum sativum*. Plant Physiol Biochem 40:407–415

Shope JC, Mott KA (2006) Membrane trafficking and osmotically induced volume changes in guard cells. Plant Physiol 57:4123–4131

Sivaramakrishnan V, Fountain SJ (2015) Evidence for extracellular ATP as a stress signal in a single-celled organism. Eukaryot Cell 14:775–782

Song C, Steinebrunner I, Wang S, Stout S, Roux SJ (2006) Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in Arabidopsis thaliana. Plant Physiol 140:1222–1232

Steinebrunner I, Jeter C, Song C, Roux SJ (2000) Molecular and biochemical comparison of two different apyrases from *Arabidopsis thaliana*. Plant Physiol Biochem 38:913–922

Steinebrunner I, Wu J, Sun Y, Corbett A, Roux SJ (2003) Disruption of apyrases inhibits pollen germination in Arabidopsis. Plant Physiol 131:1638–1647

Stokes L, Layhadi JA, Bibic L, Dhuna K, Fountain SJ (2017) P2X4 receptor function in the nervous system and current breakthroughs in pharmacology. Front Pharmacol 8:291

Sueldo DJ, Foresi NP, Casalongue CA, Lamattina L, Laxalt AM (2010) Phosphatidic acid formation is required for extracellular ATP-mediated nitric oxide production in suspension-cultured tomato cells. New Phytol 185:909–916

Summers EL, Cumming MH, Oulavallickal T, Roberts NJ, Arcus VL (2017) Structures and kinetics for plant nucleoside triphosphate diphosphohydrolases support a domain motion catalytic mechanism. Protein Sci 26:1627–1638

Sun J, Zhang C-L, Deng S-R, Lu C-F, Shen X, Zhou X-Y, Zheng X-J, Hu Z-M, Chen S-L (2012) An ATP signalling pathway in plant cells: extracellular ATP triggers programmed cell death in *Populus euphratica*. Plant Cell Environ 35:893–916

Surin AM, Gorbacheva LR, Savinkov IG, Sharipov RR, Khodorov BI, Pinelis VG (2014) Study on ATP concentration changes in cytosol of individual cultured neurons during glutamate-induced deregulation of calcium homeostasis. Biochem Mosc 79:146–157

Tanaka K, Swanson SJ, Gilroy S, Stacey G (2010) Extracellular nucleotides elicit cytosolic free calcium oscillations in Arabidopsis. Plant Physiol 154:705–719

Tang WQ, Brady SR, Sun Y, Muday GK, Roux SJ (2003) Extracellular ATP inhibits root gravitropism at concentrations that inhibit polar auxin transport. Plant Physiol 131:147–154

Tang YL, Yin Y, Miao L, Wei B, Zhai K, Ji GJ (2015) Nitric oxide enhances extracellular ATP induced Ca^{2+} oscillation in He La cells. Arch Biochem Biophys 565:68–75

Thomas C, Sun Y, Naus K, Lloyd A, Roux S (1999) Apyrase functions in plant phosphate nutrition and mobilizes phosphate from extracellular ATP. Plant Physiol 119:543–551

Thomas C, Rajagopal A, Windsor B, Dudler R, Lloyd A, Roux SJ (2000) A role for ecto-phosphatase in xenobiotic resistance. Plant Cell 12:519–534

Tong CG, Dauwalder M, Clawson GA, Hatem CL, Roux SJ (1993) The major nucleoside triphosphatase in pea (Pisum-sativum L) nuclei and in rat-liver nuclei share common epitopes also present in nuclear lamins. Plant Physiol 101:1005–1011

Torres J, Rivera A, Clark G, Roux SJ (2008) Participation of extracellular nucleotides in the wound response of *Dasycladus vermicularis* and *Acetabularia acetabulum* (Dasycladales, Chlorophyta). J Phycol 44:1504–1511

Toyoda K, Yasunaga E, Niwa M, Ohwatari Y, Nakashima A, Inagaki Y, Ichinose Y, Shiraishi T (2012) H_2O_2 production by copper amine oxidase, a component of the ecto-apyrase (ATPase)-containing protein complex(es) in the pea cell wall, is regulated by an elicitor and a suppressor from *Mycosphaerella pinodes*. J Gen Plant Pathol 78:311–315

Toyoda K, Kawakami E, Nagai H, Shiobara-Komatsu T, Tanaka K, Inagaki Y, Ichinose Y, Shiraishi T (2014) Expression of *Medicago truncatula* ecto-apyrase MtAPY1;1 in leaves of *Nicotiana benthamiana* restricts necrotic lesions induced by a virulent fungus. J Gen Plant Pathol 80:222–229

Tripathi D, Zhang T, Koo AJ, Stacey G, Tanaka K (2018) Extracellular ATP acts on jasmonate signaling to reinforce plant defense. Plant Physiol 176:511–523

Tripathy M, Weeraratne G, Clark G, Roux S (2016) Apyrase inhibitors enhance the ability of diverse fungicides to inhibit the growth of different plant pathogenic fungi. Mol Plant Pathol 7:1012–1023

Tsao HK, Chiu PH, Sun SH (2013) PKC-dependent ERK phosphorylation is essential for P2X(7) receptor-mediated neuronal differentiation of neural progenitor cells. Cell Death Dis 4:e751

Ulker P (2018) Extracellular ATP activates eNOS and increases intracellular NO generation in red blood cells. Clin Hemorheol Microcirc 68:89–101

Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE, Kinnamon SC (2013) Role of the ectonucleotidase NTPDase2 in taste bud function. Proc Natl Acad Sci U S A 110:14789–14794

Veerappa R, Slocum R, Clark G, Roux SJ (2018) Ectopic expression of psNTP9, a pea apyrase, expands root system architecture and increases nutrient uptake and seed yield in Arabidopsis and soybean. Abstract, Plant Biol 2018

Velasquez SM, Barbez E, Kleine-Vehn J, Esteves JM (2016) Auxin and cellular elongation. Plant Physiol 170:1206–1215

Verkhratsky A, Burnstock G (2014) Biology of purinergic signaling: its ancient evolutionary roots, its omnipresence and its multiple functional significance. BioEssays 36:697–705

Vlajkovic SM, Housley GD, Thorne PR, Gupta R, Enjyoji K, Cowan PJ, Liberman M, Robson SC (2009) Preservation of cochlear function in Cd39 deficient mice. Hear Res 253:77–82

Volkers L, Mechioukhi Y, Coste B (2015) Piezo channels: from structure to function. Pflugers Archiv-European J Physiol 467:95–99

Weerasinghe RR, Swanson SJ, Okada SF, Garrett MB, Kim SY, Stacey G, Boucher RC, Gilroy S, Jones AM (2009) Touch induces ATP release in Arabidopsis roots that is modulated by the heterotrimeric G-protein complex. FEBS Lett 583:2521–2526

Winkler H, Westhead E (1980) The molecular organization of adrenal chromaffin granules. Neuroscience 5:1803–1823

Wright RHG, Lioutas A, Le Dily F, Soronellas D, Pohl A, Bonet J, Nacht AS, Samino S, Font-Mateu J, Vicent GP, Wierer M, Trabado MA, Schelhorn C, Carolis C, Macias MJ, Yanes O, Oliva B, Beato M (2016) ADP-ribose-derived nuclear ATP synthesis by NUDIX5 is required for chromatin remodeling. Science 352:1221–1225

Wu J, Steinebrunner I, Sun Y, Butterfield T, Torres J, Arnold D, Gonzalez A, Jacob F, Reichler S, Roux SJ (2007) Apyrases (nucleoside triphosphate-diphosphohydrolases) play a key role in growth control in *Arabidopsis*. Plant Physiol 144:961–975

Wu S-J, Siu K-C, Wu J-Y (2011) Involvement of anion channels in elicitor-induced ATP efflux in Salvia miltiorrhiza hairy roots. J Plant Physiol 168:128–132

Wu J, Lewis AH, Grandl J (2017) Touch, tension, and transduction – the function and regulation of piezo ion channels. Trends Biochem Sci 42:57–71

Xie K, Chen J, Wang Q, Yang YO (2014) Direct phosphorylation and activation of a mitogen-activated protein kinase by a calcium-dependent protein kinase in rice. Plant Cell 26:3077–3089

Yang J (2011) Functional analyses of Arabidopsis apyrases 3 through 7. In: Molecular, cell and developmental biology. The University of Texas at Austin, Austin, p 127

Yang J, Wu J, Romanovicz D, Clark G, Roux SJ (2013) Co-regulation of exine wall patterning, pollen fertility and anther dehiscence by *Arabidopsis* apyrases 6 and 7. Plant Physiol Biochem 69:62–73

Yang X, Wang B, Farris B, Clark G, Roux SJ (2015) Modulation of root skewing in *Arabidopsis* by apyrases and extracellular ATP. Plant Cell Physiol 56:2197–2206

Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta 1783:673–694

Yegutkin GG (2014) Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. Crit Rev Biochem Mol Biol 49:473–497

Zebisch M, Krauss M, Schafer P, Strater N (2012) Crystallographic evidence for a domain motion in rat nucleoside triphosphate diphosphohydrolase (NTPDase). J Mol Biol 415:288–306

Zhang J, Zhang K, Gao Z-G, Paoletta S, Zhang D, Han G, Li T, Ma L, Zhang W, Müller CE, Yang H, Jiang H, Cherezov V, Karitch V, Jacobson KA, Stevens RC, Wu B, Zhao Q (2014) Agonist-bound structure of the human P2Y(12) receptor. Nature 509:119–122

Zhao N, Wang S, Ma X, Zhu H, Sa G, Sun J, Li N, Zhao C, Zhao R, Chen S (2016) Extracellular ATP mediates cellular K+/Na+ homeostasis in two contrasting poplar species under NaCl stress. Trees 30:825–837

Zhu R, Dong X, Hao W, Gao W, Zhang W, Xia S, Liu T, Shang Z (2017) Heterotrimeric G protein-regulated Ca^{2+} influx and PIN2 asymmetric distribution are involved in *Arabidopsis thaliana* roots' avoidance response to extracellular ATP. Front Plant Sci 8:1522

Zimmermann H (2016) Extracellular ATP and other nucleotides–ubiquitous triggers of intercellular messenger release. Purinergic Signal 12:25–57

Zimmermann H, Zebisch M, Straeter N (2012) Cellular function and molecular structure of ectonucleotidases. Purinergic Signal 8:437–502

Stanley J. Roux obtained his doctoral degree from Yale University under Professor Arthur Galston (Yale University) and Dr. William Hillman (Brookhaven National Laboratory) on light-induced structural changes in phytochrome. He carried out his postdoctoral research on ionic changes induced by phytochrome in model membranes as a National Institutes of Health Postdoctoral Fellow in the lab of Frederic Richards, Department of Molecular Biophysics and Biochemistry, at Yale University. After 5 years as an Assistant Professor of Biology at the University of Pittsburgh, he moved to the University of Texas at Austin, where he is currently a University Distinguished Teaching Professor in the Department of Molecular Biosciences. His research has focused on the early signaling changes that are induced by light and gravity and are mediated by calcium, which include those induced by extracellular ATP (eATP). His studies of eATP signaling include characterizing the structure and function of the apyrase (NTPDase) enzymes that help control the concentration of eATP. Because Professor Sopory shared an interest in phytochrome and calcium signaling with Prof. Roux, he carried out sabbatical research with him in 1983 and co-authored two papers with him in 1984.

Greg Clark obtained his Ph.D. from the University of Texas at Austin on the structure and function of plant annexins. After his Ph.D., he joined the laboratory of his Ph.D. mentor, Dr. Stanley Roux, where he is a Research Scientist and Distinguished Senior Lecturer and continues doing research on both annexins and eATP signaling.

Mammalian Neurotransmitter Are Important Signals Mediating Plant Morphogenesis

16

Lauren Alexandra Elizabeth Erland and Praveen K. Saxena

Abstract

In spite of their lack of central organized nervous system, plants possess many of the same signaling compounds which are employed in the mammalian nervous system and commonly referred to as neurotransmitters or neuromodulators. These include classes such as the indoleamines, melatonin and serotonin, and the catecholamines, dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline) and acetylcholine. These compounds, since their discoveries in plants, have been found to play important and diverse roles in plant life, including organogenesis, growth and development, flowering and reproduction, sensing environmental cues, and survival against a myriad of environmental stresses. This chapter will provide an overview of the roles these compounds play in plant life, and the mechanisms by which these compounds serve to mediate and direct growth, reproduction, and morphogenesis in plants and the as yet unidentified receptors for these compounds.

Keywords

Gamma aminobutyric acid (GABA) · Indoleamines · Melatonin · Neurotransmitters · Phytohormones · Plant morphogenesis · Serotonin · Signaling molecules

L. A. E. Erland · P. K. Saxena (✉)
Department of Plant Agriculture, Gosling Research Institute for Plant Preservation, University of Guelph, Guelph, ON, Canada
e-mail: psaxena@uoguelph.ca

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_16

16.1 Introduction

Despite the lack of discrete organs comprising a nervous system, such as that seen in mammals, plants have an amazing capacity to both sense and respond to their environment. In fact, their sessile lifestyle has necessitated the evolution of a highly complex system for constantly monitoring every facet of their environment and a diverse arsenal of chemicals with which they respond to even the most minute change. In the absence of these discrete and organized sensory organs, plants instead have a diffuse network of signals which allows them to be significantly more "tapped in" to their environment. These signals are diverse; however, this chapter aims to focus on compounds of a unique subsection, which are generally considered to be ancient in their existence and are referred to as neurotransmitters due to their signaling roles in the nervous systems of animals. There are many neurotransmitters which have been identified in the animal kingdom, and a subsection of these have been widely identified to be produced by plants. These include amino acids such as γ-aminobutyric acid (GABA) and glutamate, monoamines such as histamine, and the catecholamines such as dopamine, epinephrine, and norepinephrine, the indoleamines melatonin and serotonin, and acetylcholine. This chapter will focus specifically on the roles of the catecholamines, indoleamines, and acetylcholine on plant signaling and perception in relation to plant growth and morphogenesis (Fig. 16.1).

16.1.1 Indoleamines

The indoleamines, melatonin (N-acetyl-5-methoxytryptamine) and serotonin (5-hydroxytryptamine), are a class of monoamines which have been found to be produced ubiquitously across all forms of life. They are thought to have arisen in the first prokaryotic life forms on Earth, which used these powerful antioxidants to survive in an increasingly oxygenated world (Tan et al. 2009; Manchester et al. 2015).

The indoleamines are produced in plants from the aromatic amino acid tryptophan (Fig. 16.2), which is itself a product of the shikimate pathway. Though the main pathway of indoleamine biosynthesis was first proposed in the year 2000 (Murch et al. 2000), there are an ever-increasing number of alternate biosynthetic routes which have been and continue to be discovered (Tan et al. 2016). Diversity and redundancy of biosynthesis both pose a difficult quandary for researchers, as they impede the utility of commonly used transgenics and knockouts for molecular studies but simultaneously highlight the value placed on maintenance of this pathway by plants themselves. The primary pathway for indoleamine biosynthesis proceeds from tryptophan to tryptamine via a decarboxylation reaction mediated by tryptophan decarboxylase (TDC), an important enzyme in many plant secondary metabolite pathways, which was first isolated from *Catharanthus roseus* (L.) G. Don (De Luca et al. 1989; Kang et al. 2007). Tryptamine in then converted to serotonin via hydroxylation by tryptamine-5-hydroxylase (T-5-H) (Kang et al. 2008). This enzyme is generally considered to be one of those most active in the pathway, with TDC serving as the rate-limiting step in the production of serotonin. N-acetylserotonin (NAS) is then

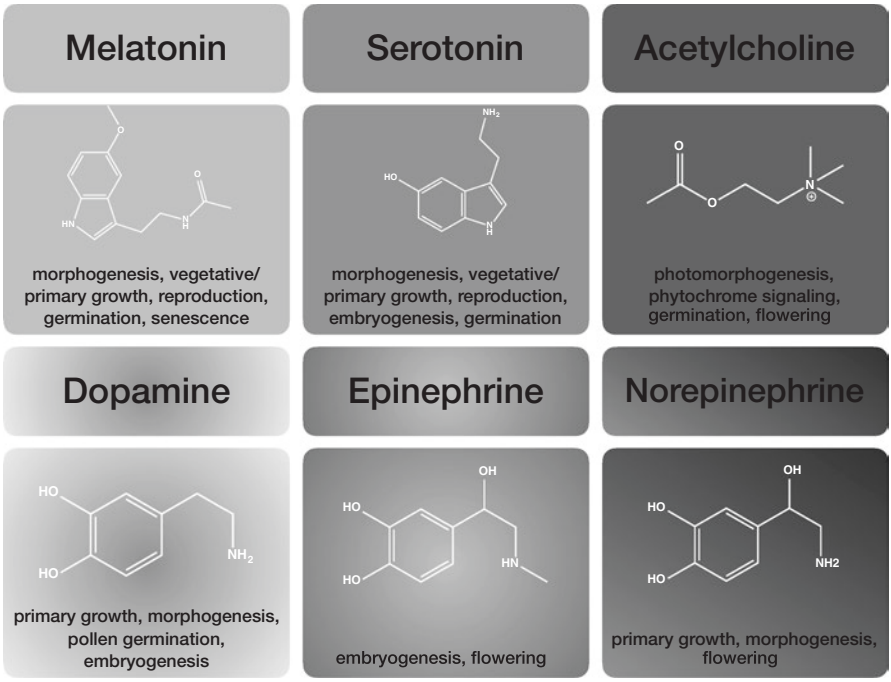

Fig. 16.1 Summary of the structure and functions of the neurotransmitters discussed in this chapter, including the indoleamines, melatonin and serotonin; the catecholamines, dopamine, epinephrine, and norepinephrine and acetylcholine

produced from serotonin by serotonin N-acetyltransferase (SNAT) (Park et al. 2014), which is then finally converted to melatonin by N-acetylserotonin-O-methyltransferase (ASMT) (Park et al. 2013). Both of these enzymes are also considered to be under strict control, making this a highly regulated biosynthetic pathway (Byeon et al. 2013a). Interestingly, the exact sites of synthesis are still not well established, though initial hypotheses implicating the mitochondria and chloroplasts, which are the results of ancient prokaryotic endosymbionts (and likely first producers of these compounds), have been supported by recent reports showing localization of some of the biosynthetic enzymes associated with these structures (Tan et al. 2012; Byeon et al. 2013a, 2014b; Back et al. 2016). Additionally, some work with isolated chloroplasts has also supported these hypotheses (Zheng et al. 2017).

In animals, melatonin is primarily produced by the pineal gland, from which it was first characterized in the 1950s, though the compound was first discovered as a skin-lightening compound in melanocytes in 1917 (Lerner et al. 1958). It is best recognized for its role as the chemical expression of darkness, due to its important role in controlling circadian rhythms (Cassone 1990). Melatonin has more recently, however, also been found to be produced in several extra-pineal locations, including the gut and reproductive systems, something that is not terribly distinct from locations of increased production in plants (Acuña-Castroviejo et al. 2014). Melatonin

was not identified in plants until the 1990s when two reports by Dubbels et al. (1995) and Hattori et al. (1995) reported melatonin in several edible plant parts. Though interest was at first slow in this field, with significant skepticism, in recent years there has been an explosion in interest in this molecule which has since been found to be produced across the breadth of the plant kingdom and to be involved in a diversity of plant responses including morphogenesis, reproductive development, and stress survival (Reiter et al. 2015; Erland et al. 2015).

Serotonin, the biosynthetic precursor of melatonin, has, however, followed a slightly different trajectory. As an important inhibitory neurotransmitter in the animal system, it was discovered more than 40 years prior to melatonin in plants, in the medicinal plant cowhage (*Mucuna pruriens* DC.) (Bowden et al. 1954). There was an initial spike in interest in serotonin in plants, with reports of high levels of serotonin in diverse plant families and several interesting papers highlighting potential interactions between serotonin, light spectrum, phytochrome signaling, and phosphatidylinositol turnover (Reynolds et al. 1985; Chandok and Sopory 1994; Raghuram and Sopory 1995; Erland et al. 2016b); however, interest lulled and has yet to invite interest to the levels seen for its metabolite, melatonin. Serotonin is often overlooked or merely lumped in with melatonin studies; however, we have proposed in our recent review that serotonin should be investigated individually as it appears to have distinct characteristics from melatonin (Erland et al. 2016a). Furthermore, we have proposed that an important balance exists between these two compounds, which is important in mediating diverse morphogenetic responses in plants (Erland et al. 2015).

16.1.2 Catecholamines

The catecholamines are another class of monoamines that are derived from another aromatic amino acid, phenylalanine (Fig. 16.2). For this reason, there has been significant interest in the relationships between catecholamine biosynthesis and the phenylpropanoid pathway, which is also derived from phenylalanine. This class comprises three compounds primarily: dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). The biosynthetic intermediate, dihydroxyphenylalanine or levodopa (L-DOPA) is generally considered a nonprotein amino acid in plants, and not a neurotransmitter, and as such, it will not be discussed in depth in this chapter.

Two biosynthetic routes for catecholamines have been discovered in plants (Kulma and Szopa 2007). Both require conversion of phenylalanine to tyrosine via L-amino acid hydroxylase/phenylalanine hydroxylase. From tyrosine, dopamine can be synthesized via conversion of tyrosine to tyramine by tyrosine decarboxylase (TD) (Facchini et al. 2000); tyramine is then converted into dopamine via monophenol hydroxylase (MH) (Rueffer and Zenk 1987). Alternatively, tyrosine can be first hydroxylated to form L-DOPA by tyrosine hydroxylase (TH) (Kong et al. 1998) and then decarboxylated to dopamine by DOPA decarboxylase (DD) (Facchini and De Luca 1994). TH is considered to be the rate-limiting step of biosynthesis. Dopamine

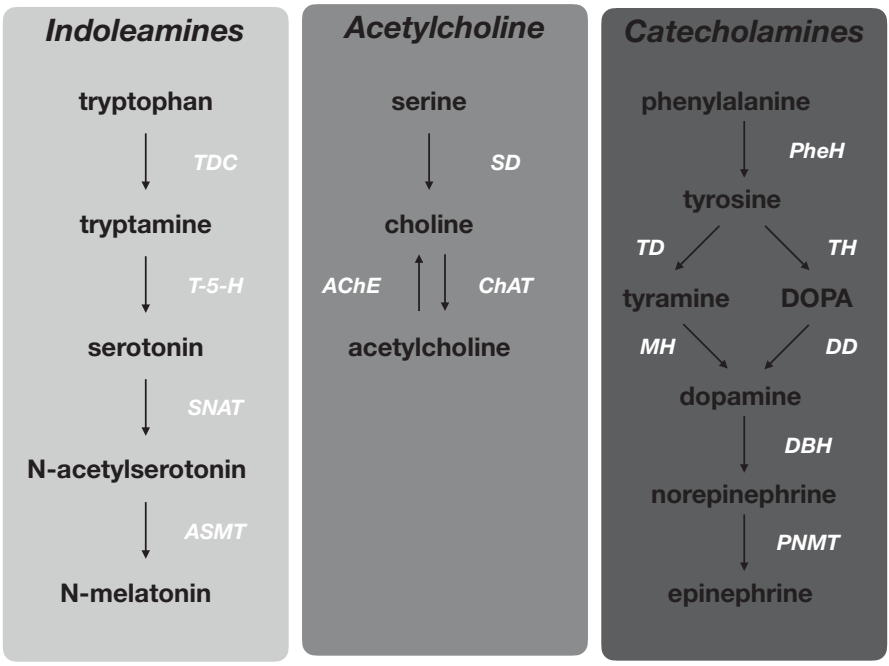

Fig. 16.2 Summary of the biosynthetic pathways of the indoleamines, catecholamines, and acetylcholine. *AChE* acetylcholinesterase, *ASMT* acetylserotonin-O-methyltransferase, *ChAT* choline acetyltransferase, *DBH* dopamine-β-hydroxylase, *DD* dopamine decarboxylase, *DOPA* dihydroxyphenylalanine, *MH* monophenol hydroxylase, *PheH* phenylalanine hydroxylase, *PNMT* phenylethanolamine-N-methyltransferase, *SD* serine decarboxylase, *SNAT* serotonin N-acetyltransferase, *TD* tyrosine decarboxylase, *TDC* tryptophan decarboxylase, *TH* tyrosine hydroxylase, *T-5-H* tryptamine-5-hydroxylase

is then hydroxylated to norepinephrine by dopamine-β-hydroxylase (DBH) and then to epinephrine by phenylethanolamine N-methyltransferase (PNMT) (Kulma and Szopa 2007). The order of reactions in the catecholamine biosynthetic pathway has many similarities to the indoleamine with the basic order reactions from tyrosine to epinephrine or tryptophan to melatonin being similar consisting of decarboxylation, hydroxylation, acetylation (indoleamine) or hydroxylation (catecholamine), and methylation.

In animals, the catecholamines are known for their role in the fight or flight response, particularly in glycogen mobilization. Generally, speaking a function in stress responses by these compounds appears to be conserved in plants, as discussed in later sections. Epinephrine was the first discovered neurotransmitter in animals, being discovered in the 1890s (Cybulski 1895). Again, its discovery lagged behind in plants, and was the last of three primary catecholamine discovered in plants, not being identified until 1972 in banana (Askar et al. 1972). Dopamine was the first catecholamine amine discovered in plants, identified in *Hermidium alipes*

(S. Watson) in 1944 (Buelow and Gisvold 1944), while norepinephrine was isolated from banana fruits in the 1950s (Waalkes et al. 1958; Udenfriend et al. 1959).

16.1.3 Acetylcholine

Acetylcholine differs in structure from the other two classes of neurotransmitters discussed in this chapter, being linear instead of aromatic; however, it is still an amino acid-derived neurotransmitter and is discussed due to some similarities in suggested signaling networks. Acetylcholine is an excitatory neurotransmitter which plays an important role in muscle contraction and generation of action potentials in animals. It was first isolated in 1914 and was later identified in the trichomes of stinging nettle (*Urtica urens* L.) in 1947 (Emmelin and Feldberg 1947). Similar to the catecholamines, acetylcholine has been closely linked with red light responses, phytochrome signaling, and photomorphogenesis (Maheshwari et al. 1982; Tretyn and Kendrick 1991).

Biosynthesis of acetylcholine is fairly simple, being the product of choline and acetyl Co-A (Fig. 16.2). Choline is produced from the amino acid serine through action of serine decarboxylase. The acetyl group is transferred by the enzyme choline acetyltransferase (ChAT) (Barlow and Dixon 1973; Smallman and Maneckjee 1981). Acetylcholine can then be rapidly recycled back to choline via action of acetylcholinesterase (AChE) (Dettbarn 1962; Kasturi 1978; Ernst and Hartmann 1980; Sagane et al. 2005). This recycling of acetylcholine plays an important role in mediating action, therefore, the acetylcholine system is generally considered to be comprised of three components: acetylcholine, ChAT, and AChE. Little is still known about localization of acetylcholine biosynthesis, though some limited reports suggest that synthesis may occur in the cytoplasm, the chloroplasts, or at the endoplasmic reticulum (Roshchina and Mukhin 1985a, b; Jaffe 1976; Hartmann 1979; Tretyn and Kendrick 1991). Acetylcholinesterase activity, however, has been strongly localized to the cell wall region in diverse species; with levels also often reported to be higher in light conditions than dark (Maheshwari et al. 1982; Tretyn and Kendrick 1991). Despite an abundance of research on acetylcholine in plants up until the early 1990s, interest in the compound has waned in recent years.

16.2 Neurotransmitters in Growth and Development

16.2.1 Vegetative Growth and Morphogenesis

Vegetative growth represents the majority of growth in a plants life. Plant signaling molecules, including established phytohormones, as well as the neurotransmitters discussed in this chapter participate in an intricate balance to mediate and direct these processes. Vegetative growth can roughly be distinguished between primary growth, i.e., growth of existing structures and morphogenesis or secondary growth, development, and differentiation of new structures, tissues, and cells.

The indoleamines are the class of neurotransmitters for which there is the greatest evidence of mediation of morphogenesis, organogenesis, and vegetative growth/primary growth in plants (Table 16.1). Melatonin and serotonin, due to their close biosynthetic relationship (Fig. 16.2), are hypothesized to exist in a balance, similar to that established for auxin and cytokinin (Skoog and Miller 1957), which helps to fine-tune the effects of other, more well-established signaling pathways. Melatonin is suggested to behave similar to auxin, promoting root growth, while serotonin is suggested to fill the role of cytokinins, promoting shoot growth (Murch et al. 2001; Erland et al. 2015). One of the first reports of indoleamine-mediated morphogenesis was in the medicinal plant St. John's wort (*Hypericum perforatum* L.), in which, melatonin and serotonin were found to improve de novo root organogenesis

Table 16.1 Summary of indoleamine-mediated effects[a]

Effect or function	Compound melatonin (Mel) or serotonin (Ser)	Species	Suggested mechanism	References
Protection developing reproductive tissues and embryos	Mel, Ser	*Vitis vinifera, Datura metel, Hypericum perforatum, Malus domestica,* edible seed plants, *Prunus avium*	Antioxidant	Manchester et al. (2000), Murch and Saxena (2002a), Murch et al. (2009, 2010), Sarropoulou et al. (2012a, b), Lei et al. (2013)
Promotion of pollen germination	Mel, Ser	*H. perforatum, Hippeastrum hybridum*	Modification of calcium distribution	Roshchina (2001a, b), Murch and Saxena (2002b)
Promotion of germination and seedling growth	Mel, Ser	*Glycine max, Cucumis sativus, Zea mays, Vigna radiata, Brassica oleracea rubrum, Phacelia tanacetifolia*	Modification of calcium/calmodulin signaling pathways; modified carbon metabolism; enhanced photosynthesis; interaction with polyamines, auxin, abscisic acid, gibberellin; antioxidant activity; modification calcium signaling	Gatineau et al. (1997), Hernández-Ruiz et al. (2004, 2005), Hernández-Ruiz and Arnao (2008), Posmyk et al. (2008, 2009), Tiryaki and Keles (2012), Byeon et al. (2013b), Janas and Posmyk (2013), Zhang et al. (2013b, 2014), Byeon and Back (2014), Mukherjee et al. (2014), Wei et al. (2015); Zhao et al. (2015a)

(continued)

Table 16.1 (continued)

Effect or function	Compound melatonin (Mel) or serotonin (Ser)	Species	Suggested mechanism	References
Promotion of de novo root organogenesis	Mel, Ser	*Arabidopsis thaliana, H. perforatum, Lupinus albus, C. sativus, Brassica juncea, Prunus* rootstocks, *Punica granatum Oryza sativa*	Interaction with auxin; Upregulation of salicylic acid and abscisic acid; Modification of expression levels of transcription factors, cell wall, and peroxidase-related genes	Murch et al. (2000, 2001), Murch and Saxena (2002b), Arnao and Hernández-Ruiz (2007), Chen et al. (2009), Park and Back (2012), Pelagio-Flores et al. (2011, 2012), Sarropoulou et al. (2012a, b), Zhang et al. (2013a, b, 2014), Koyama et al. (2013), Byeon et al. (2014a), Sarrou et al. (2014), Erland et al. (2018)
Promotion of primary root growth	Mel, Ser	*H. perforatum, V. radiata, Prunus* sp., *L. albus, O. sativa, A. thaliana, Helianthus annuus Hordeum vulgare*	Increased protein synthesis; antioxidant; increased carbohydrate metabolism; and interaction with calcium/calmodulin signaling cascades	Csaba and Pal (1982), Sarropoulou et al. (2012a, b), Szafrańska et al. (2012), Park and Back (2012), Bajwa et al. (2014), Mukherjee et al. (2014)
Promotion of de novo shoot organogenesis	Mel, Ser	*H. perforatum, Punica granatum, Vaccinium corymbosum, Mimosa pudica*	Upregulation of zeatin; Interaction with calcium signaling pathways and auxin	Murch et al. (2001), Litwinczuk and Wadas-Boron (2009), Ramakrishna et al. (2009), Sarrou et al. (2014), Erland et al. (2018)
Promotion of primary shoot growth	Mel, Ser	*H. perforatum, Prunus avium x Prunus cerasus, O. sativa, A. thaliana*	Upregulation or maintenance of protein synthesis and primary metabolic pathways, including carbon and nitrogen pathways	Park and Back (2012), Sarropoulou et al. (2012a), Bajwa et al. (2014), Zuo et al. (2014), Erland et al. (2018)

(continued)

Table 16.1 (continued)

Effect or function	Compound melatonin (Mel) or serotonin (Ser)	Species	Suggested mechanism	References
Promotion of somatic embryogenesis	Mel, Ser	*Coffea canephora*	Interaction with calcium signaling cascades	Ramakrishna et al. (2011)
Interpretation photoperiodic signals	Mel	*A. thaliana, Malus* sp., *Prunus* sp. *Eichhornia crassipes, V. vinifera, P. avium, Ulva sp, Chara australis, Chenopodium rubrum, O. sativa*	Antioxidant; support photosynthetic apparatus; light signaling	Wolf et al. (2001), Kolar et al. (2003), Tan et al. (2007, 2012), Boccalandro et al. (2011), Byeon et al. (2012), Lazár et al. (2013), Zhao et al. (2013a, b)
Mediation of floral timing	Mel	*C. rubrum, O. sativa*	Interaction with calcium/calmodulin signaling	Wolf et al. (2001), Kolar et al. (2003), Byeon and Back (2014)
Delayed senescence	Mel, Ser	*Malus* sp., *A. thaliana, H. vulgare, O. sativa*	Antioxidant; inhibition of chlorophyll degradation; upregulation of ascorbic acid and glutathione pathways; downregulation of senescence-related genes; modification of photosynthesis and sugar metabolisms; modification of nitrogen metabolism; and decreased protein degradation	Arnao and Hernández-Ruiz (2009), Kang et al. (2009), Byeon et al. (2012, 2013b), Wang et al. (2012a, b, 2013), Shi et al. (2014)

[a]All species names are included as described in the original report, synonyms, currently binomial names are included in the main text

(Murch et al. 2001). Additionally, through the use of inhibitors such as the auxin action and transport inhibitors p-chlorophenoxyisobutyric acid (PCIB) and 2,3,5-triiodobenzoic acid (TIBA), the authors suggested that this action may be due to interaction with auxin signaling (Murch et al. 2001). A follow-up study first suggested the importance of melatonin and serotonin in maintaining a balance to direct

morphogenesis toward shoot production or root production preferentially with serotonin favoring the former and melatonin the latter (Murch and Saxena 2004). Recent studies in St. John's wort, including the same wild-type strain used by Murch et al. (2001), as well as two mutants found to have modified indoleamine metabolism, found that not only is a balance of these two compounds important in dictating morphogenetic outcomes but also the pathway as a whole from tryptophan through melatonin may be important in mediating morphogenesis/organogenesis (Erland et al. 2018). Since the initial report in 2001, melatonin has been found to modify both de novo root formation and primary growth of roots in several species including *Arabidopsis thaliana* (L.) Heynh. (Pelagio-Flores et al. 2012), pomegranate (*Punica granatum* L. cv Wonderful) (Sarrou et al. 2014), *Brassica juncea* L. Czern. (Chen et al. 2009), lupin (*Lupinus albus* L.) (Arnao and Hernández-Ruiz 2007), cucumber (*Cucumis sativus* L.) (Zhang et al. 2013b), rice (*Oryza sativa* L.) (Park and Back 2012), sunflower (*Helianthus annuus* L.) (Mukherjee et al. 2014), mung bean (*Vigna radiata* L. syn. *Phaseolus aureus*) (Szafrańska et al. 2012), and *Prunus* spp., (Sarropoulou et al. 2012a, b), though this list is not exhaustive. Serotonin has also been found to promote root formation in a smaller segment of plants, including *A. thaliana* (Pelagio-Flores et al. 2011), sunflower (Mukherjee et al. 2014), walnut (*Juglans nigra x regia*) (Gatineau et al. 1997), and barley (*Hordeum vulgare* L.) (Csaba and Pal 1982), while both melatonin and serotonin have been found to promote shoot and root organogenesis in mimosa (*Mimosa pudica* L.) (Ramakrishna et al. 2009). Serotonin has also been found to have a strong effect in promoting the production of somatic embryos in *Coffea canephora* Pierre Ex Froehn (Ramakrishna et al. 2011).

The catecholamines have been found to have similar effects on primary and secondary growth as the indoleamines (Table 16.2). In hairy root cultures of *Acmella oppositifolia* (Lam.) R.K. Jansen, which do not require addition of other plant growth regulators for growth, dopamine, epinephrine, or norepinephrine treatments all lead to an increase in overall fresh weight, though de novo root organogenesis was not specifically measured (Protacio et al. 1992). In the same study, the authors also examined the effects of these three compounds on tobacco (*Nicotiana tabacum* L.) thin cell layer (TCL) cultures, where it was found that these compounds could promote both primary and secondary growth, as demonstrated by increases in callus growth, radical expansion, and overall fresh weight, accompanied by inhibition of floral and vegetative bud initiation (Protacio et al. 1992). This was associated with increased ethylene production. Epinephrine has also been found to have a promotory effect upon coapplication with indole-3-acetic acid (IAA). In orchard grass (*Dactylis glomerata* L.), coapplication led to improved incidence of somatic embryogenesis and was again associated with an increase in ethylene, though this was most noticeable at levels over 500 μM and resulted in a reversal in the growth effect (Kuklin and Conger 1995).

Epinephrine has also been found to improve primary root and shoot growth in *Vigna unguiculata* (L.) seedlings, with this effect being attributed to increased protein and carbohydrate synthesis (Kaur and Thukral 1990). Dopamine has been found to have the reverse effect in soybean seedlings, with treatment decreasing

Table 16.2 Summary of catecholamine-mediated effects[a]

Effect or function	Compound (dopamine (DA), epinephrine (E) or norepinephrine (NE)	Species	Suggested mechanism	References
Promotion of callus growth	NE, DA	*Nicotiana tabaccum*	Reduced auxin oxidation; maintenance of higher auxin levels in tissues	Protacio et al. (1992)
Promotion of primary root growth	NE, DA	*Acmella oppositifolia*, *Solanum tuberosum*, *Vigna unguiculata*	Reduced auxin oxidation; maintenance of higher auxin levels in tissues; increase protein synthesis; mobilization soluble sugars	Kaur and Thukral (1990), Protacio et al. (1992), Hourmant et al. (1998)
Promotion of primary shoot growth	DA	*Solanum tuberosum*, *V. unguiculata*, *Lactuca sativa*	Increased protein synthesis and mobilization of soluble sugars; Synergistic interaction with gibberellins	Kamisaka (1979), Kaur and Thukral (1990), Hourmant et al. (1998)
Promotion of de novo shoot organogenesis	NE, DA	*N. tabaccum*	Reduced auxin oxidation; Maintenance of higher auxin levels in tissues;	Protacio et al. (1992)
Enhanced somatic embryogenesis	E, DA	*Dactylis glomerata*	Reduced auxin oxidation; and maintenance of higher auxin levels in tissues	Kuklin and Conger (1995)
Modified floral development	E, NE	*Lemna paucicostata* 6749, *N. tabaccum*	Mimic requirement for red-light induction; modification of membrane bioelectric potential	Khurana et al. (1987), Protacio et al. (1992)
Promotion pollen germination	DA	*Equisetum arvense*, *Hippeastrum hybridum*		Roshchina, (2006), Roshchina and Yashin (2014)

[a]All species names are included as described in the original report, synonyms, currently binomial names are included in the main text

fresh weight and root length, while simultaneously having contradictory effects on antioxidant enzymes, with increases in superoxide dismutase (SOD), but decreases in peroxidase (POD). The authors suggest in this case that dopamine is functioning as an allelopathic chemical (Guidotti et al. 2013). The opposite, however, was suggested by Gomes et al. (2014) who observed an increase in root growth, associated with increased SOD and decrease POD levels associated with decreased lipid peroxidation and reactive oxygen species (ROS) levels. This suggests that another factor is possibly involved in determining these growth outcomes.

Acetylcholine's effects are primarily discussed in the following section on photomorphogenesis; however, it does appear that some effects of acetylcholine may not be dependent on phytochrome- or light-mediated effects/reactions (Table 16.3). Acetylcholine has been reported to improve de novo shoot organogenesis and inhibit callus formation in tomato (*Lycopersicon esculentum* Miller var Pusa Ruby syn *Solanum lycopersicum* L.) (Bamel et al. 2016). Acetylcholine may also play a role in gravitropism of plants, as demonstrated in a study by Momonoki (1992) in *Zea mays* L. cv Stowell's Silver Queen, which found that in response to a gravitropic stimulus (movement from vertical to horizontal), labeled acetylcholine injected into kernels was found to move from the stele into the lower cortex of horizontally oriented seedlings (Momonoki 1992). A follow-up study, where acetylcholine-hydrolyzing activity was likewise found to localize to the lower side of the horizontally oriented maize seedlings, confirmed these results. Specifically, it was found in the vascular cells surrounding the vascular stele (Momonoki 1997). This effect has also been suggested to be related to IAA regulation of gravitropism, specifically IAA-inositol synthase localization (Momonoki et al. 1998).

16.2.1.1 Photomorphogenesis

As photosynthetic organisms, the ability of plants to not only sense light but also wavelength of light, and to respond differentially, is critical to their survival. As a result, plants have evolved many and diverse means by which to sense, distinguish, and respond to light. Photoreceptors are the first line of perception for plants; however, plants also possess diverse means by which to fine-tune and mediate these responses. Despite years of research into the topic, the subtleties of the signaling cascades induced by light stimulus and downstream of photoreceptors are yet to be fully understood and represent an interesting area of research.

One of the most well-established functions of acetylcholine is its capacity to mimic red-light stimulus in plants. Generally, red and far-red light are perceived via the photoreceptor, phytochrome, which undergoes a conformational change in response to red- or far-red-light stimulus, thereby triggering a complex network of downstream signaling cascades, with far-reaching effects. Light-induced changes in de novo organ development are generally referred to as photomorphogenesis. One of the initial effects of exposure to red light is a modification in the bioelectric potential across cellular membranes (Jaffe 1968; Yunghans and Jaffe 1970). Acetylcholine treatment has been found to be capable of mimicking this response in several species, but it was first identified in mung bean (Jaffe 1970; Tanada 1972), where acetylcholine was found to mediate secondary root formation and modify

Table 16.3 Summary of acetylcholine mediated effects[a]

Effect or function	Species	Suggested mechanism	References
Inhibition root growth	*Phaseolus aureus, Lens culinaris*	Mimic red light; modification of bioelectric potential and ion flux	Jaffe (1970, 1972), Tanada (1972), Penel et al. (2008)
Promotion of germination and seedling growth	*Agropyron repens, Echinochloa crus galli, Chenopodium album, Brassica kaber, Setaria viridis, Triticum sativum, Avena sativa, Glycine max, Cucumis sativus*	mimics red-light induction; Interaction with auxin, gibberellin, and calcium	Kostir et al. (1965), Evans (1972), Dekhuijzen (1973), Verbeek and Vendrig (1977), Lawson et al. (1978), Mukherjee (1980), Hadačová et al. (1981), Bamel et al. (2016)
Inhibition of callus formation	*Lycopersicon esculentum* var Pusa Ruby		Bamel et al. (2016)
Promotion of de novo shoot organogenesis	*L. esculentum* var Pusa Ruby		Bamel et al. (2016)
Induction of pollen tube elongation	*Arachis hypogea, Hippeastrum hybridum, Lilium longiflorum*	Mimics red-light induction	Chhabra and Malik (1978), Roshchina and Melnikova (1998), Tezuka et al. (2007)
Mediation of floral timing	*Lemna gibba* L1	Replaces red-light stimuli; modification of bioelectric potential; modification of membrane permeability	Kandeler (1972), Greppin et al. (1973), Oota and Hoshino (1974), Greppin and Horwitz (1975), Oota (1977)
Regulation gravitropism	*Zea mays*	Modification of indole-3-acetic acid-inositol synthase localization	Momonoki (1992, 1997)

[a]All species names are included as described in the original report, synonyms, currently binomial names are included in the main text

bioelectric potential in root tips in a manner similar to red light exposure and which was also reversible through far-red light exposure. Acetylcholine has similarly been found to inhibit secondary root growth in light-grown pea (*Pisum sativum* L.) (Kasturi 1978) and tap root growth in lentil (*Lens culinaris* Medik. syn *Lens esculenta*) (Penel et al. 2008).

Though there is significantly less information pertaining to the interactions between the indoleamines and photomorphogenesis, there is some information to suggest that melatonin and serotonin may play a role in directing/mediating photomorphogenesis. One hypothesis is that this is via interaction with the COP1/COP9

signalosome; however, this is yet to be proven (Sanchez-Barcelo et al. 2016); there also exists some evidence suggesting that serotonin may interact with the phytochrome signaling network in a manner similar to acetylcholine as discussed in later sections. Another possibility is that the exogenous treatment of plants in dark conditions may favor morphogenetic outcomes from melatonin treatment due to enhanced stability of melatonin, as there is some evidence to suggest that melatonin may degrade under light in culture conditions (Erland et al. 2016b). For instance, in *Withania somnifera* L., melatonin (600 μM) was found to promote root induction in adventitious root cultures. This effect was enhanced in constant darkness; however, levels of melatonin in the medium were not monitored (Adil et al. 2015).

Serotonin biosynthesis has been found to be upregulated in *Sedum morganianum* E. Walther differentially in response to varying wavelengths of light (Reynolds et al. 1985). Seven light treatments were tested in this study including dark, white light (300–700 nm), red (625–725 nm), yellow (575–595 nm), green (500–550 nm), blue (400–475 nm), and violet (250–400 nm) light treatments. Serotonin levels were found to be reduced under red, yellow, and green light levels, while its biosynthetic precursor, tryptophan was found to be increased under these same three conditions. This, accompanied by a reduction in the activity of T-5-H, suggests that serotonin biosynthesis is inhibited under these conditions and that effects on growth under these conditions may be due to tryptophan action, or may merely represent depletion of the serotonin pools (Reynolds et al. 1985).

16.2.2 Reproductive Development

Neurotransmitters have been found to play important roles in mediating the timing and processes of vegetative and reproductive development in plants. This includes the induction of reproductive structures, protection of developing and germinating embryos and reproductive tissues, and modification/response of floral timing (Erland et al. 2015).

Acetylcholine has been found to be important in mediating floral timing, by replacing the requirement for photoperiodic changes or red light in floral induction in several species including spinach (*Spinacia oleracea* L.), *Perilla nankinensis* (Lour.) Britton. (syn. *Perilla frutescens* var. *nankinensis*, *P. frutescens* var. crispa, *P. arguta* Benth., *P. crispa* (Thunb.) Tan.) (Greppin et al. 1973), and two species of duckweed, including the short-day species and *Lemna perpusilla* Torr. 6746 and the long-day species *Lemna gibba* L. G1. Opposing effects have been observed in long- and short-day species. A report by Kandeler (1972) found that under continuous light, acetylcholine inhibited flowering in *L. gibba* while promoting flowering in *L. perpusilla*, though ascorbic acid was required to achieve the effect in the latter. Acetylcholine has been found to be naturally produced in *Lemna* species (Hoshino and Oota 1978). Experiments showing that addition of the AChE inhibitor, physostigmine, had the same effects on flowering as acetylcholine supplementation support a role for endogenous acetylcholine in this process (Kandeler 1972). These

results have also been confirmed under a 12 h photoperiod in *L. gibba* G1 and G3 (Oota and Hoshino 1974; Oota 1977).

The catecholamines epinephrine and norepinephrine have also been found to be effective in inducing floral induction in the short-day species *Lemna pauciostata* (Hegelm). Exposure of 10^{-4} M, prior to transfer from long-day to the short-day regime, increased number of floral primordia, improved floral development, and lead to longer duration of individual flowers (Khurana et al. 1987). These positive effects were observed up to 10^{-6} M, though concentrations lower than 10^{-4} M showed inhibitory effects. The authors further showed that treatment with a beta-adrenergic blocker, propranolol, was able to partially inhibit flowering in a manner which could be reversed by exogenous catecholamine treatment (Khurana et al. 1987). Similarly, later studies have confirmed these results, though there is some possible ambiguity as the authors have heat-treated the norepinephrine prior to application to cultures, making it unclear of the structure of the compound which was produced after this treatment (Miyawaki et al. 2014; Okatani et al. 2014). Interestingly, the opposite effect, an inhibition of floral bud development, was observed in tobacco TCL cultures. The authors of this study suggest that as opposed to *Lemna*, which lacks competence and requires an inductive stimulus for flowering, tobacco TCLs may already be competent, and in this case, treatment leads to inhibition of bud initiation (Protacio et al. 1992). Another possible explanation may be that a higher treatment level of dopamine or norepinephrine may have a positive effect, as dose dependency of neurotransmitter-mediated effects are well documented (Erland et al. 2015) and were also observed by Khurana et al. (1987).

Some information is also available on the possible roles of melatonin in mediating floral timing, though the mechanisms for this action appear to be different from acetylcholine. The first report of melatonin-mediation of floral timing was in *Chenopodium rubrum* L., a short-day plant in which melatonin was earlier found to possess a daily rhythm (Wolf et al. 2001). When melatonin was applied prior to a 12-h dark period, it was found to inhibit floral initiation (Kolar et al. 2003). In later reports, melatonin was found to delay flowering in rice, genetically modified to overproduce melatonin (Byeon and Back 2014), and the model species *A. thaliana* (Shi et al. 2016b). In *A. thaliana*, melatonin was found to be capable of stabilizing DELLA proteins, a set of transcription factors which function as repressors of the gibberellic acid pathway, which is important in inducing and mediating floral initiation. Melatonin (500 μM) was found to stabilize the DELLA protein which binds to FLOWERING LOCUS C (FLC), another transcription factor which is a strong repressor of vernalization as well as downstream DELLAs, and therefore it leads to repression of flowering (Galvão et al. 2012; Shi et al. 2016b).

Serotonin in contrast appears to be more important in processes such as gamete compatibility and pollen germination, with the primary mechanism for this action being via modulation of cyclic adenosine monophosphate (cAMP) signaling, cytoskeletal rearrangement, and also possibly modification of membrane permeability (Roshchina 2001a, b, 2005, 2006). In knight's star (*Hippeastrum hybridum* Hort.), serotonin promoted pollen germination in a manner which was reversible through treatment with cAMP inhibitors such as isobutylmethylxanthine (Roshchina 2001a,

b). In both knight's star and horsetail (*Equisetum arvense* L.), serotonin was also found to stimulate pollen germination, and in these species, it was found that the effect could be reversed through the application of anti-contractile agents which disrupted microtubulin formation (Roshchina 2005). Acetylcholine has also been found to mediate pollen germination. In knight's star, the application of acetylcholine stimulated pollen germination both in vivo and in vitro, and application of atropine, tubocuraraine (inhibitors of cholinergic receptors in animals), or physostigmine (an AChE inhibitor) inhibited this effect (Roshchina and Melnikova 1998). Similar to serotonin and acetylcholine, dopamine was also found to promote microspore germination in both knight's star and horsetail (Roshchina 2006; Roshchina and Yashin 2014). A single report has also suggested that acetylcholine can replace red-light stimulus in inducing pollen germination/pollen tube elongation in peanut (*Arachis hypogaea* L.) (Chhabra and Malik 1978). Another more recent report found that acetylcholine also promoted pollen tube elongation in *Lilium longiflorum* Thunb. cv Hinomoto after self-incompatible pollination. It was further found that during self-incompatible pollination, ChAT levels were reduced as compared with cross-pollination, suggesting that endogenous acetylcholine also plays a role in this process (Tezuka et al. 2007).

Melatonin and serotonin have been found to have specific patterns of accumulation and expression in both developing reproductive structures and embryos. The primary function of melatonin and serotonin in theses tissues is suggested to be defense of the developing embryo against oxidative damage, as melatonin and serotonin are potent antioxidants (Reiter et al. 1993; Bajwa et al. 2015). These compounds appear to often act, again, in balance with each other with serotonin levels climbing as melatonin levels drop, though this is not always the case. In St. John's wort, melatonin and serotonin were found to be differentially expressed during floral and pollen development. Where serotonin was found to be present in high concentrations at the tetrad phase of microspore development, melatonin was found at higher levels during the uninucleate stage (Murch and Saxena 2002a). Melatonin and serotonin levels, in contrast, were both found to start at high levels in undeveloped flowers of *Datura metel* (L.), with levels dropping off with advanced floral development (Murch et al. 2009).

Similar to floral development, melatonin and serotonin levels have been found to fluctuate with fruit development and ripening. In *D. metel*, despite low levels in the mature flowers, melatonin levels were found to be high in the developing fruit up until 10 days past anthesis, at which point the embryo was mature and ready for excision and levels started to drop (Murch et al. 2009). Interestingly, in wine grapes (*Vitis vinifera* L.), the trade-off in melatonin and serotonin levels observed in St. John's wort flowers was again observed, with melatonin levels being higher in pre-véraison grapes, while serotonin levels were highest post-véraison and increased as the fruit matured (Murch et al. 2010). Likewise, a similar trend was observed in sweet cherry fruits (*Prunus avium* (L.) cv Rainier and *P. avium*, cv HongDeng), with melatonin levels being highest in the green fruits, with levels dropping off with ripening and the switch to anthocyanin production (Zhao et al. 2013). Seeds and

nuts, in fact, are often the plant tissues with the highest levels of melatonin and serotonin, and they were some of the first plant tissues in which melatonin was identified (Regula 1986; Dubbels et al. 1995; Manchester et al. 2000; Zohar et al. 2011; Korkmaz et al. 2014; Sun et al. 2015). The presence and localization of serotonin has been best documented in walnut (*Juglans regia* L.), where serotonin levels are found to be highest in the endosperm rather than in the embryo itself, and were found to increase as the embryo matures (Lembeck and Skofitsch 1984), further suggesting a protective role. It has been suggested that in addition to function as an antioxidant, serotonin is also capable of detoxifying ammonium (Grosse and Artigas 1983), a function which may also be served by melatonin based on the concurrent increases in the nitrogen storage compound "GABA" which has been observed to occur concurrently with increasing melatonin concentrations in *D. metel* (Murch et al. 2009).

Acetylcholine has been found to mimic the effects of red light in promoting germination in several species including *Agropyron repens* L. (Beauv.), *Echinochloa crus-galli* (P. Beauv.), *Chenopodium album* (L.), *Brassica kaber* (DC.) Wheeler, and *Setaria viridis* (L.) P. Beauv. (Kostir et al. 1965; Hartmann and Gupta 1989). However, Tretyn et al. (1988) found more variable effects on seed germination in several species, with acetylcholine inhibiting germination in some species such as *Plantago lanceolata* L., while enhancing germination in others, including *Rumex obtusifolius*. High levels of ChAT were also found in seeds of *Allium altaicum* (Pall.) Reyse, suggesting a role in germination in this species (Hadačová et al. 1981). Acetylcholine has also been found to promote: seedling growth in wheat (*Triticum aestivum* L. cv. Juffy) (Dekhuijzen 1973), coleoptile growth in oat (*Avena sativa* L. var Victory) (Evans 1972) and wheat (Lawson et al. 1978), and hypocotyl growth in cucumber (Verbeek and Vendrig 1977), soybean (*Glycine max* L.) (Mukherjee 1980), and *Vigna sesquipedalis* (syn. *Vigna unguiculata* subsp. *sesquipedalis*, yardlong bean) (Hoshino 1983).

Though reports on the roles of melatonin and serotonin in promoting germination have primarily been examined with seeds under stress, there is significant evidence pointing toward a role of these compounds in promoting germination. Melatonin and serotonin have been found to promote seed germination in diverse species, including cucumber (Posmyk et al. 2009; Zhang et al. 2013b, 2014), soybean (Wei et al. 2015), corn (Kołodziejczyk et al. 2015), knight's star (Roshchina 2001a, b), *Phacelia tanacetifolia* Benth (Tiryaki and Keles 2012), and red cabbage (*Brassica oleracea rubrum*) (Posmyk et al. 2008). Treatment with melatonin and serotonin enhanced seedling growth in *Oryza sativa*, *Lupinus albus*, *Brassica oleracea rubrum* L., and maize (Hernández-Ruiz et al. 2004, 2005; Hernández-Ruiz and Arnao 2008; Posmyk et al. 2008; Byeon and Back 2014; Zhao et al. 2015a) and also promoted hypocotyl elongation in sunflower and lupin (Hernández-Ruiz et al. 2004; Mukherjee et al. 2014) and coleoptile growth in barley, oat, wheat, and *Phalaris canariensis* L. (Csaba and Pal 1982) (Hernández-Ruiz et al. 2005). Likewise, the catecholamines have also been implicated in seedling growth and development, with reports of improved hypocotyl elongation in lettuce (*Lactuca sativa* L.)

(Kamisaka 1979), and have been reported to be present at elevated levels in germinating seeds of *Papaver bracteatum* Lindl (Rush et al. 1985).

16.2.3 Circadian Rhythms

Due to melatonin's status as the chemical expression of darkness in animals, it is unsurprising that there has been significant interest in examining a role for melatonin in mediating circadian rhythms in plants. Though no definitive evidence has been documented demonstrating a role for melatonin in regulating the plant circadian clock, there is information to suggest that there do exist both daily and seasonal rhythms of melatonin. Unlike in animals, melatonin appears not to be important in regulating darkness, but instead in regulating light-related processes, and appears to be especially associated with photosynthesis. Daily rhythms of melatonin have been identified to date in several species including *A. thaliana* (Shi et al. 2016a), *V. vinifera* cv Malbec (Boccalandro et al. 2011), *Solanum melongena* L. (Korkmaz et al. 2017), *Eichhornia crassipes* (Mart) Solms (Tan et al. 2007), *P. avium* cv Rainier and HongDeng (Zhao et al. 2013), and *C. rubrum* (Wolf et al. 2001), though there are some conflicting reports (Hernández et al. 2015). Seasonal rhythms have also been identified primarily in developing and ripening fruits as discussed earlier but also appear to be modified with the shift to Autumn and particularly senescence and leaf fall (Byeon et al. 2012; Wang et al. 2012b, 2013; Zhao et al. 2013; Li et al. 2015; Liang et al. 2015). These rhythms can most easily be described due to the antioxidant capacity of melatonin, which helps to combat increasing ROS levels produced as a result of reduced photosynthetic efficiency in Autumn due to decreasing temperatures, shifts in photoperiod, and modification of light quality and intensity with melatonin being essential in mediating the leaf senescence process. Though there are few studies examining the seasonal changes and implications of melatonin, there is a diversity of information on the ability of melatonin to mediate and reduce senescence by improving photosynthetic efficiency (Lazár et al. 2013; Fan et al. 2015), maintaining photosynthetic pigments (Arnao and Hernández-Ruiz 2009), and quenching ROS, both directly and via upregulation of other antioxidant pathways (Boccalandro et al. 2011; Wang et al. 2012b; Liang et al. 2015), as well as interacting with other signaling pathways important in these processes, particularly abscisic acid and auxin metabolism and signaling (Lee and Back 2016).

Serotonin, though to a lesser extent than melatonin, has also been found to exist in daily and seasonal rhythms. As previously discussed, rhythms of serotonin at the seasonal level and within fruits are likely to be related to embryo defense, although a limited number of reports have also found serotonin to be capable of mitigating senescence in rice (Kang et al. 2009; Byeon et al. 2012). The presence of daily rhythms of serotonin has not been conclusively demonstrated; however, serotonin has been implicated in red light responses (Das and Sopory 1985; Chandok and Sopory 1994; Raghuram and Sopory 1995, 1999), though more research is needed on this front.

16.3 Mechanisms of Action and Signaling Networks

16.3.1 Phytochrome

Jaffe (1970) found that exposure of mung bean root tips to 4 min of red light led to an increase in endogenous acetylcholine levels and efflux of acetylcholine from secondary roots decreased production of secondary roots and a change in bioelectric potential across cellular membranes was demonstrated by adherence of the root tip to a negatively charged glass surface. The latter effect was attributed to an increase in H^+ efflux. These effects were found to be reversible through treatment with far-red light, confirming a role for phytochrome in the process. To confirm the role of acetylcholine in this effect, the author replaced red-light induction with supplementation with exogenous acetylcholine (5 mM) and found that the same effects were observed as with red-light treatment, and additionally, that far-red treatment led to reversal of this effect, represented by release of the root tip from the negatively charged surface (Jaffe 1970). Jaffe (1970) suggested that acetylcholine was acting as a local mediator of phytochrome action and that it may function via modification of ion flux across cell membranes. These results were confirmed in later work by Tanada (1972), though this report achieved results at levels as low as 0.3 mM, no effect was observed below 0.1 mM acetylcholine. Though this study confirmed the results, the author, however, disputes the conclusions of Jaffe (1970) that acetylcholine is functioning as a neurotransmitter or signaling molecule, on the basis of the requirement for high concentrations in exogenous treatment, and suggests instead that acetylcholine is interfering with another cation or signaling molecule (Tanada 1972). One possible explanation for this discrepancy is instability and bioavailability of the acetylcholine molecule, with estimation that as much as 90% of exogenously applied acetylcholine may be hydrolyzed prior to uptake (Maheshwari et al. 1982). Interestingly, one report from etiolated hypocotyls of *Phaseolus vulgaris* L. suggests that the effect of acetylcholine is similar to that of blue light rather than the red light, with hyperpolarization of membranes and decreased potassium levels with acetylcholine treatment (Hartmann 1977).

A modification in the bioelectric potential across cellular membranes is proposed as being an important mechanism of acetylcholine in mediating floral timing. In spinach and perilla, which require a switch from short-day to long-day photoperiod for induction of flowering, Greppin et al. (1973) found that treatment with acetylcholine was capable of inducing flowering and that this was linked to a bioelectric response in the far-red range, suggesting that acetylcholine was mimicking red-light induction (Greppin et al. 1973). Additionally, in follow-up studies, it was found that the bioelectric potential fluctuates daily in a cyclic manner in accordance with photoperiod and that acetylcholine was able to amplify this effect, which while having little short time or immediate effects was able to induce flowering (Greppin and Horwitz 1975). The best studied model, however, for mechanisms of floral induction by neurotransmitters is certainly *Lemna*. This tiny angiosperm is a particularly useful system, due to the existence of species which require both short- and long-day photoperiods for floral induction and its small size, making it easy to grow large

numbers of individuals. Modification of membrane permeability to ions, particularly potassium, is suggested as the mechanism by which acetylcholine appears to mimic darkness, leading to inhibition of flowering in the long-day species, *L. gibba* (Oota 1977). Floral inhibition by acetylcholine has also been suggested to be the result of interaction with cAMP signaling cascades, as demonstrated in *L. gibba* G3 (Oota and Hoshino 1974).

In conjunction with its capacity for modification of cellular membrane potential, composition, and permeability, acetylcholine has also been found to modify thylakoid membranes, energy potential (adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) levels), and oxygen uptake in several species. In mung bean root tips and isolated mitochondria, treatment with red light or acetylcholine led to an increase in phosphorus levels, O_2 uptake, and exogenous H^+, concurrent with a decrease in ATP levels, though no increase in ATP synthesis was observed. The authors, therefore, hypothesized that acetylcholine induces rapid utilization of existing ATP pools (Yunghans and Jaffe 1972). Later, labeling experiments with sodium acetate supported the hypothesis that acetylcholine increased respiration rates in mung bean (Jaffe and Thoma 1973). ATP levels were also found to be reduced by acetylcholine treatment in kidney bean (*P. vulgaris* cv Red Kidney), though this did not appear to be linked with phytochrome action (Kirshner et al. 1975). Similarly, Roshchina and Muhkhin (1985a, b) found that acetylcholine was capable of modifying ATP/NADPH ratios and ion permeability of the thylakoid membranes of isolated pea chloroplasts.

Serotonin has also been found to be capable of imitating red-light stimulus via induction of downstream signaling cascades of phytochrome. In maize protoplasts, red-light-induced calcium uptake has been found to be mediated by serotonin. Application of serotonin was found to be capable of inducing Ca^{2+} uptake in dark growing conditions to match levels observed in red-light-exposed protoplasts (Das and Sopory 1985; Huang and Kao 1992).

Both acetylcholine and serotonin have been shown to modify membrane permeability through modification of phospholipid composition and turnover in membranes, in a manner which is similar to red-light stimulus. Studies in etiolated bean hypocotyls found that treatment with acetylcholine inhibited incorporation of ^{32}P into membrane phospholipids, indicating reduced rates of turnover. Surprisingly, this is the reverse of what is observed in animals, where acetylcholine increases phospholipid turnover (Hartmann et al. 1980). Serotonin has similarly been found to increase membrane composition via modification of phospholipid turnover in maize protoplasts (Raghuram and Sopory 1995). In these experiments, this action was found to be upstream of nitrate reductase, by enhancing nitrate reductase transcript levels and inhibiting further accumulation of phytochrome (*phytI*) transcript levels. Follow-up experiments found that this also lead to generation of downstream nitrate-reduction products, including nitrite and ammonium ion, which had negative and positive feedback effects, respectively, on nitrate reductase. Serotonin, as well as, lithium, which is known to increase serotonin biosynthesis in animals, induced this effect via modulation of phosphoinositide turnover (Raghuram and Sopory 1999). This demonstrates a role for these compounds in modifying not just

bioelectric potential and ion permeability of the membrane but also phosphatidylinositol and phospholipid signaling networks in plants, which have been implicated in diverse processes including many in which these compounds have been found to function including pollen germination and root growth (Xue et al. 2009).

16.3.2 Interaction with Phytohormone Networks

To date, the phytohormone most closely associated with neurotransmitter action is auxin. This is likely due, in part, to the ubiquitous roles auxin plays in plants, in addition to its structural and biosynthetic similarities to the catechol- and indoleamines. Early hypotheses for melatonin action in plants, centered around melatonin being a minor or weak auxin, suggested that melatonin functioned through interaction with auxin receptors or auxin signaling cascades (Hernández-Ruiz et al. 2004; Arnao and Hernández-Ruiz 2007; Hernández-Ruiz and Arnao 2008; Erland et al. 2015). Recent reports have, however, demonstrated that the situation is much more complex and that though melatonin likely interacts with auxin network, it is not itself an auxin. This is best demonstrated in a study by Kim et al. (2016) which employed the classical auxin bioassay, examining effects of melatonin on maize coleoptile elongation. The authors found that melatonin was not an auxin as it had no effects on coleoptile elongation, root growth, or 1-aminocyclopropane-1-carboxylic acid (ACC) activity, while IAA application promoted the former two and inhibited the latter strongly (Kim et al. 2016). Similarly, intensive investigations into the mechanisms of melatonin promotion of adventitious and lateral root growth in *A. thaliana* utilized auxin knockout mutants, and specifically the auxin action/signaling mutant DR5, to demonstrate that melatonin did not require auxin signaling (Pelagio-Flores et al. 2012). Interestingly, an investigation of serotonin in the same system using *A. thaliana* knockout mutants for auxin transport (AUX1), action/signaling (DR5), biosynthesis (BA3), and localization and ubiquitination (AXR 1, 2 and 4) found that serotonin did interact closely with auxin. Serotonin repressed auxin activity in primary and adventitious roots as well as lateral root primordia. It was concluded that serotonin promotes growth (maturation and development) of preexisting lateral root primordia, but it does not induce production of new lateral root primordia. Additionally, though this action was independent of AUX1 and AXR 4, it was found to require AXR 1 and 2, suggesting that the primary mechanism of serotonin action in this capacity is as an auxin inhibitor (Pelagio-Flores et al. 2011). These results are supported by a transcriptomics study which also found transcripts associated with auxin signaling to be downregulated in response to melatonin treatment (Weeda et al. 2014). However, a more recent study provides some conflicting evidence suggesting that melatonin does upregulate genes associated with auxin signaling to promote lateral root growth in rice (Liang et al. 2017). This suggests that melatonin may have species-specific effects. A negative interaction has also been found between IAA and acetylcholine in floral induction in *Lemna* (*L. gibba* G3), where IAA treatment was found to mimic acetylcholine effects on floral induction but to also lower acetylcholine levels. This effect is

suggested, however, to not be due to direct interaction between the two signaling compounds, but instead due to competitive action, with both compounds acting at the same receptor/target site (Hoshino 1979).

Melatonin has also been found to interact with auxin transport and biosynthesis. Melatonin was found to suppress primary root growth and root meristem size through modification of auxin transport, particularly PIN1, 3, and 7, and downregulation of auxin biosynthesis with YUC1, 2, 5, 6, and TAR2 being decreased, though three other YUC transcripts were upregulated (Wang et al. 2016). Melatonin has also been found to be important in inducing downstream signaling cascades from auxin. In de-rooted tomato seedlings, melatonin was found to improve adventitious root formation. Promotion of adventitious root production by melatonin was associated with accumulation of nitric oxide (NO) and IAA in the hypocotyl. Interestingly, NO treatment could also enhance endogenous melatonin production in de-rooted seedlings demonstrating reciprocal regulation and interaction between the signaling network. Additionally, application of NO scavengers and gene expression analysis found that NO functioned downstream of melatonin via downregulation of S-nitrosoglutathione reductase (GSNOR), and that melatonin could mediate expression of several auxin transport and signaling genes (Wen et al. 2016).

In tobacco TCL cultures, dopamine was also demonstrated to interact with auxin action, as well as concurrently increasing ethylene production, though it is unclear if this is just a downstream effect of modification of auxin action. Interestingly, dopamine treatment when administered concurrently with auxins was found to be able to promote the effects of treatment with IAA, but not the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), leading the authors to propose that dopamine is functioning by preventing oxidation of IAA. To investigate this further, the authors examined the effects of dopamine on IAA oxidase and found that in tobacco root extracts and etiolated corn coleoptiles, dopamine was capable of inhibiting IAA oxidase activity by 60–100%. To demonstrate these effects in vivo, the authors utilized 1-^{14}C labeled IAA and found that concurrent treatment with dopamine led to a fourfold decrease in regeneration. This possible in vivo function was also demonstrated by the positive effects in *Acmella oppositifolia* hairy root cultures, which do not require addition of exogenous IAA, but in which dopamine still had an effect (Protacio et al. 1992). There are, in fact, several reports of catecholamines modifying ethylene levels. In addition to the report in tobacco, dopamine, epinephrine, and norepinephrine have all been found to increase ethylene biosynthesis, specifically increasing levels of ACC in potato cell suspension cultures. Addition of an ACC inhibitor, amino oxyacetic acid (AOA), was capable of reversing this effect (Dai et al. 1993). The role of increased ethylene production in mediating these effects is, however, ambiguous as though it certainly is a result of catecholamine treatment, it may be considered a negative side effect rather than a mechanism as it is associated with dose-dependent high-concentration catecholamine treatment and often results in either the reversal of positive effects or inhibition of morphogenesis (Kuklin and Conger 1995).

In conjunction with its capacity to modify H^+ release, acetylcholine has been suggested to function similar to auxin in cell elongation (Di Sansebastiano et al.

2014). Evans (1972) tested this hypothesis and concluded that it was likely a modification in calcium flux and does not effect on auxin or H^+ which promoted cell elongation in oat coleoptiles. These results were supported by later work which measured uptake of labeled $^{45}Ca^{2+}$ from medium by oat coleoptiles under right light or acetylcholine treatment. Both appeared to promote uptake of calcium from medium, though the effects of acetylcholine were significantly enhanced by concurrent treatment with an AChE inhibitor, eserine (Tretyn 1987). More recent work in tomato protoplasts shows that cellular elongation induced by acetylcholine may be due to modification of cellular vesicle trafficking in a manner similar to auxin, and possibly also involving sucrose signaling (Di Sansebastiano et al. 2014).

Though the majority of literature has focused on interactions with auxin, it is also apparent that interactions with other plant growth regulators are also important in mediating neurotransmitter action. A transcriptomics study found that treatment of *A. thaliana* with melatonin enhanced expression of calcium signals and stress-related hormone pathways such as salicylic acid (SA), ethylene, jasmonic acid (JA), and abscisic acid (ABA) (Weeda et al. 2014). Though these compounds are most associated with melatonin action in response to stress, ABA, SA, and gibberellic acid (GA) have all been associated with indoleamine-mediated direction of morphogenesis. Erland et al. (2018) found that treatment with indoleamines, including melatonin, serotonin, and their precursors, led to de novo shoot organogenesis in excised root segments of St. John's wort, which was associated with an increase in cytokinin (zeatin) levels. Conversely, promotion of de novo root organogenesis was associated with increases in SA and ABA levels in excised nodal segments. Melatonin has also been found to interact with gibberellic acid in induction of flowering in *A. thaliana* (Shi et al. 2016b). Acetylcholine has also been shown to inhibit gibberellin biosynthesis indirectly, in germinating barley (*H. vulgare* var Jyoti) seeds, where inhibition of AChE led to decreased GA biosynthesis (Beri and Gupta 2007). This is interesting as GA is generally considered to be a promoter of seed germination, and acetylcholine has been found to promote germination via red light stimulation-related effects.

16.3.3 Calcium and Other Signaling Networks

Calcium is an important signaling molecule in plants, which, in addition to maintaining cell wall structure and osmotic and bioelectric gradients, is a secondary messenger for many signaling cascades. Therefore, though calcium signaling and the modification of bioelectric potentials across membranes have been extensively linked with red-light responses and phytochrome action, it is not unique to the phytochrome signaling cascade. Calcium also appears to be universally important in neurotransmitter-mediated signaling.

Serotonin and the catecholamines have been found to modify membrane permeability to ions including Ca^{2+}, Mg^{2+}, and Na^+, though this has yet to be linked with phytochrome activity as has been observed for acetylcholine. Pickles and Sutcliffe (1955) found that application of serotonin to beetroot slices could inhibit uptake of

Na^+, but not K^+. Another report by Roshchina (1990), however, did not find any effect of serotonin, dopamine, epinephrine, or norepinephrine treatment on Na^+ or K^+ in isolated pea chloroplasts, but all four could stimulate efflux of Ca^{2+} and Mg^{2+}. Interestingly, acetylcholine treatment was found to have the opposite effect, inducing efflux of Na^+ or K^+ from the chloroplasts, with no effect found on Ca^{2+} or Mg^{2+} (Roshchina 1990). It seems that perhaps the catecholamines and indoleamines may function in similar manners with acetylcholine having a more divergent and often opposing effect. Another such example of this was also observed in mung bean protoplasts, produced from sieve elements, where acetylcholine was found to induce contraction of immature protoplasts, while norepinephrine was found to reverse this effect (Toriyama 1978). Acetylcholine and norepinephrine have, interestingly, also been found to have opposing effects in the induction of flowering in *Lemna*, where acetylcholine inhibits flowering in short-day species, but norepinephrine, dopamine, and epinephrine promote flowering (Khurana et al. 1987).

Due to ubiquitous importance of investigations on cellular signals and responses, a diversity of inhibitors mediating calcium signaling and action are readily available (Erland and Saxena 2018). Several studies have employed calcium inhibitors to examine the mechanisms of action of melatonin and serotonin. Studies in mimosa (*Mimosa pudica* L.) (Ramakrishna et al. 2009), echinacea (*Echinaceae purpurea* L.) (Jones et al. 2007), St. John's wort (Murch and Saxena 2002a), and coffee (Ramakrishna et al. 2011) have utilized calcium channel and signaling inhibitors as well as calcium chelators to demonstrate the importance of calcium signaling in mediating the effects of these compounds. In echinacea, treatment with (S)-Bay K8644, a calcium channel inhibitor increased auxin, melatonin, and serotonin levels concurrent with a change in cell polarity which lead to reduced regeneration of leaf discs (Jones et al. 2007). In coffee, indoleamine-induced somatic embryogenesis could be enhanced through the application of exogenous calcium, while the effect could be reversed through addition of the calcium chelator ethylene glycol-bis (b-amino ethylether)-N, N, N', N'-tetra acetic acid (EGTA), or the calcium channel inhibitor verapamil (Ramakrishna et al. 2011). Likewise, treatment with EGTA or verapamil inhibited shoot organogenesis which was induced by serotonin or melatonin treatment (Ramakrishna et al. 2009). Experiments in St. John's wort also suggest that calcium gradients may be involved in signaling the transition from tetrad to uninucleate microspore phases associated with a shift from a high-serotonin environment to a high-melatonin environment (Murch and Saxena 2002a).

Another signaling cascade, which has been found to interact with and function downstream of calcium signaling, is the mitogen-activate protein kinase (MAPK) pathway (Wurzinger et al. 2014). MAPK pathways have also been established to be important mediators of melatonin action, and in a recent study utilizing *A. thaliana* and tobacco (*Nicotiana benthamiana* Domin.), MAPK kinase (MKK) knockout mutants, MKK3 and MKK6, were identified as being directly activated by melatonin. These MAPKs could then lead to downstream activation of a further four MAPKs (MKK4, MKK5, MKK7, and MKK9). Though this research investigated the effects of melatonin on mediating response to pathogen challenge, this demonstrates a role of these signaling cascades in melatonin action, which may be relevant

in morphogenetic processes. Interestingly, the melatonin precursor and intermediate between melatonin and serotonin, NAS, was also able to induce these cascades (Lee and Back 2017). A further report demonstrated the potentially broad role of MAPKs in mediating melatonin responses and found that melatonin treatment led to down-regulation of MAPK1 in responses to drought, heat, or cold stresses. This suggests that though MAPKs may be involved in diverse melatonin-mediated processes, it is likely that the specific effects are divergent (Gong et al. 2017). Interestingly, these effects have also been found to be linked to another novel class of signaling compounds, ROS species, which is discussed in detail in the following section.

16.3.4 Reactive Oxygen Species

Both epinephrine and dopamine have also been demonstrated to be strong antioxidants, both in vivo and in vitro, though the observed effects on ROS in vitro are mixed, compared to the clear antioxidant effects of the indoleamines (Guidotti et al. 2013; Gomes et al. 2014; Kanazawa and Sakakibara 2000). Melatonin and serotonin, however, are suggested to have arisen and have been conserved in plants due to their potent antioxidant potential, both directly and via modulation of other plant antioxidant systems, including enzymes such as SOD, POD, and catalase (CAT) as well as the ascorbate-glutathione cycle. The obvious implications of this function, include quenching of excess ROS in the mitochondria and chloroplast, in developing germ tissues, and in response to stress, have been well established (Tan et al. 2012; Lazár et al. 2013; Bajwa et al. 2015; Wang et al. 2015; Reiter et al. 2016). Another novel role for melatonin in mediating ROS is in relation to the emergence of ROS as a novel class of signaling compound (Mittler et al. 2011). With the identification and characterization of NADPH oxidase as an important mediator of ROS signals, particularly hydrogen peroxide (Miller et al. 2009), it has been proposed that melatonin and serotonin are potent antioxidants which may be capable of quenching or mediating this signal (Erland et al. 2015), with melatonin having to date been demonstrated to be capable of mediating hydrogen peroxide signaling along with MAPK pathways in innate immune responses (Lee and Back 2017).

16.3.5 Primary Metabolism

In animals, the catecholamines are known for their glycogen-mobilizing effects, in response to stress induction. Though plants conserve energy in different forms, there are some limited studies which suggest that the catecholamines may also be involved in sugar mobilization in plants. In potato tubers, it has been found that dopamine, epinephrine, and norepinephrine levels decrease concurrently with soluble sugar and starch levels during storage at 4 °C, providing a correlative link (Szopa et al. 2001; Kulma and Szopa 2007). In potato cultures transformed to overproduce dopamine receptors, catecholamine content was found to be increased, and to be associated with increased sucrose synthesis and modification of enzyme activity

involved in conversion of sucrose to starch leading to increased levels of sucrose, fructose, and glucose and decreased levels of starch (Skirycz et al. 2005). In another study, transformation with a TDC from parsley led to an increase in norepinephrine levels, but a decrease in L-DOPA and dopamine levels, which were correlated with decreased starch metabolism and increased glucose and sucrose levels. Unfortunately, as TDC catalyzes products feeding into other pathways, including the indoleamines, it is possible that these responses are not specific to the modified catecholamine content (Swiedrych et al. 2004). Catecholamines have also been shown to promote primary growth in several species (Table 16.2), which has been associated with sugar metabolism and protein expression, well-established mechanisms for induction of enhanced primary organ growth (Kaur and Thukral 1990; Hourmant et al. 1998; Steward et al. 1958; Steward and Bidwell 1958).

Transcriptomic, proteomic, and metabolomics studies have also identified a role for melatonin in mediating several primary metabolic networks, including nitrogen and carbon metabolism, as well as secondary metabolites such as phenolics and anthocyanins which are involved in cell wall structure (Byeon et al. 2013b; Zhang et al. 2013a, 2016; Weeda et al. 2014; Qian et al. 2015; Shi et al. 2015c; Zhao et al. 2015a, b; Wei et al. 2016; Sun et al. 2016). Though the majority of information on melatonin and nitrogen is only available in broad transcriptomics studies, some targeted experiments have examined the links between carbon metabolism and melatonin. It is unclear if the effects of melatonin on sugar content may be due to protection of the photosynthetic apparatus through modulation of antioxidant pathways, enhanced anthocyanins, and pigment levels or if it is via some other mechanism (Lazár et al. 2013; Zhang et al. 2016; Szafrańska et al. 2016; Ding et al. 2017). Melatonin has been found to increase total carbohydrate levels in several cherry rootstocks (*Prunus cerasus* L., *P. avium* x *P. cerasus*, *P. cerasus* x *P. canescens*, and *P. avium* x *P. mahaleb*) in association with enhanced chlorophyll levels (Sarropoulou et al. 2012a, b); with increased photosynthetic pigment levels also having been associated with improved lateral rooting in cucumber (Zhang et al. 2013b). In maize melatonin enhanced overall root biomass, which was associated with enhanced leaf growth and modified carbohydrate metabolism. It was found that melatonin treatment was associated with accelerated nighttime starch metabolism, increased sucrose transport, and enhanced hexokinase activity as well as promotion of photosynthetic activity and absence of stress (Zhao et al. 2015a).

16.3.6 Modification of Gene Expression

Modification of gene expression is an area of extreme interest in plant sciences, and one means by which this is often examined is through modification of transcription factors, which control expression of genes is particularly of interest. In addition to melatonin and serotonin having been found to have broad transcriptional effects on plants in response to developmental or stress signals (Dharmawardhana et al. 2013; Zhang et al. 2013a; Weeda et al. 2014; Shi et al. 2015a; Wei et al. 2016; Hu et al. 2016), melatonin has been found to interact with diverse classes of transcription

factors including WRKY, MYB, bHLH, DELLA, and heat shock proteins (Bajwa et al. 2014; Shi and Chan 2014; Shi et al. 2014, 2015b, 2016b). The transcriptional effects of melatonin are vast, and a thorough discussion of these effects is beyond the scope of this chapter. In *A. thaliana* alone, melatonin has been found to influence more than 1300 genes, with 300 modified in cucumber and 400 in rice, with these genes being involved in processes such as auxin signaling, antioxidant pathways, development and flowering processes, stress mitigation, senescence, and primary metabolism.

16.3.7 The Search for Receptors

Despite intensive research into these classes of neurotransmitters, one large research gap still exists. To date, a receptor has yet to be identified for any of these classes of compounds, though significant information is known about their mammalian counterparts, only a few candidates have been proposed, and much work is still required to confirm their function. Several studies have employed serotonin receptor inhibitors from animals/humans to try to identify a serotonin receptor in plants; however, none has yet been identified (Murch et al. 2001). Results from studies on the mechanism of action of serotonin in pollen allelopathy have led to a hypothesis on one potential serotonin receptor type. Roshchina (2006) proposed that serotonin functions through interaction with G-protein-coupled receptors on the cell surface which upon interaction with serotonin lead to opening of ion channels modifying both cell membrane bioelectric potential and cytoskeletal structure. Though there is no direct evidence in pollen for modification of ion permeability, two reports, one in beetroot slices (Pickles and Sutcliffe 1955) and another in pea protoplasts (Roshchina 1990), found that serotonin is capable of modifying ion efflux.

Though initially it was hypothesized that melatonin may function via interaction with auxin receptors, work such as that conducted by Kim et al. (2016) demonstrating that melatonin does not possess classical auxin-activity has suggested that a separate melatonin receptor exists. With the recent cloning of a protein from *H. perforatum*, which has been found in complex with melatonin, a quinone reductase like receptor, the presence of a melatonin-specific receptor has gained additional credibility. Using X-ray crystallography with a pathogenesis-related (PR) protein, PR-10, the first plant protein in complex with melatonin has been identified. Unfortunately, downstream signaling of this complex has not be characterized, and only a crystal structure is currently available (Sliwiak et al. 2016).

The primary candidates for an acetylcholine receptor in plants are the cholinergic receptors. Addition of cholinergic receptor, inhibitors to tomato protoplasts inhibited acetylcholine-induced cellular elongation providing evidence that plant cells may possess receptors similar to those present in the animal system (Di Sansebastiano et al. 2014). Another report which used an agonist of nicotinic cholinergic receptors, galanthamine, found that application of this agonist resulted in decreased levels of acetylcholine and AChe, which was associated with decreased plant growth, further

implicating the presence of these receptors in plants, though the mode of action may differ (Turi et al. 2014).

Cytochrome b561 enzymes have been proposed as the plant catecholamine receptors. Verelst and Asard (2004) suggested that based on homology to a cytochrome present in mammals which induces ion flux in response to catecholamines, a similar enzyme may be important in plants. The authors proposed that this receptor would possess a dopamine-β-hydroxylase domain combined with a Cyt b561 domain with transmembrane electron transport, a combination which has been observed in insects (Verelst and Asard 2004).

16.4 Conclusions

Neurotransmitters, including the indoleamines, catecholamines, and acetylcholine exist in plants, and consistent efforts have been made to decipher their role at the physiological, biochemical, and molecular levels in a broad cross section of plants across diverse families. Though not a new area of study, the investigation of the roles of neurotransmitters in the lives of plants is still an under-investigated area with significant potential. These compounds have been demonstrated to have evolved with the most ancient ancestors of plants and have been demonstrated to have important roles in directing the multifaceted chemical symphony, that is, plant signaling and morphogenesis. A striking feature of these adaptive molecules is the diverse and often flexible roles they play in supporting plant throughout their life cycle. From protection of a developing embryo and interpreting light signals and inducing germination in the seed containing these embryos to determining the growth pattern of the resulting seedlings and adult plants through to mediating floral timing and back to embryo protection, the catecholamines, indoleamines, and acetylcholine play adaptive and evolving roles throughout the life cycle of plants depending on their needs and development stages. This multiplicity of functions is unique from what is observed in animals, where these compounds play unique and easily classified roles, for example, melatonin's role in the human system is often summarized into a few words "the chemical expression of darkness"; however, no such succinct description can be provided in plants, where melatonin functions as an antioxidant, a hormone, and a regulatory molecule though definitions such as "plant hormone," "plant growth regulator," and "plant master regulator" have been proposed (Erland et al. 2015; Arnao and Ruiz 2019). This difference is likely due to the sessile nature of plants, which requires plants to be much more creative, inventive, and resourceful in the use of these molecules. This has allowed plants to successfully adapt to diverse and ever-changing environments.

As plants increasingly face a changing climate, habitat loss, and increased demand for food production, the importance of understanding how plants survive and thrive will continue to increase in importance. Due to their capacity to effect almost every aspect of plant growth, as well as the ties to human consciousness and behavior, interest in these compounds is likely to grow. Much current research has focused on the molecular mechanisms underlying the action of these compounds,

particularly in stress survival. Unfortunately, this research has left significant gaps in understanding at a physiological level how these compounds are able to induce and direct changes in plant growth at the most basic levels. In fact, it seems that as literature on these compounds in plants expands, many more questions arise than are answered. This has led to some intriguing hypotheses and approaches being proposed. One such example is the proposal by Erland et al. (2018) that instead of looking at melatonin as an adaptive molecule in isolation, that it may be much more informative and fruitful to take a pathway-based approach to understanding the roles of indoleamines in plant morphogenesis. Further studies are therefore needed, which both return to the original premise of experiments in these compounds, investigating signaling pathways, chemical induction, and physiological responses, while also incorporating these novel approaches to understanding these complex adaptive molecules. With any luck, this research while continue to raise many more interesting scientific questions, helping scientists in the journey to understanding the roles of these ancient molecules in nature.

Acknowledgments The authors gratefully acknowledge the financial support of this work by the Natural Sciences and Engineering Research Council (NSERC) of Canada (grant number 46741).

References

Acuña-Castroviejo D, Escames G, Venegas C, Díaz-Casado ME, Lima-Cabello E, López LC, Rosales-Corral S, Tan DX, Reiter RJ (2014) Extrapineal melatonin: sources, regulation, and potential functions. Cell Mol Life Sci 71:2997–3025

Adil M, Abbasi BH, Khan T (2015) Interactive effects of melatonin and light on growth parameters and biochemical markers in adventitious roots of *Withania somnifera* L. Plant Cell Tissue Organ Cult 123:405–412

Arnao MB, Hernández-Ruiz J (2007) Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. J Pineal Res 42:147–152

Arnao MB, Hernández-Ruiz J (2009) Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. J Pineal Res 46:58–63

Arnao MB, Hernández-Ruiz J (2019) Melatonin: a new plant hormone and/or a plant master regulator? Trends Plant Sci 24:38–48

Askar A, Rubach K, Schormüller J (1972) Dünnschichtchromatographische Trennung der in Bananen vorkommenden Amin-Fraktion. Chem Microbiol Technol Lebensm 1:187–190

Back K, Tan D-X, Reiter RJ (2016) Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. J Pineal Res 61:426–437

Bajwa VS, Shukla MR, Sherif SM, Murch SJ, Saxena PK (2014) Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. J Pineal Res 56:238–245

Bajwa VS, Shukla MR, Sherif SM, Murch SJ, Saxena PK (2015) Identification and characterization of serotonin as an anti-browning compound of apple and pear. Postharvest Biol Technol 110:183–189

Bamel K, Gupta R, Gupta SC (2016) Acetylcholine suppresses shoot formation and callusing in leaf explants of in vitro raised seedlings of tomato, *Lycopersicon esculentum* Miller var. Pusa Ruby. Plant Signal Behav 11:e1187355

Barlow RB, Dixon RO (1973) Choline acetyltransferase in the nettle *Urtica dioica* L. Biochem J 132:15–18

Beri V, Gupta R (2007) Acetylcholinesterase inhibitors neostigmine and physostigmine inhibit induction of alpha-amylase activity during seed germination in barley, *Hordeum vulgare* var. Jyoti. Life Sci 80:2386–2388

Boccalandro HE, González CV, Wunderlin DA, Silva MF (2011) Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in *Vitis vinifera* cv Malbec: evidence of its antioxidant role in fruits. J Pineal Res 51:226–232

Bowden K, Brown BG, Batty JE (1954) 5-Hydroxytryptamine: its occurrence in cowhage. Nature 174:925–926

Buelow DW, Gisvold O (1944) A phytochemical investigation of *Hermidium alipes*. J Am Pharm Assoc 3:270–274

Byeon Y, Back K (2014) An increase in melatonin in transgenic rice causes pleiotropic phenotypes, including enhanced seedling growth, delayed flowering, and low grain yield. J Pineal Res 56:408–414

Byeon Y, Park S, Kim Y-S, Park DH, Lee S, Back K (2012) Light-regulated melatonin biosynthesis in rice during the senescence process in detached leaves. J Pineal Res 53:107–111

Byeon Y, Lee HY, Lee K, Park S, Back K (2013a) Cellular localization and kinetics of the rice melatonin biosynthetic enzymes SNAT and ASMT. J Pineal Res 56:107–114

Byeon Y, Park S, Kim Y-S, Back K (2013b) Microarray analysis of genes differentially expressed in melatonin-rich transgenic rice expressing a sheep serotonin N-acetyltransferase. J Pineal Res 55:357–363

Byeon Y, Lee HY, Lee K, Back K (2014a) A rice chloroplast transit peptide sequence does not alter the cytoplasmic localization of sheep serotonin N-acetyltransferase expressed in transgenic rice plants. J Pineal Res 57:147–154

Byeon Y, Yool Lee H, Choi D-W, Back K (2014b) Chloroplast-encoded serotonin N-acetyltransferase in the red alga *Pyropia yezoensis*: gene transition to the nucleus from chloroplasts. J Exp Bot 66:709–717

Cassone VM (1990) Effects of melatonin on vertebrate circadian systems. Trends Neurosci 13:457–464

Chandok MR, Sopory SK (1994) 5-Hydroxytryptamine affects turnover of polyphosphoinositides in maize and stimulates nitrate reductase in the absence of light. FEBS Lett 356:39–42

Chen Q, Qi W, Reiter RJ, Wei W, Wang BM (2009) Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. J Plant Physiol:324–328

Chhabra N, Malik CP (1978) Influence of spectral quality of light on pollen tube elongation in *Arachis hypogaea*. Ann Bot 42:1109–1117

Csaba G, Pal K (1982) Effects of insulin, triiodothyronine, and serotonin on plant seed development. Protoplasma 110:20–22

Cybulski N (1895) O funkcji nadnercza. Gazeta Lekarska 12:299–308

Dai Y-R, Michaels PJ, Flores HE (1993) Stimulation of ethylene production by catecholamines and phenylethylamine in potato cell suspension cultures. Plant Growth Regul 12:219–222

Das R, Sopory SK (1985) Evidence of regulation of calcium uptake by phytochrome in maize protoplasts. Biochem Biophys Res Comm 128:1455–1460

De Luca V, Marineau C, Brisson N (1989) Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase: comparison with animal dopa decarboxylases. Proc Natl Acad Sci USA 86:2582–2586

Dekhuijzen HM (1973) The effect of acetylcholine on growth and on growth inhibition by CCC in wheat seedlings. Planta 111:149–156

Dettbarn WD (1962) Acetylcholinesterase activity in *Nitella*. Nature 194:1175–1176

Dharmawardhana P, Ren L, Amarasinghe V, Moncao M, Thomason J, Ravenscroft D, McCouch S, Ware D, Jaiswal P (2013) A genome scale metabolic network for rice and accompanying analysis of tryptophan, auxin and serotonin biosynthesis regulation under biotic stress. Rice 29:15

Di Sansebastiano G-P, Fornaciari S, Barozzi F, Piro G, Arru L (2014) New insights on plant cell elongation: a role for acetylcholine. Int J Mol Sci 15:4565–4582

Ding F, Wang M, Liu B, Zhang S (2017) Exogenous melatonin mitigates photoinhibition by accelerating non-photochemical quenching in tomato seedlings exposed to moderate light during chilling. Front Plant Sci 8:244

Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwara HW, Schloot W (1995) Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J Pineal Res 18:28–31

Emmelin N, Feldberg W (1947) The mechanism of the sting of the common nettle (*Urtica urens*). J Physiol 106:440–455

Erland LAE, Saxena PK (2018) Melatonin in morphogenesis. In Vitro Cell Dev Biol Plant 54:3–24

Erland LAE, Murch SJ, Reiter RJ, Saxena PK (2015) A new balancing act: the many roles of melatonin and serotonin in plant growth and development. Plant Signal Behav 10:e1096469–e1096415

Erland LAE, Turi CE, Saxena PK (2016a) Serotonin: an ancient molecule and an important regulator of plant processes. Biotechnol Adv 8:1347–1361

Erland LAE, Chattopadhyay A, Jones AMP, Saxena PK (2016b) Melatonin in plants and plant culture systems: variability, stability and efficient quantification. Front Plant Sci 7:108

Erland LAE, Shukla MR, Singh AS, Murch SJ, Saxena PK (2018) Melatonin and serotonin: mediators in the symphony of plant morphogenesis. J Pineal Res 64:e12452

Ernst M, Hartmann E (1980) Biochemical characterization of an acetylcholine-hydrolyzing enzyme from bean seedlings. Plant Physiol 65:447–450

Evans ML (1972) Promotion of cell elongation in Avena coleoptiles by acetylcholine. Plant Physiol 50(3):414–416

Facchini PJ, De Luca V (1994) Differential and tissue-specific expression of a gene family for tyrosine/dopa decarboxylase in opium poppy. J Biol Chem 269:26684–26690

Facchini PJ, Huber-Allanach KL, Tari LW (2000) Plant aromatic L-amino acid decarboxylases: evolution, biochemistry, regulation and metabolic engineering applications. Phytochemistry 54:121–138

Fan J, Hu Z, Xie Y, Chan Z, Chen K, Amombo E, Chen L, Fu J (2015) Alleviation of cold damage to photosystem II and metabolisms by melatonin in Bermudagrass. Front Plant Sci 6:36

Galvão VC, Horrer D, Küttner F, Schmid M (2012) Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*. Development 139:4072–4082

Gatineau F, Fouché JG, Kevers C, Hausman JF, Gaspar T (1997) Quantitative variations of indolyl compounds including IAA, IAA-aspartate and serotonin in walnut microcuttings during root induction. Biol Plant 39:131–137

Gomes BR, de Siqueira-Soares RC, Dos Santos WD, Marchiosi R, Soares AR, Ferrarese-Filho O (2014) The effects of dopamine on antioxidant enzymes activities and reactive oxygen species levels in soybean roots. Plant Signal Behav 9:e977704

Gong B, Yan Y, Wen D, Shi Q (2017) Hydrogen peroxide produced by NADPH oxidase: a novel downstream signaling pathway in melatonin-induced stress tolerance in *Solanum lycopersicum*. Physiol Plant 160:396–409

Greppin H, Horwitz B (1975) Floral induction and the effect of red and far-red preillumination on the light-stimulated bioelectric response of spinach leaves. Z Pflanzenphysiol 75:243–249

Greppin H, Horwitz BA, Horwitz LP (1973) Light-stimulated bioelectric response of spinach leaves and photoperiodic induction. Z Pflanzenphysiol 68:336–345

Grosse W, Artigas F (1983) Incorporation of N-15 ammonia into serotonin in cotyledons of maturing walnuts. Z Naturforsch C J Biosci 38:1057–1058

Guidotti BB, Gomes BR, de Siqueira-Soares RC, Soares AR, Ferrarese-Filho O (2013) The effects of dopamine on root growth and enzyme activity in soybean seedlings. Plant Signal Behav 8:e25477–e25478

Hadačová V, Hofman J, de Almeida RM, Vacková K, Kutáček M, Klozová E (1981) Choliae esterases and choline acetyltransferase in the seeds of *Allium altaicum* (Pall.) Reyse. Biol Plant 23:220–227

Hartmann E (1977) Influence of acetylcholine and light on the bioelectric potential of bean (*Phaseolus vulgaris* L.) hypocotyl hook. Plant Cell Physiol 18:1203–1207

Hartmann E (1979) Attempts to demonstrate incorporation of labelled precursors into acetylcholine by *Phaseolus vulgaris* seedlings. Phytochemistry 18:1643–1646

Hartmann E, Gupta R (1989) Acetylcholine as a signaling system in plants. In: Boss WF, Morre DJ (eds) . Plant biology second messengers in plant growth and development, New York, pp 257–288

Hartmann E, Grasmück I, Lehrbach N, Müller R (1980) The influence of acetylcholine and choline on the incorporation of phosphate into phospholipids of etiolated bean hypocotyl hooks. Z Pflanzenphysiol 97:377–389

Hattori A, Migitaka H, Iigo M, Itoh M, Yamamoto K, Ohtani-Kaneko R, Suzuki T, Reiter RJ (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Biol Int 35:627–634

Hernández IG, Gomez FJV, Cerutti S, Arana MV, Silva MF (2015) Melatonin in *Arabidopsis thaliana* acts as plant growth regulator at low concentrations and preserves seed viability at high concentrations. Plant Physiol Biochem 94:191–196

Hernández-Ruiz J, Arnao MB (2008) Melatonin stimulates the expansion of etiolated lupin cotyledons. Plant Growth Regul 55:29–34

Hernández-Ruiz J, Cano A, Arnao MB (2004) Melatonin: a growth-stimulating compound present in lupin tissues. Planta 220:140–144

Hernández-Ruiz J, Cano A, Arnao MB (2005) Melatonin acts as a growth-stimulating compound in some monocot species. J Pineal Res 39:137–142

Hoshino T (1979) Simulation of acetylcholine action by β-indole acetic acid in inducing diurnal change of floral response to chilling under continuous light in *Lemna gibba* G3. Plant Cell Physiol 20:43–50

Hoshino T (1983) Effects of acetylcholine on the growth of the *Vigna* seedling. Plant Cell Physiol 24:551–556

Hoshino T, Oota Y (1978) The occurrence of acetylcholine in *Lemna gibba* G3. Plant Cell Physiol 19:769–776

Hourmant A, Rapt F, Morzadec J-M, Féray A, Caroff J (1998) Involvement of catecholic compounds in morphogenesis of in vitro potato plants effect of methylglyoxal-bis (guanylhydrazone). J Plant Physiol 152:64–69

Hu W, Kong H, Guo Y, Zhang Y, Ding Z, Tie W, Yan Y, Huang Q, Peng M, Shi H, Guo A (2016) Comparative physiological and transcriptomic analyses reveal the actions of melatonin in the delay of postharvest physiological deterioration of cassava. Front Plant Sci 7:138–112

Huang YM, Kao CH (1992) Calcium in the regulation of corn leaf senescence by light. Bot Bull Acad Sin 33:161–165

Jaffe MJ (1968) Phytochrome-mediated bioelectric potentials in mung bean seedlings. Science 162:1016–1017

Jaffe MJ (1970) Evidence for the regulation of phytochrome-mediated processes in bean roots by the neurohumor, acetylcholine. Plant Physiol 46:768–777

Jaffe MJ (1972) Acetylcholine as a native metabolic regulator of phytochrome-mediated processes in bean roots. Recent Adv Phytochem 5:81–104

Jaffe MJ (1976) Phytochrome-controlled acetylcholine synthesis at the endoplasmic reticulum. In: Smith H (ed) Light and plant development. Butterworths, London, pp 333–344

Jaffe MJ, Thoma L (1973) Rapid phytochrome-mediated changes in the uptake by bean roots of sodium acetate 1-14C and their modification by cholinergic drugs. Planta 113:283–291

Janas KM, Posmyk MM (2013) Melatonin, an underestimated natural substance with great potential for agricultural application. Acta Physiol Plant 35:3285–3292

Jones MPA, Cao J, O'Brien R, Murch SJ, Saxena PK (2007) The mode of action of thidiazuron: auxins, indoleamines, and ion channels in the regeneration of *Echinacea purpurea* L. Plant Cell Rep 26:1481–1490

Kamisaka S (1979) Catecholamine stimulation of the gibberellin action that induces lettuce hypocotyl elongation. Plant Cell Physiol 20:1199–1207

Kanazawa K, Sakakibara H (2000) High content of dopamine, a strong antioxidant, in Cavendish banana. J Agric Food Chem 48:844–848

Kandeler R (1972) The effect of acetylcholine on the photoperiodic control of flowering in Lemnaceae. Z Pflanzenphysiol 67:86–92

Kang S, Kang K, Lee K, Back K (2007) Characterization of rice tryptophan decarboxylases and their direct involvement in serotonin biosynthesis in transgenic rice. Planta 227:263–272
Kang K, Kang S, Lee K, Park M, Back K (2008) Enzymatic features of serotonin biosynthetic enzymes and serotonin biosynthesis in plants. Plant Signal Behav 3:389–390
Kang K, Kim Y-S, Park S, Back K (2009) Senescence-induced serotonin biosynthesis and its role in delaying senescence in rice leaves. Plant Physiol 150:1380–1393
Kasturi R (1978) *De novo* synthesis of acetylcholinesterase in roots of *Pisum sativum*. Phytochemistry 17:647–649
Kaur A, Thukral AK (1990) Effect of animal hormones on the growth, protein and sugar contents of *Vigna unguiculata* L. seedlings. Indian J Plant Physiol 33:259–261
Khurana JP, Tamot BK, Maheshwari N, Maheshwari SC (1987) Role of catecholamines in promotion of flowering in a short-day duckweed, *Lemna paucicostata* 6746. Plant Physiol 85:10–12
Kim M, Seo H, Park C, Park WJ (2016) Examination of the auxin hypothesis of phytomelatonin action in classical auxin assay systems in maize. J Plant Physiol 190:67–71
Kirshner RL, White JM, Pike CS (1975) Control of bean bud ATP levels by regulatory molecules and phytochrome. Physiol Plant 34:373–377
Kolar J, Johnson CH, Machackova I (2003) Exogenously applied melatonin (N-acetyl-5-methoxytryptamine) affects flowering of the short-day plant *Chenopodium rubrum*. Physiol Plant 118:605–612
Kołodziejczyk I, Bałabusta M, Szewczyk R, Posmyk MM (2015) The levels of melatonin and its metabolites in conditioned corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) seeds during storage. Acta Physiol Plant 37:105–111
Kong K-H, Lee J-L, Park H-J, Cho S-H (1998) Purification and characterization of the tyrosinase isozymes of pine needles. Biochem Mol Biol Int 45:717–724
Korkmaz A, Değer Ö, Cuci Y (2014) Profiling the melatonin content in organs of the pepper plant during different growth stages. Sci Hortic 172:242–247
Korkmaz A, Yakupoglu G, Köklü Ş, Cuci Y, Kocacinar F (2017) Determining diurnal and seasonal changes in melatonin andtryptophan contents of eggplant (*Solanum melongena* L.). Turk J Bot 41:356–366
Kostir J, Klenha J, Vyroba VJR (1965) The effect of acetylcholine on seed germination in agricultural plants. Rost Vyroba Praha 12:1239–1279
Koyama FC, Carvalho TLG, Alves E, da Silva HB, de Azevedo MF, Hemerly AS, Garcia CR (2013) The structurally related auxin and melatonin tryptophan-derivatives and their roles in *Arabidopsis thaliana* and in the human malaria parasite *Plasmodium falciparum*. J Eukaryot Microbiol 60:646–651
Kuklin AI, Conger BV (1995) Enhancement of somatic embryogenesis in orchardgrass leaf cultures by epinephrine. Plant Cell Rep 14:641–644
Kulma A, Szopa J (2007) Catecholamines are active compounds in plants. Plant Sci 172:433–440
Lawson VR, Brady RM, Campbell A, Knox GD, Walls RL (1978) Interaction of acetylcholine chloride with IAA, GA 3 and red light in the growth of excised apical coleoptile segments. Bull Torrey Bot Club 105:187
Lazár D, Murch SJ, Beilby MJ, Al Khazaaly S (2013) Exogenous melatonin affects photosynthesis in characeae *Chara australis*. Plant Signal Behav 8:e23279
Lee H-J, Back K (2016) 2-Hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). J Pineal Res:1–48
Lee HY, Back K (2017) Melatonin is required for H2O2- and NO-mediated defense signaling through MAPKKK3 and OXI1 in Arabidopsis thaliana. J Pineal Res 62:e12379
Lei Q, Wang L, Tan D-X, Zhao Y, Zheng XD, Chen H, Li QT, Zuo BX, Kong J (2013) Identification of genes for melatonin synthetic enzymes in "Red Fuji" apple (*Malus domestica* Borkh. cv.Red) and their expression and melatonin production during fruit development. J Pineal Res 55:443–451
Lembeck F, Skofitsch G (1984) Distribution of serotonin in *Juglans regia* seeds during ontogenetic development and germination. Z Pflanzenphysiol 114:349–353

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80:2587–2587

Li C, Tan D-X, Liang D, Chang C, Jia D, Ma F (2015) Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two *Malus* species under drought stress. J Exp Bot 66:669–680

Liang C, Zheng G, Li W, Wang Y, Hu B, Wang H, Wu H, Qian Y, Zhu XG, Tan DX, Chen SY, Chu C (2015) Melatonin delays leaf senescence and enhances salt stress tolerance in rice. J Pineal Res 59:91–101

Liang C, Li A, Yu H, Li W, Liang C, Guo S, Zhang R, Chu C (2017) Melatonin regulates root architecture by modulating auxin response in rice. Front Plant Sci 8:89–12

Litwinczuk W, Wadas-Boron M (2009) Development of highbush blueberry (*Vaccinium corymbosum* hort. non L.) in vitro shoot cultures under the influence of melatonin. Acta Sci Pol Hort Cult 8:3–12

Maheshwari SC, Gupta R and Gharyal PK (1982) Cholinesterases in plants. In: Sen SP (ed) Recent developments in plant science. New Delhi, pp 145–160

Manchester LC, Tan DX, Reiter RJ, Park W, Monis K, Qi W (2000) High levels of melatonin in the seeds of edible plants: possible function in germ tissue protection. Life Sci 67:3023–3029

Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, Vriend J, Tan DX, Reiter RJ (2015) Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res 59:403–419

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R (2009) The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Sci Signal 2:ra45

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F (2011) ROS signaling: the new wave? Trend Plant Sci 16:300–309

Miyawaki T, Matsumoto S, Takahashi W, Tanaka O (2014) Effect of heat-treated noradrenaline on flowering in Lemna. Biosci Biotechnol Biochem 77:1586–1588

Momonoki YS (1992) Occurrence of acetylcholine-hydrolyzing activity at the stele-cortex interface. Plant Physiol 99:130–133

Momonoki YS (1997) Asymmetric distribution of acetylcholinesterase in gravistimulated maize seedlings. Plant Physiol 114:47–53

Momonoki YS, Hineno C, Noguchi K (1998) Acetylcholine as a signaling system to environmental stimuli in plants. III. Asymmetric solute distribution controlled by ACh in gravistimulated maize seedlings. Plant Prod Sci 1:83–88

Mukherjee I (1980) The effect of acetylcholine on hypocotyl elongation in soybean. Plant Cell Physiol 21:1657–1660

Mukherjee S, David A, Yadav S, Baluska F, Bhatla SC (2014) Salt stress-induced seedling growth inhibition coincides with differential distribution of serotonin and melatonin in sunflower seedling roots and cotyledons. Physiol Plant 152:714–728

Murch SJ, Saxena PK (2002a) Mammalian neurohormones: potential significance in reproductive physiology of St. John's wort (*Hypericum perforatum* L.)? Naturwissenschaften 89:555–560

Murch SJ, Saxena PK (2002b) Melatonin: a potential regulator of plant growth and development? In Vitro Cell Dev Biol Plant 38:531–536

Murch SJ, Saxena PK (2004) Role of indoleamines in regulation of morphogenesis in *in vitro* cultures of St. John's wort (*Hypericum perforatum* L.). Acta Hortic 629:425–432

Murch SJ, Krishnaraj S, Saxena PK (2000) Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep 19:698–704

Murch SJ, Campbell SSB, Saxena PK (2001) The role of serotonin and melatonin in plant morphogenesis: Regulation of auxin-induced root organogenesis in *in vitro*-cultured explants of St. John's Wort (*Hypericum perforatum* L.). In Vitro Cell Dev Biol Plant 37:786–793

Murch SJ, Alan AR, Cao J, Saxena PK (2009) Melatonin and serotonin in flowers and fruits of *Datura metel* L. J Pineal Res 47:277–283

Murch SJ, Hall BA, Le CH, Saxena PK (2010) Changes in the levels of indoleamine phytochemicals during véraison and ripening of wine grapes. J Pineal Res 49:95–100
Okatani A, Ikegami T, Takahashi W, Tanaka O (2014) Induction and promotion of flowering by heat-treated catecholamines in *Lemna paucicostata*. Biosci Biotechnol Biochem 74:2339–2341
Oota Y (1977) Removal by chemicals of photoperiodic light requirements of *Lemna gibba* G3. Plant Cell Physiol 18:95–105
Oota Y, Hoshino T (1974) Diurnal change in temperature sensitivity *Lemna gibba* G3 induced by acetylcholine in continuous light. Plant Cell Physiol 15:1063–1072
Park S, Back K (2012) Melatonin promotes seminal root elongation and root growth in transgenic rice after germination. J Pineal Res 53:385–389
Park S, Byeon Y, Back K (2013) Functional analyses of three ASMT gene family members in rice plants. J Pineal Res 55:409–415
Park S, Byeon Y, Lee HY, Kim YS, Ahn T, Back K (2014) Cloning and characterization of a serotonin N-acetyltransferase from a gymnosperm, loblolly pine (*Pinus taeda*). J Pineal Res 57:348–355
Pelagio-Flores R, Ortíz-Castro R, Méndez-Bravo A, Macías-Rodríguez L, López-Bucio J (2011) Serotonin, a tryptophan-derived signal conserved in plants and animals, regulates root system architecture probably acting as a natural auxin inhibitor in *Arabidopsis thaliana*. Plant Cell Physiol 52:490–508
Pelagio-Flores R, Muñoz Parra E, Ortíz-Castro R, López-Bucio J (2012) Melatonin regulates *Arabidopsis* root system architecture likely acting independently of auxin signaling. J Pineal Res 53:279–288
Penel C, Darimont E, Greppin H, Gaspar TH (2008) Effect of acetylcholine on growth and isoperoxidases of the lentil (*Lens culinaris*) root. Biol Plant 18:293–298
Pickles VR, Sutcliffe JF (1955) The effects of 5-hydroxytryptamine, indole-3-acetic acid, and some other substances, on pigment effusion, sodium uptake, and potassium efflux, by slices of red beetroot *in vitro*. Biochim Biophys Acta 17:244–251
Posmyk MM, Kuran H, Marciniak K, Janas KM (2008) Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. J Pineal Res 45:24–31
Posmyk MM, Bałabusta M, Wieczorek M, Sliwinska E, Janas KM (2009) Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. J Pineal Res 46:214–223
Protacio CM, Dai Y-R, Lewis EF, Flores HE (1992) Growth stimulation by catecholamines in plant tissue/organ cultures. Plant Physiol 98:89–96
Qian Y, Tan D-X, Reiter RJ, Shi H (2015) Comparative metabolomic analysis highlights the involvement of sugars and glycerol in melatonin-mediated innate immunity against bacterial pathogen in *Arabidopsis*. Sci Rep 5:15815
Raghuram N, Sopory SK (1995) Evidence for some common signal-transduction events for opposite regulation of nitrate reductase and phytochrome-I gene-expression by light. Plant Mol Biol 29:25–35
Raghuram N, Sopory S (1999) Roles of nitrate, nitrite and ammonium ion in phytochrome regulation of nitrate reductase gene expression in maize. IUBMB Life 47:239–249
Ramakrishna A, Giridhar P, Ravishankar GA (2009) Indoleamines and calcium channels influence morphogenesis in in vitro cultures of *Mimosa pudica* L. Plant Signal Behav 4:1136–1141
Ramakrishna A, Giridhar P, Jobin M, Paulose CS, Ravishankar GA (2011) Indoleamines and calcium enhance somatic embryogenesis in *Coffea canephora* P ex Fr. Plant Cell Tissue Organ Cult 108:267–278
Regula I (1986) The presence of serotonin in the embryo of black walnut (*Juglans nigra*). Acta Bot Croat 45:91–95
Reiter RJ, Poeggeler B, Tan D-X, Chen D-L, Manchester LC, Guerrero JM (1993) Antioxidant capacity of melatonin: a novel action not requiring a receptor. Neuroendocrinol Lett 15:103–116
Reiter R, Tan D-X, Zhou Z, Cruz MH, Fuentes-Broto L, Galano A (2015) Phytomelatonin: assisting plants to survive and thrive. Molecules 20:7396–7437

Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L (2016) Melatonin as an antioxidant: under promises but over delivers. J Pineal Res 61:253–278

Reynolds JD, Kimbrough TD, Weekley LB (1985) The effect of light quality on 5-hydroxyindole metabolism in leaves of *Sedum morganianum* (Crassulaceae). Biochem Physiol Pflanzen 180:345–351

Roshchina VV (1990) Regulation of chloroplast reactions by secondary metabolites acetylcholine and biogenic amines. Acta Bot Croat 49:29–35

Roshchina VV (2001a) Molecular-cellular mechanisms in pollen allelopathy. Allelopath J 8:11–28

Roshchina VV (2001b) Neurotransmitters in plant life. Science Publishers, Enfield

Roshchina VV (2005) Contractile proteins in chemical signal transduction in plant microspores. Biol Bull Russ Acad Sci 32:229–233

Roshchina VV (2006) Chemical signaling in plant microspore cells. Biol Bull Russ Acad Sci 33:332–338

Roshchina VV, Melnikova EV (1998) Allelopathy and plant reproductive cells: participation of acetylcholine and histamine in signaling in the interactions of pollen and pistil. Allelopathy J 5:171–182

Roshchina VV, Mukhin EN (1985a) Acetylcholinesterase activity in chloroplasts and acetylcholine effects on photochemical reactions. Photosynthetica 19:164–171

Roshchina VV, Mukhin EN (1985b) Acetylcholine action on the photochemical reactions of pea chloroplasts. Plant Sci 42:95–98

Roshchina VV, Yashin VA (2014) Neurotransmitters catecholamines and histamine in allelopathy: plant cells as models in fluorescence microscopy. Allelopathy J 24:1–15

Rueffer M, Zenk MH (1987) Distant precursors of benzylisoquinoline alkaloids and their enzymatic formation. Z Naturforsch 42c:319–332

Rush MD, Kutchan TM, Coscia CJ (1985) Correlation of the appearance of morphinan alkaloids and laticifer cells in germinating *Papaver bracteatum* seedlings. Plant Cell Rep 4:237–240

Sagane Y, Nakagawa T, Yamamoto K, Michikawa S, Oguir S, Momonoki YS (2005) Molecular characterization of maize acetylcholinesterase. A novel enzyme family in the plant kingdom. Plant Physiol 138:1359–1371

Sanchez-Barcelo EJ, Mediavilla MD, Vriend J, Reiter RJ (2016) COP1 and COP9 signalosome, evolutionarily conserved photomorphogenic proteins as possible targets of melatonin. J Pineal Res 61:41–51

Sarropoulou V, Dimassi-Theriou K, Therios I, Koukourikou-Petridou M (2012a) Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). Plant Physiol Biochem 61:162–168

Sarropoulou VN, Therios IN, Dimassi-Theriou KN (2012b) Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P. mahaleb*). J Pineal Res 52:38–46

Sarrou E, Therios I, Dimassi-Theriou K (2014) Melatonin and other factors that promote rooting and sprouting of shoot cuttings in *Punica granatum* cv. Wonderful. Turk J Bot 38:293–301

Shi H, Chan Z (2014) The cysteine2/histidine2-type transcription factor ZINC FINGER OF ARABIDOPSIS THALIANA 6-activated C-REPEAT-BINDING FACTOR pathway is essential for melatonin-mediated freezing stress resistance in *Arabidopsis*. J Pineal Res 57:185–191

Shi H, Reiter RJ, Tan D-X, Chan Z (2014) INDOLE-3-ACETIC ACID INDUCIBLE 17positively modulates natural leaf senescence through melatonin-mediated pathway in *Arabidopsis*. J Pineal Res 58:26–33

Shi H, Jiang C, Ye T, Tan DX, Reiter RJ, Zhang H, Liu R, Chan Z (2015a) Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. J Exp Bot 66:681–694

Shi H, Tan D-X, Reiter RJ, Ye T, Yang F, Chan Z (2015b) Melatonin induces class A1 heat-shock factors (HSFA1s) and their possible involvement of thermotolerance in *Arabidopsis*. J Pineal Res 58:335–342

Shi H, Wang X, Tan D-X, Reiter RJ, Chan Z (2015c) Comparative physiological and proteomic analyses reveal the actions of melatonin in the reduction of oxidative stress in Bermuda grass (Cynodon dactylon (L). Pers.). J Pineal Res 59:120–131

Shi H, Wei Y, He C (2016a) Melatonin-induced CBF/DREB1s are essential for diurnal change of disease resistance and CCA1 expression in *Arabidopsis*. Plant Physiol Biochem 100:150–155

Shi H, Wei Y, Wang Q, Reiter RJ, He C (2016b) Melatonin mediates the stabilization of DELLA proteins to repress the floral transition in *Arabidopsis*. J Pineal Res 60:373–379

Skirycz A, Swiedrych A, Szopa J (2005) Expression of human dopamine receptor in potato (*Solanum tuberosum*) results in altered tuber carbon metabolism. BMC Plant Biol 5:1

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11:118–130

Sliwiak J, Dauter Z, Jaskolski M (2016) Crystal structure of Hyp-1, a *Hypericum perforatum* PR-10 protein, in complex with melatonin. Front Plant Sci 7:668

Smallman BN, Maneckjee A (1981) The synthesis of acetylcholine by plants. Biochem J 194:361

Steward FC, Bidwell RGS (1958) Nitrogen metabolism, respiration, and growth of cultured plant tissue: PART IV. The impact of growth on protein metabolism and respiration of carrot tissue explants. General discussion of results. J Exp Bot 9:285–305

Steward FC, Bidwell RGS, Yemm EW (1958) Nitrogen metabolism, respiration, and growth of cultured plant tissue: part i. experimental design, techniques, and recorded data: Part II. The interpretation of specific activity data in tracer experiments: Part III. Nitrogen metabolism and respiration of carrot tissue explants as revealed by experiments with C14-labelled substrates. J Exp Bot 1:11–51

Sun Q, Zhang N, Wang J, Zhang H, Li D, Shi J, Li R, Weeda S, Zhao B, Ren S, Guo YD (2015) Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. J Exp Bot 66:657–668

Sun Q, Zhang N, Wang J, Cao Y, Li X, Zhang H, Zhang L, Tan DX, Guo YD (2016) A label-free differential proteomics analysis reveals the effect of melatonin in promoting fruit ripening and anthocyanin accumulation upon post-harvest in tomatoes. J Pineal Res. 61:138–153

Swiedrych A, Stachowiak J, Szopa J (2004) The catecholamine potentiates starch mobilization in transgenic potato tubers. Plant Physiol Biochem 42:103–109

Szafrańska K, Glińska S, Janas KM (2012) Ameliorative effect of melatonin on meristematic cells of chilled and re-warmed *Vigna radiata* roots. Biol Plant 57:91–96

Szafrańska K, Reiter RJ, Posmyk MM (2016) Melatonin application to *Pisum sativum* L. seeds positively influences the function of the photosynthetic apparatus in growing seedlings during paraquat-induced oxidative stress. Front Plant Sci 7:789–712

Szopa J, Wilczynski G, Fiehn O, WEnczel A, Willmitzer L (2001) Identification and quantification of catecholamines in potato plants (*Solarium tuberosum*) by GC-MS. Phytochemistry 58:315–320

Tan D-X, Manchester LC, Di Mascio P, Martinez GR, Prado FM, Reiter RJ (2007) Novel rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. FASEB J 21:1724–1729

Tan D-X, Hardeland R, Manchester LC, Paredes SD, Korkmaz A, Sainz RM, Mayo JC, Fuentes-Broto L, Reiter RJ (2009) The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. Biol Rev 85:607–623

Tan D-X, Manchester LC, Liu X, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ (2012) Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. J Pineal Res 54:127–138

Tan D-X, Hardeland R, Back K, Manchester LC, Alatorre-Jimenez MA, Reiter RJ (2016) On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptamine: comparisons across species. J Pineal Res 61:27–40

Tanada T (1972) On the involvement of acetylcholine in phytochrome action. Plant Physiol 49:860–861

Tezuka T, Akita I, Yoshino N, Suzuki Y (2007) Regulation of self-incompatibility by acetylcholine and cAMP in *Lilium longiflorum*. J Plant Physiol 164:878–885

Tiryaki I, Keles H (2012) Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. J Pineal Res 52:332–339

Toriyama H (1978) Observational and experimental studies of the meristem of leguminous plants. I. Effects of acetylcholine, red light and far-red light upon the protoplasts of root tip meristem. Cytologia 43:325–337

Tretyn A (1987) Influence of red light and acetylcholine on $^{45}Ca^{2+}$ uptake by oat coleoptile cells. Cell Biol Int Rep 11:887–896

Tretyn A, Kendrick RE (1991) Acetylcholine in plants: Presence, metabolism and mechanism of action. Bot Rev 57:33–73

Tretyn A, Kopcewicz J, Ślesak E (1988) Interaction of light and the cholinergic system in the regulation of seed germination. Biol Plant 30:338–342

Turi CE, Axwik KE, Smith A, Saxena PK, Murch SJ (2014) Galanthamine, an anticholinesterase drug, effects plant growth and development in *Artemisia tridentate* Nutt. via modulation of auxin and neutrotransmitter signaling. Plant Signal Behav 9:e28645

Udenfriend S, Lovenberg W, Sjoerdsma A (1959) Physiologically active amines in common fruits and vegetables. Arch Biochem Biophys 85:487–490

Verbeek M, Vendrig JC (1977) Are acetylcholine-like cotyledon-factors involved in the growth of the cucumber hypocotyl? Z Pflanzenphysiol 83:335–340

Verelst WIM, Asard HAN (2004) Analysis of an *Arabidopsis thaliana* protein family, structurally related to cytochromes b 561 and potentially involved in catecholamine biochemistry in plants. J Plant Physiol 161:175–181

Waalkes TP, Sjoerdsma A, Creveling CR, Weissbach H, Udenfriend S (1958) Serotonin, norepinephrine, and related compounds in bananas. Science 127:648–650

Wang P, Sun X, Li C, Wei Z, Liang D, Ma F (2012a) Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. J Pineal Res 54:292–302

Wang P, Yin L, Liang D, Li C, Ma F, Yue Z (2012b) Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. J Pineal Res 53:11–20

Wang P, Sun X, Chang C, Feng F, Liang D, Cheng L, Ma F (2013) Delay in leaf senescence of *Malus hupehensis* by long-term melatonin application is associated with its regulation of metabolic status and protein degradation. J Pineal Res:424–434

Wang P, Sun X, Wang N, Tan DX, Ma F (2015) Melatonin enhances the occurrence of autophagy induced by oxidative stress in *Arabidopsis* seedlings. J Pineal Res 58:479–489

Wang Q, An B, Wei Y, Reiter RJ, Shi H, Lu H, He C (2016) Melatonin regulates root meristem by repressing auxin synthesis and polar auxin transport in *Arabidopsis*. Front Plant Sci 07:1–11

Weeda S, Zhang N, Zhao X, Ndip G, Guo Y, Buck GA, Fu C, Ren S (2014) *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. PLoS One 9:e93462

Wei W, Li Q-T, Chu Y-N, Reiter RJ, Yu XM, Zhu DH, Zhang WK, Ma B, Lin Q, Zhang JS, Chen SY (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. J Exp Bot 66:695–707

Wei Y, Zeng H, Hu W, Chen L, He C, Shi H (2016) Comparative transcriptional profiling of melatonin synthesis and catabolic genes indicates the possible role of melatonin in developmental and stress responses in rice. Front Plant Sci 7:676–615

Wen D, Gong B, Sun S, Liu S, Wang X, Wei M, Yang F, Li Y, Shi Q (2016) Promoting roles of melatonin in adventitious root development of *Solanum lycopersicum* L. by regulating auxin and nitric oxide signaling. Front Plant Sci 7:787–711

Wolf K, Kolář J, Witters E, van Dongen W, van Onckelen H, Machackova I (2001) Daily profile of melatonin levels in *Chenopodium rubrum* L. depends on photoperiod. J Plant Physiol 158:1491–1493

Wurzinger B, Mair A, Pfister B, Teige M (2014) Cross-talk of calcium-dependent protein kinase and MAP kinase signaling. Plant Signal Behav 6:8–12

Xue H-W, Chen X, Mei Y (2009) Function and regulation of phospholipid signalling in plants. Biochem J 421:145–156

Yunghans H, Jaffe MJ (1970) Phytochrome controlled adhesion of mung bean root tips to glass: a detailed characterization of the phenomenon. Physiol Plant 23:1004–1016
Yunghans H, Jaffe MJ (1972) Rapid respiratory changes due to red light or acetylcholine during the early events of phytochrome-mediated photomorphogenesis. Plant Physiol 49:1–7
Zhang N, Zhang H-J, Zhao B, Sun QQ, Cao YY, Li R, Qu XX, Weeda S, Li L, Ren S, Reiter RJ, Guo YD (2013a) The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. J Pineal Res 56:39–50
Zhang N, Zhao B, Zhang HJ, Weeda S (2013b) Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). J Pineal Res 54(1):15–23
Zhang H-J, Zhang N, Yang R-C, Wang L, Sun QQ, Li DB, Cao YY, Weeda S, Zhao B, Ren S, Guo YD (2014) Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA 4 interaction in cucumber (*Cucumis sativus* L.). J Pineal Res 57:269–279
Zhang N, Sun Q, Li H, Li X, Cao Y, Zhang H, Li S, Zhang L, Qi Y, Ren S, Zhao B, Guo YD (2016) Melatonin improved anthocyanin accumulation by regulating gene expressions and resulted in high reactive oxygen species scavenging capacity in cabbage. Front Plant Sci 7:197–117
Zhao Y, Tan D-X, Lei Q, Chen H, Wang L, Li QT, Gao Y, Kong J (2013) Melatonin and its potential biological functions in the fruits of sweet cherry. J Pineal Res 55:79–88
Zhao H, Su T, Huo L, Wei H, Jiang Y, Xu L, Ma F (2015a) Unveiling the mechanism of melatonin impacts on maize seedling growth: sugar metabolism as a case. J Pineal Res 59:255–266
Zhao H, Xu L, Su T, Jiang Y, Hu L, Ma F (2015b) Melatonin regulates carbohydrate metabolism and defenses against Pseudomonas syringae pv. tomato DC3000 infection in *Arabidopsis thaliana*. J Pineal Res 59:109–119
Zheng X, Tan DX, Allan AC, Zuo B, Zhao Y, Reiter RJ, Wang L, Wang Z, Guo Y, Zhou J, Shan D, Li Q, Han Z, Kong J (2017) Chloroplastic biosynthesis of melatonin and its involvement in protection of plants from salt stress. Sci Rep 7:41236–41212
Zohar R, Izhaki I, Koplovich A, Ben-Shlomo R (2011) Phytomelatonin in the leaves and fruits of wild perennial plants. Phytochem Lett 4:222–226
Zuo B, Zheng X, He P, Wang L, Lei Q, Feng C, Zhou J, Li Q, Han Z, Kong J (2014) Overexpression of MzASMT improves melatonin production and enhances drought tolerance in transgenic *Arabidopsis thaliana* plants. J Pineal Res 57:408–417

Lauren Alexandra Elizabeth Erland is currently completing her Ph.D. at the University of Guelph in Dr. Praveen Saxena's lab. Her doctoral work is toward understanding the roles of neurotransmitters in plant morphogenesis. She has a particular interest in establishing the indoleamines as a new class of plant growth regulators but continues to explore unusual classes of plant growth regulators for their roles in every aspect of the plant life cycle, particularly in Canadian and medicinal plant species.

Praveen K. Saxena is a Professor in the Department of Plant Agriculture, University of Guelph, and has over 30 years of experience in plant biotechnology focusing on the application of in vitro technologies for production of value-added plants for horticultural industries in Canada. A central unifying theme of his research has been to understand the control of morphogenesis in plants. This has often centered on unique, nontraditional plant growth regulators such as melatonin and serotonin to understand their role in diverse functions they modulate and their interactions with other plant-signaling networks, particularly with respect to growth, reproduction, and mitigation of abiotic and biotic stresses. Dr Saxena's research on biotechnology-based conservation of endangered plant species provides a platform for interdisciplinary research, education, and services in fundamental and applied research in conservation biology (www.gripp.ca). He also publishes the online magazine *Spiritual Botany* (www.spiritualbotany.com) to learn, envision, and disseminate information on plant-human relationship and environmental consciousness.

Part III

Information Communication and Integration

"Trees are the earths endless effort to speak to the listening heaven"

Rabindra Nath Tagore

"Autumn is a second spring when every leaf is a flower"

Albert Camus

The Plant Cell Wall: Barrier and Facilitator of Environmental Perception

17

Inder M. Saxena

Abstract

The plant cell wall is an assembly of ions, small molecules, macromolecules, and higher-order structures that surround plant cells. All plant cells start with a primary cell wall, the major components of which are polysaccharides – cellulose, hemicelluloses, and pectin. The primary cell wall is a dynamic structure that undergoes constant remodeling through synthesis, modification, and altered interactions of its macromolecular and other contents. Cells with only a primary cell wall have the ability to grow/expand or not to do so in response to a variety of intrinsic and extrinsic environmental cues through mechanisms that involve the cell wall. Depending on the environment, the cell wall may extend irreversibly with the increasing volume of an expanding cell or the cell wall may become rigid preventing the cell from expanding. How do a variety of abiotic and biotic signals interact with and influence the cell wall? Significant advances have been made in the last few years in our understanding of the physical basis of the signals, their receptors, and the downstream events that lead to remodeling of the cell wall. While some signal molecules are not cell wall-derived, for example, those from pathogens (PAMPs), in other cases, the cell wall is a source of signals, either in the form of signaling molecules (DAMPs) or changes in the composition/structure of the wall. It is believed that these signals are recognized by cell surface receptors that upon activation trigger, among other effects, change in the expression of a number of wall-related genes that code for wall-modifying proteins. In a feedback response, signals from the wall are sensed for modification of the wall. Many of the signaling pathways that utilize the cell wall as both a source of signals and a response target are the ones that operate during pattern-triggered immunity (PTI) and in the maintenance of cell wall integrity (CWI).

I. M. Saxena (✉)
Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA
e-mail: imsaxena@austin.utexas.edu

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_17

Keywords
Cell wall damage (CWD) · Cell wall integrity (CWI) · Cell wall · Environmental stress · Pattern-triggered immunity (PTI) · Receptors · Signaling

17.1 Introduction

The first impression of any wall, biological or non-biological, is that it is a physical barrier built from components that give it structure and mechanical strength. In most cases, walls are viewed as static structures that do not undergo major changes once they are built. Almost all plant cells are surrounded by a cell wall (the primary cell wall), with some cells having two cell walls (the primary cell wall and the secondary cell wall). Unlike non-biological walls, plant cell walls, and more specifically the primary cell wall, are dynamic structures that undergo constant modifications by sensing and responding to changes in both the internal and external cellular environment (Wolf et al. 2012). Recognition that plant cell walls play a sensory role has come about through studies in many fields of plant biology, and we are now at a point where there is sufficient evidence to state that the plant cell wall is not just a barrier but also a sensory structure.

Though plant cell walls may appear to be simple, they are some of the most complex biological structures (Albersheim et al. 2011). The major components of plant cell walls are polysaccharides. Additionally, walls contain a variety of proteins, and in the case of secondary cell walls, also lignin. Even as it is possible to determine the composition of plant cell walls (although not completely and not an easy task), it has yet not been possible to know exactly how the different wall components interact with each other to give rise to a structure that performs all the functions associated with the plant cell wall. This picture gets even more complicated when one considers that there are differences in the wall between different cells and even within the wall of a single cell. From the viewpoint of sensing and responding to the wide range of environmental conditions, these differences in the cell wall help different cells recognize and respond differently to changes in the environment.

Plants are sessile organisms that do not have the ability to move when the environmental conditions are not ideal or stressful. Moreover, different parts of the plant, for example, root and shoot, are exposed to different environments. Since plants are exposed to a much wider range of environmental stimuli compared to most other organisms, they have developed efficient and robust mechanisms to sense and respond to a variety of stimuli. A large number of these stimuli are environmental stresses (abiotic and biotic) that signal plant cells to respond in a defensive manner. One widely held view is that developing constitutive defense mechanisms to various environmental stresses would not be the best strategy for plants (or any organism) to cope with their environmental challenges, as it will drain resources and constrain productivity and reproduction (Bacete et al. 2018). Instead, plants have developed mechanisms to monitor a variety of stresses in the environment and elicit specific responses to adapt to their environmental challenges. The

plant cell wall is one of the environmental monitoring systems used by plants. This role of the wall is in addition to its function as a physical barrier against biotic factors and abiotic forces.

The plant cell wall covers the plant cell and in most cases is the first structure that encounters the external environment. Since it is in direct contact with the plasma membrane, it has also the ability to monitor the internal cellular environment through the plasma membrane. In addition, there are a number of transmembrane proteins that have regions extending both in the cytosol and in the cell wall, including a large number of receptors or receptor-like proteins (Shiu and Bleecker 2003). The chemical composition and the physical architecture of the wall gives it the ability to monitor the environment by undergoing changes that can be signaled downstream. In general, the environmental stimuli are monitored through changes in the wall composition and/or structure that can be directly sensed by specific membrane receptors or through the generation of molecules generated by the wall (damage-associated molecular patterns or DAMPs) that are presumably recognized by membrane receptors to trigger a response (de Azevedo Souza et al. 2017). The objective in every case is to recognize the signals and generate changes that allow plants to adapt to a wide range of environmental conditions.

Because the primary cell wall is a dynamic structure, changes occur in the wall constantly. Many of these changes are considered to be cell wall damage (CWD) that could be caused by cell elongation or abiotic and biotic stresses. There is growing evidence that plants monitor and maintain cell wall integrity (CWI) through signaling mechanisms that sense changes in the wall and respond to these changes (Hamann 2012). Plants also have mechanisms that recognize microbe-associated molecular patterns (MAMPs) (Boller and Felix 2009) as well as changes in the wall during pathogen attack and activate signaling events (Couto and Zipfel 2016) that result in pattern-triggered immunity (PTI). If the plant cell wall is a target for change during normal growth and development and also during biotic or abiotic stresses, how does a cell recognize that a change in the wall is an environmental stress signal or an event during normal growth and development?

Plant cells have the ability to detect the cause of the cell wall damage through specific mechanisms and respond in an adaptive manner. The two main mechanisms by which plant cells sense and respond to changes in the cell wall are CWI maintenance and the PTI signaling mechanisms. The robustness of cell wall signaling is highlighted in experiments that show that these two mechanisms cross talk such that the CWI maintenance mechanism acts as a backup in case the PTI mechanism is impaired (Engelsdorf et al. 2018).

The role of the plant cell wall in signaling is an exciting topic where much needs to be discovered. What do we know of the structure of the plant cell wall? What are the signals sensed by the wall? How are these signals sensed? How does the cell wall signal to the cell? How do cells respond to these signals? These questions encompass a wide range of subjects ranging from structure of polysaccharides to plant defense responses. This review will address many of these questions in an attempt to provide a dynamic view of the plant cell wall where the components of the wall are receiving signals from the cell and the environment, sending signals to the cell, and undergoing modifications.

17.2 Plant Cell Walls Are Composites of Polysaccharides, Proteins, and Lignin

Plant cell walls are multilayered structures that from the outside to the inside consist of the middle lamella, the primary cell wall, and in specialized cells the secondary cell wall. The middle lamella is derived from the cell plate formed during cytokinesis, and it is sandwiched between the primary cell walls of adjoining cells. As a cell divides, its primary cell wall is now the primary cell wall of the daughter cells, and this cell wall incorporates new wall material and undergoes remodeling during cell expansion. These two layers, the middle lamella and the primary cell wall, are part of all plant cell walls, and they contain polysaccharides and proteins that allow cell-cell adhesion, cell expansion, and determination of cell shape. The third layer, the secondary cell wall, is characterized by the presence of lignin and/or suberin and the deposition of these molecules makes the wall hydrophobic and impermeable to water, even as the outer layers of the wall (the middle lamella and primary cell wall) are hydrophilic. Secondary cell wall synthesis follows cessation of primary cell wall expansion; however, the molecular basis of this transition is not fully understood though a number of genes that are upregulated or downregulated during the switch have been identified (Li et al. 2016a). Much of the discussion on the role of the plant cell wall in signaling will be related to the primary cell wall.

In the context of cell signaling, plant cell walls are viewed not only as targets of signaling but also as generators of signals. The range of functions of plant cell walls is reflected in the variety of molecules and the amounts in which they are present in individual walls (Burton et al. 2010). Although there is wide variety in their composition, plant cell walls are built using common principles. What are these common principles? To begin with, all plant cell walls are built from two main classes of macromolecules – polysaccharides and proteins. In addition to these macromolecules, plant cell walls also contain lignin. While the plant cell walls are composed largely of the polysaccharides cellulose, hemicelluloses, and pectin, they may have about 10% protein and up to 40% lignin. These macromolecules interact with each other through covalent and non-covalent bonds to form the functional cell wall.

The plant cell wall was first viewed by Robert Hooke in 1665, but it took almost three centuries before a molecular model of the primary cell wall was proposed (Keegstra et al. 1973). In this and subsequent models, the primary cell wall was represented as a tethered network in which rigid cellulose microfibrils were separated from one another by a gel-like, hydrated pectic matrix with connections between cellulose microfibrils made by extended xyloglucan chains. In these models, xyloglucan functioned as the load-bearing tether (Carpita and Gibeaut 1993). A reevaluation of the roles of cellulose, xyloglucan, and pectin in wall structure and growth has led to a revised view of the cell wall in which cellulose microfibrils make a load-bearing network via close physical contacts with one another in bundled regions. In these revised models, cellulose microfibrils are bundled by direct contacts and by forming cellulose-xyloglucan-cellulose junctions that are the sites of wall loosening (Cosgrove 2018).

The major polysaccharide and load-bearing component in most plant cell walls is cellulose, a linear polysaccharide of β-1,4-linked glucose units, that is synthesized on the plasma membrane by cellulose synthases (CesAs) that are part of large multiprotein complexes, often referred to as cellulose-synthesizing complexes or cellulose synthase complexes (CSCs) (Turner and Kumar 2018). The glucan chains emerging from these complexes on the extracellular side assemble into cellulose microfibrils (CMFs), with each microfibril containing 18–24 glucan chains (Newman et al. 2013; Wang and Hong 2016). The arrangement of the glucan chains in CMFs creates an amphiphilic structure with both hydrophilic regions and hydrophobic regions, and these regions allow CMFs to interact with each other and with other components of the cell wall, including hemicelluloses and pectin, through non-covalent bonds (Zhao et al. 2014). Interestingly, many of the interactions between polysaccharides in the plant cell wall are believed to be non-covalent.

Synthesis of CMFs occurs at complexes on the plasma membrane that are visualized by freeze-fracture electron microscopy as rosettes with a sixfold symmetry (Mueller and Brown 1980). The rosettes are assemblies of CesAs (Kimura et al. 1999) and possibly other proteins. Each rosette contains three different CesAs, with different sets of CesAs required for cellulose synthesis during primary cell wall and secondary cell wall formation. In *Arabidopsis thaliana*, ten CesAs have been identified with CesA 1, 3, and 6-like (2, 5, 6, and 9) required for cellulose synthesis in the primary cell wall and CesA 4, 7, and 8 required for cellulose synthesis in the secondary cell wall. The stoichiometry of the three different CesAs in each rosette is 1:1:1. Based on the number of glucan chains in CMFs in plants, 36 CesAs were suggested to be present in each rosette, with each rosette subunit having 6 CesAs. The number of glucan chains and the corresponding number of CesAs in the rosette have been revised in recent years with the current view being that there may just be 18–24 CesAs in each rosette complex (Fernandes et al. 2011; Newman et al. 2013; Nixon et al. 2016).

The parallel arrangement of CMFs and cortical microtubules, observed by fluorescence microscopy, suggested the role of microtubules in determining the orientation of CMFs. A dynamic view of the movement of CesAs in the plasma membrane provided direct evidence that the direction of movement of cellulose synthases and the arrangement of cellulose microfibrils is determined by cortical microtubules (Paredez et al. 2006). A protein (CSI1/POM2) that interacts with both microtubules and cellulose synthases has now been identified (Bringmann et al. 2012; Li et al. 2012).

Unlike cellulose, many of the other polysaccharides, including hemicelluloses and pectin, present in plant cell walls are made of a number of different monosaccharides linked to each other by a variety of linkages, for example, α-1,4 linkage, β-1,4 linkage, etc. Additionally, these polysaccharides may be branched or unbranched, and it is these polysaccharides that provide diversity to the wall. In contrast to cellulose and callose (β-1,3 glucan), that appear in the extracellular space as they are synthesized by enzymes present in the plasma membrane, these polysaccharides are synthesized in the Golgi apparatus and transported via vesicles to the plasma membrane where they are released into the extracellular space and assembled into the cell wall.

Hemicelluloses, made from a variety of monosaccharide subunits, are heterogeneous polymers that include xyloglucan, xylans, mannans, glucomannans, and others. Xyloglucan consists of β-1,4-linked glucose units substituted at most C6 positions with xylose and additional glycosyl residues in many cases. Variation in the frequency and composition of these side chains affect the role of xyloglucan. Xylan is a major hemicellulosic polysaccharide in secondary cell walls and in grass primary cell walls. It is also present in reduced amounts in primary cell walls of dicotyledonous plants. Xylan is composed of a backbone of 1,4-linked β-D-xylopyranosyl (β-1,4 linked xylose) residues that may be partially glycosylated at O-2 or O-3 (C2 or C3) with arabinofuranosyl residues and/or at O-2 (C2) with 4-O-methyl glucuronosyl residues to form arabinoxylan and/or glucuronoarabinoxylan. Dicot xylan is less frequently arabinosylated, with reported arabinosylation generally at the O-2 of xylose. Xylan may also be acetylated at O-3.

Pectin is a family of galacturonic acid (GalA)-rich polysaccharides that account for 30–35% (w/w) of primary cell walls in dicots and nongraminaceous monocots but is also present in secondary walls and in grasses. The most abundant pectic polysaccharide, homogalacturonan (HG), is a linear polymer of α-1,4-linked GalA residues that may reach lengths of 100 residues. HG accounts for ~65% of pectin. The other major pectin, rhamnogalacturonan I (RG-I), comprises 20–35% of pectin and consists of a repetitive (2-α-Rha-1,4-α-GalA-1) disaccharide backbone with 20–80% of the rhamnosyl residues having side chains of 1,5-arabinans, 1,4-galactans, and type I and type II arabinogalactans. Substitution of the GalA residues of HG with four complex side chains forms rhamnogalacturonan II (RG-II), representing ~10% of wall pectin. Acetylation and methylation of pectin in vivo may further change the charge and hydrophobicity of these polysaccharides.

Proteins in the plant cell wall include enzymes and structural proteins. Arabinogalactan proteins (AGPs) are highly glycosylated hydroxyproline-rich glycoproteins (HRGPs) that consist of up to 95% carbohydrate. These proteins account for <10% of the wall matrix and are associated with a variety of functions, including plant embryogenesis and plant development. However, the molecular basis of these activities is not known. The hydroxyproline residues in AGP usually have covalently attached type II arabinogalactan (AG) chains. Individual AG chains in AGP consist of up to 150 sugar residues and are rich in Ara and Gal. An individual AG chain consists of a β-1,4-galactan backbone with β-1,6-galactosyl branches that are decorated with arabinosyl residues and occasionally with minor sugar residues, such as glucuronic acid (GlcA), rhamnose (Rha), and fucose (Fuc). Type II AGs are also found as side chains of the pectin RG-I and as free polysaccharides.

Plant cell walls harbor enzymes of the xyloglucan endotransglucosylase/hydrolase (XTH) family. Plant genomes code for about 30 members of this enzyme family that can perform transglycosylation reactions using not only xyloglucan but also cellulose and in some cases mixed-linkage β-1,3, β-1,4 glucans as donors and acceptors. Members of the XTH family have the ability to mediate post-synthetic remodeling of the cellulose-xylogucan network and thus the plant cell wall. Other enzymes in the cell wall include the pectin methylesterases (PMEs) and pectin acetylesterases (PAEs) that modify pectin or the pectin-degrading enzymes such as polygalacturonases and pectate lyase-like (Sénéchal et al. 2014).

In contrast to the non-covalent polysaccharide-polysaccharide interactions, covalent cross-links are reported between proteins (between extensins), pectins (between rhamnogalacturonan II monomers), polysaccharide and lignin (between matrix polysaccharides and phenolic moieties of lignin), and polysaccharide and protein (between matrix polysaccharides pectin and xylan and arabinogalactan proteins) in the plant cell wall (Tan et al. 2013). These interactions suggest that the plant cell wall has a much more complex architecture than originally proposed by Keegstra et al. (1973). Though many of these interactions may have a structural role, it appears that some of these covalent interactions hold many of these complex polysaccharides within the plant cell wall where they may be used in the generation of signals.

17.3 Changes Occur in the Cell Wall During Normal Growth and Development

The dynamic nature of the cell wall signifies that it undergoes constant changes during normal growth and development, and in response to environmental cues. The changes that take place in the cell wall are defined in physical terms as changes in plasticity, elasticity, and other features but also described as cell wall loosening, extensibility, stiffening, etc. Given that these features of the wall are dependent on the components of the wall, one can start to associate the physical changes in the wall to changes in the composition and/or changes in the interactions between different components of the wall.

Cell wall modification is the rearrangement of proteins and polysaccharides with respect to each other as well as the breakdown of cell wall molecules or breakdown and subsequent joining of fragments to preexisting cell wall molecules. The rearrangement of molecules involves non-covalent bonds, while the breakdown or breakdown and subsequent joining of fragments involves covalent bonds; the former may be catalyzed by proteins such as expansins and the latter by proteins such as XTHs. Both these classes of proteins are involved in cell wall loosening.

17.3.1 Auxin-Mediated Cell Expansion Involves Modification of Preexisting Wall and Addition of New Wall Material

Many of the signaling events related to the plant cell wall involve turgor-driven perception. During cell elongation, signaling is mediated by the plant hormones auxin and brassinosteroids. The role of auxin in cell expansion includes its ability to modify the wall such that water enters into the cell to increase turgor pressure for wall extension and synthesis. A feedback loop operates in the chain of events initiated by auxin, whereby cell wall extension is followed by wall compaction that utilizes signaling molecules implicated in host defense.

An increase in the concentration of auxin results in degradation of transcriptional regulators AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) by the TRANSPORT

INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX (TIR1/AFB) complex and the activation of AUXIN RESPONSE FACTORS (ARFs) that mediate a transcriptional response (Majda and Robert 2018). Auxin regulates the expression of a large number of genes, and the cell wall-related genes are just a subset of these genes. In the presence of auxin, ARFs upregulate the expression of several cell wall-related genes, including expansins, XTHs, AGPs, peroxidases, and those related to cellulose and pectin modifications, suggesting that auxin regulates cell expansion not only through acidification of the wall but also by stimulating the expression of genes that code for proteins involved in the modification of components of the wall.

Although auxin mediates a number of cellular events, the first event related to the cell wall is the acidification of the wall that is done through the activation of plasma membrane P-type H^+-ATPases – AHAs. This is the auxin-induced wall acidification. The lowering of the apoplast pH leads to the activation of a number of proteins and enzymes present in the apoplast, the major one being expansin that catalyzes the loosening of the wall by breaking the non-covalent linkages between cellulose microfibrils and xyloglucan and allowing these polysaccharide chains to be freer. Other enzymes, such as the XTHs and cellulases, are also activated in the acidic environment, and they participate in wall remodeling. Auxin-stimulated XTHs modify the polysaccharide network by cutting XG backbones and forming linkages between different XG chains. The cutting of XG loosens the wall and promotes wall rearrangement for cell elongation. More interestingly, the short XG fragments (oligosaccharins) generated by the action of XTHs have been implicated in growth promotion at high concentrations and growth inhibition at low concentrations (Albersheim et al. 2011). While the growth promotion activity of XG-derived oligosaccharins at high concentrations (above 10^{-6} M) is linked to their ability to get incorporated into the wall XG network through the activity of XTHs, the growth inhibition activity of these oligosaccharins at low concentrations (10^{-8} to 10^{-9} M) remains to be explained. These oligosaccharins inhibit auxin-stimulated growth; however, they are not known to act as elicitors, and no receptors have been identified.

At the same time that the protons are pumped into the apoplastic space, the plasma membrane is hyperpolarized, resulting in the opening of K^+ channels and the influx of K^+ into the cytosol. The increased K^+ concentration in the cytosol stimulates water uptake, generating tensile stress and forcing the cell wall to extend. The extension of the cell wall causes stretching of plasma membrane and an influx of Ca^{2+}, presumably through stretch-activated Ca^{2+} channels. The increased cytosolic Ca^{2+} concentration inhibits the H^+ proton pumps and stimulates cytosolic H^+ influx resulting in apoplast alkalization. In an alkaline environment, pectins (HGs) are demethylesterified by PMEs and deacetylated by PAEs making them more accessible for calcium cross-linking and leading to pectin compaction. PMEs also modify the HGs in a manner where cross-linking of polysaccharides and proteins (EXTs) takes place. These modifications cause wall dehydration and compaction decreasing extensibility and growth.

17.3.2 Brassinosteroid (BR) Signaling Plays a Role in Cell Wall Modification and Synthesis

Among the plant hormones that control plant growth and development, brassinosteroids play a significant role in cell expansion. Signaling events initiated by binding of brassinosteroids to cell surface receptor-like kinase (BRI1) result in the degradation of a protein kinase (BIN2) that leads to the accumulation of the BZR family of transcription factors in the nucleus to control the expression of a number of genes. In *Arabidopsis*, BZR1 and BZR2/BES1 bind to the promoter regions of a number of cell wall-related genes, including the cellulose synthase genes (Xie et al. 2011) and transcription factors (NAC and MYB) that are associated with regulation of lignin biosynthesis (Zhao and Dixon 2011).

BR signaling also directly influences cellulose synthase activity posttranscriptionally through degradation of the BIN2 protein kinase that negatively regulates the activity of the primary cell wall cellulose synthase CesA1 (Sanchez-Rodriguez et al. 2017). Additionally, BR signaling also influences cell wall remodeling by upregulating a subset of XTH and expansin genes (Kozuka et al. 2010).

The BR signaling pathway presumably also functions as a compensatory response to protect the plant when pectin is not properly modified. Inhibition of pectin de-methylesterification activates the BR signaling pathway, and a receptor-like protein (RLP) 44 is found to be essential for the compensatory response. RLP 44 mediates activation of BR signaling through direct interaction with the regulatory receptor-like kinase BAK1 (Wolf et al. 2014).

17.4 Changes in the Cell Wall Play a Role in Response to Environmental Stresses

Plants are sessile and, to survive, adapt when the environmental conditions are not favorable. In many cases, the cell wall functions in monitoring these unfavorable conditions (environmental stresses) to elicit an adaptive response that may include modification of the cell wall. Stress perception and general signaling is discussed in Chap. 7.

While some cellular responses are from primary stress signals, others are from secondary effects. For example, the secondary effects of drought and salt stresses are complex, and these secondary effects include oxidative stress which result in damage to membrane lipids, proteins, and nucleic acids as well as metabolic dysfunction. Even as salt stress and drought have unique and overlapping signals, an important feature of both is that the hyperosmotic stress (simply referred to as osmotic stress) they generate causes an accumulation of abscisic acid (ABA) which in turn elicits many adaptive responses.

Plants sense changes in their environment through various sensors (Zhu 2016). While the mechanism of sensing abiotic stresses may not be fully understood, their perception likely involves members of integral membrane receptor-like kinases. Many genes for receptor-like kinases are induced by abiotic stress. A putative

sensor of hyperosmotic response is the *Arabidopsis* OSCA1. Another putative stress sensor is COLD1 that is required for chilling resistance (0–15 °C). COLD1 is a transmembrane protein in the plasma membrane and endoplasmic reticulum (ER) membrane, and it interacts with the α subunit of the sole heterotrimeric G-protein in plants. At present it is unclear as to how COLD1-mediated calcium signaling leads to chilling tolerance.

Common plant responses include production of ROS and an increase in the activity of peroxidases, xyloglucan-modifying enzymes (XTH), and expansins, suggesting that the cell wall is affected by many abiotic stress conditions (Tenhaken 2015). ROS accumulation can cause cross-linking of phenolics and proteins in the wall resulting in its stiffening. An increase in the activity of expansins and xyloglucan-modifying enzymes remodels the wall. Expansins have a role in cell growth and adaptation to stress by loosening and modifying the cellulose and non-cellulose components of the cell wall. Pectins are often modified in plants exposed to drought stress, and an increase in side chains of rhamnogalacturonan I and II (RGI and RGII) was observed (Leucci et al. 2008). Pectins form hydrated gels and this modification may limit the damage to cells.

In *Arabidopsis*, over 500 genes respond to drought, cold, and high-salinity stress, including several members of the extensin, pectinesterase, and XTH/XET families (Seki et al. 2002). Analysis of mutants to identify the role of specific cell wall-related genes in tolerance to abiotic stress reveals that mutant alleles of *AtCesA8* (*lew2-1* and *lew2-2*) show higher tolerance to osmotic stress (exposure to NaCl and mannitol) and drought compared to the wild type, most likely through changes in the cell wall as a consequence of reduced cellulose (Chen et al. 2005). In maize root tissue, multiple cell wall-related genes are differentially expressed under salt stress, including *ZmXET1* that is thought to be involved in cell wall extension (Li et al. 2014). Other genes include the expansin genes *ZmEXPA1*, *ZmEXPA3*, *ZmEXPA5*, etc. The increased expression of cell wall-related genes is linked to an increased expression of histone acetyltransferase genes (*ZmHATB* and *ZmGCN5*) after salt stress, and this was accompanied by increased histone H3K9 and H4K5 acetylation.

Are cell wall-related responses to different abiotic stresses similar or different? For salinity alone, over 140 cell wall-related genes respond to salt stress, and sometimes these genes are different for different *Arabidopsis* ecotypes (Wang et al. 2013). In a study of responses to multiple stresses (drought, fungal, and herbivore), it was found that 12 genes (including *CslG2*) responded in the same way to all three stresses. A total of 41 cell wall-related genes responded to at least one stress. These observations indicate common transcriptional responses and possibly downstream effects on cell wall composition employed by distinct stresses (Coolen et al. 2016).

In comparison of drought-sensitive and drought-resistant cultivars in water-deficit conditions, a set of genes showed more than twofold expression change including 27 cell wall-related genes (Cal et al. 2013). Majority of the cell wall-related genes were downregulated in the drought-resistant cultivar of rice including genes for lignin production (secondary cell wall), arabinogalactan and extensin proteins, XET/XTHs, and glycosyltransferases (GTs) including two CesAs. Two genes upregulated in the drought-tolerant cultivar are members of GH28-encoding polygalacturonase.

Changes affecting the synthesis of cellulose play a role in response to stress. For example, mutations in genes coding for proteins associated with cellulose synthesis such as CesA6, POM2/CSI, and CC (companion of cellulose) enhance sensitivity to salt stress (Zhang et al. 2016). Additionally, mutations in the *KORRIGAN* gene that codes for a cellulase that is suggested to have a role in cellulose biosynthesis lead to growth arrest during salt stress (Endler et al. 2015).

How does salt stress influence cellulose biosynthesis? What is the chain of events?

The cellulose synthase complex (CSC) associates with two proteins – the cellulose synthase interacting 1 protein (CSI1) and companion of cellulose synthase protein (CC). In the plasma membrane, CC associates with the CSC (through one or more transmembrane segments) and cortical microtubules. The cytoplasmic tails of the membrane proteins CC and CSI1 bind to microtubules and promote microtubule dynamics. These proteins have a role in microtubule stability and CSC localization in the plasma membrane, and makes cells less sensitive to stress. In the absence of CC activity (*cc1 cc2* mutants) and salt stress, a stress-tolerant microtubule array is not produced, and CSCs do not repopulate the plasma membrane (Endler et al. 2015).

Identification of a number of transcription factors that regulate synthesis and remodeling of the secondary cell wall under different environmental conditions has allowed modeling of interactions between these transcription factors. Joshi et al. (2018) show a complex transcriptional circuitry for secondary cell wall development influenced by abiotic stress. Abiotic stress is sensed/relayed to *SKP2A* and the BR-signaling pathway. While *SKP2A* acts through the E2Fc pathway that is involved in synthesis of secondary cell wall components, the BR-signaling pathway influences the secondary cell wall remodeling.

17.5 The Cell Wall Integrity (CWI) Maintenance Mechanism Senses Changes in the Cell Wall Through Membrane Receptors and Ion Channels

The cell wall is a physical barrier that exhibits dynamic behavior. Cell wall dynamics occurs during normal growth and development, and it is also influenced by abiotic and biotic stresses. The dynamic behavior of the wall includes changes in the structure and composition of the cell wall through synthesis, breakdown, and modification of the different wall components. Many of these changes are recognized as cell wall damage (CWD) by cell wall integrity (CWI) maintenance mechanism that signal for compensatory responses. These responses include production of callose and lignin, accumulation of hormones (JA, SA, ABA, and ethylene), generation of reactive oxygen species (ROS) (see Chap. 14 also), and activation of Ca^{2+}-based signaling (see Chap. 11). During normal plant growth and development, the CWI maintenance mechanism maintains wall thickness and composition.

Plant cells sense changes in the wall, including CWD, through various transmembrane proteins in the plasma membrane. The transmembrane proteins may be receptor-like kinases (RLKs), wall-associated kinases (WAKs), and ion channels.

While significant progress is made in the identification and understanding the role of many of these transmembrane sensor proteins, in many cases, the signals that activate the CWI maintenance mechanism are not fully known. The most likely scenario is that CWD or cell wall stress leads to a weakening of the wall and (i) the production of DAMPs (carbohydrate and peptide signaling ligands) and (ii) turgor pressure-dependent displacement of the plasma membrane from the wall. Cells may perceive either one or both types of signals arising from the weakening of the wall through the transmembrane receptors and ion channels.

An early example of how changes in the cell wall composition lead to a response was observed in cellulose synthase mutants of *A. thaliana*. Mutation in *cesA3*, a gene that codes for a primary cell wall CesA, resulted in stunted growth, ectopic deposition of lignin, and resistance to plant pathogens (Caño-Delgado et al. 2003). These observations suggested that plant cells sense a deficiency of cellulose in the cell wall and respond to it as if this is an environmental stress, for example, a pathogen attack. A similar response is obtained when plants are treated with an inhibitor (isoxaben) of primary cell wall cellulose synthesis or treated with a mixture of cellulases and pectinases. How do plant cells sense a deficiency of cellulose in the cell wall? Cellulose is the main load-bearing component of the cell wall, and any deficiency of cellulose results in the weakening of the cell wall. Since plant cells exhibit turgor pressure, the cell wall loosening as a result of cellulose deficiency is sensed by the plant cell, triggering a response. Response to reduction in the cellulose content in the cell wall suggests that there are mechanisms that monitor CWD and respond through CWI maintenance mechanisms.

Plasma membrane-localized kinase proteins with a distinct extracellular domain are candidates for cell surface receptors. In *Arabidopsis*, approximately 600 receptor-like kinases have been identified. However, it is unclear how many cell wall components are ligands of these proteins.

17.5.1 Wall-Associated Kinases (WAKs) Have EGF-Like Repeats in Their Ectodomain and They Sense Pectin Integrity in the Wall

One of the best-studied potential CWI receptors are wall-associated kinases (WAKs). WAKs are membrane receptors with a transmembrane region, an intracellular serine/threonine kinase domain and an extracellular region that contains two EGF-like (cysteine-rich) repeats. *A. thaliana* genome has five WAK genes arranged in a gene cluster and several WAK-like genes. The different WAKs identified in *A. thaliana* show differences in the EGF repeats. WAKS are thought to be DAMP receptors, the extracellular region of which senses pectin integrity by binding to OGAs that are 10–15 units long. In *Arabidopsis*, WAK1 is identified as a receptor for OGAs (Brutus et al. 2010). Glycine-rich proteins (GRPs) also bind strongly to at least one of the WAKs (Park et al. 2001). This binding appears to activate the receptor, but downstream elements of the signaling cascade are not yet identified. WAKs may serve special functions in cells subjected to compression or expansion,

based on expression of the genes. WAK genes are also induced by pathogen infection and wounding. WAKs and WAK-like proteins are implicated in cell expansion, salt tolerance, and the coordination of solute concentrations with growth.

17.5.2 The CrRLK1-Like Receptors Have Malectin-Like Ectodomain and They Bind to Carbohydrate and Peptide Ligands

The CrRLK1-like (*Catharanthus roseus* receptor-like kinase 1-like) family of cell wall signaling receptors are candidates for CWI sensors. All CrRLK1 family members (*Arabidopsis* has 17 CrRLK1 members) have a conserved ectodomain (Boisson-Dernier et al. 2011) and so it is likely that they bind to similar ligands. For instance, they may bind to RALF-like peptides or carbohydrates as most ectodomains contain one or two "malectin" domains.

A CrRLK1-like family member, THESEUS1 (THE1) is suggested to be the sensor of CWD, as responses to CWD are absent or reduced in *the1* loss-of-function mutants and enhanced upon THE1 overexpression (Hématy et al. 2007; Denness et al. 2011). THE1 was identified in a screen for suppression of elongation defects of the cellulose-deficient mutant *cesA6*/*procuste 1-1* (*prc1-1*) (Hématy et al. 2007). Mutations in THE1 attenuate the growth inhibition and ectopic lignification of several cellulose-deficient mutants without rescuing cellulose deficiency. THE1 is also required for oxidative burst induced by isoxaben in the root. Among genes upregulated in the cellulose-deficient mutant *prc1-1*, a subset depends on THE1 signaling. Some of these genes encode ROS-detoxifying enzymes, extensins, and a peroxidase, suggesting a role in cell wall cross-linking. Other genes encode enzymes involved in synthesis of glucosinolates and other defense proteins indicating a role in pathogen defense. *the1* mutants do not show any observable change in phenotype under normal growth conditions, confirming the role of THE1 as a sensor of cell wall damage.

Other members of the CrRLK1-like family are also shown to have a role in sensing CWI. FERONIA (FER), a member of this family, in addition to its role in CWI sensing, is involved in ovule fertilization, growth control, mechanoperception, and pathogen response (Li et al. 2016b). FER has malectin-like ectodomain, and in addition to binding to cell wall components, it has also been shown to bind to the RALF family of peptides to regulate plant cell elongation (Haruta et al. 2014).

More recently, Feng et al. (2018) have shown that FER also plays a role in salt tolerance by sensing CWI. At high salt concentrations, the cell wall softens most likely by Na^+ affecting the pectin cross-links. These changes in the wall are sensed by FER through its binding to cell wall components, most likely pectin. FER activation leads to transient calcium influx, which triggers the secretion of pectin and/or the formation of calcium and boron linkages between pectin polymers.

17.5.3 The LRR Ectodomain-Containing Receptors Bind to Peptides and Are Involved in Both CWI Sensing and Immune Responses

The largest subgroup of RLKs and receptor-like proteins (RLPs) is formed by proteins with a LRR (leucine-rich repeat) ectodomain (Wolf 2017). All known LRR-containing PRRs recognize peptide ligands, and many of them play an important role in plant immunity. LRR-RLKs act by forming heterodimers with RLKs from the SOMATIC EMBRYOGENESIS RECEPTOR KINASEs (SERKs) family like BAK1/SERK1 in a ligand-dependent manner. RLPs interact with proteins that contain cytoplasmic kinase domains, such as SUPPRESSOR OF BIR1 (SOBIR1) and BAK1.

Two very similar LRR-RLKs, FEI1 and FEI2, known to be involved in CWD responses, were first identified on the basis of sucrose-dependent swollen-root phenotype of *fei1 fei2* double mutant seedlings that is similar to that observed in the cellulose synthase mutant *prc1-1* and in isoxaben-treated seedlings (Xu et al. 2008). FEI1 and FEI2 control cellulose biosynthesis and anisotropic growth under high-sucrose and high-salinity conditions, acting together with SALT OVERLY SENSITIVE5 (SOS5) (Shi et al. 2003; Xu et al. 2008).

MIK2 is another LRR-RLK that has a role in CWI maintenance by sensing cell wall perturbations. MIK2 was identified in a screen of mutants insensitive to inhibitors of cellulose biosynthesis (Van der Does et al. 2017). Loss-of-function *mik2* mutants are affected in immune marker gene expression, JA production, and lignin deposition. Interestingly, MIK2 has both overlapping and distinct functions with THE1 in response to inhibition of cellulose biosynthesis.

In addition to FEI1, FEI2, and MIK2 that are involved in CWI sensing upon CWD, two LRR-RLKs – PEPR1 and PEPR2 – are linked to cell wall-mediated immune responses through binding of the Pep peptides (considered DAMPs) that are released during CWD. The peptide Pep1 is processed from the Pep1 precursor that is encoded by the *PROPEP1* gene, and this gene is upregulated in the presence of the cellulose synthase inhibitor isoxaben (Engelsdorf et al. 2018) and during pathogen infection and wounding (Huffaker et al. 2006). Pep1 is recognized by PEPR1 and PEPR2, and it functions as a PTI response enhancer (Bartels and Boller 2015). Oligogalacturonides (OGs) are also shown to activate the expression of *PROPEP2* and *PROPEP3* genes (Gravino et al. 2017). The precursor peptides encoded by these two genes are processed to Pep 2 and Pep3, and these peptides interact with PEPR1 and PEPR2 resulting in the upregulation of the *PATHOGENESIS RELATED 1* (*PR1*) gene and enhanced resistance to a fungal pathogen. Interestingly, the upregulation of *PROPEP2*, but not of *PROPEP3*, is ethylene dependent, linking Pep2 as a component in the pathway from OG perception to ethylene and the downstream responses (Gravino et al. 2017).

17.5.4 Osmosensors Sense Direct and Indirect Effects of Osmotic Imbalance Across a Membrane

CWD responses induced by different stimuli are osmosensitive. While osmosensitivity distinguishes CWI signaling from DAMP- and PAMP-dependent responses, our understanding of osmosensing is rather limited. In plants, "osmosensing" includes both the direct perception of osmotic imbalance across a membrane (by yet to identify mechanisms) as well as the perception of indirect effects of osmotic imbalance on the membrane, cell wall, or membrane-cell wall system (Haswell and Verslues 2015). An osmosensor identified in plants is the *Arabidopsis* histidine kinase AHK1, that along with other AHKs was identified following complementation of the osmosensing-deficient mutant impaired in SLN1 in yeast (Urao et al. 1999). Even as the mechanism of osmosensing by AHK1 is not understood, studies in *Arabidopsis* show that AHK1 is a positive regulator of drought and salt stress responses and abscisic acid (ABA) signaling (Tran et al. 2007). Other recent studies on osmosensing has been also covered in Chap. 10.

17.5.5 Mechanoreceptors and Mechanosensitive Ion Channels Sense Turgor Pressure and Mechanical Stimulus

Plant cells respond to mechanical signals that may originate internally or externally. If one excludes the mechanical perturbations coming from the environment, like wind, the intrinsic cause of mechanical stress comes down to turgor pressure only (Hamant and Haswell 2017). Moreover, mechanical stimuli reflect not only the strain of the growing cell but also those caused by growth of other cells in a tissue. Two classes of proteins perform mechanical sensing in plant membranes – RLKs that perceive CWD and mechanosensitive (MS) ion channels.

The CrRLK1L family member FER has a role in mechanical signal transduction in *Arabidopsis* seedlings (Shih et al. 2014). FER is required for sensing intrinsic mechanical signals associated with growth, and it likely suppresses the strain rate fluctuations observed during cell expansion. In an *Arabidopsis* mutant lacking FER, Ca^{2+} signaling and growth responses to various forms of mechanical perturbations were altered. *fer* mutants exhibit impaired growth phenotypes such as biased root skewing, an inability to penetrate hard agar layers, and abnormal growth responses to impenetrable obstacles (Shih et al. 2014).

Over 20 different MS ion channel activities have been identified in plant membranes (Hamilton et al. 2015). In plants, the role of MS ion channels has been proposed for a number of functions including the perception of gravity, vibration, touch, hyperosmotic and hypoosmotic stress, pathogenic invasion, interaction with commensal microbes, and pollen tube growth. These channels may open by increased membrane tension or via interaction with intracellular or extracellular structures. However, it is not known fully as to how mechanical forces are sensed and how cells discriminate between mechanical noise and mechanical signals (Hamant and Haswell 2017).

Five families of likely plant MS ion channels include MSL (MscS-like), MCA (Mid1-complementing activity), TPK (two pore potassium), OSCA (reduced hyperosmolality-induced [Ca^{2+}] increase), and Piezo channel families with MCA, OSCA, and Piezo involved predominantly in Ca^{2+} flux, MSL in Cl^- flux, and TPK in K^+ flux (Hamant and Haswell 2017). The MCA1 and MCA2 are localized in the plasma membrane in plant cells where they mediate Ca^{2+} influx when triggered by mechanical stimulus or hypoosmotic pressure (Kurusu et al. 2013).

Recent data suggests that both RLKs and MS ion channels work together to regulate CWD responses. The LRR-RLK FEI1 and MCA1 function downstream of THE1, triggering Ca^{2+} influx, ROS production, and JA and SA production and modulating immune-related gene expression (Engelsdorf et al. 2018).

17.6 Signaling Pathways Downstream of CWI-Sensing Receptors Involve Rho of Plants (ROP), Reactive Oxygen Species (ROS), Ca^{2+} Influx, and MAP Kinase Cascades

A wide range of signaling pathways are activated by CWD, and they overlap in large part with responses to abiotic and biotic stresses (Fig. 17.1). CWD induced by inhibition of cellulose biosynthesis stimulates production of callose and lignin; accumulation of JA, SA, and ethylene; generation of reactive oxygen species (ROS); and activation of Ca^{2+}-based signaling (Engelsdorf and Hamann 2014). Even as responses to CWD and various stresses are known, the signaling cascades leading to the observed changes are not fully understood.

The RLK FER is shown to act upstream of several GEFs (ROPGEFs) activating Rho-like GTPases (RAC/ROPs) and leading to ROS-mediated responses (Duan et al. 2010; Huang et al. 2013). While most plant RLKs require kinase function for their activity, a few do not, suggesting that these RLKs perform their function without kinase activity. Interestingly, no direct targets of phosphorylation by candidate CWI-monitoring RLKs have been identified (Engelsdorf and Hamann 2014). Moreover, in the case of FER, it was shown that while the kinase domain is necessary for its function, kinase activity is not necessary (Kessler et al. 2015). Instead of directly phosphorylating downstream components of its signal transduction pathway, FER probably functions as part of a complex with another RLK as a co-receptor to enhance the activity of another kinase that transduces the signal. FER could thus act as a scaffolding protein to bring other components such as ROPGEFs into a complex so that signal transduction can occur (Kessler et al. 2015). Phosphorylation of ROPGEFs in the C-terminal domain relieves autoinhibition of these proteins making them active. ROPGEFs activate ROPs that in turn regulate a variety of events including organization and dynamics of actin and microtubule networks, endocytosis and exocytosis, activation of NADPH oxidase (for ROS production), intracellular kinase cascade, and cell wall sensing during cell growth (Feiguelman et al. 2018).

The peptide RALF1 binds to FER, stimulates its phosphorylation, and regulates its functions. However, it is not clear where RALF1 binds in the extracellular

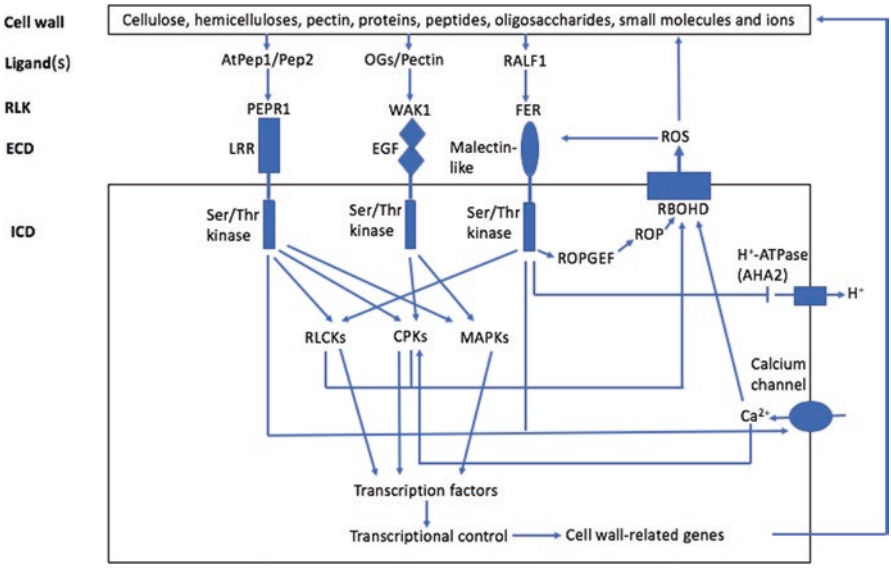

Fig. 17.1 Cell wall-derived signals, receptors, and signaling pathways. The plant cell wall is a complex compartment present outside the plasma membrane. The cell wall is made of mostly polysaccharides – cellulose, hemicelluloses, and pectin. In addition, there are proteins (structural proteins and enzymes) and peptides secreted from the cell that comprise the wall and the apoplast. Cell wall-derived signals may include oligosaccharides (breakdown products or secreted), peptides, polysaccharides, proteins, small molecules, and ions. Many of these signals are recognized by cell surface receptors. Representatives of a few of these receptor classes are shown in the figure. The ectodomain or extracellular domain (ECD), intracellular domain (ICD), and the ligands that are known to bind to these receptors are also indicated. All the receptors shown here have a cytosolic serine/threonine kinase domain. Binding of the ligand promotes assembly of a receptor-signaling complex that involves phosphorylation of the receptor and downstream targets. A number of cytoplasmic protein kinases (RLCKs, CPKs, and MAPKs) play a significant role in downstream phosphorylation events, phosphorylating among others transcription factors, NADPH oxidase (RBOHD), H^+-ATPase (AHA2), and calcium channels. Phosphorylation of transcription factors results in changes in gene expression, including changes in the expression of cell wall-related genes, that may result in cell wall changes (e.g., ectopic lignin deposition or synthesis of callose). Signaling pathways also include an influx of calcium through activation of calcium channels. Activation of NADPH oxidase through phosphorylation and calcium binding or through interaction with ROPs results in production of reactive oxygen species (ROS) in the apoplast. ROS in the apoplast may influence signaling by affecting the ECD of the receptors or the signal molecules

domain of FER or how it induces FER phosphorylation and direct the consequences of FER phosphorylation (Li et al. 2016b). RALF1 treatment enhances phosphorylation of an *Arabidopsis* H^+-ATPase (AHA2) that results in downregulation of its activity and alkalization of the medium and growth suppression (Haruta et al. 2014). It is not known if RALF1-stimulated phosphorylation of AHA2 requires FER, given that kinase inactive forms of FER are shown to be adequate for specific responses (Kessler et al. 2015; Shih et al. 2014). These observations raise the possibility that there may be bifurcation in FER signaling, with some downstream processes

dependent on its kinase activity and others that are independent of the kinase activity, but dependent on other molecules that may be recruited to the FER signaling complex (Li et al. 2016b). FER also plays a role in mechanosensing through a biphasic Ca^{2+} increase (Shih et al. 2014). However, it is not known as to how these Ca^{2+} responses mediate downstream changes.

The LRR-RLKs, PEPR1/PEPR2, recruit receptor-like cytoplasmic kinases (RLCKs) BIK1 and PBL1 for activation of multiple downstream pathways. Pattern recognition by PEPR1/2 triggers a number of cellular events, including production of ROS (Boller and Felix 2009). In *Arabidopsis*, the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) is essential for pattern-triggered ROS production. BIK1 phosphorylates RBOHD at multiple sites, and this is required for ROS production. Also, CALCIUM-DEPENDENT PROTEIN KINASEs (CPKs) are necessary to phosphorylate additional sites in RBOHD for activation (Tang et al. 2017).

ROS production is a frequent result of RLK signaling in a multitude of cellular processes, and RBOH isoforms (RBOHD and RBOHF) play major roles in responses to abiotic and biotic stresses (Kimura et al. 2017). As mentioned earlier, the two classes of signaling components by which RLK activation controls RBOH activity are RLCKs and RAC/ROPs. Apoplastic ROS perception is thought to occur by two modes – direct or indirect. The direct ROS perception model assumes that apoplast-localized soluble or membrane-associated proteins function as sensors and effectors by continuously monitoring the redox status of the apoplast and directly relaying the signal to downstream signaling components. This could involve the direct oxidation of RLK ectodomains and the subsequent activation of signaling functions. The indirect ROS perception model assumes that extracellular peptides or metabolites exist that, upon oxidation, bind to RLKs. Alternatively, the oxidized ROS sensor proteins may oxidize RLK ectodomains via a redox relay mechanism. So far, no targets for apoplastic ROS have been identified (Kimura et al. 2017).

WAKs are the only receptor class implicated in cell wall signaling for which binding to wall components has been documented. WAK1 has been experimentally characterized as an OG receptor (Brutus et al. 2010). Downstream signaling regulated by OGs includes Ca^{2+} influx, calcium-dependent protein kinase (CPK) activation, and phosphorylation of MAPK3 and MAPK6. Three members of the ARABIDOPSIS NPK1-RELATED PROTEIN KINASE (ANP) MAP kinase kinase kinases (MAP 3Ks) family, ANP1, ANP2, and ANP3, are required for OG-triggered signal transduction and ROS production (Savatin et al. 2014).

17.7 Cross Talk Between the CWI Maintenance Mechanisms and PTI Signaling Mechanisms

Changes in the composition and structure of the plant cell wall occur during normal growth and development, as well as a consequence of environmental stresses (abiotic and biotic). While changes in the wall during normal growth and development should not elicit responses that negatively affect growth of plants, many

environmental stresses do tend to influence growth, suggesting that cells have mechanisms that allow them to distinguish between the different signals and respond in an adaptive manner. Changes in the wall are treated as CWD, and these can occur as a result of intrinsic (e.g., if there are mutations that alter cell wall synthesis) or extrinsic (abiotic or biotic) factors. The CWI maintenance mechanism senses and responds to CWD, and in the case of abiotic stimuli, this response is sufficient. In many cases, the response is a compensatory response that includes ectopic production of cell wall components, accumulation of hormones (JA, SA, and ethylene), generation of ROS, and activation of Ca^{2+}-based signaling. However, when pathogens attack plants, cells not only sense CWD (through DAMPs) but also recognize certain pathogen-associated molecular patterns (PAMPs) and effectors to elicit an immune response. Pattern recognition receptors (PRRs) recognize PAMPs and DAMPs and trigger PRR-triggered immunity (PTI) against non-adapted pathogens (Couto and Zipfel 2016). Downstream signaling involves activation of RBOHD and the generation of reactive O_2 species, influx of Ca^{2+}, and the phosphorylation of MAPKs and CDKs. Late PTI responses include the inhibition of seedling growth and deposition of callose which in the form of papillae is important for immunity as it reinforces the cell wall at the points of fungal infection. The final consequence of PTI is induction of resistance to prevent microbial colonization.

Given that the main goal of PTI is to develop resistance to a pathogen, how do the CWI maintenance mechanism and PTI signaling mechanism interact with each other? A recent study by Engelsdorf et al. (2018) shows that the CrRLK1L member THE1 is a key signaling element mediating CWD-induced responses, but not PAMP-induced responses. Through a mechanism that is independent of THE1, CWD and pathogen-derived PAMPs induce production of peptides AtPep1 and AtPep3 that bind to the LRR-RLKs PEPR1 and PEPR2 to generate the PTI response in *A. thaliana*. The AtPep peptides enhance expression of their own *PROPEP* genes creating a positive feedback loop and PTI-controlled defense responses. Coordination between CWI and PTI is mediated by AtPep1 and AtPep3. These peptides, through PEPR1 and PEPR2, repress CWD-induced phytohormone accumulation and therefore function as repressors of CWI. If CWD occurs as a consequence of developmental events or abiotic stresses, there are no PAMPs, and activation of *PROPEP* genes may not be enhanced. As a result, the CWI mechanism is not suppressed, and the responses are mediated by the CWI mechanism. However, if CWD is mediated by a cell wall-degrading pathogen, *PROPEP* gene activation will be enhanced by PAMPs, resulting in increased amounts of AtPep1 and AtPep3 followed by an increased activation of the PTI pathway and suppression of the CWI pathway. Thus, PTI and CWI mechanisms detect CWD in different ways and modulate responses in an adaptive manner where the CWI maintenance mechanism acts as a backup in case the PTI mechanism is impaired.

17.8 Plant Cell Wall Signaling: Promises and Challenges

To monitor the environment, plant cells have the ability to sense a wide range of signals. Evidence from a variety of experimental approaches shows that the plant cell wall participates in signaling actively by contributing signals and as a target of response during normal growth and development, as well as during environmental stresses. Dissecting the various plant processes reveals that while there can be overlaps, as expected, the signals and response to changes in the cell wall during normal growth and development to non-pathogen CWD to pathogen attack become increasingly more complex (Fig. 17.2).

One key plant cell wall-related phenomenon that has gained prominence in the last few years is CWI sensing and signaling in response to CWD. Even as there is considerable information on the nature of receptors and sensors involved in monitoring CWI, mostly through analysis of mutants and the use of inhibitors, not much is known of the ligands that are recognized by these receptors and sensors. The cell wall is a complex structure and a rich source of signals, but so far only a few cell wall-derived signal molecules have been identified as ligands for the many cell surface receptors that are implicated in sensing changes in the wall. Identification of carbohydrate-based signaling molecules is a challenge mainly due to the complexity of carbohydrate chemistry (Wolf 2017; Bacete et al. 2018). Even where signal

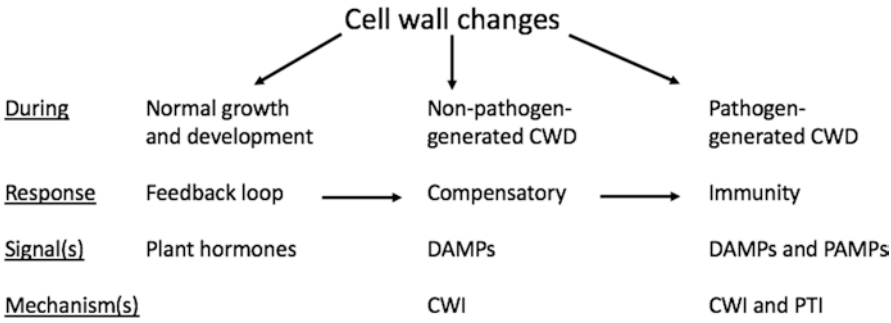

Fig. 17.2 An increasing complexity in signals and response to cell wall changes. The plant cell wall is dynamic, and changes in the wall occur during normal growth and development as well as during environmental stresses. During normal growth and development, the signals are mostly plant hormones, and the wall goes through stages of softening and rebuilding. When CWD occurs as a result of a non-pathogen-generated event (e.g., a mutation in a gene for synthesis of a cell wall component or an abiotic stress), the CWI maintenance mechanism is invoked for a compensatory response such that the wall can still function. In this case DAMPs (OGs or secreted peptides), osmotic signals, and/or mechanical signals are sensed by cell surface receptors and ion channels. Interestingly, the CWI maintenance mechanism leads to reduced growth and resistance against pathogens, even when there is no pathogen attack. During pathogen attack, the cell recognizes additional signals (PAMPs), and though CWI maintenance may be invoked, recognition of PAMPs results in the PTI system taking over resulting in host immunity. This view suggests that while changes in the cell wall may be more or less similar in the three types of events, the ability to recognize additional signals allows plants to differentiate between these events and respond in an adaptive manner

molecules are identified through in vitro binding assays, it will be interesting to determine if these signal molecules actually function in signaling in vivo, given the wide range of interactions and turnover that occurs in the wall. It is the dynamic behavior of the wall that makes it such an exciting area of research for not only those who are interested in determining the structure of the plant cell wall but also for those who consider the wall to be more than a barrier!

References

Albersheim P, Darvill A, Roberts K, Sederoff R, Staehelin A (2011) Plant cell walls. Garland Science, New York

Bacete L, Mélida H, Miedes E, Molina A (2018) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant J 93:614–636

Bartels S, Boller T (2015) Quo vadis, Pep? Plant elicitor peptides at the crossroads of immunity, stress, and development. J Exp Bot 66:5183–5193

Boisson-Dernier A, Kessler SA, Grossniklaus U (2011) The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals. J Exp Bot 62:1581–1591

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Bringmann M, Li E, Sampathkumar A, Kocabek T, Hauser M-T, Persson S (2012) POM-POM2/CELLULOSE SYNTHASE INTERACTING1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*. Plant Cell 24:163–177

Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proc Natl Acad Sci U S A 107:9452–9457

Burton RA, Gidley MJ, Fincher GB (2010) Heterogeneity in the chemistry, structure and function of plant cell walls. Nat Chem Biol 6:724–732

Cal AJ, Liu D, Mauleon R, Hsing YC, Serraj R (2013) Transcriptome profiling of leaf elongation zone under drought in contrasting rice cultivars. PLoS One 8:e54537

Caño-Delgado A, Penfield S, Smith C, Catley M, Bevan M (2003) Reduced cellulose synthesis invokes lignification and defense responses in *Arabidopsis thaliana*. Plant J 34:351–362

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3:1–30

Chen Z, Hong X, Zhang H, Wang Y, Li X, Zhu J-K, Gong Z (2005) Disruption of the cellulose synthase gene, *AtCesA8/IRX1*, enhances drought and osmotic stress tolerance in Arabidopsis. Plant J 43:273–283

Coolen S, Proietti S, Hickman R, Olivas NHD, Huang P-P, Van Verk MC, Van Pelt JA, Wittenberg AHJ, De Vos M, Prins M, Van Loon JJA, Aarts MGM, Dicke M, Pieterse CMJ, Van Wees SCM (2016) Transcriptome dynamics of Arabidopsis during sequential biotic and abiotic stresses. Plant J 86:249–267

Cosgrove DJ (2018) Diffuse growth of plant cell walls. Plant Physiol 176:16–27

Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16:537–552

de Azevedo Souza C, Li S, Lin AZ, Boutrot F, Grossmann G, Zipfel C, Somerville S (2017) Cellulose-derived oligomers act as damage-associated molecular patterns and trigger defense-like responses. Plant Physiol 173:2383–2398

Denness L, McKenna JF, Segonzac C, Wormit A, Madhou P, Bennett M, Mansfield J, Zipfel C, Hamann T (2011) Cell wall damage-induced lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process in Arabidopsis. Plant Physiol 156:1364–1374

Duan Q, Kita D, Li C, Cheung AY, Wu H-M (2010) FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. Proc Natl Acad Sci U S A 107:17821–17826
Endler A, Kesten C, Schneider R, Zhang Y, Ivakov A, Froehlich A, Funke N, Persson N (2015) A mechanism for sustained cellulose synthesis during salt stress. Cell 162:1353–1364
Engelsdorf T, Hamann T (2014) An update on receptor-like kinase involvement in the maintenance of plant cell wall integrity. Ann Bot 114:1339–1347
Engelsdorf T, Gigli-Bisceglia N, Veerabagu M, McKenna JF, Vaahtera L, Augstein F, Van der Does D, Zipfel C, Hamann T (2018) The plant cell wall integrity maintenance and immune signaling systems cooperate to control stress responses in *Arabidopsis thaliana*. Sci Signal 11:eaao3070
Feiguelman G, Fu Y, Yalovsky S (2018) ROP GTPases structure-function and signaling pathways. Plant Physiol 176:57–79
Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, Duan Q, Liu M-C, Maman J, Steinhorst L, Schmitz-Thom I, Yvon R, Kudla J, Wu H-M, Cheung AY, Dinneny JR (2018) The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. Curr Biol 28:666–675
Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC (2011) Nanostructure of cellulose microfibrils in spruce wood. Proc Natl Acad Sci U S A 108:1195–1203
Gravino M, Locci F, Tundo S, Cervone F, Savatin DV, De Lorenzo G (2017) Immune responses induced by oligogalacturonides are differentially affected by AvrPto and loss of BAK1/BKK1 and PEPR1/PEPR2. Mol Plant Pathol 18:582–595
Hamann T (2012) Plant cell wall integrity maintenance as an essential component of biotic stress response mechanisms. Front Plant Sci 3:77
Hamant O, Haswell ES (2017) Life behind the wall: sensing mechanical cues in plants. BMC Biol 15:59
Hamilton ES, Schlegel AM, Haswell ES (2015) United in diversity: mechanosensitive ion channels in plants. Annu Rev Plant Biol 66:113–137
Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR (2014) A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science 343:408–411
Haswell ES, Verslues PE (2015) The ongoing search for the molecular basis of plant osmosensing. J Gen Physiol 145:389–394
Hématy K, Sado P-E, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou J-P, Höfte H (2007) A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. Curr Biol 17:922–931
Huang GQ, Li E, Ge FR, Li S, Wang Q, Zhang CQ, Zhang Y (2013) Arabidopsis RopGEF4 and RopGEF10 are important for FERONIA-mediated developmental but not environmental regulation of root hair growth. New Phytol 200:1089–1101
Huffaker A, Pearce G, Ryan CA (2006) An endogenous peptide signal in *Arabidopsis* activates components of the innate immune response. Proc Natl Acad Sci U S A 103:10098–10103
Joshi R, Singla-Pareek SL, Pareek A (2018) Engineering abiotic stress response in plants for biomass production. J Biol Chem 293:5035–5043
Keegstra K, Talmadge KW, Bauer WD, Albersheim P (1973) The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol 51:188–197
Kessler SA, Lindner H, Jones DS, Grossniklaus U (2015) Functional analysis of related CrRLK1L receptor-like kinases in pollen tube reception. EMBO Rep 16:107–115
Kimura S, Laosinchai W, Itoh T, Cui X, Linder CR, Brown RM Jr (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. Plant Cell 11:2075–2085
Kimura S, Waszczak C, Hunter K, Wrzaczek M (2017) Bound by fate: the role of reactive oxygen species in receptor-like kinase signaling. Plant Cell 29:638–654
Kozuka T, Kobayashi J, Horiguchi G, Demura T, Sakakibara H, Tsukaya H, Nagatani A (2010) Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol 153:1608–1618

Kurusu T, Kuchitsu K, Nakano M, Nakayama Y, Iida H (2013) Plant mechanosensing and Ca^{2+} transport. Trends Plant Sci 18:227–233

Leucci MR, Lenucci MS, Piro G, Dalessandro G (2008) Water stress and cell wall polysaccharides in the apical root zone of wheat cultivars varying in drought tolerance. J Plant Physiol 165:1168–1180

Li S, Lei L, Somerville CR, Gu Y (2012) Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. Proc Natl Acad Sci U S A 109:185–190

Li H, Yan S, Zhao L, Tan J, Zhang Q, Gao F, Wang P, Hou H, Li L (2014) Histone acetylation associated up-regulation of the cell wall related genes is involved in salt stress induced maize root swelling. BMC Plant Biol 14:105

Li Z, Omranian N, Neumetzler L, Wang T, Herter T, Usadel B, Demura T, Giavalisco P, Nikoloski Z, Persson S (2016a) A transcriptional and metabolic framework for secondary wall formation in Arabidopsis. Plant Physiol 172:1334–1351

Li C, Wu H-M, Cheung AY (2016b) FERONIA and her pals: functions and mechanisms. Plant Physiol 171:2379–2392

Majda M, Robert S (2018) The role of auxin in cell wall expansion. Int J Mol Sci 19:951

Mueller SC, Brown RM Jr (1980) Evidence for an intramembrane component associated with a cellulose microfibril-synthesizing complex in higher plants. J Cell Biol 84:315–326

Newman RH, Hill SJ, Harris PJ (2013) Wide-angle X-ray scattering and solid-state nuclear magnetic resonance data combined to test models for cellulose microfibrils in mung bean cell walls. Plant Physiol 163:1558–1567

Nixon BT, Mansouri K, Singh A, Du J, Davis JK, Lee J-G, Slabaugh E, Vandavasi VG, O'Neill H, Roberts EM, Roberts AW, Yingling YG, Haigler CH (2016) Comparative structural and computational analysis supports eighteen cellulose synthases in the plant cellulose synthesis complex. Sci Rep 6:28696

Paredez AR, Somerville CR, Ehrhardt DW (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312:1491–1495

Park AR, Cho SK, Yun UJ, Jin MY, Lee SH, Sachetto-Martins G, Park OK (2001) Interaction of the *Arabidopsis* receptor protein kinase Wak1 with a glycine-rich protein, AtGRP-3. J Biol Chem 276:26688–26693

Sanchez-Rodriguez C, Ketelaar K, Schneider R, Villalobos JA, Somerville CR, Persson S, Wallace IS (2017) BRASSINOSTEROID INSENSITIVE2 negatively regulates cellulose synthesis in *Arabidopsis* by phosphorylating cellulose synthase 1. Proc Natl Acad Sci U S A 114:3533–3538

Savatin DV, Bisceglia NG, Marti L, Fabbri C, Cervone F, De Lorenzo G (2014) The Arabidopsis NUCLEUS-AND PHRAGMOPLAST-LOCALIZED KINASE-related protein kinases are required for elicitor-induced oxidative burst and immunity. Plant Physiol 165:1188–1202

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002) Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31:279–292

Sénéchal F, Wattier C, Rustérucci C, Pelloux J (2014) Homogalacturonan-modifying enzymes: structure, expression, and roles in plants. J Exp Bot 65:5125–5160

Shi H, Kim YS, Guo Y, Stevenson B, Zhu J-K (2003) The Arabidopsis *SOS5* locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. Plant Cell 15:19–32

Shih H-W, Miller ND, Dai C, Spalding EP, Monshausen GB (2014) The receptor-like kinase FERONIA is required for mechanical signal transduction in *Arabidopsis* seedlings. Curr Biol 24:1887–1892

Shiu SH, Bleecker AB (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. Plant Physiol 132:530–543

Tan L, Eberhard S, Pattathil S, Warder C, Glushka J, Yuan C, Hao Z, Zhu X, Avci U, Miller JS, Baldwin D, Pham C, Orlando R, Darvill A, Hahn MG, Kieliszewski MJ, Mohnen D (2013) An

Arabidopsis cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. Plant Cell 25:270–287
Tang D, Wang G, Zhou J-M (2017) Receptor kinases in plant-pathogen interactions: more than pattern recognition. Plant Cell 29:618–637
Tenhaken R (2015) Cell wall remodeling under abiotic stress. Front Plant Sci 5:771
Tran L-SP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci U S A 104:20623–20628
Turner S, Kumar M (2018) Cellulose synthase complex organization and cellulose microfibril structure. Phil Trans R Soc A 376:20170048
Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell 11:1743–1754
Van der Does D, Boutrot F, Engelsdorf T, Rhodes J, McKenna JF, Vernhettes S, Koevoets I, Tintor N, Veerabagu M, Miedes E, Segonzac C, Roux M, Breda AS, Hardtke CS, Molina A, Rep M, Testerink C, Mouille G, Höfte H, Hamann T, Zipfel C (2017) The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. PLoS Genet 13:e1006832
Wang T, Hong M (2016) Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls. J Exp Bot 67:503–514
Wang Y, Yang L, Zheng Z, Grumet R, Loescher W, Zhu J-K, Yang P, Hu Y, Chan Z (2013) Transcriptomic and physiological variations of three Arabidopsis ecotypes in response to salt stress. PLoS One 8:e69036
Wolf S (2017) Plant cell wall signaling and receptor-like kinases. Biochem J 474:471–492
Wolf S, Hématy K, Höfte H (2012) Growth control and cell wall signaling in plants. Annu Rev Plant Biol 63:381–407
Wolf S, van der Does D, Ladwig F, Sticht C, Kolbeck A, Schürholz A-K, Augustin S, Keinath N, Rausch T, Greiner S, Schumacher K, Harter K, Zipfel C, Höfte H (2014) A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling. Proc Natl Acad Sci U S A 111:15261–15266
Xie L, Yang C, Wang X (2011) Brassinosteroids can regulate cellulose biosynthesis by controlling the expression of *CESA* genes in *Arabidopsis*. J Exp Bot 62:4495–4506
Xu S-L, Rahman A, Baskin TI, Kieber JJ (2008) Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in *Arabidopsis*. Plant Cell 20:3065–3079
Zhang SS, Sun L, Dong X, Lu SJ, Tian W, Liu JX (2016) Cellulose synthesis genes CESA6 and CSI1 are important for salt stress tolerance in Arabidopsis. J Integr Plant Biol 58:623–626
Zhao Q, Dixon RA (2011) Transcriptional networks for lignin biosynthesis: more complex than we thought? Trends Plant Sci 16:227–233
Zhao Z, Crespi VH, Kubicki JD, Cosgrove DJ, Zhong L (2014) Molecular dynamics simulation study of xyloglucan adsorption on cellulose surfaces: effects of surface hydrophobicity and side-chain variation. Cellulose 21:1025–1039
Zhu J-K (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324

Inder M. Saxena did his Ph.D. with Prof. Sipra-Guha Mukherjee and with the Editor at JNU, New Delhi, where he worked on the uptake and integration of foreign DNA in plant cells. After his Ph.D. he worked as a Research Associate in a project with the Editor. In 1986, he went to The University of Texas at Austin (UT Austin) as a Postdoctoral Fellow and then worked as a Research Associate in a project on the identification and characterization of genes for cellulose biosynthesis in the lab of Prof. R.M Brown. In 1990, they simultaneously reported the first identification and cloning of the cellulose synthase gene from any organism. Since 1986, he continues to work and teach at UT Austin.

Plastid Retrograde Signals: More to Discover

18

Jeannette Pfalz and Ralf Oelmüller

Abstract

DNA and the machinery for gene expression have been discovered in chloroplasts during the 1960s. It was soon evident that the chloroplast genome is small, that many genes for chloroplast-localized proteins must reside in the nucleus, and that the expression of the genes in both cellular compartments must be coordinated. In the 1970s, the first evidence for plastid signals controlling nuclear gene expression was provided for plastid ribosome-deficient mutants. This review describes the discovery and the first studies on plastid-to-nucleus signaling. Today, many retrograde signals are known, which coordinate plastid and nuclear gene expression during the development of the organelle and in response to environmental changes. The nucleus receives information about the flux through the heme branch of the tetrapyrrole pathway, the expression of plastid genes, the metabolite stage in the organelle, and the efficiency of the photosynthetic electron flow. Singlet oxygen generated during light stress and breakdown products of carotenoids initiate signaling events in the organelle which alter nuclear gene expression. Operational signals permanently coordinate gene expression in both organelles. The biosynthesis of phytohormones like jasmonic, salicylic, and abscisic acids or cytokinins starts in the plastids, and these hormones became crucial players in coordinating plastid and nuclear gene expression under stress. Methylerythritol cyclodiphosphate, a biochemical intermediate of the methylerythritol phosphate pathway, alters the chromatin structure in the nucleus which in turn affects the expression of a particular subset of stress-inducible genes. Dual targeted proteins with plastid and nuclear locations participate in the interorganellar communication. We discuss our current knowledge about retrograde signaling and address open questions.

J. Pfalz · R. Oelmüller (✉)
Matthias-Schleiden-Institute, Friedrich-Schiller-University Jena, Jena, Germany
e-mail: b7oera@uni-jena.de

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_18

Keywords
Jasmonic acid · Photosynthesis-associated nuclear genes · Plastids · Redox · Salicylic acid · Signaling · Singlet oxygen · Tetrapyrroles

Abbreviations

ABI4	abscisic acid insensitive 4
β-CC	β-cyclocitral
GLK1/2	golden 2-like 1/2
GUN1/4/5	genomes uncoupled 1/4/5
EX1/EX2	executer 1/2
HDS1	hydroxymethylbutenyl diphosphate synthase
LHCB	gene-encoding photosystem II chlorophyll a/b binding protein
MEcPP	methylerythritol cyclodiphosphate
Mg-protop-IX	Mg-protoporphyrin IX
ΔPET	impairment of photosynthetic electron transport chain
PGE	plastid gene expression
PhANG	photosynthesis-Associated Nuclear Genes
PQ	plastoquinone
PRIN2	plastid redox-insensitive 2
PSI	photosystem I
ROS	reactive oxygen species
SAL1	inositol polyphosphate 1-phosphatase
TFs	transcription factors
STN7	thylakoid protein kinase 7
WHY1	whirly 1

18.1 Discovery of Plastid Retrograde Signals and Early Steps in Their Function

In the 1970s, it became clear that many genes for plastid proteins must be located in the nucleus, because the genetic information in the organelle is too small for the huge amount of functions that chloroplasts, etioplasts, leucoplasts, amyloplast, or chromoplasts fulfill in their different cellular environments (Kirk and Tilney-Bassett 1967; Kirk 1971; Börner et al. 1973; Bogorad 1975; Taylor 1989). More than 3000 different proteins were identified in plastids, and it is estimated that more than 95% of them are encoded by nuclear genes (Leister 2005, 2016; Tiller and Bock 2014). The plastome of higher plants contains only approximately 100 genes for photosynthesis, fatty acid biosynthesis, components of the import machinery, ribosomal proteins, and RNA polymerase subunits as well as rRNAs and tRNAs. Thus, the expression of the genes in both compartments have to be coordinated (Brunkard and Burch-Smith 2018; Van Dingenen et al. 2016; Greiner and Bock 2013). An obvious

idea was that the expression of these genes in the nucleus are only expressed when the gene products are required in the organelles, therefore the nucleus should be informed about the stage of the plastids in a particular organ, tissue, or cell. The first hints for the existence of such a control mechanism came from mutants defective in plastid development (Börner 2017). Plastid-ribosome-deficient mutants do not only lack the plastid-encoded components of multiprotein complexes (such as the ribulose-1,5-bisphosphate carboxylase, the photosynthesis complexes, or the 70S ribosomes of the plastids) but also the nuclear-encoded partners. Further analyses of these mutants, as well as plants which were chemically or physically treated to inhibit plastid gene expression or development showed that the absence of the nuclear-encoded proteins of these multiprotein complexes is caused by the absence or reduction of their expression. Tom Börner (2017) recently summarized early steps of the discovery of plastid retrograde signals and focused on the genetic evidence based on mutants with lesions in plastid functions. The historical overview also described the contribution of the researchers in this field and their interaction across the iron curtain. We only summarize a few additional historical aspects which were not in the main focus of Börner's review.

With the knowledge that the small subunit of ribulose-1,5-bisphosphate carboxylase is nuclear- and the large subunit plastid-encoded, early research focused on the identification of the mechanisms of how the expression of the genes in the two genetic compartments is coordinated (Bradbeer et al. 1979; Criddle et al. 1970; Givan and Criddle 1972; Chan and Wildman 1972; Blair and Ellis 1973; Ellis 1975, 1977; Börner et al. 1972, 1973, 1974, 1976; Hagemann and Börner 1987; Reichenbächer et al. 1978). Finally, mRNA measurements for *RBCS* transcript levels (for the small subunit of ribulose-1,5-bisphosphate carboxylase) in mutants impaired in plastid functions let to the hypothesis that the expression of the nuclear *RBCS* genes is controlled by signals from the plastids (Mayfield and Taylor 1984, 1987; Oelmüller and Mohr 1986; Harpster et al. 1984; Batschauer et al. 1986; Oelmüller et al. 1986a, b; Burgess and Taylor 1988; Giuliano and Scolnik 1988). The studies were extended to other nuclear-encoded genes for plastid proteins, with a main focus on genes for light-harvesting chlorophyll-*a/b*-binding proteins (LHCPs) (Mayfield and Taylor 1984; Oelmüller et al. 1986b; Oelmüller and Schuster 1987; Johanningmeier and Howell 1984). Physiological experiments initially demonstrated that *LHCP* expression is far more sensitive to photooxidative damage of the plastids than *RBCS* gene expression, and comparable differences were observed when plastids recovered from photodamage (Schuster et al. 1988). It appeared that more than one signal might be involved in the interorganellar cross talk and that there might be specificity for individual genes in their response to the information deriving from the plastids. Intermediates of chlorophyll biosynthesis have been postulated as signaling molecules mediating plastid-to-nucleus signaling, with the main focus on *LHCP* expression (Johanningmeier and Howell 1984; Kropat et al. 1997). Furthermore, also etioplasts are able to inform the nucleus about the stage of the organelle, as shown with inhibitor studies in etiolated mustard seedlings (Oelmüller et al. 1986b).

Tom Börner (2017) already described the interesting observation that also the activity and expression of nitrate reductase, an enzyme located in the cytoplasm, is decreased in leaves with impaired plastids, suggesting that the organelle also controls non-plastidal enzymes which require functional plastids (Börner et al. 1986; Oelmüller et al. 1988; Mohr et al. 1992; Hess et al. 1994; Oelmüller 1989; Oelmüller and Briggs 1990; Sherameti et al. 2002b). Nitrate reductase activity and expression is induced by nitrate and light, and both stimuli are only active when functional plastids are present. Besides effects in the cytoplasm (Reiss et al. 1983), also peroxisomal enzyme activities are controlled by the state of the plastids (Bajracharya et al. 1987). How the interorganellar signaling could occur, how specific such a signal has to operate, and which are the targets of plastid-derived signals in the nucleus/cytoplasm or peroxisomes were a matter of intensive discussion. The original studies were performed with plants in which chloroplast development was severely impaired by either mutation (Börner 2017; Bradbeer and Börner 1978; Hagemann and Börner 1987; Bradbeer et al. 1979), chemical (Oelmüller 1989) or heat (Feierabend 1977; Feierabend and Schrader-Reichhardt 1976; Feierabend and Mikus 1977) treatments. It was difficult to imagine that these badly damaged organelles, often without any detectable organelle structure, repress nuclear gene expression highly specifically, and that only one signaling molecule is responsible for the altered gene expression in the nucleus. Therefore, the discussions about the nature of the information flow from the organelle to the nucleus ranged from organellar cross talk with information exchange at many levels and multiple actors to highly specific plastid-derived signals which control the expression of individual genes in the nucleus. Quite early, it became obvious that the regulatory scenario must be somehow coupled to light signaling, since all known plastid-responsive genes were also light regulated (cf. Lepistö and Rintamäki 2012; Lepistö et al. 2012). However, at that time, we were only at the beginning to understand which signaling molecules mediate light responsiveness, and nobody could envision at that time that light-, hormone-, and other signaling processes share common signaling compounds, cross talk to each other and integrate the information from internal and external sources (e.g., Gollan et al. 2015).

During the discovery of plastid retrograde signaling, a similar process was already discussed intensively for mitochondria, based on studies with *petite* mutants from yeast. These mutants were impaired in mitochondrial functions and had severe alterations in the nuclear/cytoplasm cross talk, including altered expression of nuclear genes. The *petite* mutants were already discovered in the 1950 (summarized in Bernardi 1979) in yeast, and besides mitochondrial retrograde signals which control nuclear gene expression, also many other processes in the cytoplasm were affected. The available information about these mutants stimulated the discussion about a comparable role of plastids for nuclear gene expression and plastid-related enzymes located in the cytosol. Even now, plant researchers can still learn from the cross talk between the mitochondria and the nucleus/cytoplasm, in particular with regard to signaling components which transfer the information from the plastids to the nucleus and integrate organelle information with those from other sources.

Butow and Avadhani (2004) described "mitochondrial retrograde signaling as a pathway of communication from mitochondria to the nucleus that influences many cellular and organismal activities under both normal and pathophysiological conditions. In yeast it is used as a sensor of mitochondrial dysfunction that initiates readjustments of carbohydrate and nitrogen metabolism. In both yeast and animal cells, retrograde signaling is linked to TOR signaling, but the precise connections are unclear. In mammalian cells, mitochondrial dysfunction sets off signaling cascades through altered Ca^{2+} dynamics, which activate factors such as NFκB, NFAT, and ATF. Retrograde signaling also induces invasive behavior in otherwise nontumorigenic cells implying a role in tumor progression." This short description by Butow and Avadhani (2004) also highlights that much more has to be discovered for plastid retrograde signaling even now (cf. Pesaresi et al. 2006, 2007).

Initially, the expression levels of nuclear genes for plastid proteins were detected by their translatability in vitro, Northern analyses, and run-on transcription assays. In particular, the experiments by Batschauer et al. (1986) demonstrated that the plastid-derived signals must control transcriptional events in the nucleus. This implies the involvement of nuclear-localized transcription factors and responsive *cis*-regulatory elements in the promoters of the responding genes as targets of the signals from the plastids. Since light-responsive *cis*-regulatory elements in the promoter regions of light-inducible genes were studied in many laboratories at that time, one research direction focused on the identification of plastid-responsive elements in the promoters of genes for plastid proteins. The overall results of these studies uncovered that light-responsive and plastid-responsive elements were either identical or at least overlapping. Apparently, signals from the plastids and those from light converge before regulation the expression of their target genes in the nucleus (Kusnetsov et al. 1996). For example, Bolle et al. (1996a) showed that the spinach AtpC and AtpD genes for two of the three nuclear-encoded proteins of the plastid ATP synthase contain elements for light-regulated, plastid-dependent, and organ-specific expressions in the vicinity of the transcription start sites. Bolle et al. (1996b) also demonstrated that intron sequences are involved in the plastid- and light-dependent expression of the spinach PsaD gene. A number of quite short additional promoter sequence of genes for thylakoid proteins were identified to be involved in plastid-dependent expression (Kusnetsov et al. 1996, 1999; Oelmüller et al. 1993; Lübberstedt et al. 1994; Bolle et al. 1994); however a common plastid-responsive element which is present in the promoter region of more than one gene for plastid proteins could not be identified (Oelmüller et al. 1993; Bolle et al. 1996a, b). Overall, it appears that quite different target sequences are coupled to the signals from the plastids and that light signals and plastid-derived signals merge before controlling nuclear gene expression (Bolle et al. 1994; Kusnetsov et al. 1996). Finally, Kusnetsov et al. (1999) showed that the assembly of the CAAT-box-binding complex at a photosynthesis gene promoter is regulated by light, cytokinin, and the stage of the plastids. Apparently, also hormone signals target the same or similar *cis*-elements as plastid signals.

While these studies support transcriptional control by retrograde signals from the plastids, Sherameti et al. (2002a) investigated polyribosome loading of spinach mRNA species. They found that in light-grown, but not in dark-grown, spinach seedlings, the mRNAs for the nuclear-encoded photosystem (PS) I subunits D, F, and L are associated with polyribosomes, and this association is prevented by the application of 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU), an inhibitor of the photosynthetic electron transport. To identify the *cis*-elements which are responsible for this regulation, they generated a series of chimeric *PsaD* constructs and tested them in transgenic tobacco. The spinach *PsaD* 5′-untranslated region is sufficient to confer light- and photosynthesis-dependent polyribosome association onto a reporter gene, while the tobacco *PsaD* 5′-untranslated region directs constitutive polyribosome association. These results suggest that signals from photosynthetic electron flow control also posttranslational events. Thus, retrograde signals may be involved in quite different steps of nuclear gene expression, from transcription in the nucleus to the efficiency of the translation of specific mRNAs in the cytoplasm. Since the main focus on the research was directed toward transcriptional control, and the nature of the signals from the plastids, posttranscriptional events controlling the translatability and stability of specific RNA species were only considered much later.

A breakthrough in the research on retrograde signaling came with the identification of the *gun* (*genomes uncoupled*) mutants in Joanne Chory's laboratory (Susek et al. 1993). They used *Arabidopsis* plants with an *LHCP* reporter gene construct and screened for mutants which express the nuclear gene in seedlings in which plastids were destroyed by photooxidative damage due to inhibition of carotenoid biosynthesis with Norflurazon, an inhibitor that blocks carotenoid biosynthesis and thus leads to photooxidative destruction of the plastids. The herbicide treatment results in the downregulation of *LHCP* gene expression, and the mutants thus uncouple the expression from the state of the plastids. Ultimately, six *GUN* genes were identified, five of them are related to tetrapyrrole biosynthesis. This showed that at least one retrograde pathway is based on Mg-ProtoporphyrinIX, the first intermediate in the chlorophyll branch of the tetrapyrrole biosynthetic pathway (Nott et al. 2006; Pogson et al. 2008; Woodson et al. 2011). The sixth protein, GUN1, is a chloroplast-localized PPR protein (Nott et al. 2006, cf. below).

18.2 Nature of the Plastid Signal

Quite early after the discovery of chloroplast retrograde signaling, four different starting points in the organelle have been postulated: components of the tetrapyrrole biosynthesis, products deriving from chloroplast gene expression, chloroplast redox homeostasis, and photosynthesis-derived reactive oxygen species (ROS). Later, after the discovery that the whole scenario is more complex than anticipated that far, the retrograde signals were classified as those exerting biogenic control during early chloroplast development in seedlings which leads to the transition from etioplast to chloroplast development, and operational signals that inform the nucleus about the

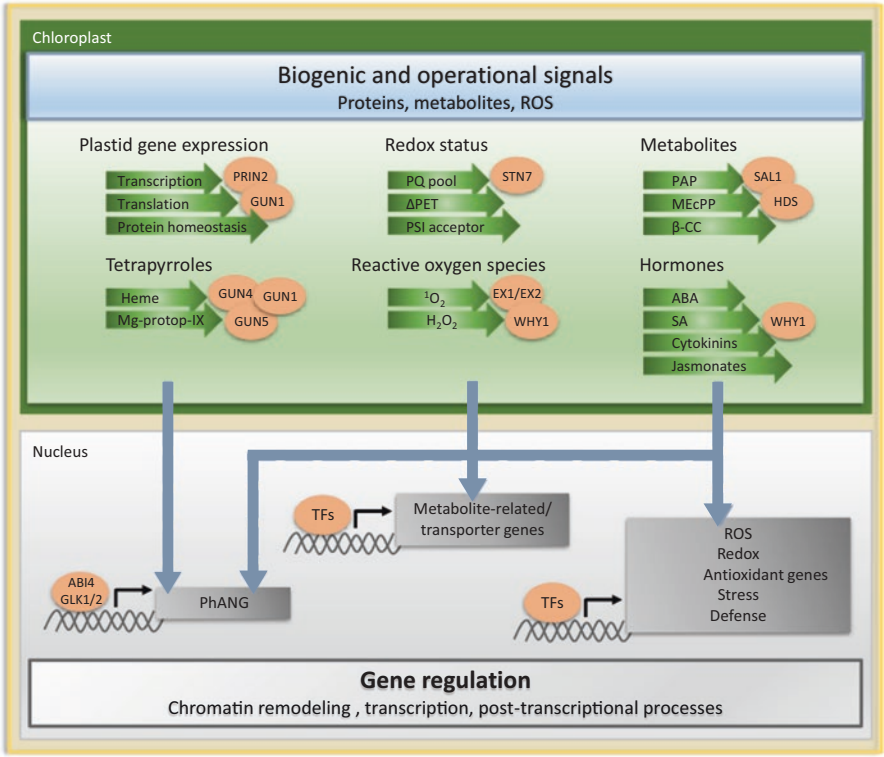

Fig. 18.1 Schematic diagram depicting the retrograde signaling pathways originating from chloroplasts. Plastid-to-nucleus retrograde signaling can be classified into two processes: "biogenic signals" that are relayed to the nucleus during early chloroplast development and "operational signals" that inform the nucleus about the state of the mature and functional organelle. Sensing and processing of plastid signals are mediated by diverse pathways, some of which appear to be interconnected through proteins, metabolites, and/or ROS. The pathways include various components of plastid genes expression, tetrapyrrole synthesis, redox state of photosynthetic electron transport, and chloroplast metabolite stage as well as different kinds of reactive oxygen species and hormones (green arrowheads). Several regulatory proteins have been found to be involved in signal transduction (orange). The signals cause transcriptional responses and may influence chromatin modeling and/or post-transcriptional processes in the nucleus and cytoplasm. Targets of nuclear gene regulation (gray boxes) frequently include transcription factors (orange)

state of the mature and functional organelle (e.g., in Brunkard and Burch-Smith 2018; Kleine and Leister 2016; de Souza et al. 2017). This includes the efficiency of photosynthetic electron transport but also metabolite requirements of the cell from the plastids or compounds such as hormone precursors and secondary metabolites including volatiles to respond to stress or pathogens. Ultimately, with the identification of specific metabolites as retrograde signals, such as methylerythritol cyclodiphosphate (MEcPP) (Xiao et al. 2012), the role of plastidal control on phytohormone synthesis and signaling for biotic stress responses became an important facet in the cross talk scenario. The nature of the plastid signals and the cross talk with nucleus in regulating the expression of genes is depicted in Fig. 18.1.

18.2.1 GUN1, a Biogenic Control Signal

Functional plastid gene expression (PGE) is crucial to initiate the expression of Photosynthesis Associated Nuclear Genes (PhANG) during early chloroplast development (Koussevitzky et al. 2007). In this process, perturbation of plastid gene expression triggers retrograde signals that control nuclear gene expression. Evidence for this type of regulation comes from studies with inhibitors of plastid translation and transcription (Oelmüller et al. 1986a, b; Gray et al. 1995; Woodson et al. 2013). The inhibitory effect can be attributed to a decline in protein synthesis rate in plastids or a blockade in chloroplast development. Genetic analysis placed GUN1 in the PGE pathway as an important factor (Koussevitzky et al. 2007). GUN1 is a pentatricopeptide repeat protein (PPR) that was originally identified in a screen with other *gun* mutants which were involved in the tetrapyrrole biosynthesis (Susek et al. 1993). However, GUN1 is not involved in this pathway and operates differently. It has been shown that only in *gun1* mutants, mRNA levels of the photosynthesis-related genes *LHCB1* and *RBCS* are altered in the presence of lincomycin, whereas these genes are sensitive to the treatment in the *gun2*, *gun3*, *gun4*, and *gun5* mutants (Susek et al. 1993; Mochizuki et al. 2001; Larkin et al. 2003).

Based on its evolutionary relationships with other members of the PPR family, a role in nucleic acid recognition can be assigned (Lurin et al. 2004; Barkan and Small 2014), although the experimental evidence of such conclusion remains scarce (Koussevitzky et al. 2007; Tadini et al. 2016). Recent studies found now that it interacts with multiple proteins, likely in a transient manner. Among the interacting partners are those involved in plastid transcription, translation, and protein homeostasis as well as tetrapyrrole biosynthesis enzymes (Tadini et al. 2016). According to this work, GUN1 appears to modulate the formation of protein complexes in the chloroplast. The authors further suggested that retrograde signaling might be linked to GUN1-dependent formation of protein complexes (Tadini et al. 2016; Colombo et al. 2016).

The GUN1 protein was associated with signals which are based on perturbations of plastid translation and transcription, as well as oxidative stress induced by carotenoid deficiency. The current model proposes that GUN1 integrates several signals originating from chloroplasts (e.g., signals related to the tetrapyrrole biosynthesis pathway, PGE-triggered retrograde signals, signals derived from the photosynthetic electron transport chain) and subsequently controls downstream nuclear gene expression (Koussevitzky et al. 2007; Woodson et al. 2011; Kindgren et al. 2012; Pfalz et al. 2012; Hernández-Verdeja and Strand 2018; Colombo et al. 2016). However, the exact mechanism of signal transduction by GUN1 and downstream components has not yet been fully understood. Recent work suggested that plastid-derived signals upon stress induction direct the plant homeodomain transcription factor PTM from the chloroplast outer envelope membrane into the nucleus, where it regulates PhANG expression. Furthermore, genetic analysis provided a molecular link to GUN1-mediated responses (Sun et al. 2011), although some controversy remains (Page et al. 2017). Downstream, the nucleus-localized transcription factors ABA INSENSITIVE 4 (ABI4) and Golden 2-Like1/2 (GLK1/GLK2) appear to be

major determinants for transcription (Brunkard and Burch-Smith 2018). GUN1 activates ABI4, an ERF/AP2 transcription factor which negatively regulates the expression of PhANGs (Koussevitzky et al. 2007). GUN1 also represses *glk1/2* transcription, which positively regulate expression of PhANGs and promote photomorphogenesis by antagonizing PHYTOCHROME-INTERACTING FACTORs (PIFs) (Waters et al. 2009; Martin et al. 2016). PIFs promote skotomorphogenetic development in dark-grown seedlings. Based on recent genetic information, activities of ABI4 and GLK1/2 represent two independent GUN1-mediated signaling events, in which phytochrome and retrograde signals converge antagonistically to control nuclear transcription during dark-to-light transition (Martin et al. 2016).

18.2.2 Redox, an Operational Signal

Imbalanced energy distribution between PSII and PSI generates redox signals within the plastoquinone (PQ) pool that controls both nuclear and plastid gene expression (Pfannschmidt et al. 1999; Fey et al. 2005; Dietzel et al. 2015). Likewise, it has been shown that redox states of acceptor or donor components of the PSI induce changes in the expression of nuclear genes for plastid proteins (Baier et al. 2004; Piippo et al. 2006; Barajas-López et al. 2013). Tuning gene expression to fluctuating light condition is necessary to maintain the efficiency of photosynthesis and metabolism and allows plants to survive unfavorable conditions. In this perspective, plants have developed mechanisms for both short- and long-term regulatory adaptations. A rapid reaction, a so-called short-term response, is state transition for balancing light energy distribution between the PSs by lateral movement of the LHCII antenna (Bellafiore et al. 2005; Bonardi et al. 2005). It takes place in a range of seconds or a few minutes. The details of molecular processes during short-term adaptation have been reviewed elsewhere (Dietzel et al. 2008; Rochaix 2013a, b). Longer term acclimation responses, which proceed at a slower tempo, are related to cellular strategies keeping PS stoichiometry adjusted to external light variations. This includes complex sensing and signaling pathways which regulate gene expression. Here, we focus on the role of redox signals from photosynthesis in regulation of nuclear gene expression. For details of the redox-regulatory mechanism controlling plastid gene expression see reviews by Barajas-López et al. (2013) and Dietzel et al. (2008).

Light acclimation and the molecular mechanism underlying this process have been an intense focus in recent years (Karpiński et al. 2013). Early evidence that redox-signals emanating from the photosynthetic electron transport chain regulate nuclear gene expression (e.g., genes associated with photosynthesis) was first demonstrated in the green algae *Dunaliella tertiolecta* (Escoubas et al. 1995; Maxwell et al. 1995). Escoubas et al. (1995) showed that light intensity alters the transcriptional activity of *LHCB* genes during photoacclimation and concluded that the changes in gene expression are associated with changes of the redox state of the PQ pool, as *LHCB* expression levels increased or decreased upon application of the selective chemical photosynthesis inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyl

urea (DCMU) or 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), respectively. A redox-regulatory mechanism on the expression of nuclear genes by the redox state of the PQ pool was also found in higher plants. In *Arabidopsis*, for example, an increase in transcript abundance of two cytosolic ascorbate peroxidase genes (APX1 and APX2) was measured in response to high light and DCMU treatments (Karpinski et al. 1997). Subsequent studies revealed a link between cytosolic defense mechanism and the redox state of the PQ pool by which H_2O_2 might act as a systemic signal molecule (Karpinski et al. 1999). In the following decade, a few single nuclear genes related to photosynthesis have been identified to be regulated in response to light intensity as well as light quality by photosynthetic redox signals (Petracek et al. 1998; Pursiheimo et al. 2001; Eguchi et al. 2002; Pfannschmidt et al. 2001). These signals effect nuclear gene expression on almost all levels, including the regulation of transcription, stability, and translational efficiency (Pfannschmidt et al. 2003). The application of array-based technologies combined with physiological and genetic analyses have facilitated discovery of redox-responsive genes through comparison of the expression profiles of *Arabidopsis* plants exposed to wavelengths that preferentially excited either PSII or PSI (Fey et al. 2005; Piippo et al. 2006; Bräutigam et al. 2009; Pesaresi et al. 2009). Besides transcriptional control of photosynthesis-related genes, light quality shifts also effected the expression of genes involved in regulation, signal transduction, gene expression, stress responses, transport, and metabolism. According to the observed dynamics of transcriptional changes, redox signals rapidly (within 30 min to 2 h) alter the transcriptome pattern, with significant temporal changes during the period of 48 h light acclimation (Bräutigam et al. 2009). Related efforts by Dietzel et al. (2015) exhibited a set of early regulated genes. They fell into functional groups with defined processes including genes for the mitochondrial electron transport chain, tetrapyrrole biosynthesis, photosynthesis, and lipid metabolism. The light shift experiments showed expression profiles that were clearly different from those in plants exposed to high light treatments (Jung et al. 2013). In summary, these studies emphasize that the mechanism triggering the changes in expression of nuclear genes involves diverse redox signals emanating from the photosynthetic electron transport chain (Barajas-López et al. 2013; Hernández-Verdeja and Strand 2018). The existence of different sets of regulatory genes suggest a complex relationship between sensing, signaling, gene expression, and adaptation to the environment and may reflect a high degree of variability in light acclimation capabilities.

Efforts in understanding the transduction pathway of signals in response to the redox state of the photosynthetic machinery have combined multiple genetic and physiological analyses, but an answer still remains elusive. In this context, a phosphorylation-mediated signal cascade has been suggested. Among the components to be discovered, the STN7 kinase, which induces state transition to ensure balanced excitation within the photosynthetic system (Bellafiore et al. 2005; Bonardi et al. 2005), has been proposed to transduce signals due to its redox-sensitive kinase activity (Pesaresi et al. 2009, 2011; Bräutigam et al. 2009). However, studies by Tikkanen et al. (2012) have shown that the genetic disruption of *stn7* in *Arabidopsis* does not fully inactivate the redox signaling pathway, indicating that STN7 is not

essential for this process. In this work, STN7 was proposed to exert its signaling effect by maintaining the steady-state phosphorylation of the light-harvesting II proteins and the redox balance in the thylakoid membrane, thereby controlling chloroplast ROS homeostasis. In turn, alterations in redox homeostasis trigger signals that regulate the entire cellular network, probably by modification of hormone-mediated pathways (Tikkanen et al. 2012).

18.2.3 Metabolite Stage of Cell in Retrograde Signaling

Besides highly specific signaling molecules (cf. below) which potentially leave the organelle and control nuclear gene expression, changes in metabolite concentrations or intermediates of biochemical pathways are likely to be involved in the interorganellar cross talk (Estavillo et al. 2013; Brunkard and Burch-Smith 2018). The metabolite state in the cell or in a subcellular compartment permanently changes and is redirected according to the requirements of the organism. These changes result in the alteration of expression of the genes which are involved in the redirection of the metabolite pathways. Metabolite changes in the organelle, caused by, for instance, changes in light conditions, externally applied abiotic or biotic stresses or nutrient shortages, pathogen attack, and also developmental processes which result in a specific metabolite requirement at a particular place, time and organ, or circadian rhythm, cause appropriate changes in the metabolite profiles outside of the plastid in the cytoplasm, and consequently altered expression of responsive nuclear genes (Kleine and Leister 2016). Therefore, it is reasonable to assume that the nucleus is permanently informed about metabolite alterations in the organelle, either directly or indirectly due to metabolite adjustments between the plastid and cytoplasmic compartments, and adjusts its gene expression profile according to the metabolomic situation. This is particularly striking since many essential metabolites required for cellular functions and plant development are synthesized in the plastids and are exported into the cytoplasm. Obviously, metabolite concentrations represent an additional source of retrograde signaling during plant growth and upon responses to stress (Chi et al. 2013, 2015). Metabolite fluxes with plastidal involvement have been reviewed repeatedly and include carbon (Demmig-Adams et al. 2017; Tamoi and Shigeoka 2015), sulfur (Przybyla-Toscano et al. 2018; Eisenhut et al. 2015; Hanke and Mulo 2013; Tripathy et al. 2010; Hawkesford and De Kok 2006), nitrogen (Otori et al. 2017; Dörmann et al. 2014), and phosphorous (Karlsson et al. 2015; Rausch and Bucher 2002). Recently, de Souza et al. (2017) summarized the cross talk of multiple signaling events from mitochondria and plastids to coordinate nuclear gene expression and proposed that retrograde signals act as integrators of interorganellar communication and orchestrators of plant development. Interorganellar communication signals mediate reallocation of metabolic resources and energy currencies to balance growth and development against adaptive responses. Kleine and Leister (2016) highlight genetic screens which have already been performed and should be extended in the future to identify additional components in the cross talk. Metabolite profiling combined with bioinformatic tools is

also a promising approach to identify novel players which are directly involved in retrograde signaling. Overall, it is reasonable to assume that changes in metabolite concentrations integrate information from the plastids, peroxisomes, mitochondria, the cytosol, as well as extracellular regions to regulate the activity of already existing signaling pathways and molecules to adjust nuclear gene expression.

Metabolite transporters in the plastid envelope membrane play a crucial role in the connection of plastidal and cytoplasmic metabolite pools. One would expect that they are of prokaryotic origin; however, the story appears to be more complex (cf. Weber and Linka 2011). A connection between the organellar metabolism and the host cell was probably an important issue after establishment of the symbiosis, and it must have been established early in evolution. The plastidic phosphate translocators were the first transporters identified in the plastid envelope. The discovery of triose phosphate/phosphate translocator, glucose 6-phosphate/phosphate translocator, xylulose 5-phosphate/phosphate translocators, and phosphoenolpyruvate/phosphate transporter highlights the important role of phosphate homeostasis between organelles and cytoplasm. Nucleotide carriers facilitate exchange of this essential metabolite across the organellar membrane. ADP/glucose, folate, S-adenoylmethionine, ATP and NAD carriers, dicarboxylate, glycolate and glycerate, maltose and glucose, as well as amino acid transporters are well known. Some of them are members of the mitochondrial carrier family and were redirected to the plastid envelop in the evolution. The function and evolution of these transporters are summarized by Weber and Linka (2011). This also highlights the importance of the metabolite exchange between the plastids, cytoplasm, and other cellular subcompartments, which consequently affects the expression of metabolite-related genes in the nucleus (Eisenhut et al. 2015; Mehrshahi et al. 2014; Linka and Theodoulou 2013; Flügge et al. 2011; Linka and Weber 2010; Weber and Fischer 2007; Hawkesford and De Kok 2006; Weber 2004). Thus, plastid metabolite levels might have an indirect effect on nuclear gene expression.

18.3 Specific Plastid Metabolites Control Specific Sets of Nuclear Genes

18.3.1 Tetrapyrroles

The role of more specific metabolites located in the plastids for the expression of nuclear genes has been investigated intensively. As mentioned above, five *gun* (*gun2–6*) mutants affect the branch point in the tetrapyrrole pathway (Susek et al. 1993; Larkin et al. 2003; Strand et al. 2003; Mochizuki et al. 2001, 2008; Moulin et al. 2008; Woodson et al. 2011; Thomas and Weinstein 1990). Protoporphyrin IX is chelated with iron by the ferrochelatase 1 or 2. The Fe-containing heme either remains in the plastids or further metabolizes to phytochromobilin, which is exported and associated with the apoprotein of phytochromes in the cytoplasm. The *gun2* and *gun3* mutants are affected in the conversion of heme to phytochromobilin. Alternatively, protoporphyrin IX is chelated with magnesium for chlorophyll

biosynthesis. The *gun4* and *gun5* mutations prevent the insertion of magnesium. GUN5 is the H subunit of Mg-chelatase, and GUN4 binds the substrate of the Mg-chelatase reaction and activates the enzyme. Independent evidence of the involvement of chlorophyll precursors in the retrograde signaling came from the analysis of *LHCP* gene expression in *Chlamydomonas* (Johanningmeier and Howell 1984; Kropat et al. 1997, 2000). Whether one of the intermediates of the pathway triggers retrograde signaling and if so which of them is involved in it remains an open question. In the heme branch of the tetrapyrrole biosynthesis, the plastid ferrochelatase 1 synthesizes heme which results in the stimulation of nuclear gene expression. *gun6* overexpresses the plastid-localized ferrochelatase 1, stimulates the flux through the heme branch of the tetrapyrrole pathway and the expression of the responsive genes in the nucleus. Therefore, it has been postulated that heme is a positively acting retrograde signal for nuclear genes (Woodson et al. 2011). Heme is also known to be released from the organelle (Thomas and Weinstein 1990), which further supports the idea. Finally, algae like Chlamydomonas synthesize bilin, which might have a similar signaling function (discussed in Duanmu et al. 2013). In contrast, Mg-protoporphyrin IX represses the responding genes in the nucleus. Whether Mg-protoporphyrin IX acts as negative signal (Strand et al. 2003) or heme as positive signal (Woodson et al. 2011), or both metabolites are involved, remain an open question. Currently, it appears more likely that the flux through the two branches of the pathway might activate so far unknown signaling compounds in the plastids, which trigger retrograde signaling.

18.3.2 Singlet Oxygen ($^1O_2$) and Carotenoids

It is long known that reactive oxygen species (ROS) trigger nuclear gene expression (Galvez-Valdivieso and Mullineaux 2010), whereas the responding genes depend largely on the amount of location of ROS in and around the cell: low ROS levels have often signaling functions whereas high ROS levels are lethal. In photosynthetically highly active chloroplasts, singlet oxygen is produced in huge amounts, which is associated with the damage at the thylakoid membrane and altered gene expression in the nucleus (e.g., Kim and Apel 2013a; Ramel et al. 2012; Laloi et al. 2006). Originally proposed as retrograde signal, the short half-life of singlet oxygen suggests that it is unable to leave the organelle; however, it reacts with numerous compounds in its direct environment including carotenoids which have ROS-quenching functions (Ramel et al. 2012; 2013a). One of the carotenoid oxidation products is β-cyclocitral (β-CC), a volatile, which induces massive alteration of nuclear gene expression when applied to leaves in physiologically relevant concentrations (Ramel et al. 2012). The list includes $^1O_2$-responsive genes (Ramel et al. 2012, 2013b), genes involved in light-stress acclimation (Lv et al. 2015), but also *ISOCHORISMATE SYNTHASE 1* (*ICS1*), which synthesizes salicylic acid (SA) in the organelle. Elevated SA levels in the cell stimulate nuclear localization of NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1 (NPR1) which in turn activates SA-responsive genes (Lv et al. 2015). We are only

at the beginning to understand how the abiotic and biotic stress acclimation responses are linked (cf. Maruta et al. 2012, 2016; Padmanabhan and Dinesh-Kumar 2010) and what is the exact role of events in the plastid that affect the expression of the genes in the nucleus. Nevertheless, as lipid-soluble volatile β-CC appears to be an ideal candidate for retrograde signaling, β-CC is not the only or the most important singlet oxygen-derived signaling compound. Apocarotenoids as enzymatic cleavage products of carotenoids may also have signaling functions (Auldridge et al. 2006; Avendaño-Vázquez et al. 2014). However, there must be additional pathways involved in the cross talk between the two organelles which become activated after singlet oxygen generation. Klaus Apel's group demonstrated that the nuclear-encoded and plastid-localized EXECUTER1 and EXECUTER2 (Lee et al. 2007) are required for the activation of an independent plastid-localized signaling pathway by singlet oxygen, and the target genes in the nucleus differ from those responding to β-CC (Lee et al. 2007; Ramel et al. 2012). Single oxygen plays a crucial role in programmed cell death (PCD). Green leaves initiate PCD to restrict pathogen growth and distribution, a process that is stimulated by or even dependent on light perceived by photosynthesis. The *fluorescent* (*flu*) mutants show these lesions in the absence of any pathogen in light, but not in the dark. They accumulate excess protochlorophyllide in the dark, which are photosensitizing agents after transfer of the plants from the dark to light where they synthesize the toxic single oxygen leading to PCD phenotypes (Meskauskiene et al. 2001; op den Camp et al. 2003; Kim and Apel 2013a, b). EXECUTER1 and EXECUTER2 are required for the transduction of the single oxygen signal to the nucleus to initiate the PCD responses (Wagner et al. 2004). EXCECUTER1 is degraded in the *flu* mutants by the FtsH2 protease (Wang et al. 2016; Dogra et al. 2017). Obviously, high EXECUTOR1 levels are necessary for retrograde signaling from the plastids to the nucleus (Wang et al. 2016; Dogra et al. 2017) and are crucial for the survival of a cell.

18.3.3 3′-Phosphoadenosine 5′-Phosphate

3′-Phosphoadenosine 5′-phosphate (PAP) is proposed as a retrograde-active metabolite and accumulates, under stress conditions such as drought or high light, in plastids (Estavillo et al. 2011). The plastid- and mitochondria-localized enzymes SAL1 dephosphorylate PAP to AMP (Klein and Papenbrock 2004; Wilson et al. 2009) and a mutant of the plastid SAL1 protein accumulate high levels of PAP, similar to exposure of wild-type plants to stress (Rossel et al. 2006; Estavillo et al. 2011). In contrast, constitutively high levels of SAL1 in either the nucleus or the plastids result in lower PAP levels, even when the enzyme is expressed in the other compartment, suggesting that the metabolite can travel in the cell. Based on these and additional studies, it was proposed that accumulation of PAP stimulates the expression of nuclear-encoded stress genes, in particular those for antioxidant enzymes, including ascorbate peroxidase 2 (*APX2*), which was used for an initial mutant screen (Rossel et al. 2006). Targeting of SAL1 to either the nucleus or

chloroplasts decreased the PAP levels and consequently *APX2* expression (Estavillo et al. 2011). Since PAP appears to move between the plastid and cytoplasm, probably by a specific transporter (Gigolashvili et al. 2012), it fulfills a major criteria as retrograde signal. PAP is also produced during sulfonation reactions, whereby sulfate is transferred from PAPS to different metabolic substrates (Klein and Papenbrock 2004), and PAP is released during this reaction. However, quite interesting is the observation that PAP binds irreversibly to yeast 5′-3′ exoribonucleases and inhibits their activities (van Dijk et al. 2011). It appears that also in plants, PAP can alter RNA metabolism and thus acts posttranscriptionally. Although there is no doubt that PAP fulfills all criteria to transfer stress information from the plastids to the nuclear/cytoplasmic compartment, there might be many more such metabolites with similar functions.

18.4 Methylerythritol Cyclodiphosphate (MEcPP) as Defense-Related Retrograde Signal

MEcPP is a biochemical intermediate of the methylerythritol phosphate (MEP) pathway for the isoprenoid synthesis in chloroplasts (Vranova et al. 2013; Banerjee and Sharkey 2014). Not surprisingly, inhibition of this pathway leads to severe lesions in growth and development. The stress-inducible metabolite was identified as a plastid retrograde signal, which alters the chromatin structure in the nucleus that in turn affects the expression of a particular subset of stress-inducible genes (Xiao et al. 2012, 2013). Expression of the hydroperoxide lyase (HPL) and isochorismate synthase1 (ICS1) genes is altered in isolated mutants, and this results in increased SA levels, a phytohormone which confers resistance against biotrophic pathogens such as *Pseudomonas syringae* (Xiao et al. 2012). The authors showed that SA accumulation and the induction of the *HPL* gene are caused by the plastidal metabolite MEcPP and are not due to a general stress response due to the manipulation of the MEP pathway in the mutants (Xiao et al. 2012). MEcPP application also regulates *HPL* expression directly, confirming that the metabolite is active and plays a role as stress sensor in plastids. MEcPP is also present in bacteria and accumulates upon exposure to oxidative stress (Ostrovsky et al. 1992, 1998), suggesting a conserved mechanism of its occurrence and action during abiotic stresses (Walley et al. 2015; Xiao et al. 2012, 2013). Interestingly, MEcPP can disrupt histone H1-like protein interaction with DNA, which suggests that the metabolite remodels the chromatin structure to allow expression of stress-related genes (Grieshaber et al. 2004, 2006). MEcPP is probably the most direct evidence for the existence of metabolites in the plastid that control transcription in the nucleus. Besides functional conservation in evolution, it also differs from tetrapyrrole signaling, for which changes in flux rates play an important role for signal initiation. However, how MEcPP travels from the organelle to the nucleus is not known yet. Furthermore, MEcPP also highlights the important role of the plastid for biotic stress responses, in which SA and jasmonic acid (JA) are crucial phytohormones (Nomura et al. 2012; cf. below).

18.5 Dual Targeted Proteins in Plastids and Nucleus: Function as Transmitters or Integrators of Information?

Retrograde signal transduction is initiated by signaling molecules that are produced in and exported from plastids and then enter the nucleus to regulate the expression of appropriate genes. Signal transduction from plastids (and/or mitochondria) to the nucleus may also occur through the movement of proteins (Krause et al. 2012), such as transcription factors like PTM (for PHD type transcription factor with transmembrane domains), a chloroplast envelope-bound plant homeodomain transcription factor with transmembrane domains (Sun et al. 2011), PEND, a plastid envelope DNA-binding protein (Terasawa and Sato 2009), or WHIRLY1 (WHY1; Miao et al. 2013; Ren et al. 2017; Desveaux et al. 2004; Foyer et al. 2014; Isemer et al. 2012), a protein with specific functions in both organelles. Distinct retrograde signals may converge at PTM in the plastids, which then transmit common signals to the nucleus (Sun et al. 2011). In the nucleus, PTM promotes ABI4 transcription upon high light treatments. ABI4 was proposed to be involved in the integration of three plastids as well as mitochondrial retrograde signals (Koussevitzky et al. 2007). Retrograde signaling via members of the AP2/EREBP transcription factor gene family plays a role in the connection of metabolic, hormonal, and environmental signals during stress acclimation (Dietz et al. 2010). These examples demonstrate that signal information can also be transferred from plastids to the nucleus by traveling proteins. How this occurs is a matter of discussion. They might participate in signal integration in the plastids before transfer of the information to the nucleus (Koussevitzky et al. 2007). Others are part of signaling pathways or respond to them which are activated by different stimuli from outside of the plastids. This allows them to integrate information from plastids with those from other extraplastidic sources. Some of the proteins like WHY1 have defined functions in each of the organelle (Desveaux et al. 2004; Miao et al. 2013; Foyer et al. 2014; Isemer et al. 2012; Ren et al. 2017). As mentioned above, dual targeted proteins are often transcription factors or regulators of gene expression when they are in the nucleus. Since more and more dual targeted proteins with quite different functions are described (cf. Krause and Krupinska 2009; Nevarez et al. 2017; Mazzoleni et al. 2015; Gile et al. 2015; Langner et al. 2014; Ge et al. 2014; Berglund et al. 2009; Rokov-Plavec et al. 2008; Millar et al. 2006), it appears that there is a need for intensive investigations, including the import of nuclear-encoded proteins into the organelle (Inaba 2010; Inaba et al. 2011).

A well-studied example for a dual-targeted protein is WHY1. Like other members of the WHIRLY protein family, they perform numerous cellular functions in both locations (Krause et al. 2005; Grabowski et al. 2008; Miao et al. 2013; Ren et al. 2017; Foyer et al. 2014). These proteins were first discovered as nuclear transcriptional activators binding an elicitor response element in the promoter regions of pathogenesis-related genes in potato and *Arabidopsis* (Desveaux et al. 2000, 2004). They bind to various DNA sequences, including telomeres (Yoo et al. 2007), a distal element upstream of a kinesin gene (Xiong et al. 2009), the promoter region of the early senescence marker gene *WRKY53* in a development-dependent manner in *Arabidopsis* (Miao et al. 2013), and the promoter region of the senescence-associated

gene *HvS40* which was induced during natural and stress-related senescence in barley (Krupinska et al. 2013). In plastids, WHY1 is present in the transcriptional active chromosome (TAC, Pfalz et al. 2006) and nucleoid preparations although it can be purified away from the transcriptional activity (Melonek et al. 2010) and binds to both single-stranded DNA and RNA with a role in intron splicing in maize chloroplasts (e.g., Prikryl et al. 2008). In barley chloroplasts, WHY1 also was found to be associated with intron-containing RNAs (Melonek et al. 2010). Moreover, the Brission group demonstrated that WHY proteins in organelles function as antirecombinant proteins favoring accurate DNA repair to maintain organellar genome stability (Cappadocia et al. 2010, 2012; Lepage et al. 2013). These results suggest that WHY proteins might function differently depending on their intracellular localization and/or the developmental stage of the plant (Ren et al. 2017). Recently, the Miao group constructed "compartmental mutants" of WHY1 that differentially accumulate different isoforms of the WHY1 protein in plastids (pWHY1) or nuclei (nWHY1) of *Arabidopsis*. Based on these mutants, the group identified differentially expressed nuclear genes in plants with constitutive and inducible pWHY1 or nWHY1 versions. The results shine new light on the role of WHY1 in integrating metabolic, hormonal, and environmental signals in retrograde signaling. In particular, the group demonstrates that WHY1-mediated retrograde signals involve ROS (H_2O_2)- and SA-dependent compounds and are integrated into known signaling events. The quite strong phenotypes of the compartmentalized WHY1 mutants generated in the Miao lab in response to external signals will be important tools to unravel the function of the dual targeted protein in the interorganellar cross talk.

18.6 The Role of Plastids in Stress Response: Importance for Retrograde Signaling?

Biogenic control signals inform the nucleus about developmental changes of the organelles, such as the development of chloroplasts from etioplasts or proplastids. Operational signals, such as redox signals inform the nucleus about the events that occur in functional plastids/chloroplasts such as the efficiency of the photosynthetic electron transport. Dramatic changes in nuclear gene expression occur also when the plants are exposed to stress (Fernández and Strand 2008). Abiotic stresses such as drought are counteracted by the synthesis of the phytohormone abscisic acid, biotic stresses involve SA and JA. Other plastid-related hormones such as cytokinins also participate in defense responses (Chan et al. 2010, 2016). Since the synthesis of these hormones starts in the plastids (SA is also synthesized in the cytoplasm), and is strongly stimulated upon stress, the organelle plays the essential role in the response of the cell to stress. Furthermore, SA accumulates in response to the retrograde signaling metabolite MEcPP and in response to the plastid-localized isoform of WHY1, connecting phytohormones to other retrograde signaling. Finally, MEcPP is a regulator of SA and JA cross talk (Lemos et al. 2016). Since these phytohormones strongly activate defense genes in the nucleus upon stress or pathogen attack, phytohormones also play a crucial role in retrograde signaling.

18.6.1 Salicylic Acid

Salicylic acid (SA) in plants is synthesized via two biosynthetic pathways: the plastid-localized isochorismate synthase (ICS) and the cytosolic phenylalanine ammonia lyase (PAL) pathways. Both pathways use chorismate as precursor, which is synthesized via the shikimate pathway in plastids (Poulsen and Verpoorte 1991; Schmid and Amrhein 1995). The plastid-localized isochorismate pathway is the main source of SA upon exposure of the plant to abiotic stress or pathogen attacks (Vlot et al. 2009; Dempsey et al. 2011). Furthermore, SA is the main defense hormone upon attack of plant by biotrophic pathogens, while necrotrophic pathogens activate the JA defense pathway. SA is also involved in a number of developmental processes (Martínez et al. 2004; Morris et al. 2000; Zhang et al. 2013; Abreu and Munné-Bosch 2009; Seguel et al. 2018) in which not only chloroplasts but also other types of plastids participate. The plastid-localized enzyme ICS1 (Strawn et al. 2007) converts chorismate to isochorismate which is subsequently converted to SA by a so-far unknown organellar enzyme. The SA biosynthesis is negatively regulated by an autoinhibitory feedback loop operating around ICS1. Export of SA from the chloroplast to the cytoplasm is mediated by the multidrug and toxin-extrusion transporter ENHANCED DISEASE SUSCEPTIBILITY5 (EDS5) in the chloroplast envelope. Interestingly, analysis of the *eds5* mutant in *Arabidopsis* has demonstrated that SA is trapped in the chloroplast of the mutant and inhibits its own accumulation by the autoinhibitory feedback mechanism which couples SA export to its synthesis (Serrano et al. 2013; Yamasaki et al. 2013).

The cross talk between plastids and cytoplasm is a result of the evolution of the two pathways. In *Arabidopsis*, the basal SA level is produced via the PAL pathway (Huang et al. 2010), whereas under pathogen attack or abiotic stress, the vast majority of the SA is synthesized by the isochorismate pathway in the plastids (Wildermuth et al. 2001; Garcion et al. 2008). This appears to be species specific, since in soybean, both pathways contribute equally to the SA production upon pathogen attack (Shine et al. 2016). *Arabidopsis* and soybean contain two genes for the key enzyme of the plastid ICS pathway. In other species, different ICS isoforms are produced by alternative splicing of a single *ICS* gene (Macaulay et al. 2017). Apparently, the plastid-localized pathway for SA is highly sophisticated and an evolutionary result of intensive cross talk between the two organelles.

18.6.2 Jasmonic Acid

It is long known that Jasmonic acid (JA) precursors and, in particular, the JA precursor 12-oxo-phytodienoic acid (OPDA) are synthesized in plastids. Jasmonates are derived from the α-linolenic acid (18:3) or 7(*Z*)-, 10(*Z*)-, and 13(*Z*)-hexadecatrienoic acid (16:3). A lipoxygenase catalyzes the addition of molecular oxygen to α-linolenic acid which initiates JA biosynthesis by providing the substrate for the formation of an allene oxide by the allene oxide synthase (AOS), which is further converted to OPDA. The reactions until OPDA formation take place in plastids, while the subsequent steps in the JA biosynthesis occur in peroxisomes. In the plastids, OPDA can

also be esterified to lipids. JA is converted to jasmonoyl-isoleucine (JA-Ile) in the cytoplasm, and after binding to its receptor, JAR1 activates specific defense genes in the nucleus (Huang et al. 2017; Zhang et al. 2017; Han 2017; Wasternack and Song 2017). Thus, besides being integrated into a complex hormone network, jasmonate also functions as retrograde signals in concert with other signals and plastid metabolites.

Lemos et al. (2016) showed that the plastidial retrograde signal methyl erythritol cyclopyrophosphate is a regulator of SA and JA cross talk. Wang et al. (2018) identified two ABA-responsive plastid-localized lipases which are involved in JA biosynthesis (cf. Mach 2018). Farmer and Mueller (2013), among others, proposed a link between jasmonate and ROS signaling. Thus, JA, SA, and ABA appear to be coupled to retrograde-active signals. Since not even the cross talk between the phytohormones is completely understood, it appears that their involvement in the cross talk between plastids and nucleus will become an interesting research field in the near future.

18.7 Concluding Remarks

Obviously, there is much more to be discovered in the interorganellar cross talk (cf. Godoy Herz et al. 2014). For instance, metabolites specifically responding to singlet oxygen in the organelle need to be identified. The redox signaling network is likely important for the distribution of information within the cell and entire organisms (Dietz 2016; Dietz et al. 2016) and does not only include redox signals from the photosynthetic electron transport but also other metabolic processes which are regulated by internal and environmental signals. The flux rate in the tetrapyrrole pathway needs to be translated into traveling metabolites or signals. Although much work has been performed to understand the role of light stress for retrograde signaling, there are many open questions to be addressed with novel tools (Szechyńska-Hebda and Karpiński 2013). For instance, little is known about processes balancing energy distribution and stress responses (Woodson 2016). Information transfer between organelles involves reversible phosphorylation events and Ca^{2+} signaling, and they have been barely investigated in this scenario (Chandok et al. 2001; Pesaresi et al. 2011; Guo et al. 2016). Whether proteins or peptides leave the organelle and inform the nucleus is also an open question. Finally, plastids play an essential role in phytohormone functions. They have a tremendous influence on gene expression profiles and developmental strategies (cf. Li et al. 2013; Serrano et al. 2016). Phytohormones determine the response of the plant to environmental signals and the decision of the plant to invest in either growth and productivity or defense. Not all concepts could be covered in this brief overview. For instance, Burch-Smith et al. (2011) proposed an organelle-nucleus cross talk via plasmodesmata. Signaling via Ca^{2+} levels coordinates many responses and integrates cell's internal and external information (Guo et al. 2016; de Souza et al. 2017). The Ca^{2+} signaling network is well known to participate in mitochondrial retrograde signaling (cf. Butow and Avadhani 2004). Many volatiles and secondary metabolites are partially synthesized in plastids and have tremendous influences on nuclear gene expression. Considering the central role of plastids for all processes in the plant cell and entire plant, there

are probably many more communication systems that will be discovered in the future. Finally, the cross talk between plastid- and mitochondria-derived signals has been little investigated (Van Aken and Pogson 2017).

References

Abreu ME, Munné-Bosch S (2009) Salicylic acid deficiency in *NahG* transgenic lines and *sid2* mutants increases seed yield in the annual plant *Arabidopsis thaliana*. J Exp Bot 60:1261–1271

Auldridge ME, McCarty DR, Klee HJ (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol 9:315–321

Avendaño-Vázquez AO, Cordoba E, Llamas E, San Román C, Nisar N, De la Torre S, Ramos-Vega M, de la Luz Gutiérrez-Nava M, Cazzonelli CI, Pogson BJ, León P (2014) An uncharacterized apocarotenoid-derived signal generated in ζ-carotene desaturase mutants regulates leaf development and the expression of chloroplast and nuclear genes in Arabidopsis. Plant Cell 26:2524–2537

Baier M, Stroher E, Dietz KJ (2004) The acceptor availability at photosystem I and ABA control nuclear expression of 2-Cys peroxiredoxin-A in *Arabidopsis thaliana*. Plant Cell Physiol 45:997–1006

Bajracharya D, Bergfield R, Hatzfeld W-D, Klein S, Schopfer P (1987) Regulatory involvement of plastids in the development of peroxisomal enzymes in the cotyledons of mustard (*Sinapis alba* L.) seedlings. J Plant Physiol 126:421–436

Banerjee A, Sharkey TD (2014) Methylerythritol 4-phosphate (MEP) pathway metabolic regulation. Nat Prod Rep 31:1043–1055

Barajas-López J de D, Blanco NE, Strand Å (2013) Plastid-to-nucleus communication, signals controlling the running of the plant cell. Biochim Biophys Acta 1833:425–437

Barkan A, Small I (2014) Pentatricopeptide repeat proteins in plants. Annu Rev Plant Biol 65:415–442

Batschauer A, Mösinger E, Kreuz K, Dörr I, Apel K (1986) The implications of a plastid-derived factor in the transcriptional control of nuclear genes encoding the light-harvesting chlorophyll *a/b* protein. Eur J Biochem 154:625–634

Bellafiore S, Barneche F, Peltier G, Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433:892–895

Berglund AK, Spånning E, Biverståhl H, Maddalo G, Tellgren-Roth C, Mäler L, Glaser E (2009) Dual targeting to mitochondria and chloroplasts: characterization of Thr-tRNA synthetase targeting peptide. Mol Plant 2:1298–1309

Bernardi G (1979) The petite mutation in yeast. Trends Biochem Sci 4:197–201

Blair GE, Ellis RJ (1973) Protein synthesis in chloroplasts. I. Light-driven synthesis of the large subunit of fraction I protein by isolated pea chloroplasts. Biochim Biophys Acta 319:223–234

Bogorad L (1975) Evolution of organelles and eukaryotic genomes. Science 188:891–898

Bolle C, Sopory S, Lübberstedt T, Klösgen RB, Herrmann RG, Oelmüller R (1994) The role of plastids in the expression of nuclear genes for thylakoid proteins studied with chimeric β-glucuronidase gene fusions. Plant Physiol 105:1355–1364

Bolle C, Kusnetsov VV, Herrmann RG, Oelmüller R (1996a) The spinach AtpC and AtpD genes contain elements for light-regulated, plastid-dependent and organ-specific expression in the vicinity of the transcription start sites. Plant J 9:21–30

Bolle C, Herrmann RG, Oelmüller R (1996b) Intron sequences are involved in the plastid- and light-dependent expression of the spinach PsaD gene. Plant J 10:919–924

Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T, Jahns P, Leister D (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437:1179–1182

Börner T (2017) The discovery of plastid-to-nucleus retrograde signaling-a personal perspective. Protoplasma 254:1845–1855

Börner T, Knoth R, Herrmann F, Hagemann R (1972) Struktur und Funktion der genetischen Information in den Plastiden. V. Das Fehlen von ribosomaler RNS in den Plastiden der Plastommutante "Mrs. Parker" von *Pelargonium zonale* Ait. Theor Appl Genet 42:3–11

Börner T, Herrmann FH, Hagemann R (1973) Plastid ribosome deficient mutants in *Pelargonium zonale*. FEBS Lett 37:17–19

Börner T, Knoth R, Herrmann F, Hagemann R (1974) Struktur und Funktion der genetischen Information in den Plastiden. X. Das Fehlen von Fraktion-I-Protein in den weißen Plastiden einiger Sorten von *Pelargonium zonale*. Biochem Physiol Pflanz 165:429–432

Börner T, Schumann B, Hagemann R (1976) Biochemical studies on a plastid ribosome-deficient mutant of *Hordeum vulgare*. In: Bücher T, Neupert W, Sebald W, Werner S (eds) Genetics and biogenesis of chloroplasts and mitochondria. Elsevier/North-Holland Medical Press, Amsterdam, pp 41–48

Börner T, Mendel RR, Schiemann J (1986) Nitrate reductase is not accumulated in chloroplast-ribosome-deficient mutants of higher plants. Planta 169:202–207

Bradbeer JW, Börner T (1978) Activities of glyceraldehyde-dephosphate dehydrogenase ($NADP^+$) and phosphoribulokinase in two barley mutants deficient in chloroplast ribosomes. In: Akoyunoglou G, Argyroudi-Akoyunoglou JG (eds) Chloroplast development. North Holland, Amsterdam, pp 727–732

Bradbeer JW, Atkinson YE, Börner T, Hagemann R (1979) Cytoplasmic synthesis of plastid polypeptides may be controlled by plastid-synthesised RNA. Nature 279:816–817

Bräutigam K, Dietzel L, Kleine T, Ströher E, Wormuth D, Dietz KJ, Radke D, Wirtz M, Hell R, Dörmann P, Nunes-Nesi A, Schauer N, Fernie AR, Oliver SN, Geigenberger P, Leister D, Pfannschmidt T (2009) Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. Plant Cell 21:2715–2732

Brunkard JO, Burch-Smith TM (2018) Ties that bind: the integration of plastid signalling pathways in plant cell metabolism. Essays Biochem 62:95–107

Burch-Smith TM, Brunkard JO, Choi YG, Zambryski PC (2011) Organelle-nucleus cross-talk regulates plant intercellular communication via plasmodesmata. Proc Natl Acad Sci U S A 108:E1451–E1460

Burgess DG, Taylor WC (1988) Chloroplast photooxidation affects the transcription of a nuclear gene family. Mol Gen Genet 214:89–96

Butow RA, Avadhani NG (2004) Mitochondrial signaling: the retrograde response. Mol Cell 14:1–15

Cappadocia L, Maréchal A, Parent JS, Lepage E, Sygusch J, Brisson N (2010) Crystal structures of DNA-Whirly complexes and their role in *Arabidopsis* organelle genome repair. Plant Cell 22:1849–1867

Cappadocia L, Parent JS, Zampini E, Lepage E, Sygusch J, Brisson N (2012) A conserved lysine residue of plant Whirly proteins is necessary for higher order protein assembly and protection against DNA damage. Nucleic Acids Res 40:258–269

Chan PH, Wildman SG (1972) Chloroplast DNA codes for the primary structure of the large subunit of fraction I protein. Biochim Biophys Acta 277:677–680

Chan KX, Crisp PA, Estavillo GM, Pogson BJ (2010) Chloroplast-to-nucleus communication: current knowledge, experimental strategies and relationship to drought stress signaling. Plant Signal Behav 5:1575–1582

Chan KX, Phua SY, Crisp P, McQuinn R, Pogson BJ (2016) Learning the languages of the chloroplast: retrograde signaling and beyond. Annu Rev Plant Biol 67:25–53

Chandok MR, Sopory SK, Oelmüller R (2001) Cytoplasmic kinase and phosphatase activities can induce *PsaF* gene expression in the absence of functional plastids: evidence that phosphorylation/dephosphorylation events are involved in interorganellar crosstalk. Mol Gen Genet 264:819–826

Chi W, Sun X, Zhang L (2013) Intracellular signaling from plastid to nucleus. Annu Rev Plant Biol 64:559–582

Chi W, Feng P, Ma J, Zhang L (2015) Metabolites and chloroplast retrograde signaling. Curr Opin Plant Biol 25:32–38

Colombo M, Tadini L, Peracchio C, Ferrari R, Pesaresi P (2016) GUN1, a Jack-of-all-trades in chloroplast protein homeostasis and signaling. Front Plant Sci 7:1427

Criddle RS, Dau B, Kleinkopf DE, Huffaker RC (1970) Differential synthesis of ribulose diphosphate subunits. Biochem Biophys Res Commun 41:621–627

de Souza A, Wang JZ, Dehesh K (2017) Retrograde signals: integrators of interorganellar communication and orchestrators of plant development. Annu Rev Plant Biol 68:85–108

Demmig-Adams B, Stewart JJ, Adams WW 3rd (2017) Environmental regulation of intrinsic photosynthetic capacity: an integrated view. Curr Opin Plant Biol 37:34–41

Dempsey DA, Vlot AC, Wildermuth MC, Klessig DF (2011) Salicylic acid biosynthesis and metabolism. Arabidopsis Book 9:e0156

Desveaux D, Després C, Joyeux A, Subramaniam R, Brisson N (2000) PBF-2 is a novel single-stranded DNA binding factor implicated in PR-10a gene activation in potato. Plant Cell 12:1477–1489

Desveaux D, Subramaniam R, Després C, Mess JN, Lévesque C, Fobert PR, Dangl JL, Brisson N (2004) A "Whirly" transcription factor is required for salicylic acid-dependent disease resistance in Arabidopsis. Dev Cell 6:229–240

Dietz KJ (2016) Thiol-Based peroxidases and ascorbate peroxidases: why plants rely on multiple peroxidase systems in the photosynthesizing chloroplast? Mol Cells 39:20–25

Dietz KJ, Vogel MO, Viehhauser A (2010) AP2/EREBP transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. Protoplasma 245:3–14

Dietz KJ, Turkan I, Krieger-Liszkay A (2016) Redox- and reactive oxygen species-dependent signaling into and out of the photosynthesizing chloroplast. Plant Physiol 171:1541–1550

Dietzel L, Bräutigam K, Pfannschmidt T (2008) Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry – functional relationships between short-term and long-term light quality acclimation in plants. FEBS J 275:1080–1088

Dietzel L, Gläßer C, Liebers M, Hiekel S, Courtois F, Czarnecki O, Schlicke H, Zubo Y, Börner T, Mayer K, Grimm B, Pfannschmidt T (2015) Identification of early nuclear target genes of plastidial redox signals that trigger the long-term response of Arabidopsis to light quality shifts. Mol Plant 8:1237–1252

Dogra V, Duan J, Lee KP, Lv S, Liu R, Kim C (2017) FtsH2-dependent proteolysis of EXECUTER1 Is essential in mediating singlet oxygen-triggered retrograde signaling in *Arabidopsis thaliana*. Front Plant Sci 8:1145

Dörmann P, Kim H, Ott T, Schulze-Lefert P, Trujillo M, Wewer V, Hückelhoven R (2014) Cell-autonomous defense, re-organization and trafficking of membranes in plant-microbe interactions. New Phytol 204:815–822

Duanmu D, Casero D, Dent RM, Gallaher S, Yang W, Rockwell NC, Martin SS, Pellegrini M, Niyogi KK, Merchant SS, Grossman AR, Lagarias JC (2013) Retrograde bilin signaling enables *Chlamydomonas* greening and phototrophic survival. Proc Natl Acad Sci U S A 110:3621–3626

Eguchi S, Takano H, Ono K, Takio S (2002) Photosynthetic electron transport regulates the stability of the transcript for the protochlorophyllide oxidoreductase gene in the liverwort, *Marchantia paleacea* var. *diptera*. Plant Cell Physiol 43:573–577

Eisenhut M, Hocken N, Weber AP (2015) Plastidial metabolite transporters integrate photorespiration with carbon, nitrogen, and sulfur metabolism. Cell Calcium 58:98–104

Ellis RJ (1975) Inhibition of plastid protein synthesis by lincomycin and 2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide. Phytochemistry 14:89–93

Ellis RJ (1977) Protein synthesis by isolated chloroplasts. Biochim Biophys Acta 463:285–315

Escoubas JM, Lomas M, LaRoche J, Falkowski PG (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. Proc Natl Acad Sci U S A 92:10237–11041

Estavillo GM, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, Carrie C, Giraud E, Whelan J, David P, Javot H, Brearley C, Hell R, Marin E, Pogson BJ (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. Plant Cell 23:3992–4012

Estavillo GM, Chan KX, Phua SY, Pogson BJ (2013) Reconsidering the nature and mode of action of metabolite retrograde signals from the chloroplast. Front Plant Sci 3:300
Farmer EE, Mueller MJ (2013) ROS-mediated lipid peroxidation and RES-activated signaling. Annu Rev Plant Biol 64:429–450
Feierabend J (1977) Capacity for chlorophyll synthesis in heat-bleached 70S ribosome-deficient rye leaves. Planta 135:83–88
Feierabend J, Mikus M (1977) Occurrence of a high temperature sensitivity of chloroplast ribosome formation in several higher plants. Plant Physiol 59:863–867
Feierabend J, Schrader-Reichhardt U (1976) Biochemical differentiation of plastids and other organelles in rye leaves with a high-temperature-induced deficiency of plastid ribosomes. Planta 129:133–145
Fernández AP, Strand A (2008) Retrograde signaling and plant stress: plastid signals initiate cellular stress responses. Curr Opin Plant Biol 11:509–513
Fey V, Wagner R, Braütigam K, Wirtz M, Hell R, Dietzmann A, Leister D, Oelmüller R, Pfannschmidt T (2005) Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of Arabidopsis thaliana. J Biol Chem 280:5318–5328
Flügge UI, Häusler RE, Ludewig F, Gierth M (2011) The role of transporters in supplying energy to plant plastids. J Exp Bot 62:2381–2392
Foyer CH, Karpinska B, Krupinska K (2014) The functions of WHIRLY1 and REDOX-RESPONSIVE TRANSCRIPTION FACTOR 1 in cross tolerance responses in plants: a hypothesis. Philos Trans R Soc Lond Ser B Biol Sci 369:20130226
Galvez-Valdivieso G, Mullineaux PM (2010) The role of reactive oxygen species in signalling from chloroplasts to the nucleus. Physiol Plant 138:430–439
Garcion C, Lohmann A, Lamodière E, Catinot J, Buchala A, Doermann P, Métraux JP (2008) Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of Arabidopsis. Plant Physiol 147:1279–1287
Ge C, Spånning E, Glaser E, Wieslander A (2014) Import determinants of organelle-specific and dual targeting peptides of mitochondria and chloroplasts in *Arabidopsis thaliana*. Mol Plant 7:121–136
Gigolashvili T, Geier M, Ashykhmina N, Frerigmann H, Wulfert S, Krueger S, Mugford SG, Kopriva S, Haferkamp I, Flügge UI (2012) Much more than a thylakoid ADP/ATP carrier–enlightening a role of TAAC in plastidic phosphoadenosine 50-phosphosulfate (PAPS) supply to the cytosol. Plant Cell 24:4187–4204
Gile GH, Moog D, Slamovits CH, Maier UG, Archibald JM (2015) Dual organellar targeting of aminoacyl-tRNA synthetases in diatoms and cryptophytes. Genome Biol Evol 7:1728–1742
Giuliano G, Scolnik PA (1988) Transcription of two photosynthesis-associated nuclear gene families correlates with the presence of chloroplasts in leaves of the variegated tomato *ghost* mutant. Plant Physiol 86:7–9
Givan AL, Criddle RS (1972) Ribulose diphosphate carboxylase from *Chlamydomonas reinhardi*: purification, properties and mode of synthesis in the cell. Arch Biochem Biophys 149:153–154
Godoy Herz MA, Kornblihtt AR, Barta A, Kalyna M, Petrillo E (2014) Shedding light on the chloroplast as a remote control of nuclear gene expression. Plant Signal Behav 9:e976150
Gollan PJ, Tikkanen M, Aro EM (2015) Photosynthetic light reactions: integral to chloroplast retrograde signalling. Curr Opin Plant Biol 27:180–191
Grabowski E, Miao Y, Mulisch M, Krupinska K (2008) Single-stranded DNA-binding protein Whirly1 in barley leaves is located in plastids and the nucleus of the same cell. Plant Physiol 147:1800–1804
Gray JC, Sornarajah R, Zabron AA, Duckett CM, Khan MS (1995) Chloroplast control of nuclear gene expression. In: Mathis P (ed) Photosynthesis, from light to biosphere. Kluwer, Dordrecht, pp 543–550
Greiner S, Bock R (2013) Tuning a menage a trois: co-evolution and co-adaptation of nuclear and organellar genomes in plants. BioEssays 35:354–365
Grieshaber NA, Fischer ER, Mead DJ, Dooley CA, Hackstadt T (2004) Chlamydial histone-DNA interactions are disrupted by a metabolite in the methylerythritol phosphate pathway of isoprenoid biosynthesis. Proc Natl Acad Sci U S A 101:7451–7756

Grieshaber NA, Sager JB, Dooley CA, Hayes SF, Hackstadt T (2006) Regulation of the *Chlamydia trachomatis* histone H1-like protein Hc2 is IspE dependent and IhtA independent. J Bacteriol 188:5289–5292

Guo H, Feng P, Chi W, Sun X, Xu X, Li Y, Ren D, Lu C, Rochaix JD, Leister D, Zhang L (2016) Plastid-nucleus communication involves calcium-modulated MAPK signalling. Nat Commun 7:12173

Hagemann R, Börner T (1987) Plastid ribosome-deficient mutants of higher plants as a tool in studying chloroplast biogenesis. In: Akoyunoglou G, Argyroudi-Akoyunoglou JG (eds) Chloroplast development. North Holland, Amsterdam, pp 709–720

Han GZ (2017) Evolution of jasmonate biosynthesis and signaling mechanisms. J Exp Bot 68:1323–1331

Hanke G, Mulo P (2013) Plant type ferredoxins and ferredoxin-dependent metabolism. Plant Cell Environ 36:1071–1084

Harpster MH, Mayfield SP, Taylor WC (1984) Effects of pigment-deficient mutants on the accumulation of photosynthetic proteins in maize. Plant Mol Biol 3:258–263

Hawkesford MJ, De Kok LJ (2006) Managing sulphur metabolism in plants. Plant Cell Environ 29:382–395

Hernández-Verdeja T, Strand Å (2018) Retrograde signals navigate the path to chloroplast development. Plant Physiol 176:967–976

Hess WR, Müller A, Nagy F, Börner T (1994) Ribosome-deficient plastids affect transcription of light-induced nuclear genes: genetic evidence for a plastid-derived signal. Mol Gen Genet 242:305–312

Huang J, Gu M, Lai Z, Fan B, Shi K, Zhou YH, Yu JQ, Chen Z (2010) Functional analysis of the Arabidopsis *PAL* gene family in plant growth, development, and response to environmental stress. Plant Physiol 153:1526–1538

Huang H, Liu B, Liu L, Song S (2017) Jasmonate action in plant growth and development. J Exp Bot 68:1349–1359

Inaba T (2010) Bilateral communication between plastid and the nucleus: plastid protein import and plastid-to-nucleus retrograde signaling. Biosci Biotechnol Biochem 74:471–476

Inaba T, Yazu F, Ito-Inaba Y, Kakizaki T, Nakayama K (2011) Retrograde signaling pathway from plastid to nucleus. Int Rev Cell Mol Biol 290:167–204

Isemer R, Mulisch M, Schafer A, Kirchner S, Koop HU, Krupinska K (2012) Recombinant Whirly1 translocates from transplastomic chloroplasts to the nucleus. FEBS Lett 586:85–88

Johanningmeier U, Howell SH (1984) Regulation of light-harvesting chlorophyll-binding protein messenger-RNA accumulation in *Chlamydomonas rheinhardi* – possible involvement of chlorophyll synthesis precursors. J Biol Chem 259:3541–3549

Jung HS, Crisp PA, Estavillo GM, Cole B, Hong F, Mockler TC, Pogson BJ, Chory J (2013) Subset of heat-shock transcription factors required for the early response of Arabidopsis to excess light. Proc Natl Acad Sci U S A 110:1447–1479

Karlsson PM, Herdean A, Adolfsson L, Beebo A, Nziengui H, Irigoyen S, Ünnep R, Zsiros O, Nagy G, Garab G, Aronsson H, Versaw WK, Spetea C (2015) The Arabidopsis thylakoid transporter PHT4;1 influences phosphate availability for ATP synthesis and plant growth. Plant J 84:99–110

Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in Arabidopsis during excess light stress. Plant Cell 9:627–640

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science 284:654–657

Karpiński S, Szechyńska-Hebda M, Wituszyńska W, Burdiak P (2013) Light acclimation, retrograde signalling, cell death and immune defences in plants. Plant Cell Environ 36:736–744

Kim C, Apel K (2013a) Singlet oxygen-mediated signaling in plants: moving from *flu* to wild type reveals an increasing complexity. Photosynth Res 116:455–464

Kim C, Apel K (2013b) O_2-mediated and EXECUTER-dependent retrograde plastid-to-nucleus signaling in norflurazon-treated seedlings of *Arabidopsis thaliana*. Mol Plant 6:1580–1591

Kindgren P, Kremnev D, Blanco NE, de Dios Barajas López J, Fernández AP, Tellgren-Roth C, Kleine T, Small I, Strand A (2012) The plastid redox insensitive 2 mutant of Arabidopsis is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. Plant J 70:279–291

Kirk JTO (1971) Chloroplast structure and biogenesis. Annu Rev Biochem 40:161–196

Kirk JTO, Tilney-Bassett RAE (1967) The plastids. Freeman, London

Klein M, Papenbrock J (2004) The multi-protein family of Arabidopsis sulphotransferases and their relatives in other plant species. J Exp Bot 55:1809–1820

Kleine T, Leister D (2016) Retrograde signaling: organelles go networking. Biochim Biophys Acta 1857:1313–1325

Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, Lim J, Mittler R, Chory J (2007) Signals from chloroplasts converge to regulate nuclear gene expression. Science 316:715–719

Krause K, Krupinska K (2009) Nuclear regulators with a second home in organelles. Trends Plant Sci 14:194–199

Krause K, Kilbienski I, Mulisch M, Rödiger A, Schäfer A, Krupinska K (2005) DNA-binding proteins of the Whirly family in *Arabidopsis thaliana* are targeted to the organelles. FEBS Lett 579:3707–3712

Krause K, Oetke S, Krupinska K (2012) Dual targeting and retrograde translocation: regulators of plant nuclear gene expression can be sequestered by plastids. Int J Mol Sci 13:11085–11101

Kropat J, Oster U, Rüdiger W, Beck CF (1997) Chlorophyll precursors are signals of chloroplast origin involved in light induction of nuclear heat-shock genes. Proc Natl Acad Sci U S A 94:14168–14172

Kropat J, Oster U, Rüdiger W, Beck CF (2000) Chloroplast signalling in the light induction of nuclear HSP70 genes requires the accumulation of chlorophyll precursors and their accessibility to cytoplasm/nucleus. Plant J 24:523–531

Krupinska K, Dähnhardt D, Fischer-Kilbienski I, Kucharewicz W, Scharrenberg C, Trösch M, Buck F (2013) Identification of WHIRLY1 as a factor binding to the promoter of the stress and senescence-associated gene HvS40. J Plant Growth Regul 33:91

Kusnetsov V, Bolle C, Lübberstedt T, Sopory S, Herrmann RG, Oelmüller R (1996) Evidence that the plastid signal and light operate via the same cis-acting elements in the promoters of nuclear genes for plastid proteins. Mol Gen Genet 252:631–639

Kusnetsov V, Landsberger M, Meurer J, Oelmüller R (1999) The assembly of the CAAT-box binding complex at a photosynthesis gene promoter is regulated by light, cytokinin, and the stage of the plastids. J Biol Chem 274:36009–36014

Laloi C, Przybyla D, Apel K (2006) A genetic approach towards elucidating the biological activity of different reactive oxygen species in *Arabidopsis thaliana*. J Exp Bot 57:1719–1724

Langner U, Baudisch B, Klösgen RB (2014) Organelle import of proteins with dual targeting properties into mitochondria and chloroplasts takes place by the general import pathways. Plant Signal Behav 9:e29301

Larkin RM, Alonso JM, Ecker JR, Chory J (2003) GUN4, a regulator of chlorophyll synthesis and intracellular signaling. Science 299:902–906

Lee KP, Kim C, Landgraf F, Apel K (2007) EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 104:10270–10275

Leister D (2005) Genomics-based dissection of the cross-talk of chloroplasts with the nucleus and mitochondria in Arabidopsis. Gene 354:110–116

Leister D (2016) Towards understanding the evolution and functional diversification of DNA-containing plant organelles. F1000 Res 5:330

Lemos M, Xiao Y, Bjornson M, Wang JZ, Hicks D, Ad S, Wang CQ, Yang P, Ma S, Dinesh-Kumar S, Dehesh K (2016) The plastidial retrograde signal methyl erythritol cyclopyrophosphate is a regulator of salicylic acid and jasmonic acid crosstalk. J Exp Bot 67:1557–1566

Lepage É, Zampini É, Brisson N (2013) Plastid genome instability leads to reactive oxygen species production and plastid-to-nucleus retrograde signaling in *Arabidopsis*. Plant Physiol 163:867–881

Lepistö A, Rintamäki E (2012) Coordination of plastid and light signaling pathways upon development of Arabidopsis leaves under various photoperiods. Mol Plant 5:799–816

Lepistö A, Toivola J, Nikkanen L, Rintamäki E (2012) Retrograde signaling from functionally heterogeneous plastids. Front Plant Sci 3:286

Li B, Kronzucker HJ, Shi W (2013) Molecular components of stress-responsive plastid retrograde signaling networks and their involvement in ammonium stress. Plant Signal Behav 8:e23107

Linka N, Theodoulou FL (2013) Metabolite transporters of the plant peroxisomal membrane: known and unknown. Subcell Biochem 69:169–194

Linka N, Weber AP (2010) Intracellular metabolite transporters in plants. Mol Plant 3:21–53

Lübberstedt T, Oelmüller R, Wanner G, Herrmann RG (1994) The role of plastids in the expression of nuclear genes for thylakoid proteins studied with chimeric β-glucuronidase gene fusions. Plant Physiol 105:1355–1364

Lurin C, Andrés C, Aubourg S, Bellaoui M, Bitton F, Bruyère C, Caboche M, Debast C, Gualberto J, Hoffmann B, Lecharny A, Le Ret M, Martin-Magniette ML, Mireau H, Peeters N, Renou JP, Szurek B, Taconnat L, Small I (2004) Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell 16:2089–2103

Lv F, Zhou J, Zeng L, Xing D (2015) β-Cyclocitral upregulates salicylic acid signalling to enhance excess light acclimation in *Arabidopsis*. J Exp Bot 66:4719–4732

Macaulay KM, Heath GA, Ciulli A, Murphy AM, Abell C, Carr JP, Smith AG (2017) The biochemical properties of the two *Arabidopsis thaliana* isochorismate synthases. Biochem J 474:1579–1590

Mach J (2018) The lipase Link: abscisic acid induces PLASTID LIPASES, which produce Jasmonic acid precursors. Plant Cell 30(5):948–949

Martin G, Leivar P, Ludevid D, Tepperman JM, Quail PH, Monte E (2016) Phytochrome and retrograde signalling pathways converge to antagonistically regulate a light-induced transcriptional network. Nat Commun 7:11431

Martínez C, Pons E, Prats G, León J (2004) Salicylic acid regulates flowering time and links defence responses and reproductive development. Plant J 37:209–217

Maruta T, Noshi M, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2012) H_2O_2-triggered retrograde signaling from chloroplasts to nucleus plays specific role in response to stress. J Biol Chem 287:11717–11729

Maruta T, Sawa Y, Shigeoka S, Ishikawa T (2016) Diversity and evolution of ascorbate peroxidase functions in chloroplasts: more than just a classical antioxidant enzyme? Plant Cell Physiol 57:1377–1386

Maxwell DP, Laudenbach DE, Huner N (1995) Redox regulation of light-harvesting complex II and cab mRNA abundance in Dunaliella salina. Plant Physiol 109:787–795

Mayfield SP, Taylor WC (1984) Carotenoid-deficient maize seedlings fail to accumulate light-harvesting chlorophyll a/b binding protein (LHCP) mRNA. Eur J Biochem 144:79–84

Mayfield SP, Taylor WV (1987) Chloroplast photooxidation inhibits the expression of a set of nuclear genes. Mol Gen Genet 208:309–314

Mazzoleni M, Figuet S, Martin-Laffon J, Mininno M, Gilgen A, Leroux M, Brugière S, Tardif M, Alban C, Ravanel S (2015) Dual targeting of the protein methyltransferase PrmA contributes to both chloroplastic and mitochondrial ribosomal protein L11 methylation in Arabidopsis. Plant Cell Physiol 56:1697–1710

Mehrshahi P, Johnny C, DellaPenna D (2014) Redefining the metabolic continuity of chloroplasts and ER. Trends Plant Sci 19:501–507

Melonek J, Mulisch M, Schmitz-Linneweber C, Grabowski E, Hensel G, Krupinska K (2010) Whirly1 in chloroplasts associates with intron containing RNAs and rarely co-localizes with nucleoids. Planta 232:471–481

Meskauskiene R, Nater M, Goslings D, Kessler F, op den Camp R, Apel K (2001) FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 98:12826–12831

Miao Y, Jiang J, Ren Y, Zhao Z (2013) The single-stranded DNA-binding protein WHIRLY1 represses WRKY53 expression and delays leaf senescence in a developmental stage-dependent manner in Arabidopsis. Plant Physiol 163:746–756

Millar AH, Whelan J, Small I (2006) Recent surprises in protein targeting to mitochondria and plastids. Curr Opin Plant Biol 9:610–615

Mochizuki N, Brusslan JA, Larkin R, Nagatani A, Chory J (2001) Arabidopsis genomes uncoupled 5 (*GUN5*) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. Proc Natl Acad Sci U S A 98:2053–2058

Mochizuki N, Tanaka R, Tanaka A, Masuda T, Nagatani A (2008) The steady-state level of Mg-protoporphyrin IX is not a determinant of plastid-to-nucleus signaling in Arabidopsis. Proc Natl Acad Sci U S A 105:15184–15189

Mohr H, Neininger A, Seith B (1992) Control of nitrate reductase and nitrite reductase gene expression by light, nitrate and a plastidic factor. Bot Acta 105:81–89

Morris K, MacKerness SA, Page T, John CF, Murphy AM, Carr JP, Buchanan-Wollaston V (2000) Salicylic acid has a role in regulating gene expression during leaf senescence. Plant J 23:677–685

Moulin M, McCormac AC, Terry MJ, Smith AG (2008) Tetrapyrrole profiling in *Arabidopsis* seedlings reveals that retrograde plastid nuclear signaling is not due to Mg-protoporphyrin IX accumulation. Proc Natl Acad Sci U S A 105:15178–15183

Nevarez PA, Qiu Y, Inoue H, Yoo CY, Benfey PN, Schnell DJ, Chen M (2017) Mechanism of dual targeting of the phytochrome signaling component HEMERA/pTAC12 to plastids and the nucleus. Plant Physiol 173:1953–1966

Nomura H, Komori T, Uemura S, Kanda Y, Shimotani K, Nakai K, Furuichi T, Takebayashi K, Sugimoto T, Sano S, Suwastika IN, Fukusaki E, Yoshioka H, Nakahira Y, Shiina T (2012) Chloroplast-mediated activation of plant immune signalling in *Arabidopsis*. Nat Commun 3:926

Nott A, Jung HS, Koussevitzky S, Chory J (2006) Plastid-to-nucleus retrograde signaling. Annu Rev Plant Biol 57:739–759

Oelmüller R (1989) Photooxidative destruction of chloroplasts and its effect on nuclear gene expression and extraplastidic enzyme levels. Photochem Photobiol l49:229–239

Oelmüller R, Briggs WR (1990) Intact plastids are required for nitrate- and light-induced accumulation of nitrate reductase activity and mRNA in squash cotyledons. Plant Physiol 92:434–439

Oelmüller R, Mohr H (1986) Photooxidative destruction of chloroplasts and its consequences for expression of nuclear genes. Planta 167:106–113

Oelmüller R, Schuster C (1987) Inhibition and promotion by light of the accumulation of translatable mRNA of the light-harvesting chlorophyll *a/b*-binding protein of photosystem II. Planta 172:60–70

Oelmüller R, Dietrich G, Link G, Mohr H (1986a) Regulatory factors involved in gene expression (subunits of ribulose-1,5-bisphosphate carboxylase) in mustard (*Sinapis alba* L.) cotyledons. Planta 169:260–266

Oelmüller R, Levitan I, Bergfeld R, Rajasekhar VK, Mohr H (1986b) Expression of nuclear genes as affected by treatments acting on the plastids. Planta 168:482–492

Oelmüller R, Schuster C, Mohr H (1988) Physiological characterization of a plastidic signal required for nitrate-induced appearance of nitrate and nitrite reductases. Planta 174:75–83

Oelmüller R, Bolle C, Tyagi AK, Niekrawietz N, Breit S, Herrmann RG (1993) Characterization of the promoter from the single-copy gene encoding ferredoxin-$NADP^+$-oxidoreductase from spinach. Mol Gen Genet 237:261–272

op den Camp R, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Göbel C, Feussner I, Nater M, Apel K (2003) Rapid induction of distinct stress responses after the release of singlet oxygen in *Arabidopsis*. Plant Cell 15:2320–2332

Ostrovsky D, Kharatian E, Malarova I, Shipanova I, Sibeldina L, Shashkov A, Tantsirev G (1992) Synthesis of a new organic pyrophosphate in large quantities is induced in some bacteria by oxidative stress. Biofactors 3:261–264

Ostrovsky D, Diomina G, Lysak E, Matveeva E, Ogrel O, Trutko S (1998) Effect of oxidative stress on the biosynthesis of 2-*C*-methyl-d-erythritol-2,4-cyclopyrophosphate and isoprenoids by several bacterial strains. Arch Microbiol 171:69–72

Otori K, Tanabe N, Maruyama T, Sato S, Yanagisawa S, Tamoi M, Shigeoka S (2017) Enhanced photosynthetic capacity increases nitrogen metabolism through the coordinated regulation of carbon and nitrogen assimilation in Arabidopsis thaliana. J Plant Res 130:909–927

Padmanabhan MS, Dinesh-Kumar SP (2010) All hands on deck—the role of chloroplasts, endoplasmic reticulum and the nucleus in driving plant innate immunity. Mol Plant-Microbe Interact 23:1368–1380

Page MT, Kacprzak SM, Mochizuki N, Okamoto H, Smith AG, Terry MJ (2017) Seedlings lacking the PTM protein do not show a *genomes uncoupled* (*gun*) mutant phenotype. Plant Physiol 174:21–26

Pesaresi P, Masiero S, Eubel H, Braun HP, Bhushan S, Glaser E, Salamini F, Leister D (2006) Nuclear photosynthetic gene expression is synergistically modulated by rates of protein synthesis in chloroplasts and mitochondria. Plant Cell 18:970–991

Pesaresi P, Schneider A, Kleine T, Leister D (2007) Interorganellar communication. Curr Opin Plant Biol 10:600–606

Pesaresi P, Hertle A, Pribil M, Kleine T, Wagner R, Strissel H, Ihnatowicz A, Bonardi V, Scharfenberg M, Schneider A, Pfannschmidt P, Leister D (2009) Arabidopsis STN7 kinase provides a link between short- and long-term photosynthetic acclimation. Plant Cell 21:2402–2423

Pesaresi P, Pribil M, Wunder T, Leister D (2011) Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. Biochim Biophys Acta 1807:887–896

Petracek ME, Dickey LF, Nguyen TT, Gatz C, Sowinski DA, Allen GC, Thompson WF (1998) Ferredoxin-1 mRNA is destabilized by changes in photosynthetic electron transport. Proc Natl Acad Sci U S A 95:9009–9013

Pfalz J, Liere K, Kandlbinder A, Dietz KJ, Oelmüller R (2006) pTAC2, -6 and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 18:176–197

Pfalz J, Liebers M, Hirth M, Grübler B, Holtzegel U, Schröter Y, Dietzel L, Pfannschmidt T (2012) Environmental control of plant nuclear gene expression by chloroplast redox signals. Front Plant Sci 3:257

Pfannschmidt T, Nilsson A, Tullberg A, Link G, Allen JF (1999) Direct transcriptional control of the chloroplast genes psbA and psaAB adjusts photosynthesis to light energy distribution in plants. IUBMB Life 48:271–276

Pfannschmidt T, Schütze K, Brost M, Oelmüller R (2001) A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. J Biol Chem 276:36125–36130

Pfannschmidt T, Schütze K, Fey V, Sherameti I, Oelmüller R (2003) Chloroplast redox control of nuclear gene expression – a new class of plastid signals in interorganellar communication. Antioxid Redox Signal 5:95–101

Piippo M, Allahverdiyeva Y, Paakkarinen V, Suoranta UM, Battchikova N, Aro EM (2006) Chloroplast-mediated regulation of nuclear genes in Arabidopsis thaliana in the absence of light stress. Physiol Genomics 25:142–152

Pogson BJ, Woo NS, Förster B, Small ID (2008) Plastid signalling to the nucleus and beyond. Trends Plant Sci 13:602–609

Poulsen C, Verpoorte R (1991) Roles of chorismate mutase, isochorismate synthase and anthranilate synthase in plants. Phytochemistry 30:377–386

Prikryl J, Watkins KP, Friso G, Van Wijk KJ, Barkan A (2008) A member of the Whirly family is a multifunctional RNA- and DNA-binding protein that is essential for chloroplast biogenesis. Nucleic Acids Res 36:5152–5165

Przybyla-Toscano J, Roland M, Gaymard F, Couturier J, Rouhier N (2018) Roles and maturation of iron-sulfur proteins in plastids. J Biol Inorg Chem 23(4):545–566

Pursiheimo S, Mulo P, Rintamaki E, Aro EM (2001) Coregulation of light-harvesting complex II phosphorylation and *lhcb* mRNA accumulation in winter rye. Plant J 26:317–327

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M (2012) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci U S A 109:5535–5540

Ramel F, Ksas B, Akkari E, Mialoundama AS, Monnet F, Krieger-Liszkay A, Ravanat JL, Mueller MJ, Bouvier F, Havaux M (2013a) Light-induced acclimation of the *Arabidopsis chlorina1* mutant to singlet oxygen. Plant Cell 25:1445–1462

Ramel F, Mialoundama AS, Havaux M (2013b) Nonenzymatic carotenoid oxidation and photooxidative stress signalling in plants. J Exp Bot 64:799–805

Rausch C, Bucher M (2002) Molecular mechanisms of phosphate transport in plants. Planta 216:23–37

Reichenbächer D, Börner T, Richter J (1978) Untersuchungen am Fraktion-I-Protein der Gerste mit Hilfe quantitativer Immunelektrophoresen. Biochem Physiol Pflanz 172:53–60

Reiss T, Bergfeld R, Link G, Thien W, Mohr H (1983) Photooxidative destruction of chloroplasts and its consequences for cytosolic enzyme levels and plant development. Planta 159:518–528

Ren Y, Li Y, Jiang Y, Wu B, Miao Y (2017) Phosphorylation of WHIRLY1 by CIPK14 shifts its localization and dual functions in Arabidopsis. Mol Plant 10:749–763

Rochaix JD (2013a) Plant science. Fine-tuning photosynthesis. Science 342:50–51

Rochaix JD (2013b) Redox regulation of thylakoid protein kinases and photosynthetic gene expression. Antioxid Redox Signal 18:2184–2201

Rokov-Plavec J, Dulic M, Duchêne AM, Weygand-Durasevic I (2008) Dual targeting of organellar seryl-tRNA synthetase to maize mitochondria and chloroplasts. Plant Cell Rep 27:1157–1168

Rossel JB, Walter PB, Hendrickson L, Chow WS, Poole A, Mullineaux PM, Poson BJ (2006) A mutation affecting ASCORBATE PEROXIDASE 2 gene expression reveals a link between responses to high light and drought tolerance. Plant Cell Environ 29:269–281

Schmid J, Amrhein N (1995) Molecular organization of the shikimate pathway in higher plants. Phytochemistry 39:737–749

Schuster C, Oelmüller R, Bergfeld R, Mohr H (1988) Recovery of plastids from photooxidative damage: significance of a plastidic factor. Planta 174:289–297

Seguel A, Jelenska J, Herrera-Vásquez A, Marr SK, Joyce MB, Gagesch KR, Shakoor N, Jiang S-C, Fonseca A, Wildermuth MC, Greenberg JT, Holuigue L (2018) PROHIBITIN3 forms complexes with ISOCHORISMATE SYNTHASE1 to regulate stress-induced salicylic acid biosynthesis in Arabidopsis. Plant Physiol 176:2515–2531

Serrano M, Wang B, Aryal B, Garcion C, Abou-Mansour E, Heck S, Geisler M, Mauch F, Nawrath C, Métraux JP (2013) Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5. Plant Physiol 162:1815–1821

Serrano I, Audran C, Rivas S (2016) Chloroplasts at work during plant innate immunity. J Exp Bot 67:3845–3854

Sherameti I, Nakamura M, Yamamoto YY, Pfannschmidt T, Obokata J, Oelmüller R (2002a) Polyribosome loading of spinach mRNAs for photosystem I subunits is controlled by photosynthetic electron transport. Plant J 32:631–639

Sherameti I, Sopory SK, Trebicka A, Pfannschmidt T, Oelmuller R (2002b) Photosynthetic electron transport determines nitrate reductase gene expression and activity in higher plants. J Biol Chem 277:46594–46600

Shine MB, Yang JW, El-Habbak M, Nagyabhyru P, Fu DQ, Navarre D, Ghabrial S, Kachroo P, Kachroo A (2016) Cooperative functioning between phenylalanine ammonia lyase and isochorismate synthase activities contributes to salicylic acid biosynthesis in soybean. New Phytol 212:627–636

Strand Å, Asami T, Alonso J, Ecker JR, Chory J (2003) Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX. Nature 421:79–83

Strawn MA, Marr SK, Inoue K, Inada N, Zubieta C, Wildermuth MC (2007) Arabidopsis isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits properties consistent with a role in diverse stress responses. J Biol Chem 282:5919–5933

Sun X, Feng P, Xu X, Guo H, Ma J, Chi W, Lin R, Lu C, Zhang (2011) A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. Nat Commun 2:477

Susek RE, Ausubel FM, Chory J (1993) Signal-transduction mutants of *Arabidopsis* uncouple nuclear *CAB* and *RBCS* gene-expression from chloroplast development. Cell 74:787–799

Szechyńska-Hebda M, Karpiński S (2013) Light intensity-dependent retrograde signalling in higher plants. J Plant Physiol 170:1501–1516

Tadini L, Pesaresi P, Kleine T, Rossi F, Guljamow A, Sommer F, Mühlhaus T, Schroda M, Masiero S, Pribil M, Rothbart M, Hedtke B, Grimm B, Leister D (2016) GUN1 controls accumulation of the plastid ribosomal protein S1 at the protein level and interacts with proteins involved in plastid protein homeostasis. Plant Physiol 170:1817–1830

Tamoi M, Shigeoka S (2015) Diversity of regulatory mechanisms of photosynthetic carbon metabolism in plants and algae. Biosci Biotechnol Biochem 79:870–876

Taylor WC (1989) Regulatory interactions between nuclear and plastid genomes. Annu Rev Plan Physiol Plant Mol Biol 40:211–233

Terasawa K, Sato N (2009) Plastid localization of the PEND protein is mediated by a noncanonical transit peptide. FEBS J 276:1709–1719

Thomas J, Weinstein JD (1990) Measurement of heme efflux and heme content in isolated developing chloroplasts. Plant Physiol 94:1414–1423

Tikkanen M, Gollan PJ, Suorsa M, Kangasjärvi S, Aro EM (2012) STN7 operates in retrograde signaling through controlling redox balance in the electron transfer chain. Front Plant Sci 3:277

Tiller N, Bock R (2014) The translational apparatus of plastids and its role in plant development. Mol Plant 7:1105–1120

Tripathy BC, Sherameti I, Oelmüller R (2010) Siroheme: an essential component for life on earth. Plant Signal Behav 5:14–20

Van Aken O, Pogson BJ (2017) Convergence of mitochondrial and chloroplastic ANAC017/PAP-dependent retrograde signalling pathways and suppression of programmed cell death. Cell Death Differ 24:955–960

van Dijk EL, Chen CL, D'Aubenton-Carafa Y, Gourvennec S, Kwapisz M, Roche V, Bertrand C, Silvain M, Legoix-Né P, Loeillet S (2011) XUTs are a class of Xrn1-sensitive antisense regulatory non-coding RNA in yeast. Nature 475:114–117

Van Dingenen J, Blomme J, Gonzalez N, Inze D (2016) Plants grow with a little help from their organelle friends. J Exp Bot 67:6267–6281

Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177–206

Vranova E, Coman D, Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol 64:665–700

Wagner D, Przybyla D, op den Camp R, Kim C, Landgraf F, Lee KP, Würsch M, Laloi C, Nater M, Hideg E, Apel K (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science 306:1183–1185

Walley JW, Xiao Y, Wang JZ, Baidoo EE, Keasling JD, Shen Z, Briggs SP, Dehesh K (2015) Plastid-produced interorgannellar stress signal MEcPP potentiates induction of the unfolded protein response in endoplasmic reticulum. Proc Natl Acad Sci U S A 112:6212–6217

Wang L, Kim C, Xu X, Piskurewicz U, Dogra V, Singh S, Mahler H, Apel K (2016) Singlet oxygen- and EXECUTER1-mediated signaling is initiated in grana margins and depends on the protease FtsH2. Proc Natl Acad Sci U S A 113:E3792–E3800

Wang K, Guo Q, Froehlich JE, Hersh HL, Zienkiewicz A, Howe GA, Benning C (2018) Two abscisic acid responsive plastid lipase genes involved in jasmonic acid biosynthesis in *Arabidopsis thaliana*. Plant Cell 29:1678–1696

Wasternack C, Song S (2017) Jasmonates: biosynthesis, metabolism and signaling by proteins activating and repressing transcription. J Exp Bot 68:1303–1321

Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA (2009) GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. Plant Cell 21:1109–1128

Weber AP (2004) Solute transporters as connecting elements between cytosol and plastid stroma. Curr Opin Plant Biol 7:247–253

Weber AP, Fischer K (2007) Making the connections – the crucial role of metabolite transporters at the interface between chloroplast and cytosol. FEBS Lett 581:2215–2222

Weber AP, Linka N (2011) Connecting the plastid: transporters of the plastid envelope and their role in linking plastidial with cytosolic metabolism. Annu Rev Plant Biol 62:53–77
Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562–565
Wilson PB, Estavillo GM, Field KJ, Pornsiriwong W, Carroll AJ, Howell KA, Woo NS, Lake JA, Smith SM, Harvey Millar A, von Caemmerer S, Pogson BJ (2009) The nucleotidase/phosphatase SAL1 is a negative regulator of drought tolerance in Arabidopsis. Plant J 58:299–317
Woodson JD (2016) Chloroplast quality control – balancing energy production and stress. New Phytol 212:36–41
Woodson JD, Perez-Ruiz JM, Chory J (2011) Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. Curr Biol 21:897–903
Woodson JD, Perez-Ruiz JM, Schmitz RJ, Ecker JR, Chory J (2013) Sigma factor-mediated plastid retrograde signals control nuclear gene expression. Plant J 73:1–13
Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, Tolstikov V, Corwin JA, Kliebenstein DJ, Keasling JD, Dehesh K (2012) Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell 149:1525–1535
Xiao Y, Wang J, Dehesh K (2013) Review of stress specific organelles-to-nucleus metabolic signal molecules in plants. Plant Sci 212:102–107
Xiong JY, Lai CX, Qu Z, Yang XY, Qin XH, Liu GQ (2009) Recruitment of AtWHY1 and AtWHY3 by a distal element upstream of the kinesin gene AtKP1 to mediate transcriptional repression. Plant Mol Biol 71:437–449
Yamasaki K, Motomura Y, Yagi Y, Nomura H, Kikuchi S, Nakai M, Shiina T (2013) Chloroplast envelope localization of EDS5, an essential factor for salicylic acid biosynthesis in Arabidopsis thaliana. Plant Signal Behav 8:e23603
Yoo HH, Kwon C, Lee MM, Chung IK (2007) Single-stranded DNA binding factor AtWHY1 modulates telomere length homeostasis in *Arabidopsis*. Plant J 49:442–451
Zhang K, Halitschke R, Yin C, Liu CJ, Gan SS (2013) Salicylic acid 3-hydroxylase regulates Arabidopsis leaf longevity by mediating salicylic acid catabolism. Proc Natl Acad Sci U S A 110:14807–14812
Zhang L, Zhang F, Melotto M, Yao J, He SY (2017) Jasmonate signaling and manipulation by pathogens and insects. J Exp Bot 68:1371–1385

Jeannette Pfalz obtained her Ph.D. from Friedrich Schiller University Jena (Germany) on the isolation and characterization of the transcriptionally active chromosome in plastids under Prof. Oelmüller. She performed her postdoctoral research at the University of Oregon (USA) in the lab of Prof. Alice Barkan, where she investigated the role of PPR proteins for the stability of plastid mRNAs. After her return to Jena, she continued with the investigation on molecular factors and mechanisms involved in plastid gene expression.

Ralf Oelmüller obtained his doctoral degree from Albert Ludwig University of Freiburg (Germany) under Prof. Hans Mohr, where he investigated the interaction of phytochrome and blue-light photoreceptors. He discovered that the expression of nuclear genes for plastid proteins is controlled by signals from the plastids. He carried out his postdoctoral research on chromatic adaptation of *Fremyella diplosiphon* as an Alexander von Humboldt fellow in the lab of Profs. Winslow Briggs and Arthur Grossman at the Carnegie Institution of Washington (Stanford University, USA). He became Assistant Professor at the Botanical Institute of Ludwig Maximilian University in Munich (Germany) where he investigates regulatory elements in genes for thylakoid proteins. Around this time he and the Editor worked together and published jointly. Since 1998, he is Full Professor of Botany at Friedrich Schiller University Jena (Germany). His work focuses on nuclear control of plastid gene expression and the molecular basis of beneficial symbiotic interactions between root-colonizing fungi and the model plant *Arabidopsis*.

Electric Signaling and Long-Distance Communication in Plants

19

Neeti Sanan-Mishra

Abstract

Plants seem to have different modes of cell-to-cell and long-distance communication. The transmission of information involves phytohormones, organic transmitters and movement of macromolecules. There is also substantial evidence on the existence of electric signals in higher plants that converge on contact nodes similar to the immunological synapses found in animals. The origin, nature and mechanism of conduction of these signals are largely unknown. It was suggested that electrical potentials play an important role in inter- and intracellular cross talk; however, the mechanism through which plants decipher and act upon these signals is also a black box. Here we have covered the historical purview of electrical signaling in plants including the nature of electrical signals, mechanism of electrical conduction, and pathways for transmission. A brief description of other mobile molecular and cellular transmitters operative in long-distance communication is also provided.

Keywords

Action potential · Conduction mechanism · Excitation transmitters · Systemic potential · Transmission mode · Variation potential

N. Sanan-Mishra (✉)
Plant RNAi Biology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
e-mail: neeti@icgeb.res.in

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_19

19.1 Introduction

Plants by nature seem to be sessile and silent, but in reality they have the ability to move and communicate. Their limited capacity to move is visible in case of leaf movements in the sensitive plant *Mimosa* or "spontaneous" gyration of the lateral leaflets in the telegraph plant, *Desmodium*, or movement of specific organs as seen in the insect-trapping carnivorous plants. Specific small movements like that seen in stomata occur in response to the environmental cues, whereas extremely slow movements related to growth have been captured by time-lapse camera.

Plants also exhibit limited commune to "touch or contact" or other sensory cues as demonstrated by a range of investigations. For instance, greenhouse-grown lettuce and celery seedlings raised close together in seed trays grow tall and "skinny," whereas plants that were exposed to wind grow more stunted. In early 1973, Mordechai Jaffe at Ohio University observed that gentle stroking of garden pea tendrils triggered their coiling. Similarly stroking the plant stem for a few seconds a day induced stunting of stem and widening its girth. He noted that the stem girth began to thicken just 30 minutes after the plants were rubbed. Interestingly this stunting response helped the plants to withstand the buffeting of the wind (Jaffe 1973). Later it was observed that simply spraying the plants with water stunted their growth by about a third. "Contact" could also stimulate plants to cut down their water loss by closing their leaf pores, to delay flower production, and to increase metabolism and chlorophyll production (Biddington and Dearman 1985; Braam and Davis 1990).

An attempt to understand these phenomena led to the discovery of cell-to-cell and long-distance communication in plants. Normal plant growth and development is a genetically programmed phenomenon that is directed by environmental cues like light, water, gravity, and temperature. Plant cells have mechanisms to perceive these stimuli and alter their gene expression machinery accordingly. It is believed that specific sensors present in any plant organ can sense the environmental cues, but the signals may be transmitted to the whole plant to elicit a suitable response.

In plants the cells seem to have different modes of information exchange with each other. The route of transmission may be intracellular, i.e., within the cell, from membrane to nucleus or membrane to organelles like chloroplast. This may involve chemical second messengers like inositol phosphate, calcium ions (Ca^{2+}), etc. Communication over short and long distances involves transmission from one cell to the other or one organ to the other, respectively. The transmission of information may involve phytohormones like auxins, abscisic acid (ABA), etc. or organic transmitters like serotonin, biological amines, etc. or macromolecules like proteins and small RNAs. There are also observations that indicate the existence of electric signals in higher plants that may regulate a wide variety of physiological responses (Wayne 1994; Shepherd et al. 2001). Plants seem to assemble adhesive contacts similar to the immunological synapses found in animals for facilitating cell-to-cell communication. So far, the natural conditions that cause the plants to generate these signals and the mechanism of their conduction are largely unknown. It is also not known as to how plants decipher and act upon these signals.

19.2 Electrical Signaling in Plants

Just like the neuronal responses seen in animals, plants also exhibit rapid response to touch. For example when insects attack a plant, a quick alarm is sent out throughout the plant to trigger the systemic processes that activate the defense genes. In some plants this results in release of specific enzymes that can disrupt the digestive system of the insects so that it will stop eating the plant. In another case, it may result in release of specific hormones that attract "friendly bugs" which in turn predate on these insects and help the plants. An investigation at the molecular level indicated that the wound response caused by the insect activates the proteinase inhibitor genes resulting in accumulation of *pin* proteins at the local site of injury. In parallel, a similar response was systemically generated throughout the unwounded aerial regions of the plant.

For many years it has been known that plants have the ability to rapidly communicate over long distances. The rapid transmission of oxidative and nitrosative stress signals between root and shoot apices appear to be necessary for establishment of plant immunity (Capone et al. 2004). Some of these signals were considered to be transmitted using classical action potentials. Likewise the plant roots appear to communicate and are able to discriminate between "self" and "non-self" in a manner similar to that seen at the neuronal synapses in animals. Interesting sets of findings indicate that plants may have a complex social life mediated preferentially via their root systems (Bais et al. 2004; Baluška et al. 2004; Gruntman and Novoplansky 2004).

It was also demonstrated that plants synthesize numerous neuronal molecules and fulfill some criteria for intelligent behavior (Roshchina 2001; Brenner et al. 2003). Studies related to physiology and ecology have shed light on different aspects of plant intelligence (Trewavas 2003). Plants perceive and process information related to their environment, including information from neighboring plants and microorganisms. This information is also stored for memory-based learning, which allows them to benefit from trial-and-error guided and experience-driven behavior.

19.2.1 Electrical Signals

The work done by several research groups has shown that bio-electro-chemical signals that look like nerve impulses exist in all plants. The preliminary observations, which indicated that plants also communicate feelings, were not only intriguing but also generated controversy and outrage among the religiously inclined people. The main reason behind this being the strong belief was that plants were considered nonmotile and passive organisms, so they were not in need of rapid long-distance communications and excitability. Nonetheless the great scientific minds in that era predicted the ubiquitous presence of mechanisms for perception and fast reaction in plants. It was even proposed that multi-functional electric signals were primarily responsible for coordinating plant responses to the environment (Darwin 1966; Davies 1987; Pickard 1973; Sibaoka 1969; Wayne 1993).

The first such instance was reported over 145 years ago, when Prof. Claude Bernard predicted the existence of a common mechanism, in all organisms, for perception of external stimulus and generation of a fast reaction. He demonstrated that volatile anesthetics, such as ether and chloroform, inhibited several processes in plants including plant movements, seed germination, and photosynthesis (Bernard 1878; Grémiaux et al. 2014).

Later Dr. John Burdon-Sanderson at the University College of London attempted to prove Darwin's belief on the presence of a central nervous system that guided the shutting of trap in Venus flytrap (*Dionaea muscipula*). He attached electrodes to the surface of the trap lobes and observed that a wave of electrical activity was elicited each time the insect touched the trigger hairs (Burdon-Sanderson 1873). A single touch generated a limited receptor potential, almost identical to the nerve impulses or action potentials produced by animal neurons, in sensor cells. This fired a fast-moving electrical wave that spread across the trap leaf lobes (Hodick and Sievers 1989). The trap did not move but "remembered" being touched, and when the second electrical wave was fired, the cells on the inside walls of the trap become flaccid by transferring water to the outside walls. Striking two sensory hairs once or a single hair twice within an interval from 0.75 to 20 s could elicit the same response. Brown and Sharp (1910) found that at high temperature of 35–40 °C, usually only one mechanical stimulus was required.

He repeated the same experiment with the curling of the tentacles in sundew plant (*Drosera*). In this case mechanical stimulation induced by the insect generated an action potential that induced a hormonal signal at the tip of the tentacles (Williams and Pickard 1972; Williams and Spanswick 1976). As a result the marginal cells in the tentacles enlarged resulting in their bending toward the prey.

The great Indian scientist, Sir Jagadish Chandra Bose, performed biophysical experiments on telegraph plant, *Desmodium* (Bose 1913), and discovered electrical "pulsations" or oscillations in electric potentials in plant cells. He hypothesized that the regular wave-like 'pulsations' in electric potential and turgor pressure were coupled with rhythmic movements and represented an endogenous form of signaling. He put forth a radical theory that bioelectric and environmental phenomena were inseparable and the mechanism of the ascent of sap is based on the electromechanical activities of living cells.

This prompted measurements of electrophysiological recordings in single-celled algae (Findlay 1961; Hope and Walker 1975; Gradmann 1976; Dziubinska et al. 1983) and insectivorous plants (Williams and Pickard 1980) and during leaf movements of *Mimosa pudica* (Sibaoka 1962, 1979), *Aldrovanda vesiculosa* (Iijima and Sibaoka 1981), and *Dionaea muscipula* (Hodick and Sievers 1988). Touch-sensitive movements occur in more than a thousand species of flowering plants spread across 17 families. It is likely that these too probably depend on electrical impulses. It was observed that touch-induced action potentials caused a transient increase in the rate of respiration of pumpkin stems (Gunar and Sinyukhin 1963), increase in the respiration rate of ovary during pollination in *Incarvillea grandiflora* (Fromm et al. 1995), and inhibition in growth in *Luffa cylindrica* (Shiina and Tazawa 1986).

Mechanically stimulated depolarizing transients provided evidence for electrical activity as a mechanism of signal propagation during regulation of diverse physiological and biochemical responses in plants (Davies 1987; Thain and Wildon 1996).

Pickard (1973) and Davies (1987) proposed that the wound signal, which induced the *pin* gene during pathogen resistance in tomato, could be electrical rather than chemical. This was later confirmed by measurements of the transmitted action potentials (Wildon et al. 1992). The physiological basis for plant movements has been investigated at the levels of both long- and short-distance electrical signaling in plants. Sunflowers (*Helianthus annuus* L) use both action potentials and slow-wave potentials as separate electric signals for their long-distance communication (Zawadzki et al. 1991; Stankovic et al. 1997). It was shown that older plants (16–22 days) exhibited high excitability levels, requiring stimulus of minimum 2 V, 1 s, and the plants of similar size, shape, and age that were grown under identical conditions exhibited high variability in the degree of excitation.

It was also shown that light could trigger the bioelectrical activity of plants (Haake 1892). Changes in the light conditions such as a dark/light transition could trigger proton extrusion via the H^+-ATPase resulting in potential variations of the guard cell membrane that regulated the stomatal movements (Assmann et al. 1985; Dietrich et al. 2001). Transition from dark to light also evoked transient membrane depolarization in the epidermal and mesophyll cells in leaves (Spalding and Cosgrove 1992; Johannes et al. 1997). Our group provided evidence for electrical signaling in root-shoot interactions during early stages of growth and establishment of seedlings. The primary leaf emergence and expansion in *Sorghum bicolor* is a light-dependent process. Providing a short photo-exposure to the roots alone also induced leaf opening over a similar time scale; however, any injury to the primary root inhibited leaf formation. The rapid transmission of the signal involved generation and transduction of the electrical impulses (Sanan et al. 2000). Electrical stimulus given to the root medium could overcome the requirement of photo-exposure to induce primary leaf formation in etiolated seedlings. To characterize the excitable properties and capability of fast conduction of electrical stimulus, non-damaging electrical stimuli were applied to the seedlings. The stimulus given in the root region produced a characteristic response, which could be recorded in the shoot tissue. The extracellular propagation of electrical signal suggested that *S. bicolor* exhibits typical excitable properties comparable to neural tissues. The young seedlings (5–7 days) were highly excitable and exhibited a consistency in the response; however, with age the tissues lost the excitability.

Recently, it was demonstrated that application of anesthesia stops autonomous and touch-induced movements in plants by inhibiting the generation of electrical signals. Currently three types of electrical signals are recognized in plants: action potentials (AP), variation potentials (VP) and systemic potentials (SP). AP and VP depolarize the membrane (Fromm and Lautner 2007) while SP is the self-propagating hyperpolarization of membrane (Zimmermann et al. 2016). The strength of physiological response depends on number and frequency in the case of AP or amplitude in the case of VP (Fromm and Lautner 2007; Böhm et al. 2016).

19.2.1.1 Action Potentials

An AP is generated by a non-damaging stimulus strong enough to reach a specific threshold and generate a wave involving depolarization, repolarization and hyperpolarization phases. It is the fastest known form of electrical communication in plants. It is rapidly propagated, within a few seconds, over a long distance (Fromm and Bauer 1994; Fromm and Lautner 2007) and follows an all-or-nothing character; that is, after a stimulus reaches a certain threshold, further increase in the stimulus strength does not change its amplitude. In plants, stimuli such as chilling, heating, cutting, touching, electric stimulus, or changes in external osmolarity result in action potentials. Transient depolarizations of cell membrane are electronically transmitted at rates of 10–40 mm/s and resemble primitive nerve action potentials. This indicated that cells of most, perhaps all, plants are excitable, though neurons (as we understand from animal systems) are not present in plants.

In his experiments, Sir J.C. Bose observed that mechanical stimulation of *Mimosa* and *Desmodium* plants could be mimicked by electrical stimulation. The earliest recordings were measured using a device akin to the modern chart recorder, the resonant recorder, and the oscillating recorder (Bose 1913). The leaf movements were measured at time intervals of less than 1–2 s. Different parts of the plants were electrically stimulated with feeble stimulating current pulse using miniature electrodes, and the electrical responses of the plant were recorded with an electric probe (Bose 1926). He showed that in absence of mechanical stimulation, strong electric stimulation in the *Mimosa* pulvinus made the leaves dip. Likewise a cut in *Desmodium* stalk prevented the rhythmic leaf movements, but an electric current passing through the pulvinus restored these rhythms. The transmission of stimulus was electrotonic since an electronic block (two electrodes placed 5 mm apart in between the pulvinus and the point of stimulation, with a constant current maintained between them) stopped the response.

The excitatory response in *Mimosa* and rhythmic movements in *Desmodium* were lost by repeated stimulation or by application of KCN, $CuSO_4$, and anesthetics such as chloroform or ether and sudden changes of temperature such as application of ice water. The velocity of transmission was affected by season, temperature, light, vigor of the plants, and age of the organ where it was measured. Based on these observations, Sir Bose generalized that all strong stimuli produced a decrease in turgor pressure, a contraction of cells, a transient diminution of growth rate, a negative mechanical response (such as dropping of leaves), and an electric response of "galvanometric negativity" (Shepherd et al. 1999). Feeble stimuli produced directly opposite effects, increase of turgor, expansion of cells, transient increase in growth rate, and an electric response of "galvanometric positivity."

The electrical stimuli were also measured in single characean cells by laser interferometry (Sandlin et al. 1968). Later, experiments were performed on several higher plants using intracellular microelectrodes and surface-contact electrodes (Thain 1995). The characteristics of action potentials have been studied in shoots of *Lupinus angustifolius* (Paszewski and Zawadzki 1994; Zawadzki 1980), *Helianthus annuus* (Zawadzki et al. 1991), *Salix viminalis* (Fromm and Spanswick 1993), and *Sorghum bicolor* (Sanan et al. 2000). The characteristic properties of an excitable

tissue entail that it responds to threshold stimulus, follows all-or-none law, has a characteristic strength-duration curve, and on stimulation with supra-threshold stimulus can propagate an impulse.

The action potentials were also associated with growth and development in plants. Electrical activity was recorded during phloem unloading in *Mimosa pudica* (Eschrich et al. 1988; Fromm 1991) and ovarian respiration in *Hibiscus rosa-sinensis* (Fromm et al. 1995). It was shown that an electric potential was generated when a sperm penetrates a Fucus egg. The electric current driven through the egg appeared to help establish embryo polarity. The first cell division was always at right angles to the direction of flow of current. A similar correlation was observed between transmitted action potentials and plant development during shoot-apex formation in *Bidens pilosu* (Frachisse et al. 1985). Eschrich et al. (1988) found that an electrical signal transmitted through the phloem moves between fruit and petiole in zucchini.

The action potential showed a definite temperature dependency in *Nitella* (Blatt 1974). The electrophysiological properties of plant cells also changed seasonally and with age. In cells of *Chara* the cell membrane potential difference was significantly less hyperpolarized (less negative) in winter (Shephard and Goodwin 1992; Hodick and Sievers 1989). This correlated with changes in the cell-to-cell communications between vegetative and reproductive life cycles that varied seasonally depending on sucrose concentration and potassium ion (K^+) content (Shephard and Goodwin 1992; Kirst et al. 1988).

In another alga, *Eremosphaera*, illumination followed by darkness caused transient hyperpolarization of the cell potential difference resulting in divalent cation and anion currents (Glebicki et al. 1989). The photosignal was also shown to mediate changes in the membrane potentials in maize (Racusen and Galston 1980), oat coleoptiles (Newman 1981), expanding leaves of pea (Staal et al. 1994) and rosette leaf of *Arabidopsis* (Spalding 1995). Light caused rapid changes in the membrane potential of plant cells by altering the activities of ion pumps and channels at the plasma membrane that generated a photomorphogenetic signal. For instance blue light induced a large, transient membrane depolarisation in the hypocotyls of etiolated seedlings (Spalding and Cosgrove 1989). The underlying changes in ion transport were thought to be part of a transduction chain that linked the blue light receptor to inhibition of hypocotyl growth.

19.2.1.2 Variation and Systemic Potential

VP is a slow propagating type of signal, which is generated upon an injurious stress treatment. Such wound-induced electrical signals are also known as "slow wave potentials" (Stahlberg et al. 2006). The rapid (<2 s) and massive (>50 mV) membrane depolarization are followed by slow (>5 min) repolarization. So the cycle takes several minutes. In Arabidopsis, severe damage triggers electrical activity that propagates from leaf to leaf with apparent velocities in the range of a few centimeters per minute. The VP is initiated with changes in the hydraulic pressure via mechanosensitive ion channels, mainly in xylem vessels, and fades away with distance from the point of origin (Malone 1996; Stankovic et al. 1997; Stahlberg et al. 2005) or in response to transport of a chemical signal via ligand-activated channels

(Malone 1996). Its amplitude positively correlates with the stimulus strength. The underlying mechanism of VP employs mainly perturbations of H^+-ATPase activity (Stahlberg et al. 2006). VP affects hormone emission and gene expression (Wildon et al. 1992; Dziubinska et al. 2003) through mechanosensitive ion channels (Mancuso 1999) or ligand-activated channels (Malone 1996).

In SP primary polarity is reversed with all-or-nothing character, and the changes are not caused by a hydraulic pressure surge, unlike the initial depolarization that accompanies the generation of AP and VP (Zimmermann et al. 2009). SP can be evoked by wounding as well as heat stimulation (scorching), and its induction and spread depend mainly on cations (Zimmermann and Mithöfer 2013). It is noteworthy that a close relationship between SP propagation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was recently reported as plants devoid of functional NADPH oxidase (rbohD) had a suppressed capability to mediate SP (Miller et al. 2009; Suzuki et al. 2013). This finding suggested a close link and cross talk between ROS (reactive oxygen species) and this type of electrical signaling.

19.2.1.3 Mechanism of Electrical Conduction

When a plant is disturbed some changes occur in receptor cells, which rapidly send out an alarm throughout the plant using electrical ions. Both Burdon-Sanderson and Darwin thought that plants have some sort of rudimentary neural system, similar to that of animals, through which they could communicate electrically. The touch sensing receptor cells had the ability to generate electrical impulses that are transmitted through cell membranes with voltage-sensitive pores for triggering an appropriate response. Thus, such plants were considered close to the coelenterates, like sea anemones and jellyfish, which have a network of touch sensors, nerves and muscles that are connected without synapse.

However other leading biologists and botanists of the time discounted this evidence as ridiculous because plants did not have any of the usual components of an animal nervous system. Moreover, in most animals electrical impulses travel along nerve fibers at speeds between 1 and 100 meters per second, whereas the impulses of most plants travel at speeds between 1 and 10 centimeters per second. Later, J.C. Bose demonstrated that like animals, plants had receptors for perceiving a stimulus, which was electrically propagated to the terminal motor organ (Bose 1913, 1926). Modern-day plant electrophysiologists have confirmed that the electric impulses were indeed action potentials and much similar to those detected in animals.

During the late 1960s, Stuart Jacobson, an insect physiologist at Carlton University Ottawa, discovered that touching of a trigger hair in Venus fly trap is translated into an electrical signal in the form of a reduction in the voltage across the membranes of cells at the base of the hair. The degree of depolarization correlated with the intensity of stimulus until eventually it reached a critical threshold and triggered the action potential that signaled the trap to close (Jacobson 1965). Further experiments showed that currents of the same ions produce all action potentials in similar touch-sensitive plants.

In 1981, two German scientists Erwin Neher and Bert Sakmann invented the "patch clamp technique" to identify the molecular channels associated with the

spread of action potentials along the fibers of neurons (Simons 1992). The technique involved removing a tiny piece of cell membrane with the end of an exceptionally fine-tipped electrode and applying voltage to such "patches." It was found that opening of specific voltage-sensitive channels in the membrane triggered ion currents across the patches. For instance, opening of sodium (Na^+) channels leads to entry of Na^+ ions at a certain point along a nerve fiber. This causes a fall in the voltage further down, encouraging more Na channels to open. The effect is transmitted along the nerve fiber resulting in a wave of voltage change.

The existence of voltage-sensitive and pressure-sensitive ion channels has been demonstrated in both plant and animal cells. Using the patch clamp technique, three types of stretch-sensitive channels, specific for chloride (Cl^-), K^+, or Ca^{2+}, were discovered (Cosgrave and Hedrich 1991). In each species, a rapid influx of Ca^{2+} into cells seemed to trigger the action potential and an efflux of K^+ and Cl^- appeared to sustain it as it traveled from pore to pore. The action potentials in animal neurons are produced in a similar way as they are usually triggered by Na^+, not Ca^{2+}, while epithelial tissues use Ca^{2+} instead of Na^+. The embryonic cells destined to become nerves or muscles can change their preference for Na^+ or Ca^{2+} as they develop.

Studies in wheat and bean plants led to the identification of voltage-sensitive channels in leaf cells (Moran et al. 1984). Most observations on ion channels were made on guard cells using the whole-cell patch clamp technique (Hedrich et al. 1990). It was demonstrated that the voltage-dependent anion channels in the plasma membrane of guard cells are activated by a rise in cytoplasmic Ca^{2+} in the presence of nucleotides. This stimulates an efflux of Cl^-, followed by a voltage-dependent efflux of K^+ (Beilby 1984). It is thus understood that in plants action potentials result from an abrupt depolarization of cell membrane potential difference followed by a slower decay to the negative resting potential difference. This can be explained by the co-operative kinetics of Ca^{2+}, Cl^- and K^+ ion channels in plasmalemma and tonoplast (Wayne 1994; Lunevsky et al. 1983; Kikuyama and Tazawa 1998). Depolarization was coupled with withdrawal of water and loss of turgor (Zimmermann and Beckers 1978) followed a transitory contraction of the cell. It can thus be produced mechanically (by touch, injury, chilling, heating) or electrically (by introducing a depolarizing current). Prolonged stimulation by depolarizing voltages results in the inactivation of the anion current ($t1/2 = 10–12$ s).

Subsequently a wide variety of plants were found to possess voltage-sensitive ion channels. It was hypothesized that action potentials in touch-sensitive plants like the Venus flytrap also depend on voltage-sensitive channels; however, no such studies have been validated so far, probably due to the difficulty in isolating the excitable cells. The involvement of ion channels in detecting pressure and mechanical stress during growth and development was also predicted. For example, in embryos the channels "sense" the time for initiating cell division, while in young plants the presence of water triggers the channels for activating division. It was observed that many algal cells, such as Chara and Nitella, pass action potentials when they swell or deflate with water. These impulses are triggered through stretch-sensitive ion channels (Shepherd et al. 2001)**.** Chara or Nitella cells also exhibit cytoplasmic streaming movement that suddenly stops whenever the cell is touched. The stimulus

triggered Ca^{2+} to flow into the cell, dramatically altering the voltage across the cell's membrane and driving in yet more Ca^{2+}. This Ca^{2+} flood blocks the actin and myosin protein filaments that power the movements of cells and all their internal components. In animals, a similar sequence of events leads to the contraction of muscle cells.

Turgor pressure was also linked to the electrical signals, as hypotonic shock in the cells of *Lamprothamnium*, a salt-tolerant charophyte, resulted in opening of Ca^{2+} channels and efflux of Cl^- and then of K^+, resulting in depolarization (Beilby and Shepherd 1996). The process varied in cells of different age or from different environments (Beilby et al. 1999; Shepherd et al. 1999). An association between electrical signals induced by heat stimulation of a leaf and transient photosynthesis changes in mimosa, poplar and tobacco were also reported (Koziolek et al. 2003; Lautner et al. 2005; Hlaváčková et al. 2006). Scientists are now beginning to unravel the molecular and cellular reasons underlying these processes. It has been demonstrated that electrical signals are followed by changes in intracellular Ca^{2+} concentration and generation of ROS like H_2O_2 (Maffei et al. 2007; Kiep et al. 2015).

19.2.2 Pathways and Mode of Transmission

Many animal cells also possess sensors that convert mechanical stimuli such as touch into electrical signals. Neuronal communication in animals consists of specialized nerve fibers and networks of neuron cells, which communicate rapidly due to the chemical versatility of synapses. As the ion potential reaches the end of the nerve fibers, it releases neurotransmitters, which fuse across the synapse and trigger an electrical response in the neuron at the opposite end. Using a variety of different types of neurotransmitters and neurons, a nervous system can efficiently process signals and route them to different parts of the body while constantly inverting electrical signals into chemical ones and vice versa. Unlike conventional excitable tissues (nerves or muscles), most animal epithelial and embryonic tissues pass action potentials using the gap junctions.

It was proposed that mechanical disturbance of plants induces amino acid changes in the receptor cells, which initiate an electric signal that can travel from cell to cell across the system. This mechanism draws parallel with the glutamate receptors in human neurons, which are used to control neural signal transmission by changing the levels of two amino acids. Frank Turano, a molecular biologist at the Agricultural Research Service in Beltsville, has identified and cloned a dozen genes responsible for nerve-like signals in plants (Lacombe et al. 2001). The neuromotor components in plants include acetylcholine neurotransmitter, cellular messenger calmodulin, cellular motors actin and myosin, voltage-gated channels, and sensors for touch, light, gravity, and temperature.

The transmission of action potentials in plants is akin to the embryonic mode, and these are conducted through ordinary cells, which are connected at the membranes through the plasmodesmata. The signals comprise of currents of ions moving to and fro across cell membranes (Ellison and Gotelli 2009). Conduction through

plasmodesmata (and gap junctions) is a relatively slow process and the signals can only be sent down one route to perform one action. For example, if a Venus flytrap is touched but does not catch anything it will close very quickly and it will be several hours before the chemical trigger wears off and it reopens.

Earlier Sir J.C. Bose had proposed that the excitatory response in plants was a wave of protoplasmic, electrotonic excitation, which depended on living cells (Bose 1926). He presented evidence for a simple neural network where action potentials traveled predominantly in the phloem through the plasma membrane and plasmodesmata. It was later shown that patterns of light-induced "spiking" were transmitted through the apoplast to the unilluminated parts of several different plant species (Glebicki et al. 1989). It is generalized that plants need pathways for electrical signal transmission to respond rapidly to environmental stress factors. Different environmental stimuli evoke specific responses in living cells, which have the capacity to transmit a signal to the responding region. Today it is hypothesized that action potentials can travel both intracellularly and extracellularly (apoplastically) and similar patterns of electrical regulation of growth may be universal to all fungi, plants and animals.

There is no consensus regarding the cell populations that are necessary for organ-to-organ electrical signaling in plants. The dead xylem vessels have been proposed to play an essential, if not exclusive, role in long-distance propagation of electrical signals (Evans and Morris 2017). The hydraulic signals in the xylem have been proposed to underlie slow-wave propagation (Stankovic and Davies 1998). The role for the phloem is implicated in leaf-to-leaf electrical signaling (Rhodes et al. 1996; Hedrich et al. 2016), and wound response-related electrical signals have been detected directly in sieve elements (Salvador-Recatalà et al. 2014). Both phloem and xylem-associated cells were found to be highly excitable in *M. pudica* (Sibaoka 1962).

The vascular system plays other important roles in long-distance communication networks by allowing plants to integrate environmental cues into physiological and developmental responses (Lough and Lucas 2006). Environmental changes are sensed by mature organs and the signals are then transported to the meristematic regions where newly formed organs adopt a development fate to better adapt to the environment in which they will develop and function.

Most plant cells have characteristic tubular shapes, and these are in tight contact at non-growing cross walls through numerous plasmodesmata. These cross walls, enriched in F-actin and plant-specific unconventional class VIII myosin, constitute the end-poles of the cells (Baluška 2003; Barlow and Baluška 2004) representing a unique "plant developmental synapse." It is proposed that the synapses are actively involved in driving polar auxin transport through actin-driven endocytosis, endosomal sorting, and vesicular recycling (Geldner et al. 2003; Baluška et al. 2003). The short photoperiod-mediated initiation of dormancy in aspen plants occurs due to the ABA-dependent closure of plasmodesmata. This blocks the symplastic intercellular communication and limits the transport of growth promotive signals to SAM. Reopening of closed plasmodesmata in dormant buds occurs slowly and only after prolonged exposure to low temperature. On the other hand, in the absence of dormancy and plasmodesmatal closure, growth arrest can be quickly reversed. Hence, dormancy prevents precocious activation of growth and thus ensures

perennial survival and longevity in the face of changing seasons (Tylewicz et al. 2018). Plants are also capable of forming such junctions with cells of microorganisms like fungi, algae, or bacteria that may correspond to immunological synapse which might entail suitable responses to ward off infection or develop symbiotic associations.

19.3 Long-Distance Transmission of Signals

19.3.1 Excitation Transmitters

The natural conditions that cause plants to generate the electric signals are largely unknown. It is also not known as to how plants decipher and act upon these signals. Modern-day plant physiologists are now beginning to unravel the molecular and cellular reasons underlying these processes. Spontaneous changes in temperature, light, touch or wounding can induce electrical signals at any site of the symplastic continuum. Events within the first seconds to minutes, which are responsible for recognition and triggering of signal transduction pathways, are still poorly understood. The plasma membrane of cells is the only compartment with a direct contact to the environment and represents the sensing element able to recognize changes and to initiate cascades of events eventually leading to specific responses (Maffei et al. 2007).

The changes in transmembrane potential or modulation of ion fluxes at the plasma membrane level are the first cellular responses to biotic and abiotic stresses in plants followed by a cascade of downstream reactions (Maffei et al. 2007). During different kinds of stresses in plants, the ionic composition is altered which in turn changes the cell electrical potential. It has been reported that, in saline conditions, most salt-tolerant plants accumulate lower amount of sodium in their leaves than salt-sensitive plants (Coleman 1986). Hebbar and Sinha (2000) reported a difference in the surface electrical potentials of salt-tolerant and sensitive-wheat varieties. Herbivory-induced changes of membrane potentials are also followed by a fast electrical signal that travels through the entire plant from the point of origin of the perceived input (Mousavi et al. 2013).

The plant hormones ABA and jasmonic acid (JA) play a predominant role in mediating the changes in plant gene expression in response to environmental signals. Studies on wounding in tomato suggested that an increase in endogenous ABA and JA levels follows electrical current-induced *pin2* gene expression upon wounding (Peña-Cortés et al. 1991, 1995; Farmer and Ryan 1992; Herde et al. 1999). The wounding of sundew leaves induced the accumulation of ABA (Flokova et al. 2014). Even burning of leaves triggered the electrical currents that activated *pin2* gene expression. This involved biosynthesis of JA via an alternative pathway that is independent of endogenous ABA levels (Herde et al. 1999). Interactions between ABA and JA are both antagonistic (through the ERF transcription factor) and synergistic (through the MYC transcription factor). It seems that ABA is required for JA biosynthesis and JA-dependent defense gene expression in response to wounding or

pathogen attack (Adie et al. 2007), but the induction of some JA-regulated genes is prevented by ABA (Anderson et al. 2004). The molecular mechanism of the JA-ABA interaction has been described (Lackman et al. 2011).

At the sites that receive the electrical signals like the xylem contact cells, jasmonates accumulate, and jasmonate-mediated gene expression is turned on to initiate defense-responsive gene expression (Mousavi et al. 2013; Kiep et al. 2015; Gilroy et al. 2016). The isoleucine conjugate of jasmonic acid (JA-Ile) is the only jasmonate for which the molecular basis of its gene-regulatory activity has been elucidated. The binding of JA-Ile to the *Coronatine Insensitive 1* (CoI1) receptor mediates the ubiquitin-dependent degradation of jasmonate zim-domain (JAZ) repressors, resulting in the activation of jasmonate-dependent gene expression (Thines et al. 2007; Fonseca et al. 2009; Sheard et al. 2010). However, signaling activity has been demonstrated for other jasmonate molecules for which the molecular mechanism is largely unknown, such as cis-(+)-12-oxo-phytodienoic acid (cis-OPDA) or 12-hydroxyjasmonic acid glucoside (Stelmach et al. 1998; Stintzi et al. 2001; Nakamura et al. 2011). It has become clear that jasmonates act as elicitors of the production of secondary metabolites some of which may act as warning signals in plants (De Geyter et al. 2012). The triggering response in Venus flytrap also accumulates high concentration of JA, JA-Ile, and cis-OPDA which activates the expression of carnivory-related genes like chitinases, cysteine protease, etc. (Libiaková et al. 2014; Böhm et al. 2016; Bemm et al. 2016). The biosynthetic pathway of JA is equated to those of the mammalian eicosanoids, i.e., prostaglandins (sensitize spinal neurons to pain).

19.3.2 Bioactive Signals

The information-processing network in plants is not based on neurons and synapses. However, they have characteristic plasmodesmata that constitute the end poles of the cells (Baluška 2003; Barlow and Baluška 2004) and form a functional "plant developmental synapse." The cellular activities here are fundamentally similar to the information-processing system operative at the neuronal synapse. Plants use several bioactive molecules for activating Ca^{2+}-regulated signaling that are known to be involved in transmitter-mediated cell-to-cell communication at neuronal synapses.

Several properties of auxin- and pectin-derived molecules suggest that they act as plant-specific excitatory transmitter in cell-to-cell communication (Baluška et al. 2005). Exogenously applied auxin can induce rapid Ca^{2+} transients and may elicit rapid electric responses in plant cells. Auxin application also activates plasma membrane H^+-ATPase and other ion channels thereby initiating wave-like stimulation of the polar auxin transport along the longitudinal axis of plant organs (Baluška et al. 2004). The polar transport of auxin mechanistically links stimuli sensing with the multifarious response, thus influencing the whole plant body (Friml 2003; Swarup and Bennett 2003).

The carotenoid derivatives, strigolactones, act as a mobile branching signal (Gomez-Roldan et al. 2008; Umehara et al. 2008). There are two widely accepted hypothesis related to the function of strigolactone function in plants. According to one view they play an important role in auxin transport (Domagalska and Leyser 2011). It was shown that strigolactones can repress the expression and accumulation of PIN auxin transporters (Crawford et al. 2010), and this repression limits auxin flow from buds (Prusinkiewicz et al. 2009; Balla et al. 2011). The second view discusses the role of auxin in regulating strigolactones (Brewer et al. 2009), as expression of genes required for strigolactone biosynthesis is suppressed in the absence of auxin (Arite et al. 2009). However, it is still not known which strigolactone-related molecules are transported and what is the effect of this signaling.

The precursor of ethylene, ACC (1-aminocyclopropane-1-carboxylic acid), is also implicated as a possible mobile factor responsible for long-distance communication. In waterlogged tomato plants, ACC produced in the roots is translocated to the aerial parts of the plant where conversion to ethylene occurs, resulting in epinastic leaf curvature (Bradford and Yang 1980). Root-to-shoot translocation of ACC is suggested to be involved in pathogenic symptom expression in tomato after root-knot nematode infection (Glazer et al. 1984) and in leaf abscission in water-stressed citrus plants following dehydration (Tudela and Primo-Millo 1992). ACC concentrations increase in different flower parts following pollination or stigma wounding indicated that ACC might act as a mobile factor initiating pollination-induced senescence in flowers (Nichols and Frost 1985).

Oligogalacturonides (OGAs) are bioactive signal molecules, released from homogalacturonan pectins, which are rapidly transported throughout plant bodies. They exert numerous regulatory effects on plant growth and physiology, most of which are antagonistic to auxin (Ridley et al. 2001; Baluška et al. 2005). It is thus hypothesized that OGAs act as plant-specific inhibitory transmitters of cell-to-cell communication. Exogenously added OGAs induce depolarization of the plasma membrane, activate a phospholipase C-like enzyme, release hydrogen peroxide (H_2O_2) and rapidly promote transient mobilization of cytoplasmic Ca^{2+} combined with cytosolic acidification (Baluška et al. 2005). Moreover, OGAs rapidly induce systemic wound responses and systemic acquired resistance (SAR) via complex signaling cascades that may even involve electrical long-distance communication (Ridley et al. 2001).

Plants also possess and use several classical neuronal transmitters, receptors and interacting molecules in their rapid cell-to-cell communication. These include compounds such as glutamate, ionotropic glutamate receptors, glycine, gamma-aminobutyric acid (GABA), N-arachidonylethanolamine (NAE) anandamide, acetylcholine and ATP that control Ca^{2+}-regulated exocytosis and vesicle recycling at neuronal synapses (Roshchina 2001). Glutamate behaves as an excitatory transmitter while GABA seems to act as an inhibitory transmitter in plants. Glutamate and glycine act on Ca^{2+}-permeable channels in plants and rapidly depolarize the plasma membrane in a process mediated by specific receptors (Baluška et al. 2004). GABA is rapidly produced and transported from cell-to-cell across plant tissues under diverse stress situations. Many of the neurotransmitters are derived from

amino acids and may therefore utilize the conserved amino acid transporters for transmission (Wipf et al. 2002).

19.3.3 Biomolecular Signals

Long-distance signaling mediates diverse developmental and physiological processes including photoperiodic flowering (Zeevaart 1976), tuberization (Jackson 1999), nodulation (Oka-Kira and Kawaguchi 2006), leaf development (Haywood et al. 2005; Kim et al. 2001), shoot branching (Beveridge 2006), and defense against pathogens (Palauqui et al. 1997). Investigations in the content of the phloem sap have revealed the presence of numerous RNA transcripts (mRNAs), proteins, and regulatory small RNAs (Lough and Lucas 2006) indicating their role in cell-to-cell communication (Melnyk et al. 2011; Spiegelman et al. 2013). This communication may be apoplastic or intracellular and symplastic or intercellular.

In the apoplastic pathway, a cell secretes a protein ligand that migrates within the cell walls to reach the surface of the target cell, where it interacts with a plasma membrane-localized receptor to initiate a signaling cascade. This is best characterized by CLAVATA-mediated signaling, which regulates the stem cell population in the shoot apical meristem of Arabidopsis (Somssich et al. 2016). The apoplastic movement of macromolecules from the companion cells (CCs) or adjacent parenchyma cells to the sieve elements (SEs) is tightly regulated and takes place through a series of carriers and pumps, present on their respective membranes, or through the pore plasmodesmata units at the CC-SE interface (Turgeon and Wolf 2009; Dinant and Lemoine 2010). The entry of macromolecules can be either selective or passive via diffusion in a size-dependent manner. The mRNA profile in the SE is unique and does not reflect the transcript profile in the neighboring CC.

In symplastic pathway, the proteins, mainly transcription factors and RNA, are trafficked via plasmodesmata and the phloem to regulate gene expression in neighboring or distant cells. Plants have evolved a definite cellular machinery to restrict the diffusion of small molecules while facilitating the trafficking of selective endogenous macromolecules through the same plasmodesmata between cellular domains so that they can perform important functions. The transport of molecules through the phloem provides the most important long-distance transport pathway. This movement is regulated at multiple checkpoints including phloem entry, transport and exit, and targeting to specific organs, as exemplified by the selective RNA trafficking into the shoot apical meristem (Foster et al. 2002). The turgor gradient creates a hydraulic pressure that provides the driving force for long-distance transport (Knoblauch and Peters 2010). It has been proposed (van Bel et al. 2011) that the role of the phloem also encompasses modulation and amplification of signals along the long-distance transport conduit.

Recently it was shown by Chen et al. (2016) that bZIP transcription factor, ELONGATED HYPOCOTYL5 (HY5), serves as a shoot-to-root mobile signal to mediate light-responsive coupling of shoot growth in Arabidopsis. HY5 is also known to integrate multiple phytohormonal (e.g., ABA) and environmental (e.g., low temperature) signaling to control plant growth and development (Catalá et al.

2011; Xu et al. 2014). HY5 regulates the transcription of a large number of genes by directly binding to cis-regulatory elements. Its movement contributes to regulation of C fixation in the shoot and via sucrose-enhanced promotion of HY5-dependent N uptake in the roots, to maintain a homeostatic balance between C and N metabolism in response to a fluctuating environment. It was recently shown that light can be efficiently conducted through the stems to the photoactivated phytochrome B (phyB) in the roots to trigger the expression and accumulation of HY5. Mutations in roots expressing HY5 led to alterations in root growth and gravitropism in response to shoot illumination (Lee et al. 2016).

Likewise it was proposed that several inducers and repressors, including phytohormones and photosynthates, regulate flowering time by ordering the vegetative to reproductive phase transition through the flowering signal or florigen. A key component of this is a small globular protein encoded by the Flowering locus T (FLT) gene. Mutations in FLT caused a considerable delay in flowering (Koornneef 1991), while overexpression of FLT caused precocious flowering, indicating that FLT is necessary and sufficient for the acceleration of the floral transition (Kardailsky et al. 1999; Kobayashi et al. 1999). FLT translocates from the leaves to the shoot apex through the phloem to activate the FD transcription factor to convert leaf meristems to floral ones (Takada and Goto 2003). FLT, along with two other key genes, Leafy (LFY) and Suppressor of Overexpression of Constans (SOC1), constitute the floral pathway integrator (FPI) genes for incorporating flowering information (Simpson and Dean 2002). In the leaf, Tempranillo1 and Tempranillo2 have been shown to repress FLT (Castillejo and Pelaz 2008). According to a recent hypothesis, floral initiation can only be triggered when FLT and other limiting determinants, which include different genetic, biochemical, and physiological factors, are present at the SAM, at the right dose and time. The accumulation of these factors is also influenced by genotypes and/or under diverse abiotic and biotic conditions. miR172 also plays a key role in repressing floral repressor, AP2 transcription factors (TOE and SMZ/SNZ), which have been shown to directly repress FLT expression (Mathieu et al. 2007).

Recent studies using plant pathogens as model systems have shown that not only proteins but small non-coding RNAs (21–24 nucleotide) can also move systemically within plants (Subramanian 2019). The small non-coding RNAs including the microRNAs (miRNAs) and small interfering RNAs (siRNAs) have emerged as important regulators of gene expression. The majority of plant miRNAs target transcription factors and are therefore hypothesized to regulate several developmental processes. Though most miRNAs are considered to act in a locally restricted manner, they have been shown to move intercellularly and data suggest that this movement occurs through plasmodesmata (Brosnan and Voinnet 2011; Lim et al. 2011; Melnyk et al. 2011).

The first proposal that miRNA can translocate to adjacent cells came from studies of miR165/166 and miR390 (together with TAS3/tasiR-ARFs) in maize and *Arabidopsis thaliana* (Juarez et al. 2004; Kidner and Martienssen 2004). These miRNAs localize in complementary domains in young leaf primordial of maize (Nogueira et al. 2009). It is proposed that the intercellular movement of miR390 determines the production of tasiR-ARFs on the adaxial side of the leaf. These small

RNAs move from the adaxial to the abaxial leaf domain, establishing a gradient that accurately defines the adaxial/abaxial boundary. This limits the spatial localization of ARF proteins and miR166 to the abaxial side. ARFs specify abaxial fate, and miR166 restricts the HD-ZIP III transcription factors, which specify adaxial fate, to the adaxial domain (Chitwood et al. 2009). Expression of a viral protein that affects cell-to-cell trafficking causes severe defects in leaf polarity (Foster et al. 2004), which is consistent with the notion that small RNAs move in leaves. The small RNAs make good signaling molecules due to their high degree of specificity, rapid and direct mode of action, and the ability to exert a gradient response (Skopelitis et al. 2018).

Although long-distance movement of mRNAs through the phloem is well documented in diverse species (Lough and Lucas 2006; Kehr and Buhtz 2008), less is known about mechanisms and regulation of miRNA movement. The indications on the systemic movement of miRNAs were obtained from reports on their presence in phloem exudates of pumpkin, cucumber, castor bean, and yucca (Yoo et al. 2004). miRNAs present in phloem sap were later detected in rice (Sasaki et al. 1998), rapeseed (Buhtz et al. 2008), barley (Gaupels et al. 2008), apple (Varkonyi-Gasic et al. 2010) and field lupine (Rodriguez-Medina et al. 2011). Phloem exudates are enriched in specific miRNAs, like miR156, miR168, miR169, miR390, miR395 and miR399, when compared with other tissues. Specific molecules like miR167 and miR171 are consistently found to be under-represented in exudates. A substantial proportion of the miRNAs detected in phloem sap targeted genes involved in processes that require systemic signals, such as flowering (e.g., miR156, miR159, and miR172) (Poethig 2009), nutrient homeostasis (e.g., miR169, miR395, miR398, and miR399) (Liu and Chen 2009), and nodulation (e.g., miR169) (Combier et al. 2006). This also suggests that they might coordinate responses between the shoot and the root (Pant et al. 2009).

miR399 has been demonstrated as a long-distance signal for phosphate homeostasis by specific grafting experiments. It regulates the inorganic phosphate (Pi) homeostasis by targeting PHO2 transcripts. Mature miR399 accumulates to high levels in roots under Pi deficiency and suppresses the accumulation of PHO2, to promote Pi uptake and translocation (Chiou and Lin 2011). miR399 primary transcripts show much stronger upregulation in shoots than roots, and mature miR399 is present in phloem exudates in Pi-starved plants (Bari et al. 2006). Recently it was demonstrated that the pool of mature miR399 in the root might derive in part from the shoot via phloem transport (Pant et al. 2008). Likewise phloem sap contains a specific set of miRNAs that respond to the lack of essential nutrients, such as miR395 associated with sulfate deficiency and miR398 associated with copper or iron deficiency (Buhtz et al. 2010).

Recently, miR2111 was identified as a long-distance signal that regulates nitrogen acquisition through nodulation in lotus (Tsikou et al. 2018). Under nitrogen-deficient conditions, miR2111 is expressed in the leaves, and it travels to the roots to silence the expression of Too Much Love (TML), a kelch-repeat F-box protein, that suppresses nodule emergence. This enables rhizobium to infect the roots and nodule emergence. Two miRNAs present in phloem exudates, miR162 and miR168,

target DCL1 and AGO1, respectively. This suggests that the production of small RNAs might itself be modulated by mobile signals.

It can be extrapolated that the mobile 21-nt miRNAs are likely to regulate target gene expression post-transcriptionally via target mRNA cleavage or translation repression. The mobile 22-nt miRNAs could induce mRNA cleavage to initiate the production of secondary siRNAs (tasiRNAs), while the 24-nt miRNAs can direct epigenetic modifications (DNA methylation) in the genome of the recipient cells. The accumulation of miRNAs in the phloem sap suggests that they are mobile and may function as possible systemic signals. However, it is also possible that they non-specifically move from cell to cell and those expressed in phloem companion cells simply leak into sieve elements and may not necessarily play a role in systemic signaling.

19.4 Conclusion

Plants receive, store and process large amounts of information about their environment. This information is used for memory-based learning, which allows for an experience-driven learning response. Communication or signaling in plants seems to be an integral part of their immune system as it enables detecting dangers and invaders. The rapid communication system can be employed to warn other parts of the same plant, other plans of the same species, or other nearby plants of different species of an impending danger and taking prompt defensive actions.

The electrical and biomolecular signaling in plant cells seem to play many important roles, and therefore it is of great interest for plant scientists and has several implications of general interest. Plant cells have been shown to generate propagating action potentials in response to external stimuli. Electrical signals or waves can propagate over long distances and are involved in intercellular cross talk by regulating a wide variety of physiological responses in plants, including elongation growth, respiration (Fromm and Spanswick 1993), water uptake, phloem unloading, activation of genes, and gas exchange (Fromm et al. 1995). Ion channels that regulate efflux of Cl^- and K^+ and influx of Ca^{2+} facilitate the generation of electrical signals. The nature of electrical signaling in plants is very complex, and it seems to be in active cross talk with some of the other main components of rapid signaling such as ROS and Ca^{2+} waves (Gilroy et al. 2016). Though complete understanding of the phenomenon is still a black box, its presence seems to be common and probably ubiquitous. It is plausible that plants like animals inherited the ability to sense and communicate from a common ancestor. In fact it has been shown that bacteria, the forebears of all protists, plant and animal life, appear to be capable of responding to stimuli by producing electrical signals (Martinac et al. 1987). The knowledge gained so far about rapid communication in plant cells has built a strong case for further studies toward elucidating their biological significance and to unravel the associated metabolic pathways.

Accumulating evidence also indicates that environmental factors including light also influence growth and development through the release of signaling molecules

that can travel from the shoot to the root. Communication via direct protein and RNA transport is also unique to plants, and overall the findings suggest that complex mechanisms of short- and long-distance regulation do exist. The studies have been aided by grafting experiments to test movement of a mutated miRNA from a donor plant to a receptor carrying a complementarily mutated target gene and use of mutants affected in intercellular trafficking of macromolecules. Interesting observations undoubtedly demonstrate the movement of proteins and endogenous miRNAs, but additional experiments are required to prove that this movement is required for acting at a distance. The understanding of mechanisms, regulation and functions of the transport will provide essential clues to solving the mystery behind plant communication.

Recent reports show that underground parts can directly sense stem-piped light under natural conditions to monitor the aerial (light) environment during plant environmental adaptation (Lee et al. 2016). Scientists are striving to get more insights into the process so that farmers and gardeners can exploit the communication systems to control different stages of development and ease plant care. Plant signals may have strong implications for identifying and understanding health-producing phyto-substances for creating sustainable agriculture.

Acknowledgments There is a vast literature in the field, so we offer our apologies to researchers whose work could not be cited here. The authors performed their experiments on electrical conductance in the laboratory of Prof. B.N. Mallick.

References

Adie BA, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA, Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell 19:1665–1681

Anderson JP, Badruzsaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, Maclean DJ, Ebert PR, Kazan K (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16:3460–3479

Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J (2009) d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiol 50:1416–1424

Assmann SM, Simoncini L, Schroeder JI (1985) Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. Nature 318:285–287

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Balla J, Kalousek P, Reinöhl V, Friml J, Procházka S (2011) Competitive canalization of PIN-dependent auxin flow from axillary buds controls pea bud outgrowth. Plant J 65:571–577

Baluška F (2003) Polar transport of auxin: carrier-mediated flux across the plasma membrane or neurotransmitter-like secretion? Trends Cell Biol 13:282–285

Baluška F, Šamaj J, Wojtaszek P, Volkmann D, Menzel D (2003) Cytoskeleton – plasma membrane – cell wall continuum: emerging links revisited. Plant Physiol 133:482–491

Baluška F, Mancuso S, Volkmann D, Barlow P (2004) Root apices as plant command centres: the unique "brain-like" status of the root apex transition zone. Biologia 59:7–19

Baluška F, Volkmann D, Menzel D (2005) Plant synapses: actin based domains for cell-to-cell communication. Trends Plant Sci 10:106–111
Bari R, Pant BD, Stitt M, Scheible W-R (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. Plant Physiol 141:988–999
Barlow PW, Baluška F (2004) Polarity in roots. In: Lindsey K (ed) Polarity in plants. Blackwell, Oxford, pp 192–241
Beilby MJ (1984) Calcium and plant action potentials. Plant Cell Environ 7:415–412
Beilby MJ, Shepherd VA (1996) Turgor regulation in *Lamprothamnium papulosum.* I I/V analysis and pharmacological dissection of the hypotonic effect. Plant Cell Environ 19:837–847
Beilby MJ, Cherry CA, Shepherd VA (1999) Dual turgor regulation response to hypotonic stress in *Lamprothamnium papulosum*. Plant Cell Environ 22:347–361
Bemm F, Becker D, Larisch C, Kreuzer I, Escalante-Perez M, Schulze WX, Ankenbrand M, Van de Weyer A-L, Krol E, Al-Rasheid KA et al (2016) Venus flytrap carnivorous lifestyle builds on herbivore defense strategies. Genome Res 26:812–825
Bernard C (1878) La sciences experimentale. Hachette Livre-BNF, Paris, pp 218–236
Beveridge CA (2006) Advances in the control of axillary bud outgrowth: sending a message. Curr Opin Plant Biol 9:35–40
Biddington NL, Dearman AS (1985) The effect of mechanically induced stress on the growth of cauliflower, lettuce and celery seedlings. Ann Bot 55:109–119
Blatt FJ (1974) Temperature dependence of the action potential in *Nitella flexilis*. Biochim Biophys Acta 339:382–389
Böhm J, Scherzer S, Krol E, Kreuzer I, Meyer K, Lorey C, Mueller TD, Shabala L, Monte I, Solano R et al (2016) The Venus flytrap *Dionaea muscipula* counts prey-induced action potentials to induce sodium uptake. Curr Biol 26:286–295
Bose JC (1913) Researches on irritability of plants. Longmans, Green and Co, London
Bose JC (1926) The nervous mechanisms of plants. Longmans, Green and Co, London
Braam J, Davis RW (1990) Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell 60:357–364
Bradford KJ, Yang SF (1980) Xylem transport of 1-aminocyclopropane-1-carboxylicacid, and ethylene precursor, in water logged tomato plants. Plant Physiol 65:322–326
Brenner ED, Stevenson DW, Twigg RW (2003) Cycads: evolutionary innovations and the role of plant-derived neurotoxins. Trends Plant Sci 8:446–452
Brewer PB, Dun EA, Ferguson BJ, Rameau C, Beveridge CA (2009) Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and Arabidopsis. Plant Physiol 150:482–493
Brosnan CA, Voinnet O (2011) Cell-to-cell and long-distance siRNA movement in plants: mechanisms and biological implications. Curr Opin Plant Biol 14:580–587
Brown WH, Sharp LW (1910) The closing response in *Dionaea*. Bot Gaz 49:290–302
Buhtz A, Springer F, Chappell L, Baulcombe DC, Kehr J (2008) Identification and characterization of small RNAs from the phloem of *Brassica napus*. Plant J 53:739–749
Buhtz A, Pieritz J, Springer F, Kehr J (2010) Phloem small RNAs, nutrient stress responses, and systemic mobility. BMC Plant Biol 10:64
Burdon-Sanderson J (1873) Note on the electrical phenomenon which accompany stimulation of the leaf of *Dionaea muscipula*. Proc Roy Soc London 21:495–496
Capone R, Tiwari BS, Levine A (2004) Rapid transmission of oxidative and nitrosative stress signals from roots to shoots in Arabidopsis. Plant Physiol Biochem 42:425–428
Castillejo C, Pelaz S (2008 Sep 9) The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. Curr Biol 18(17):1338–1343
Catalá R, Medina J, Salinas J (2011) Integration of low temperature and light signaling during cold acclimation response in Arabidopsis. Proc Natl Acad Sci U S A 108:16475–16480
Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X (2016) Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr Biol 26:640–646
Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol 62:185–206

Chitwood DH, Nogueira FT, Howell MD, Montgomery TA, Carrington JC, Timmermans MC (2009) Pattern formation via small RNA mobility. Genes Dev 23:549–554
Coleman HA (1986) Chloride currents in *Chara*—a patch-clamp study. J Membr Biol 93:55–61
Combier JP, Frugier F, de Billy F, Boualem A, El-Yahyaoui F, Moreau S, Vernié T, Ott T, Gamas P, Crespi M, Niebel A (2006) MtHAP2-1 is a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 in *Medicago truncatula*. Genes Dev 20:3084–30888
Cosgrave DJ, Hedrich R (1991) Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. Planta 186:143–153
Crawford S, Shinohara N, Sieberer T, Williamson L, George G, Hepworth J, Müller DMA, Leyser O (2010) Strigolactones enhance competition between shoot branches by dampening auxin transport. Development 137:2905–2913
Darwin C (1966) The power of movements in plants. Da Capo Press, New York
Davies E (1987) Action potentials as multifunctional signals in plants, a unifying hypothesis to explain apparently disparate wound responses. Plant Cell Enviorn 10:623–631
De Geyter N, Gholami A, Goormachtig S, Goossens A (2012) Transcriptional machineries in jasmonate-elicited plant secondary metabolism. Trends Plant Sci 17:349–359
Dietrich P, Sanders D, Hedrich R (2001) The role of ion channels in light dependent stomatal opening. J Exp Bot 52:1959–1967
Dinant S, Lemoine R (2010) The phloem pathway: new issues and old debates. C R Biol 333:307–319
Domagalska MA, Leyser O (2011) Signal integration in the control of shoot branching. Nat Rev Mol Cell Biol 12:211–221
Dziubinska H, Paszewski A, Trebacz K, Zawadzki T (1983) The effect of excitation on the rate of respiration in the liverwort *Conocephalum conicu*. Physiol Plant 75:417–423
Dziubinska H, Filek M, Koscielniak J, Trebacz K (2003) Variation and action potentials evoked by thermal stimuli accompany enhancement of ethylene emission in distant non-stimulated leaves of *Vicia faba* minor seedlings. J Plant Physiol 160:1203–1210
Ellison A, Gotelli N (2009) Energetics and the evolution of carnivorous plants–Darwin's 'most wonderful plants in the world'. J Exp Bot 60(1):19–42
Eschrich W, Fromm J, Evert RF (1988) Transmission of electrical signals in sieve tubes of zucchini plants. Bot Acta 101:327–331
Evans MJ, Morris RJ (2017) Chemical agents transported by xylem mass flow propagate variation potentials. Plant J 91:1029–1037
Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4:129–134
Findlay GP (1961) Voltage-clamp experiments with Nitella. Nature 191:812–814
Flokova K, Tarkowska D, Miersch O, Strnad M, Wasternack C, Novak O (2014) UHPLC-MS/MS based target profiling of stress-induced phytohormones. Phytochemistry 105:147–157
Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol 12:539–547
Foster TM, Lough TJ, Emerson SJ, Lee RH, Bowman JL, Forster RLS, Lucas WJ (2002) A surveillance system regulates selective entry of RNA into the shoot apex. Plant Cell 14:1497–1508
Foster TP, Melancon JM, Baines JD, Kousoulas KG (2004) The herpes simplex virus type 1 UL20 protein modulates membrane fusion events during cytoplasmic virion morphogenesis and virus-induced cell fusion. J Virol 78:5347–5357
Frachisse JM, Desbiez MO, Champagnat P, Thellier M (1985) Transmission of a traumatic signal via a wave of electric depolarization, and induction of correlations between the cotyledonary buds in *Bidens pilosus*. Physiol Plant 64:48–52
Friml J (2003) Auxin transport – shaping the plant. Curr Opin Plant Biol 6:7–12
Fromm J (1991) Control of phloem unloading by action potentials in *Mimosa*. Physiol Plant 83:529–533
Fromm J, Bauer T (1994) Action potentials in maize sieve tubes change phloem translocation. J Exp Bot 45:463–469

Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. Plant Cell Environ 30:249–257

Fromm J, Spanswick R (1993) Characteristics of action potentials in willow (*Salix viminalis* L.). J Exp Bot 44:1119–1125

Fromm J, Hajirezaei M, Wilke I (1995) The biochemical response of electrical signalling in the reproductive system of *Hibiscus* plants. Plant Physiol 109:375–384

Gaupels F, Furch AC, Will T, Mur LA, Kogel KH, van Bel AJ (2008) Nitric oxide generation in *Vicia faba* phloem cells reveals them to be sensitive detectors as well as possible systemic transducers of stress signals. New Phytol 178:634–646

Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003) The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112:219–230

Gilroy S, Białasek M, Suzuki N, Górecka M, Devireddy A, Karpinski S et al (2016) ROS, calcium and electric signals: key mediators of rapid systemic signaling in plants. Plant Physiol 171:1606–1615

Glazer I, Orion D, Apelbaum A (1984) Interrelationships between ethylene production, gall formation, and root-knot nematode development in tomato plants infected with *Meloidogyne javanica*. J Nematol 15:539–544

Glebicki K, Hejnowicz Z, Pijanowski A (1989) Induced fluctuations of electric potentials in the apoplast of leaves. Planta 180:1–4

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194

Gradmann D (1976) "Metabolic" action potentials in *Acetabularia*. J Membr Biol 29:23–45

Grémiaux A, Yokawa K, Mancuso S, Baluška F (2014) Plant anesthesia supports similarities between animals and plants: Claude Bernard's forgotten studies. Plant Signal Behav 9(1):e27886

Gruntman M, Novoplansky A (2004) Physiologically mediated self/non-self discrimination in roots. Proc Natl Acad Sci U S A 101:3863–3867

Gunar II, Sinyukhin AM (1963) Functional significance of action currents affecting the gas exchange of higher plants. Sov Plant Physiol 10:219–226

Haake O (1892) Über die ursachen electrischer ströme in pflanzen. Flora 75:455–487

Haywood V, Yu TS, Huang NC, Lucas WJ (2005) Phloem long-distance trafficking of GIBBERELLIC ACID-INSENSITIVE RNA regulates leaf development. Plant J 42:49–68

Hebbar KB, Sinha SK (2000) Surface electrical potential changes of salt tolerant and sensitive wheat varieties differ with sodium chloride treatment. Curr Sci 78:76–78

Hedrich R, Busch H, Raschke K (1990) Ca^{2+} and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. EMBO J 9:3889–3892

Hedrich R, Salvador-Recatalà V, Dreyer I (2016) Electrical wiring and long-distance plant communication. Trends Plant Sci 21:376–387

Herde O, Cortés OP, Wasternack C, Willmitzer L, Fisahn J (1999) Electric signaling and *Pin2* gene expression on different abiotic stimuli depend on a distinct threshold level of endogenous abscisic acid in several abscisic acid-deficient tomato mutants. Plant Physiol 119:213–218

Hlaváčková V, Krchňák P, Nauš J, Novák O, Špundová M, Strnad M (2006) Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. Planta 225:235–244

Hodick D, Sievers A (1988) The action potential of *Dionaea muscipula* Ellis. Planta 174:8–18

Hodick D, Sievers A (1989) On the mechanism of trap closure of Venus flytrap (*Dionaea muscipula Ellis*). Planta 179:32–42

Hope AB, Walker NA (1975) The physiology of giant algal cells. Cambridge University Press, Cambridge

Iijima T, Sibaoka T (1981) Action potential in the trap-lobes of *Aldrovanda vesiculosa*. Plant Cell Physiol 22:1595–1601

Jackson SD (1999) Multiple signaling pathways control tuber induction in potato. Plant Physiol 119:1–8

Jacobson SL (1965) Receptor response in Venus fly-trap. J Gen Physiol 49:117–129

Jaffe MJ (1973) Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation. Planta 114:143–157

Johannes E, Ermolayeva E, Sanders D (1997) Red light-induced membrane potential transients in the moss *Physcomitrella patens*: ion channel interaction in phytochrome signalling. J Exp Bot 48:599–608

Juarez MT, Kui JS, Thomas J, Heller BA, Timmermans MCP (2004) microRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. Nature 428:84–88

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science 286:1962–1965

Kehr J, Buhtz A (2008) Long distance transport and movement of RNA through the phloem. J Exp Bot 59:85–92

Kidner CA, Martienssen RA (2004) Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. Nature 428:81–84

Kiep V, Vadassery J, Lattke J, Maaß JP, Boland W, Peiter E, Mithöfer A (2015) Systemic cytosolic Ca(2+) elevation is activated upon wounding and herbivory in Arabidopsis. New Phytol 207:996–1004

Kikuyama M, Tazawa M (1998) Temporal relationship between action potential and Ca^{2+} transient in characean cells. Plant Cell Physiol 39:1359–1366

Kim SA, Kwak JM, Jae SK, Wang MH, Nam HG (2001) Overexpression of the AtGluR2 gene encoding an Arabidopsis homolog of mammalian glutamate receptors impairs calcium utilization and sensitivity to ionic stress in transgenic plants. Plant Cell Physiol 42:74–84

Kirst GO, Janssen MIB, Winter U (1988) Ecophysiological investigations of *Chara vulgaris* L. grown in a brackish water lake: ionic changes and accumulation of sucrose in the vacuolar sap during sexual reproduction. Plant Cell Environ 11:55–61

Knoblauch M, Peters WS (2010) Munch, morphology, microfluidics—our structural problem with the phloem. Plant Cell Environ 33:1439–1452

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science 286:1960–1962

Koornneef M (1991) Isolation of higher plant developmental mutants. Symp Soc Exp Biol 45:1–19

Koziolek C, Grams TEE, Schreiber U, Matyssek R, Fromm J (2003) Transient knockout of photosynthesis mediated by electrical signals. New Phytol 161:715–722

Lackman P, González-Guzmán M, Tilleman S, Carqueijeiro I, Pèrez AC, Moses T, Seo M, Kanno Y, Häkkinen ST, Van Montagu MC et al (2011) Jasmonate signaling involves the abscisic acid receptor PYL4 to regulate metabolic reprogramming in Arabidopsis and tobacco. Proc Natl Acad Sci U S A 108:5891–5896

Lacombe B, Becker D, Hedrich R, DeSalle R, Hollmann M, Kwak JM, Schroeder JI, Le Novere N, Nam H-G, Spalding EP, Tester M, Turano FJ, Chiu J, Coruzzi GM (2001) On the identity of plant glutamate receptors. Science 292:1486–1487

Lautner S, Grams TEE, Matyssek R, Fromm J (2005) Characteristics of electrical signals in poplar and responses in photosynthesis. Plant Physiol 138:2200–2209

Lee HJ, Ha JH, Kim SG, Choi HK, Kim ZH, Han YJ, Kim JI, Oh Y, Fragoso V, Shin K, Hyeon T, Choi HG, Oh KH, Baldwin IT, Park CM (2016) Stem-piped light activates phytochrome B to trigger light responses in Arabidopsis thaliana roots. Sci Signal 9:RA106

Libiaková M, Floková K, Novák O, Slováková L, Pavlovič A (2014) Abundance of cysteine endopeptidase dionain in digestive fluid of Venus flytrap *Dionaea muscipula* Ellis is regulated by different stimuli from prey through jasmonates. PLoS One 9:e104424

Lim GH, Shine MB, de Lorenzo L, Yu K, Cui W, Navarre D, Hunt AG, Lee JY, Kachroo A, Kachroo P (2011) Plasmodesmata localizing proteins regulate transport and signaling during systemic acquired immunity in plants. Cell Host Microbe 19:541–549

Liu Q, Chen YQ (2009) Insights into the mechanism of plant development: interactions of miRNAs pathway with phytohormone response. Biochem Biophys Res Commun 384:1–5

Lough TJ, Lucas WJ (2006) Integrative plant biology: role of phloem long-distance macromolecular trafficking. Annu Rev Plant Biol 57:203–232
Lunevsky VZ, Zheralova OM, Vostrikov IY, Berestovsky GN (1983) Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels. J Membr Biol 72:43–58
Maffei ME, Mithöffer A, Boland W (2007) Before gene expression: early events in plant–insect interaction. Trends Plant Sci 12:310–316
Malone M (1996) Rapid, long-distance signal transmission in higher plants. Adv Bot Res 22:163–228
Mancuso S (1999) Hydraulic and electrical transmission of wound-induced signals in *Vitis vinifera*. Aust J Plant Physiol 26:55–61
Martinac B, Buechner M, Delcour AH, Adler J, Kung C (1987) Pressure-sensitive ion channel in *Escherichia coli*. Proc Natl Acad Sci U S A 84:2297–2301
Mathieu J, Warthmann N, Kuttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol 17:1055–1060
Melnyk CW, Molnar A, Bassett A, Baulcombe DC (2011) Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. Curr Biol 21:1678–1683
Miller G, Honig A, Stein H, Suzuki N, Mittler R, Zilberstein A (2009) Unraveling Δ1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. J Biol Chem 284:26482–26492
Moran N, Ehrenstein G, Iwasa K, Bare C, Mischke C (1984) Ion channels in plasmalemma of wheat protoplasts. Science 226:835–838
Mousavi SAR, Chauvin A, Pascaud F, Kellenberger S, Farmer EE (2013) Glutamate receptor-like genes mediate leaf-to-leaf wound signals. Nature 500:422–426
Nakamura Y, Mithöfer A, Kombrink E, Boland W, Hamamoto S, Uozumi N, Tohma K, Ueda M (2011) 12-Hydroxyjasmonic acid glucoside is a COI1-JAZindependent activator of leaf-closing movement in *Samanea saman*. Plant Physiol 155:1226–1236
Newman IA (1981) Rapid electric responses of oats to phytochrome show membrane processes unrelated to pelletabilty. Plant Physiol 68:1494–1499
Nichols R, Frost CE (1985) Wound-induced production of 1-aminocyclopropane-1-carboxylic acid and accelerated senescence of *Petunia corollas*. Sci Hortic 26:47–55
Nogueira FT, Chitwood DH, Madi S, Ohtsu K, Schnable PS, Scanlon MJ, Timmermans MC (2009) Regulation of small RNA accumulation in the maize shoot apex. PLoS Genet 5:e1000320
Oka-Kira E, Kawaguchi M (2006) Long-distance signaling to control root nodule number. Curr Opin Plant Biol 9:496–502
Palauqui JC, Elmayan T, Pollien JM, Vaucheret H (1997) Systemic acquired silencing: transgene-specific posttranscriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J 1617:4738–4745
Pant BD, Buhtz A, Kehr J, Scheible WR (2008) MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. Plant J 53:731–738
Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, Kehr J, Walther D, Scheible WR (2009) Identification of nutrient responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. Plant Physiol 150:1541–1555
Paszewski A, Zawadzki T (1994) Action potentials in *Lupinus angustifolius* L. shoots. J Exp Bot 25:1097–1103
Peña-Cortés H, Willmitzer L, Sanchez-Serrano JJ (1991) Abscisic acid mediates wound induction but not developmental-specific expression of the proteinase inhibitor II gene family. Plant Cell 3:963–972
Peña-Cortés H, Fisahn J, Willmitzer L (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proc Natl Acad Sci 92:4106–4113
Pickard BG (1973) Action potentials in higher plants. Bot Rev 39:172–201
Poethig RS (2009) Small RNAs and developmental timing in plants. Curr Opin Genet Dev 19:374–378

Prusinkiewicz P, Crawford S, Smith RS, Ljung K, Bennett T, Ongaro V, Leyser O (2009) Control of bud activation by an auxin transport switch. Proc Natl Acad Sci U S A 106:17431–17436
Racusen RH, Galston TJ (1980) Phytochrome modifies blue-light-induced electrical changes in corn coleoptiles. Plant Physiol 70:331–333
Rhodes JD, Thain JF, Wildon DC (1996) The pathway for systemic electrical signal conduction in the wounded tomato plant. Planta 200:50–57
Ridley BL, O'Neill MA, Mohnen D (2001) Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57:929–967
Rodriguez-Medina C, Atkins CA, Mann AJ, Jordan ME, Smith PMC (2011) Macromolecular composition of phloem exudate from white lupin (*Lupinus albus* L.). BMC Plant Biol 11:1–19
Roshchina VV (2001) Neurotransmitters in plant life. Science Publishers, Enfield
Salvador-Recatalà V, Tjallingii WF, Farmer EE (2014) Realtime, in vivo intracellular recordings of caterpillar-induced depolarization waves in sieve elements using aphid electrodes. New Phytol 203:674–684
Sanan N, Mallick BN, Sopory SK (2000) Electrical signal from root to shoot in *Sorghum bicolor*: induction of leaf opening and evidence for fast extracellular propagation. Plant Sci 160:237–245
Sandlin R, Lerman L, Barry W, Tasaki I (1968) Application of laser interferometry to physiological studies of excitable tissues. Nature 217:575–576
Sasaki T, Chino M, Hayashi H, Fujiwara T (1998) Detection of several mRNA species in rice phloem sap. Plant Cell Physiol 39:895–897
Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu FF, Sharon M, Browse J, He SY, Rizo J, Howe GA, Zheng N (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405
Shephard VA, Goodwin PB (1992) Seasonal patterns of cell-to-cell communication in *Chara corallina*. Klein ex Willd. I. Cell-to-cell communication in vegetative lateral branches during winter and spring. Plant Cell Environ 15:137–150
Shepherd VA, Beilby MJ, Heslop D (1999) Ecophysiology of the hypotonic response in the salt-tolerant alga *Lamprothamnium papulosum*. Plant Cell Environ 22:333–346
Shepherd VA, Shimrnen T, Beilby MJ (2001) Mechanosensory ion channels in Chara: the influence of cell turgor pressure on touch-activated receptor potentials and action potentials. Aust J Plant Physiol 28:551–566
Shiina T, Tazawa M (1986) Action potentials in *Luffa cylindrica* and its effects on elongation growth. Plant Cell Physiol 27:33–39
Sibaoka T (1962) Excitable cells in Mimosa. Science 137:226
Sibaoka T (1969) Physiology of raid movements in higher plants. Annu Rev Plant Physiol 20:165–184
Sibaoka T (1979) Action potentials and rapid plant movements. In: Skoog F (ed) Plant growth substances 1979. Springer, Berlin, pp 462–469
Simons P (1992) The action plant. Blackwell, Oxford
Simpson GG, Dean C (2002) Arabidopsis, the Rosetta stone of flowering time? Science 296:285–289
Skopelitis DS, Hill K, Klesen S, Marco CF, von Born P, Chitwood DH, Timmermans MCP (2018) Gating of miRNA movement at defined cell-cell interfaces governs their impact as positional signals. Nat Commun 9:3107
Somssich M, Je BI, Simon R, Jackson D (2016) CLAVATA-WUSCHEL signaling in the shoot meristem. Development 143:3238–3248
Spalding EP (1995) An apparatus for studying rapid electrophysiological responses to light demonstrated on Arabidopsis leaves. Photochem Photobiol 62:934–939
Spalding EP, Cosgrove DJ (1989) Large plasma-membrane depolarization precedes rapid blue-light induced growth inhibition in cucumber. Planta 178:407–410
Spalding EP, Cosgrove DJ (1992) Mechanism of blue-light-induced plasma-membrane depolarization in etiolated cucumber hypocotyls. Planta 188:199–205
Spiegelman Z, Golan G, Wolf S (2013) Don't kill the messenger: long-distance trafficking of mRNA molecules. Plant Sci 213:1–8

Staal M, Elzenga TM, van Elk AG, Prins HBA, Van-Volkenburgh E (1994) Red and blue-stimulated proton efflux by epidermal leaf cells of the argenteum mutant of *Pisum sativum*. J Exp Bot 54:1213–1218

Stahlberg R, Cleland RE, Van Volkenburgh E (2005) Decrement and amplification of slow wave potentials during their propagation in *Helianthus annuus* L. shoots. Planta 220:550–558

Stahlberg R, Cleland R, Van Volkenburgh E (2006) Slow wave potentials-a propagating electrical signal unique to higher plants. In: Baluška F, Mancuso S, Volkmann D (eds) Communication in plants. Springer, Berlin, pp 291–308

Stankovic B, Davies E (1998) Communication within plant cells. In: Sahi VP, Baluška F (eds) Concepts in cell biology – history and evolution, Plant cell monographs, vol 23. Springer, Berlin

Stankovic B, Zawadzki T, Davies E (1997) Characterization of the variation potential in sunflower. Plant Physiol 115:1083–1088

Stelmach BA, Müller A, Hennig P, Laudert D, Andert L, Weiler EW (1998) Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signalling compound in plant mechanotransduction. Phytochemistry 47:539–546

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plants defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci U S A 98:12837–12842

Subramanian S (2019) Little RNAs go a long way: long-distance signaling by microRNAs. Mol Plant 12:18–20

Suzuki N, Miller G, Salazar C, Mondal HA, Shulaev E, Cortes DF, Shuman JL, Luo X, Shah J, Schlauch K, Shulaev V, Mittler R (2013) Temporal–spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. Plant Cell 25:3553–3569

Swarup R, Bennett M (2003) Auxin transport: the fountain of life in plants? Dev Cell 5:824–826

Takada S, Goto K (2003) Terminal flower2, an Arabidopsis homolog of heterochromatin protein1, counteracts the activation of flowering locus T by constans in the vascular tissues of leaves to regulate flowering time. Plant Cell 15:2856–2865

Thain JF (1995) Electrophysiology. In: Gallbraith DW, Bohnert HJ, Bourque DP (eds) Methods in cell biology, vol 49. Academic, San Diego, pp 259–274

Thain JF, Wildon DC (1996) Electrical signalling in plants. In: Smallwood M, Knox JP, Bowles DJ (eds) Membranes: specialized functions in plants. BIOS Scientific Publisher, Oxford, pp 301–317

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signaling. Nature 448:661–665

Trewavas A (2003) Aspects of plant intelligence. Ann Bot (Lond) 92:1–20

Tsikou D, Yan Z, Holt DB, Abel NB, Reid DE, Madsen LH, Bhasin H, Sexauer M, Stougaard J, Markmann K (2018) Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA. Science 362:233–236

Tudela D, Primo-Millo E (1992) 1-Aminocyclopropane-1-carboxylic acid transported from roots to shoots promotes leaf abscission in *Cleopatra Mandarin* (Citrus reshni Hort. ex Tan.) seedlings rehydrated after water stress. Plant Physiol 100:131–137

Turgeon R, Wolf S (2009) Phloem transport: cellular pathways and molecular trafficking. Annu Rev Plant Biol 60:207–221

Tylewicz S, Petterle A, Marttila S, Miskolczi P, Azeez A, Singh RK, Immanen J, Mähler N, Hvidsten TR, Eklund DM, Bowman JL, Helariutta Y, Bhalerao RP (2018) Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science 360:212

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195–200

van Bel AJE, Furch ACU, Hafke JB, Knoblauch M, Patrick JW (2011) (Questions)n on phloem biology: 2. Mass flow, molecular hopping, distribution patterns and macromolecular signalling. Plant Sci 181:325–330

Varkonyi-Gasic E, Gould N, Sandanayaka M, Sutherland P, MacDiarmid RM (2010) Characterisation of microRNAs from apple (*Malus domestica* 'Royal Gala') vascular tissue and phloem sap. BMC Plant Biol 10:159

Wayne R (1993) The excitability of plant cells. Am Sci 81:140–151

Wayne R (1994) The excitability of plant cells: with a special emphasis on characean internodal cells. Bot Rev 60:265–267

Wildon DC, Thain JF, Minchin PEH, Gubb IR, Reilly AJ, Skipper YD, Doherty HM, O'Donnell PJ, Bowles DJ (1992) Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. Nature 360:62–65

Williams SE, Pickard BG (1972) Receptor potentials and action potentials in Drosera tentacles. Planta 103:193–221

Williams SE, Pickard BG (1980) The role of action potentials in the control of capture movements of Drosera and Dionaea. In: Skoog F (ed) Plant growth substances. Springer, Berlin, pp 470–480

Williams SE, Spanswick RM (1976) Propagation of the neuroid action potential of the carnivorous plant Drosera. J Comp Physiol 108:211–223

Wipf D, Ludewig U, Tegeder M, Rentsch D, Koch W, Frommer WB (2002) Amino acid/neurotransmitter transporters are highly conserved between fungi, plants and animals. Trends Biochem Sci 27:139–147

Xu D, Li J, Gangappa SN, Hettiarachchi C, Lin F, Andersson MX, Jiang Y, Deng XW, Holm M (2014) Convergence of light and ABA signaling on the ABI5 promoter. PLoS Genet 10:e1004197

Yoo BC, Kragler F, Varkonyi-Gasic E et al (2004) A systemic small RNA signaling system in plants. Plant Cell 16:1979–2000

Zawadzki T (1980) Action potentials in *Lupinus augustifolius* L. shoots. V. Spread of excitation in the stem, leaves and root. J Exp Bot 31:1371–1377

Zawadzki T, Davies E, Dziubinska H, Trebacz K (1991) Characteristics of action potentials in *Helianthus annuus*. Physiol Plant 83:601–604

Zeevaart AJ (1976) Some effects of fumigating plants for short periods with NO2. Environ Pollut 11:97–108

Zimmermann U, Beckers F (1978) Generation of action potentials in *Chara corallina* by turgor pressure changes. Planta 138:173–179

Zimmermann MR, Mithöfer A (2013) Electrical long-distance signaling in plants. In: Baluška F (ed) Long-distance systemic signaling and communication in plants. Springer, Berlin, pp 291–308

Zimmermann MR, Maischak H, Mithöfer A, Boland W, Felle HH (2009) System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. Plant Physiol 149:1593–1600

Zimmermann MR, Mithöfer A, Will T, Felle HH, Furch AC (2016) Herbivore triggered electrophysiological reactions: candidates for systemic signals in higher plants and the challenge of their identification. Plant Physiol 170:2407–2419

Neeti Sanan-Mishra obtained her doctoral degree from JNU, New Delhi (with the Editor), on phytochrome control of leaf opening in *Sorghum bicolor*: GTP-binding proteins, calcium, and protein phosphorylation. Subsequently she joined the Centre for Plant Molecular Biology in the lab of Prof. Anil Grover at the University of Delhi. She is currently Group Leader, Plant RNAi Biology at ICGEB, New Delhi, India. Her research has focused on understanding the abiotic stress responses in plants. Her current research interests are on understanding the miRNA networks involved in regulating the rice plant development in response to virus infection and abiotic stress.

How Plants Respond to Pathogen Attack: Interaction and Communication

20

Srayan Ghosh, Kamal Kumar Malukani, Ravindra Kumar Chandan, Ramesh V. Sonti, and Gopaljee Jha

Abstract

Plants are exposed to a plethora of microorganisms in their environment. A number of these microorganisms are plant pathogens. In order to defend themselves against pathogen attack, plants have evolved specialized sensory receptors to recognize some of the conserved molecular features (PAMPs, DAMPs, HAMPs, and NAMPs) as well as secreted effector molecules of pathogens. A cascade of signal transduction events are triggered which causes transcriptional rewiring leading to activation of defense responses. Closure of stomata, strengthening of cell wall along with accumulation of secondary metabolites, and induction of a hypersensitive response (HR) and pathogenesis-related (PR) proteins are some of the key defense strategies of the host. Interestingly, through secretion of volatile organic compounds (VOCs), plants have the ability to induce defense responses in uninfected tissues as well as surrounding plants. In this chapter, we elaborate on the mechanisms by which plants perceive pathogen attack and transduce the signal to downstream signaling molecules, culminating in the activation of defense responses.

Authors Srayan Ghosh and Kamal Kumar Malukani have equally contributed to this chapter.

S. Ghosh · R. K. Chandan · G. Jha (✉)
Plant Microbe Interactions Laboratory, National Institute of Plant Genome Research, New Delhi, India
e-mail: jmsgopal@nipgr.ac.in

K. K. Malukani
CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

R. V. Sonti
Plant Microbe Interactions Laboratory, National Institute of Plant Genome Research, New Delhi, India

CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_20

Keywords
Defense hormones · Effector-triggered immunity · Pathogen perception · Pathogen-triggered immunity · Plant defense responses · Resistance genes · Secondary messengers

20.1 Introduction

Plants are constantly exposed to a diverse array of microorganisms. Among them some are pathogenic on the host, whereas others grow in harmony with the host without causing any damage. Plants possess a proficient and dynamic sensory system to distinguish between them. In case of beneficial interactions, plants have adapted to harbor these microorganisms in specialized compartments, thus maintaining a suitable niche inside their tissue (Oldroyd 2013; Jones et al. 2007). However, in case of a negative interaction, the microorganism tries to forcefully colonize to obtain nutrients from the host plant. Plants being sessile cannot evade from such harmful interactions but possess several robust defense mechanisms to inhibit the growth of such pathogenic organisms.

The first step in mounting an immune response lies in the ability of host to perceive the pathogen attack, and this is achieved via a wide array of specialized extracellular receptors that are present on the plant cell membrane. Generally, plants recognize bacterial pathogens by conserved structural components such as flagellin, lipopolysaccharides (LPS), peptidoglycans (PG), etc. or bacterial molecules such as EfTu or RaxX that are released into the extracellular milieu (Couto and Zipfel 2016). Fungal pathogens are sensed by the recognition of chitin or fungal secreted proteins such as NLPs (NEP1 like proteins) (Kaku et al. 2006). These conserved microbe-specific molecules are known as PAMPs/MAMPs (pathogen-/microbe-associated molecular patterns). Herbivory is perceived by the presence of certain herbivore-associated molecular patterns (HAMPs) present in the oral secretion of the insect at the time of attack (Mithofer and Boland 2008). Nematodes also secrete molecules that are known to elicit plant defense responses, and these molecules are known as nematode-associated molecular patterns (NAMPs) (Mendy et al. 2017). Besides these signals, plants can also sense molecules that are released from their own cells as a consequence of pathogen attack and use them as cues to mount an immune response (Bacete et al. 2018). These molecules are known as DAMPs (damage-associated molecular patterns). Classic examples of DAMPs are degradation products that are released following the action of microbial enzymes on various components of the plant cell wall. Also, plants have cytoplasmic receptors to sense effector molecules secreted by potential pathogens to mount a robust immune response (Schreiber et al. 2016).

Plants possess a two-tiered detection system against pathogens (Zipfel 2014). The first tier comprises of receptors present on the surface of a cell called PRRs (pattern recognition receptors) that recognizes PAMPs, DAMPs, HAMPs or NAMPs. The PRRs can broadly be classified under two types, receptor-like kinases

(RLKs; comprising of a ligand-binding ectodomain, a transmembrane domain, and a cytoplasmic kinase domain) and receptor-like proteins (RLPs; comprising of a ligand-binding ectodomain and a transmembrane domain). The immune responses that are mounted upon recognition of the pathogen by PRRs are referred to as pathogen-triggered immunity (PTI). Moreover, the immune responses that are induced following recognition of DAMPs are known as DAMP-triggered immunity (DTI). The second tier of the pathogen recognition system comprises of intracellular immune receptors that can sense secreted pathogenic effectors either directly or indirectly. The immune responses that are mounted upon recognition of these effectors are referred to as effector-triggered immunity (ETI). The receptors are classified into two types: nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins and Toll-like receptor (TLR) proteins. The major difference in the signaling events during PTI and ETI is the duration and amplitude of the defense response, which is more in ETI as compared to PTI. In this chapter, we elaborate on how plants recognize various phytopathogens (bacteria and fungi) as well as herbivores and nematodes. We have described various players involved in the signal transduction events associated with pathogen perception and how the perceived signal is transduced to regulate host defense response pathways in host.

20.2 Perception of Pathogen Attack

Perception of danger is a key step in the activation of immune responses. However, induction of immune responses is an energy-consuming process that involves activation/deactivation of many molecular pathways, synthesis of new molecules, and alterations in basic metabolic processes (Andolfo and Ercolano 2015; Duan et al. 2013). Hence, it is crucial for plants to distinguish between a potential pathogen/pest and a random visitor to mount an appropriate immune response (Table 20.1).

20.2.1 Recognition of Bacterial Pathogens

Plants can recognize various structural components of bacteria or their secreted compounds to mount an immune response. Flagellin-Sensing 2 (FLS2), a LRR repeat domain-containing receptor-like kinase in Arabidopsis can recognize a 22-amino acid long peptide named flg22 derived from the flagellin of *Pseudomonas syringae* (Gómez-Gómez and Boller 2000). The flg22 peptide binds to the extracellular N-terminal domain of FLS2 and acts as a molecular glue between FLS2 and its co-receptor somatic embryogenesis receptor kinase 3 (SERK3) [also called as BAK1 (BRI1-associated receptor kinase 1)] (Meindl 2000; Sun et al. 2013). This complex phosphorylates downstream interacting partners and activates the immune response (Couto and Zipfel 2016). Interestingly, different plant species have evolved diverse receptors to recognize different epitopes on flagellin. Solanaceous plants such as pepper, potato and tomato recognize flgII-28 (the flagellin peptide derived from *Pseudomonas syringae*) by another type of LRR receptor, FLS3 (Hind et al. 2016).

Table 20.1 List of some known receptors of MAMPs, DAMPs and effectors perceived by plants

Source of ligand	Receptor	Plants	Ligand	Type of receptor	Type of Immune Response	Reference
Bacteria	FLS2	Arabidopsis	Flg22 (flagellin)	LRR-RLK	PTI	Gómez-Gómez and Boller (2000)
	FLS3	*Solanaceae*	FlgII-28 (flagellin)	LRR-RLK	PTI	Hind et al. (2016)
	EFR	Brassicaceae	Elf18 (EF-Tu)	LRR-RLK	PTI	Zipfel et al. (2006)
	Xa21	Rice	RaxX (putative)	LRR-RLK	PTI	Pruitt et al. (2015)
	OsLYM4/ OsLYM6	Rice	Peptidoglycan (bacterial cell wall)	LysM-RLP	PTI	Liu et al. (2013a, b)
	AtLYM1/ AtLYM3	Arabidopsis		LysM-RLP	PTI	Willmann et al. (2011)
	AtLORE (Putative)	Arabidopsis	Lipooligosaccharide (bacterial cell wall)	Lectin-RLK	PTI	Ranf et al. (2015)
Fungal/oomycete	OsCEBiP	Rice	Chitin	LysM-RLP	PTI	Kouzai et al. (2014)
	AtCERK1/ AtLYK5	Arabidopsis		LysM-RLK	PTI	Cao et al. (2014)
	LeEix1/LeEix2	Tomato	EIX (fungal xylenase)	LRR-RLP	PTI	Rotblat et al. (2002)
	RLP23	Arabidopsis	Nlp20 (various pathogens)	LRR-RLP	PTI	Albert et al. (2015)
	RGA5	Rice	AVR-Pia (magnaporthe effector)	NB-LRR	ETI	Ortiz et al. (2017)
	RPP1	Arabidopsis	ATR1 (*Hyaloperono-spora arabidopsidis*)		ETI	Krasileva et al. (2010)
	L5/ L6	Flax	AvrL567 (*Melampsora lini*)		ETI	Ravensdale et al. (2012)
Nematodes	NILR1	Arabidopsis	Ascarosides	LRR-RLK	PTI	Mendy et al. (2017)
Cell wall degraded products	WAK1	Arabidopsis	Oligogalacturonides (OG)	EGF-RLK	DTI	Brutus et al. (2010)
	DORN1	Extracellular ATP	eATP	Lectin-RLK	DTI	Choi et al. (2014)
	PEPR1/PEPR2	Arabidopsis	pep	LRR-RLK	PTI	Krol et al. (2010)

Plants can also recognize peptidoglycan (PG) and lipopolysaccharide (LPS) that are major components of either bacterial cell wall or the outer membrane, respectively. Exogenous treatment with either LPS or PG activates plant immune responses (Erbs et al. 2010; Gust et al. 2007). PG is a polymer of N-acetylglucosamine and N-acetylmuramic acid linked by oligopeptides (Gust et al. 2007). Plants possess LysM domain (lysin motif)-containing proteins that can recognize glycans in N-acetylglucosamine (Gust et al. 2012). In Arabidopsis, PG is recognized by receptor-like proteins AtLYM1 and AtLYM3 where chitin receptor AtCERK1 serves as a key component in PG recognition (Willmann et al. 2011). In rice, OsLYP4 and OsLYP6 are known to interact with both chitin oligomers as well as peptidoglycan (Liu et al. 2012). Additionally, OsCERK1 appears to be a key receptor/co-receptor for LPS perception in rice (Desaki et al. 2017). In Arabidopsis, bulb-type (B-type) lectin S-domain (SD)-1 containing RLK protein LORE (lipooligosaccharide-specific reduced elicitation) is thought to be the putative LPS receptor (Ranf et al. 2015). However, the physical interaction between LPS and putative receptors are yet to be established.

Plants can also sense various bacterial secreted proteins/peptides. Elongation factor Tu (Ef-Tu) is an abundant bacterial protein that is released upon cell lysis. Ef-Tu acts as an elicitor of immune responses in various plant species (Kunze 2004). Members of the Brassicaceae family recognize a conserved 18-aa long peptide (elf18) present at the N-terminal of EF-Tu by the LRR-RLK Ef-Tu receptor (EFR) (Zipfel et al. 2006). Rice recognizes EFa50, comprising of a 50aa long peptide sequence from the middle of Ef-Tu amino acid sequence (Furukawa et al. 2014). Another secreted peptide recognized by plants is RexX21-sY, a sulfated peptide secreted by *Xanthomonas oryzae* pv. *oryzae* (Xoo) type 1 secretion system (Pruitt et al. 2015). This is recognized by rice LRR-RLK receptor Xa21. Here it is worth mentioning that Xa21 has been widely used to breed rice for bacterial blight resistance (Williams et al. 1996).

20.2.2 Recognition of Fungal/Oomycete Pathogens

Chitin, a polymer of N-acetyl-D-glucosamine (GlcNAc), is a major component of fungal cell walls. Plants can identify chitin oligomers by 40aa long globular LysM motif-containing receptor proteins (Kaku et al. 2006; Miya et al. 2007; Wan et al. 2008). In Arabidopsis, AtCERK1/AtLYK1 (chitin elicitor receptor kinase) recognizes chitin oligomers and mounts defense responses. Binding of 7–8-residue long chitin oligomer with *AtCERK1* causes receptor homodimerization and transphosphorylation that lead to activation of defense signaling cascade (Liu et al. 2012). Rice recognizes chitin by a GPI-anchored RLP protein, OsCEBiP (chitin elicitor binding protein), that contains three extracellular LysM domains but lacks an intracellular kinase domain (Kaku et al. 2006; Kouzai et al. 2014). Ligand (GlcNAc)8 binding causes homodimerization of OsCEBiP and OsCERK1 leading to the formation of a $GlcNAc_8$-2CEBiP-2CERK1 complex which in turn activates immune responses (Hayafune et al. 2014). Other plant receptors such as AtLYK4 (RLK),

OsLYP4 and OsLYP6 (both RLP) can also recognize chitin (Liu et al. 2012; Petutschnig et al. 2010; Wan et al. 2012).

Some plants can sense presence of fungal xylanases to mount immune responses. A fungal protein ethylene-inducing xylanase (EIX) was found to activate plant immune responses in various host species (Bailey et al. 1990, 1993; Fuchs et al. 1989; Ron et al. 2000). In tomato, LeEIX is recognized by LRR-RLP LeEix2 leading to activation of immune responses (Bar and Avni 2009; Bar et al. 2009). Similarly, in Arabidopsis, LRR-RLP receptor AtRLP42 recognizes fungal endopolygalacturonases (PGs) and activates its immune responses (Zhang et al. 2014).

20.2.3 Recognition of Herbivores

The plants are exposed to different insects, some of which feed upon plant parts by a process known as insect herbivory. Herbivorous insects can activate plant defense mechanisms either through mechanical wounding caused during the process of chewing or by their oral secretions. Mechanical wounding caused during herbivory induces either the activation of defense mechanisms or secretion of plant volatiles. Production of chemical factors or relaying of electrical signals across distal parts of the host tissues are some of the early plant responses generated immediately after wounding (Maffei et al. 2007).

The herbivore-associated molecular patterns (HAMPs) that are present in the oral secretions of insects are recognized by plants (Mithofer and Boland 2008). Some orally secreted compounds like fatty acid amino conjugates (FACs) act as elicitors in priming of plant defense responses (Bonaventure et al. 2011). Perception of FACs induces a MAPK signaling cascade including SIPK (salicylic acid-induced protein kinase) and WIPK (wound-induced protein kinase) along with activation of NPR1 signaling (Wu et al. 2007; Seo et al. 2007; Bonaventure and Baldwin 2010), culminating in the activation of defense responses.

20.2.4 Recognition of Nematodes

Plants are continuously exposed to a plethora of microorganisms surrounding their rhizosphere. The different varieties of root exudates secreted by the plants may either attract or deter away these microorganisms. Plants secrete flavonoid compounds that can attract symbiotic microbes like Rhizobia in case of beneficial interactions, phytoalexins to deter pathogen growth or allelopathic phenolic compounds to alter the growth of other plants (Hirsch et al. 2003). However, plant parasitic nematodes like root-knot nematode and potato cyst nematode can sense these host-derived signals. Following penetration inside the host tissue, the nematode migrates to its feeding site inside the root, wherein it feeds upon the host nutrients resulting in altered root architecture and reduced crop yield. Since long it had been speculated that plants could also mount a PTI response against nematodes, however not much was known about the compounds which elicit plant defense response.

Recently, a nematode pheromone, ascaroside has been identified that is perceived by host plants as a NAMP to mount a PTI response including activation of MAP kinase cascade, upregulation of plant defense hormones such as salicylic acid and jasmonic acid, and induction of defense responses (Manosalva et al. 2015; Holbein et al. 2016; Choi et al. 2016). Moreover, a nematode immune receptor NILR1 (nematode-induced LRR-RLK 1) belonging to the LRR-RLK has been identified in Arabidopsis that perceives NAMP and mounts PTI responses (Mendy et al. 2017).

20.2.5 Recognition of DAMPs

The plant cell wall serves as a formidable barrier against pathogens. Pathogen secretes various proteins to degrade different components of the plant cell (Jha et al. 2005). Moreover, plants have evolved the ability to sense this damage by recognition of the cell wall degradation products. Treatment of Arabidopsis with cellulose degradation products such as cellobiose, cellotriose, etc. or cellulose synthesis inhibitors (Engelsdorf et al. 2017) activates the host immune responses (Souza et al. 2017). Similarly, the treatment of plant tissue with pectin degradation products such as oligogalacturonides (OG) can activate the host immune responses (Ferrari 2013). In Arabidopsis, wall-associated kinases (AtWAK1 and AtWAK2) can perceive pectin and pectin degradation products (OG) (Brutus et al. 2010; Decreux and Messiaen 2005; Decreux et al. 2006). The activation of immune responses by WAKs has also been reported in other plant species such as rice and maize (Delteil et al. 2016; Zuo et al. 2015; Hu et al. 2017).

In response to pathogen/damage perception, plants secrete various peptides and nucleotides in their apoplast to amplify the immune response and trigger an elaborate defense mechanism in their neighboring cells (Boutrot and Zipfel 2017). Release of plant elicitor peptides (Peps, also known as danger peptides) derived from PROPEPs (precursor proteins) has been reported in Arabidopsis upon pathogen attack (Bartels et al. 2013; Klauser et al. 2015). Arabidopsis secretes 23aa long endogenous elicitor peptides known as *At*Pep1, which are recognized via LRR-RLK PEP receptor (PEPR) (Krol et al. 2010). Moreover, it has been reported that extracellular ATP (eATP) can act as a DAMP in Arabidopsis (Weerasinghe et al. 2009; Wu et al. 2008). The eATP is recognized by a lectin receptor kinase-I.9 (LecRK-I.9) named DORN1 (Does not Respond to Nucleotides 1) in Arabidopsis which activates downstream defense-responsive genes (Choi et al. 2014).

20.2.6 Recognition of Effectors

PTI and DTI form the first layer of plant immune responses. Pathogens can suppress these immune responses by secreting effector molecules directly into plant cells via the type-III-secretion system (Alfano and Collmer 2004). However, plants have evolved *R* gene-encoded proteins to recognize effector proteins to activate effector-triggered defense (ETD) response (Dodds and Rathjen 2010). Plants can

either directly recognize effector molecules via NB-LRR (or NLR) domain-containing receptor proteins or can indirectly sense their presence by monitoring their activity (Kourelis and van der Hoorn 2018). In both cases, plants mount a robust immune response that usually culminates in a hypersensitive response and localized death of plant tissue to limit spread of the pathogen. The NLR receptor proteins are usually comprised of either coiled-coil (CC) domain or toll/interleukin-1 receptor (TIR) domains at their N-terminal (Cui et al. 2015; Schreiber et al. 2016). However, there are exceptions, wherein certain effector proteins are not directly recognized by receptor proteins, instead are recognized when bound to an accessory protein (guardee). The guard model has been proposed to explain this phenomenon (Dangl and Jones 2001). Further, a modification of this hypothesis has been proposed as a decoy model, wherein certain effector targets have evolved to function as decoys (co-receptor) which bind to the effectors and cause activation of the defense response (van der Hoorn and Kamoun 2008). Due to a few limitations in the decoy model, an improved bait-and-switch model was proposed. In this model, a two-step recognition has been proposed wherein the accessory protein (bait) associated with the receptor protein interacts with the effector protein to mount a defense response (Collier and Moffett 2009). The current hypothesis states that the receptor protein instead of recognizing the accessory protein directly recognizes the effector protein only when it is bound with its accessory protein (Dodds and Rathjen 2010). We will now provide an outline of the different effector molecules that are secreted in different pathosystems and how plants are able to recognize them.

20.2.6.1 Bacterial Effector Recognition

The AvrPto and AvrPtoB (also known as HopAB2) effectors secreted by pathogenic strains of *P. syringae* (Abramovitch et al. 2003; Ronald et al. 1992) are recognized by plants to mount immune responses. AvrPto and AvrPtoB bind to various PTI receptors and suppress immune responses. For example, AvrPto binds to various PTI receptors like FLS2 and EFR while AvrPtoB binds to FLS2, BAK1 and LysM receptor kinases and suppress immune responses (Cheng et al. 2011; Gimenez-Ibanez et al. 2009; Göhre et al. 2008; Shan et al. 2008; Xiang et al. 2008; Zeng et al. 2012). Prf/Pto protein complex recognizes the presence of both of these effector molecules, whereas Pto has binding sites for both AvrPto and AvrPtoB as well as Prf. Prf acts as a positive regulator of ETI. In the native state, Pto binds to Prf along with some other kinases to form a large macromolecular complex that keeps Prf in its inactive state (Ntoukakis et al. 2013). In presence of cognate effectors, Pto binds to the effector, gets released from Prf/Pto complex and in turn activates ETI (Abramovitch et al. 2003; Dong et al. 2009; Mathieu et al. 2014).

Rin4 (RPM1 interacting protein 4) is a membrane-localized protein that lacks any functional domain but is a part of many PRR complexes (Selote and Kachroo 2010). Rin4 can activate as well as suppress PTI depending on the phosphorylation status of the protein (Chung et al. 2014). Pathogens have evolved effector molecules such as AvrB, AvrRpt2, AvrRpm1, and HopF2 to directly or indirectly target Rin4 to suppress PTI

(Lee et al. 2015; Russell et al. 2015; Wang et al. 2010; Wilton et al. 2010). In response, plants have also evolved R genes such as RPS2 (resistance to *P. syringae*) and RPM1 (resistance to *P. syringae* pv. *maculicola*) to sense the activity of effectors on Rin4 and mount defense responses (Chung et al. 2014; Coaker et al. 2005; Kim et al. 2005).

20.2.6.2 Fungal Effector Recognition

Fungal pathogens are also known to produce effector molecules which can either be secreted into the host cytoplasm or localized into the apoplastic space (Giraldo et al. 2013; Stotz et al. 2014). The recognition of apoplastic effectors is mediated by integral membrane proteins (RLPs) containing an extracellular leucine-rich repeat (eLRR) (Stergiopoulos and de Wit 2009). Induction of RLPs has been reported in tomato, apple, and oilseed rape against fungal pathogens like *Cladosporium fulvum*, *Venturia inaequalis*, and *Leptosphaeria maculans*, respectively (Rouxel and Balesdent 2013; Belfanti et al. 2004). However cytoplasmic effectors secreted by pathogens like *Blumeria graminis*, *Bremia lactucae*, *Puccinia striiformis*, *Magnaporthe grisea*, and *Phytophthora infestans* are recognized by NBS-LRR receptors that are present in the cytoplasm of respective host species (Bozkurt et al. 2010; Bai et al. 2012; Bonardi et al. 2012; Larkan et al. 2013; Rooney et al. 2005).

20.2.6.3 Nematode Effector Recognition

Plants utilize NB-LRR immune receptors to recognize effectors secreted from root or cyst nematodes to activate host defense responses. Some common examples of immune receptors against nematodes are Gpa2, Gro1-4 and Hero (Goverse and Smant 2014). It has been observed that root-knot nematodes secrete a diffusible compound called NemF that is very similar to NF (nodulation factor) secreted by symbiotic bacteria. The NemF signal is perceived by the plant through primary receptor kinases NFR1 and NFR5 along with secondary receptor kinase SYMRK. Signal perception leads to root hair branching and waviness which in turn facilitate nematode penetration (Weerasinghe et al. 2005). Plants also encode R genes to recognize effector proteins secreted by herbivores (Hogenhout and Bos 2011). Examples of R genes which confer resistance against herbivores are Mi-1.2 (Meloidogyne 1.2), Vat (Virus aphid transmission resistance) and Bph14 (Brown planthopper 14).

20.2.6.4 Miscellaneous

Necrosis and ethylene-inducing peptide 1-like proteins (NLPs) are plant immunogenic proteins with cytotoxic activity produced by a vast variety of bacterial, fungal, and oomycete species (Oome et al. 2014). Plants belonging to Brassicaceae family can recognize a conserved 20aa long fragment of NLP called nlp20 to activate their immune responses (Böhm et al. 2014; Oome et al. 2014; Oome and Van den Ackerveken 2014). In Arabidopsis, the LRR-RLP AtRLP23 recognizes nlp20 and activates immune responses by making a tripartite complex with two LRR-RLK, BAK1 (brassinosteroid insensitive 1 (BRI1)-associated kinase) and SOBIR1 (Albert et al. 2015).

20.3 Players Involved in Transduction of a Perceived Signal

The PRR proteins present on the plant cell surface can recognize pathogen attack and mount a defense response against the pathogen. However, induction of defense responses involves an intricate signaling network that transduces the signal to downstream molecular players to trigger immune responses. These signaling molecules include protein kinases (CDPKs, MAPKs), Ca^{2+} burst, ROS burst, NO, lipids, 14-3-3 proteins and various phytohormones (such as SA, JA and ethylene) (Bigeard et al. 2015).

20.3.1 Phosphorylation Events

Phosphorylation and dephosphorylation of proteins by kinases and phosphatases play an important role in the signal transduction process. After ligand binding, conformational changes in protein/binding with co-receptors lead to phosphorylation of the receptor. Somatic embryogenesis receptor kinase (SERK) family usually works as a co-receptor for many receptor kinases such as FLS2, EFR, BRI1, Xa21, PEPR, PSKR, etc. (Ma et al. 2016). In Arabidopsis, SERK3 [also called bri1-associated receptor kinase 1 (BAK1)] is a key co-receptor for many receptor kinases and is required for proper induction of immune responses (Ma et al. 2016). In rice, OsSERK2 interacts with Xa21, Xa3 and FLS2 (Chen et al. 2014) and is required for receptor-mediated resistance against Xoo. SERKs are also involved in RLP-mediated activation of immune responses such as nlp20-triggered immunity in Arabidopsis, csp22-triggered immunity in Nicotiana, Avr4- and Avr9-induced HR in tomato (Albert et al. 2015; Postma et al. 2016; Saur et al. 2016).

20.3.1.1 MAP Kinases

Mitogen-activated protein kinases (MAPKs) form signaling modules, which translate extracellular stimuli of pathogen attack into appropriate defense responses. MAPK cascade typically contains three sequential kinases (Rasmussen et al. 2012):

- MAP kinase kinase kinase (MAPKKK or MEKK)
- MAP kinase kinase (MAPKK or MKK)
- MAP kinase (MAPK or MPK)

Usually receptor/co-receptor phosphorylates MAPKKK that phosphorylates MAPKK which phosphorylates MAPK. MAPK then phosphorylates downstream signaling components such as transcription factors and modulates defense responses (Meng and Zhang 2013). In a recent study, it has been shown that phosphorylation of OsMKK3-OsMPK7-OsWRKY30 leads to transcriptional activation of defense responses against *X. oryzae* in rice (Jalmi and Sinha 2016). Interestingly, in order to suppress PTI response, pathogens have evolved effector molecules that majorly target MAPK modules due to their primary role in defense signaling of plants (Feng et al. 2012).

20.3.1.2 CDPKs

Calcium-dependent protein kinases (CDPKs) have a serine/threonine protein kinase domain at their N-terminal and CaM-like domain with EF-hand calcium-binding sites at their C-terminal (Boudsocq and Sheen 2013). They act as Ca^{2+} sensors and decode the signal to generate a swift response to the external stimulus (Seybold et al. 2014, 2017). CDPK response was found to be associated with changes in host physiology such as transcriptional reprogramming, ROS accumulation, and alteration of phytohormone levels. CDPKs together with MAPKs have been found to orchestrate the transcriptional regulation of defense genes under pathogen attack (Boudsocq et al. 2010). Another group of kinases called AGC kinases, comprising of cAMP-dependent protein kinase 1 (PKA) and cGMP-dependent protein kinase (PKG) along with protein kinase C (PKC), have been shown to regulate MAPK signaling cascade upon pathogen attack (Garcia et al. 2012).

20.3.1.3 14-3-3

14-3-3 proteins act as phosphosensors which bind to phosphorylated proteins and regulate their functions. 14-3-3 proteins aid in phosphorylation of proteins thereby activating them (Chevalier et al. 2009). They play a crucial role in strengthening plant defense mechanisms by interacting with MAPKK proteins involved in the defense signal transduction pathway (Oh et al. 2010; Oh and Martin 2011). Induction of 14-3-3 proteins was found primarily in the penetration stage and upper epidermis of barley infected with *Blumeria graminis* suggesting its involvement in early signaling events (Lozano-Durán et al. 2015). 14-3-3 proteins have been found to interact with plant immune-responsive proteins such as receptor kinase BAK1 and WRKY transcription factor along with few R genes (Chang et al. 2009). 14-3-3 proteins have also been reported to regulate phytohormone levels in infected plants culminating in enhanced immune responses. (Chang et al. 2009; Camoni et al. 2018).

20.3.1.4 Heterotrimeric G proteins

G proteins have been found to play a critical role in defense signaling in animals. However plants lack the canonical G protein structure as observed in animals (Urano and Jones 2014). G proteins are known to activate plant defense signaling responses mediated by the action of multiple RLKs (Liu et al. 2013a, b; Maruta et al. 2015). The signals received from RLKs by G proteins are transduced downstream to different MAPKs and ROS signaling genes (Nitta et al. 2015; Cheng et al. 2015). Studies have revealed direct physical association between the Gα, Gγ1, and Gγ2 subunits and RD-type kinases CERK1, BAK1, and BIR1 to activate the plant defense network (Aranda-Sicilia et al. 2015).

20.3.2 Regulation of Immune Responses

Plant immune responses are metabolically costly affair; plants regulate the processes in a tight manner to avoid non-specific activation and dampen the responses when they are no longer required. This is usually achieved by dephosphorylation or

degradation of receptors. After activation of immune responses, protein phosphatases (PP) such as PP2C and PP2A dephosphorylate the receptor and other intermediate kinases to negatively regulate immune responses (Durian et al. 2016; Fuchs et al. 2013). Some examples of PP2C involvement in immunity include kinase-associated protein phosphatase (KAPP), PLL4 and PLL5 of Arabidopsis, and XB15 of rice (Holton et al. 2015; Park et al. 2008).

Another approach to regulate immune response is via vesicle-mediated internalization of activated receptors or degradation of the receptor/signaling intermediate (Wang et al. 2016a, b). These proteins are polyubiquitinated by E3 ubiquitin ligases and degraded by 26S proteasomes. Some examples of this pathway include XB3 of rice and PUB12 and PUB13 of Arabidopsis (Lu et al. 2011; Wang et al. 2006).

20.3.3 Transcriptional Regulation

Activation of immune responses involves rapid transcriptional and translational changes (Li et al. 2016). Transcriptional events are modulated by transcription factors (TFs) which get activated by MAP kinases, Ca^{2+} signaling or hormonal response (Kang et al. 2015; Li et al. 2016). Some key TF families involved in defense responses include WRKY, MYC, TCP, ZIP, MVQ, AP2/ERF, etc. (Birkenbihl et al. 2017). TFs enhance expression of various defense genes such as PR genes, secondary metabolism, and hormone biosynthesis as well as regulation of related genes.

20.3.4 Secondary Signaling Molecules

Many non-proteinaceous molecules are key signaling intermediates in plant innate immunity. These molecules include Ca^{2+}, ROS, NO, etc.

20.3.4.1 Burst of Ca^{2+}

Ca^{2+} ions play an important role in defense signaling during pathogen attack. Ca^{2+} burst occurs when MAMPs/DAMPs are perceived and Ca^{2+} from the extracellular milieu is transported into the cytoplasm (Jeworutzki et al. 2010; Ranf et al. 2011). The permeability of plasma membrane to Ca^{2+} is mediated by elicitor responsive ion channels. The calcium levels accumulate in distinct signature patterns and generate a particular defense response pathway against the pathogen (Lecourieux et al. 2006). Influx of Ca^{2+} is followed by opening of other membrane ion transporters such as H^+, K^+, Cl^-, and NO_3^- channels which lead to alkalization of extracellular space and membrane depolarization (Jeworutzki et al. 2010).

EF-hand motif-containing proteins are known to bind with calcium and serve as sensors of Ca^{2+} concentration (Schulz et al. 2013). These proteins mainly include Ca^{2+}-dependent protein kinases (CDPK) and calmodulin (CaM). Ca^{2+} binding causes conformational changes in structure of these proteins leading either to phosphorylation or binding with downstream signaling intermediates (Ishida and Vogel 2006; Wernimont et al. 2010).

20.3.4.2 ROS Burst

Production of extracellular reactive oxygen species (ROS) also referred to as ROS burst has been found to be associated with pathogen attack (Ranf et al. 2011; Chinchilla et al. 2007; Nühse et al. 2007). MAMP perception is often associated with ROS production by respiratory burst oxidase homolog D (RBOHD), a member of NADPH oxidase family in Arabidopsis (Bigeard et al. 2015). ROS can be present in membranes as impermeable superoxide (O^{2-}) or as permeable hydrogen peroxide (H_2O_2) and it can be readily translocated from one cell to another. Also it is often associated with elevated Ca^{2+} levels in the cytosol (Ranf et al. 2011; Bigeard et al. 2015). ROS signaling is accompanied by alteration in plant defense hormone levels such as JA, SA, and ethylene indicating a complex crosstalk between different pathways (Baxter et al. 2014).

20.3.4.3 NO Signaling

Nitric oxide (NO) along with its derivatives has also been involved in signal transduction pathway upon perception of pathogen attack. The role of NO in activating plant defense was first reported in tobacco mosaic virus infection wherein increase in NO synthase (NOS) resulted in activation of several downstream defense genes (Klessig et al. 2000). Interestingly, NO together with ROS plays a synergistic role in activation of plant defense responses (Domingos et al. 2015). NO can cause a rapid change in cellular glutathione levels in the cell associated with accumulation of SA and activation of NPR1-mediated defense responses (Kovacs et al. 2015).

20.3.4.4 Lipid Signaling

Lipid-based signaling molecules are also known to play a crucial role in defense signaling upon pathogen attack. These lipid molecules are produced as a result of degradation/destabilization of the cell wall upon pathogen attack. For example, phosphatidic acid (PA) and ceramides have been found to be involved in signal transduction upon pathogen infection (Okazaki and Saito 2014). PA is also involved in release of other signaling intermediates such as DAG, free fatty acids, and lysoPA which in turn induce downstream defense signaling (Wang 2004). Phospholipase A (PLA) which catalyzes the hydrolysis of phospholipids is involved in release of free fatty acids which are utilized during biosynthesis of defense hormone jasmonic acid (Shah 2005).

20.3.4.5 Hormonal Signaling

Major phytohormones such as jasmonic acid (JA), salicylic acid (SA) and ethylene have been found to play an important role in coordinating cell-to-cell communication during perception of pathogen attack. Each of these phytohormones activates its own downstream targets which lead to diverse immune and signaling events. There are also reports that other phytohormones such as auxin, cytokinin, abscisic acid, gibberellins and brassinosteroids are involved in plant immunity. There is a complex crosstalk among different phytohormones occurring at the cellular level that tailors a specific defense response upon attack by a specific pathogen. Here we summarize the role of some key defense-related phytohormones.

20.3.4.5.1 Salicylic Acid

SA is a phenolic hormone that is synthesized from chorismate via phenylalanine ammonia pathway (PAL) or isochorismate synthase (ICS) pathway (Chen et al. 2009). It is a key component of PTI as well as ETI and is known to enhance tolerance against various biotrophic, hemi-biotrophic, and viral infections (Dodds and Rathjen 2010; Malamy et al. 1990; Shigenaga and Argueso 2016). It is also necessary for the activation of various PR genes. Arabidopsis ICS1 mutant (*ics1*), also called SA deficient 2 (*sid2*), was compromised in SA-mediated immune response (Dewdney et al. 2000; Wildermuth et al. 2001). Interestingly, the non-expressor of PR genes 1 (NPR1) acts as a transcription co-activator and plays a key role in SA-mediated immune responses (Cao 1994). Generally at normal SA levels, NPR1 is localized in the cytoplasm in oligomeric form (Mou et al. 2003). However, at elevated SA level, the NPR1 binds to SA, adopts monomeric form, and gets transported to the nucleus (Kinkema et al. 2000; Mou et al. 2003). In the nucleus, NPR1 binds to TGA transcription factors and activates expression of defense-related genes including PR genes (Kesarwani et al. 2007). Infection studies on *ics1* mutant (that fails to increase SA level), NahG (salicylate hydroxylase that degrades SA) expressing transgenic lines (that fail to accumulate SA), and *npr1* mutant (that does not respond to SA) indicate that although SA can enhance tolerance towards biotrophic and hemi-biotrophic pathogens, it reduces resistance towards necrotrophic pathogens (Delaney et al. 1994; Glazebrook et al. 1996; Thomma et al. 1998). It is worth noting that phytopathogens utilizes various effectors (such as HopJ, HaRxL44, HopM1 and PsIcs1) to target SA signaling pathway during host colonization (Caillaud et al. 2013; DebRoy et al. 2004; Liu et al. 2014).

20.3.4.5.2 Jasmonic Acid

JA is a lipid-derived hormone that is involved in many developmental and defense response pathways (Santino et al. 2013; Carvalhais et al., 2017). JA is synthesized by oxygenation of α-linolenic by lipoxygenase (Lox) enzymes and is converted into JA-Ile (JA-isoleucine; the active form of JA) by JA amido synthetase (JAR1) (Staswick 2004; Wasternack and Hause 2013). Coronatine insensitive 1 (COI1), an E3 ubiquitin ligase, is a receptor of JA, and a transcription factor jasmonate ZIM domain 1 (JAZ1) is a negative regulator of JA pathway (Sheard et al. 2010; Yan et al. 2009). At low JA levels, JAZ1 represses JA-responsive genes (Pauwels et al. 2010). After perception of pathogen attack, JA-Ile binds to COI1, which ubiquitinates JAZ1 leading to degradation of JAZ1. Degradation of JAZ1 leads to enhanced expression of JA-responsive genes (Thines et al. 2007).

JA and SA are believed to play antagonistic roles against each other in very complex plant defense response-activating pathways depending on the nature of the pathogen (Thaler et al. 2012; Robert-Seilaniantz et al. 2011). Pathogens have evolved mechanisms to utilize this crosstalk to suppress plant immune responses (Pieterse et al. 2012). A well-studied example is synthesis of the JA mimic molecule coronatine (COR) by *Pseudomonas* sp. COR activates the JA pathway and suppresses SA pathway leading to increased susceptibility towards biotrophic and hemi-biotrophic pathogens including *Pseudomonas* (Zheng et al. 2012). Interestingly

a hemi-biotrophic pathogen, i.e., *Pseudomonas*, utilizes effector molecules such as HopZ1 and HopX1 to induce JA pathway during pathogenicity process (Gimenez-Ibanez et al. 2014; Jiang et al. 2013).

20.3.4.5.3 Ethylene

Ethylene is a gaseous plant hormone known for its role in fruit ripening. However, it is also known to be involved in plant defense responses. ET and JA phytohormones work in a synergistic manner (Robert-Seilaniantz et al. 2011). The activation of JA pathway leads to enhanced expression of ET pathway genes (Penninckx et al. 1998; Zhu et al. 2011). Alike JA, ET also enhances tolerance towards necrotrophic pathogens but increases susceptibility towards biotrophic pathogens (Lawton et al. 1994, 1995). Similar to other phytohormones, ET pathway is also targeted by pathogens to overcome immunity. For example, AvrPto and AvrPtoB effectors of *Pseudomonas* sp. and XopD effector of *Xanthomonas* sp. have been found to alter the ET pathway (Cohn and Martin 2005; Kim et al. 2013).

20.4 Plant Defense Responses

Upon perception of pathogen attack, plants mount a strong immune response to restrict the spread of pathogen/predator. These immune responses involve strengthening of the cell wall, localized cell death, production of antimicrobial compounds, etc. The strength of the immune response depends upon the type of danger. Many pathogens have evolved mechanisms to suppress PTI/ETI directly by secreting effector molecules into the plant cell. This is known as effector-triggered susceptibility (ETS). However, recognition of effectors by host R genes leads to activation of ETI that includes robust defense responses such as programmed cell death to restrict the growth of the pathogen at the site of infection.

20.4.1 Stomatal Closure

Several phytopathogens use stomata to enter inside the host. Closure of stomata is one of the early defense responses used by the host to prevent pathogens from colonization. Upon perception of pathogen cues (flg22, elf18, elf26, LPS, chitin, oligogalacturonan, etc.), plants close their stomata (Arnaud and Hwang 2015; Murata et al. 2015). This process involves various signaling events including activation of MAP kinase pathway, synthesis of hormones, Ca^{2+} influx, ROS and NO production, etc. (Desclos-Theveniau et al. 2012; Melotto et al. 2006, 2017). SA and ABA pathways are known to promote stomatal closure while JA-Ile serves as a negative regulator of stomatal closure.

However, successful phytopathogens have evolved various mechanisms to avoid plant stomatal closure. For example, *P. syringae* secretes various effectors such as

HopM1, HopF2, HopZ1, HopZ1a, Hopx1 and AvrB to suppress closure of stomata (Gimenez-Ibanez et al. 2014; Hurley et al. 2014; Jiang et al. 2013; Lozano-Durán et al. 2014; Zhou et al. 2014, 2015). XopR, a Xoo-secreted effector, suppresses flg22-induced stomatal closure in rice (Wang et al. 2016a, b). On the other hand, some of the bacterial pathogens secrete phytotoxins to open stomatal pores to assist colonization. Some of the notable phytotoxins used by bacterium to open stomata are coronatin (COR) (Bender et al. 1999) and syringolin A secreted by *P. syringae* (Groll et al. 2008), plant natriuretic peptide-like (Gottig et al. 2008) and diffusible signaling factor (DSF) (Gudesblat et al. 2008) molecules secreted by *Xanthomonas* species.

20.4.2 Cell Wall Strengthening

Cell wall serves as a key barrier to phytopathogens. Pathogens need to degrade the cell wall to gain access to nutrients that are inside the plant cell. Strengthening of the cell wall is achieved by deposition of callose (β-1,3 glucan) and lignin (phenolic polymers). This is one of the basic mechanisms used by the host plant to suppress the growth of pathogen (Malinovsky et al. 2014). Treatment with various MAMPs, DAMPs or avirulent pathogen strains causes callose deposition in the infected tissues (Luna et al. 2011). Synthesis of callose usually leads to papillae formation that contains antimicrobial compounds such as thionins, H_2O_2, etc. (McLusky et al. 1999; Thordal-Christensen et al. 1997; Voigt 2016). Besides callose, lignin is also deposited at the secondary cell wall to provide mechanical strength (Malinovsky et al. 2014). Loss of function mutations in various genes involved in lignin synthesis pathway makes the plants more susceptible to pathogens (Miedes et al. 2014).

20.4.3 Pathogenesis-Related Proteins

Expression of pathogenesis-related (PR) proteins is upregulated in plants after pathogen infection. These proteins are key components of plant immune responses. Many PR proteins are also observed to be upregulated after MAMP and DAMP treatment, wounding, ETI activation, and treatment with immune response-associated hormones (Sels et al. 2008). PR genes encode diverse classes of proteins which can be classified under 17 different families (van Loon et al. 2006). Most of the PR proteins have antimicrobial activities. PR3, PR4, PR8 and PR10 are chitinases which can degrade fungal cell wall, while PR2 proteins are β-1,3-glucanases. PR1 is a most common PR protein accumulated in various plant species upon pathogen attack and is known to have antimicrobial activity (Ménard et al. 2005; Segarra et al. 2013; Song et al. 2015). Some PR genes encode small peptides such as the PR6 family containing proteinase inhibitor peptides (Green and Ryan 1972), PR12s are cysteine-rich defensins (Terras 1995), PR13 encodes thionins (Epple et al. 1995) and PR14 codes for lipid transfer proteins (LPT) (García-Olmedo et al. 1995).

Interestingly, AtPR1, AtPR2, and AtPR5 are SA-responsive genes known to provide resistance against biotrophic and hemi-biotrophic pathogens in Arabidopsis. AtPR3 and AtPR4 are JA-responsive genes and provide tolerance against necrotrophic pathogens and herbivores (van Loon et al. 2006).

20.4.4 Secondary Metabolites

Plants produce various types of secondary metabolites upon infection by phytopathogens (Piasecka et al. 2015). These metabolites usually have antimicrobial activity and have a toxic effect on phytopathogens. One type of secondary metabolites that are constitutively produced are called phytoanticipins (VanEtten 1994). These are usually produced in an inactive form and are activated by hydrolysis after perception of danger. Plants produce various kinds of phytoanticipins including saponins such as α-tomatine and avenacin, glucosinolates, cyanogenic glucosides and benzoazinone glucoside compounds (Faizal and Geelen 2013; Halkier and Gershenzon 2006; Burkhardt et al. 1964; Papadopoulou et al. 1999; Sandrock and VanEtten 1998). Secondary metabolites that are de novo synthesized in response to biotic stress are called phytoalexins. The major types of phytoalexins include camalexins, phenylalanine-derived phytoalexins, and terpenoids (VanEtten 1994). Mutations in secondary metabolite synthetic genes make plants more susceptible to pathogens (Toyomasu et al. 2014; Xu et al. 2012).

20.4.5 Hypersensitive Response

Sometimes, plants undergo programmed cell death in the infected area to restrict the spread of a pathogen. This process is called hypersensitive response (HR). PCD is generally involved in developmental processes and stress responses including tolerance towards biotic stress (Bozhkov and Lam 2011; Pennell 1997). Upon pathogen perception by the host R gene, an intricate signaling cascade is triggered that leads to HR. This signaling cascade involves MAP kinase activation, SA production, ROS production, NO accumulation, cytosolic Ca^{2+} increase, membrane depolarization, etc. (Kadota et al. 2004; Kärkönen and Kuchitsu 2015; Kurusu et al. 2011). HR can be observed as the lesion phenotypes during infection or elicitor treatment or as lesion mimic phenotype if the immune response is constitutively activated (Coll et al. 2011; Lorrain et al. 2003). Although HR is a strong immune response, sometimes it can act like a double-edged sword for plants as necrotrophic pathogens that flourish on dead plant tissues have evolved various pathways to utilize this immune response of plants to colonize host tissues (Mukhtar et al. 2016). These pathogens modulate plant signaling to enhance ROS production and induce HR (Shetty et al. 2008).

20.4.6 Secretion of Volatile Compounds

Upon pathogen attack, plants often emit gaseous compounds known as VOCs (volatile organic compounds). Emission of volatile derivatives of certain plant hormones such as jasmonic acid and ethylene have been found to be responsible for the systemic activation of plant defense responses (Champigny and Cameron 2009; Fiers et al. 2013; Wasternack and Kombrink 2010; Tamogami et al. 2008). Plants also secrete volatile components that can attract predators or parasitoids such as parasitic wasps to forage upon the feeding insects or induce a systemic defense response in distal uninfected plant parts (Heil 2008; Heil and Silva Bueno 2007; Frost et al. 2007). Volatile compounds that are thus secreted are known as herbivore-induced plant volatiles (HIPV). Lima bean plants secrete certain compounds which are not only involved in attraction of predatory insects (natural enemies of herbivores) but also in production of certain extrafloral nectars (EFN) which serve as a food source for these predatory insects (Choh and Takabayashi 2010). Apple plants have been found to emit certain VOCs upon infection by the bacterial pathogen *Erwinia amylovora* which can activate the defense responses even in surrounding healthy uninfected plants (Cellini et al. 2018). Interestingly, it has been observed that VOCs produced upon infection by fungal pathogen *Colletotrichum lindemuthianum* in resistant bean plants can trigger defense responses in neighbouring susceptible plants (Quintana-Rodriguez et al. 2015).

20.5 Conclusion

Plants have specialized receptors to sense pathogen attack and mount potent defense responses. Various conserved structural components, damaged cell wall products or effector molecules produced by the pathogens are recognized by these receptors. Generally, these receptor proteins are maintained in a dephosphorylated inactive state and get activated at the time of pathogen attack. Various signaling intermediates like MAPKs, CDPKs, 14-3-3 and heterotrimeric G proteins participate in interception, amplification and transduction of the signal from the receptor to the target defense genes. Also several secondary messengers such as ROS, Ca^{2+}, NO, lipids, and hormones help in the relay of signal (Fig. 20.1). Interestingly, the induction of defense responses is not merely restricted to the infected tissue but it is also elaborated in uninfected as well as distal parts of the plant. Interestingly, phytohormones such as salicylic acid, jasmonic acid and ethylene play a significant role in activation of immune responses.

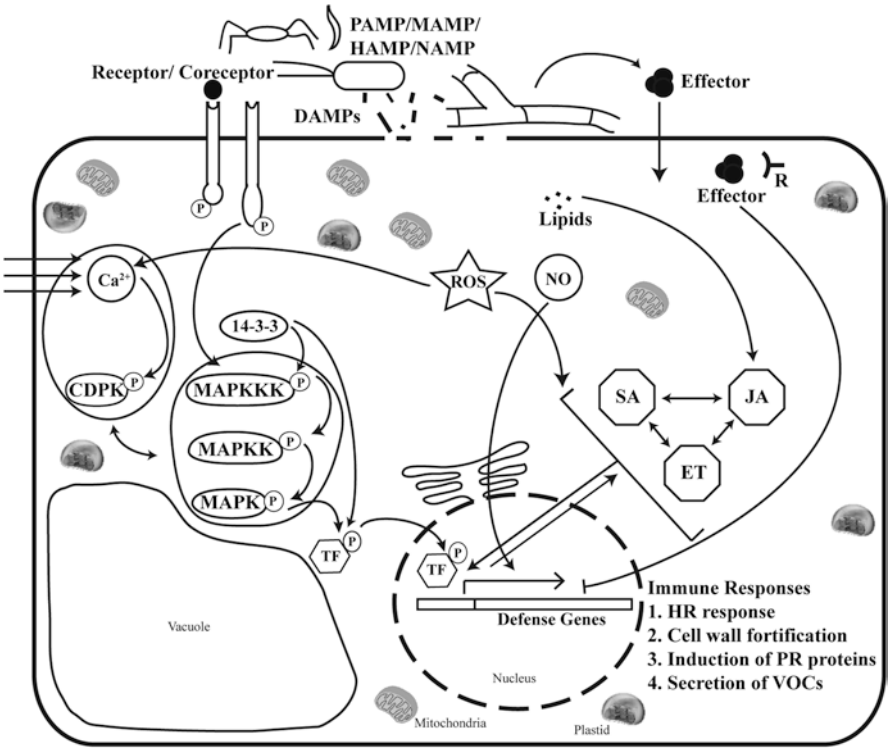

Fig. 20.1 Schematic overview of cellular responses induced in host upon perception of pathogen attack. The pathogen possesses certain conserved structural components (MAMPS, DAMPs, HAMPs, NAMPs, etc.) which are recognized by cognate receptors present in host plant. Upon signal perception, a cascade of signal transduction events including induction of phosphorylation events (involving MAP kinases, CDPKs, etc.) as well as secondary signaling molecules (such as calcium, NO, ROS, etc.) are triggered. All these events culminate in activation of potent immune responses which combat most of the potential pathogens to cause disease. Notably, several phytohormones such as SA, JA, ET, etc. also play important roles in elucidation of plant defense responses. Additionally, pathogens secrete effector molecules to inhibit plant immune responses, but plants have evolved resistance genes (R genes) to directly or indirectly recognize them and mount a strong defense response

Acknowledgments The authors acknowledge various researchers who have significantly contributed in this field, but due to lack of space, their work has not been cited in this book chapter. SG and KM are supported by fellowship from the Council of Scientific and Industrial Research (Govt. of India). GJ was supported by core research grant from the National Institute of Plant Genome Research, India, and research funding from DBT, Government of India. RVS was supported by core research grants from the National Institute of Plant Genome Research, and Centre for Cellular and Molecular Biology, India, along with research funding from DBT, ICAR, and CSIR, Government of India. RVS is also supported by a J C Bose fellowship from the Science and Engineering Research Board, Government of India.

References

Abramovitch RB, Kim YJ, Chen S, Dickman MB, Martin GB (2003) Pseudomonas type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J 22:60–69

Albert I, Böhm H, Albert M, Feiler CE, Imkampe J, Wallmeroth N, Brancato C, Raaymakers TM, Oome S, Zhang H, Krol E, Grefen C, Gust AA, Chai J, Hedrich R, Van Den Ackerveken G, Nürnberger T (2015) An RLP23-SOBIR1-BAK1 complex mediates NLP-triggered immunity. Nat Plants 1:15140

Alfano JR, Collmer A (2004) TYPE III secretion system effector proteins: double agents in bacterial disease and plant defense. Annu Rev Phytopathol 42:385–414

Andolfo G, Ercolano MR (2015) Plant innate immunity multicomponent model. Front Plant Sci 6:987

Aranda-Sicilia MN, Trusov Y, Maruta N, Chakravorty D, Zhang Y, Botella JR (2015) Heterotrimeric G proteins interact with defense-related receptor-like kinases in Arabidopsis. J Plant Physiol 188:44–48

Arnaud D, Hwang I (2015) A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. Mol Plant 8:566–581

Bacete L, Mélida H, Miedes E, Molina A (2018) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant J 93:614–636

Bai S, Liu J, Chang C, Zhang L, Maekawa T, Wang Q, Xiao W, Liu Y, Chai J, Takken FLW, Schulze-Lefert P, Shen QH (2012) Structure-function analysis of barley NLR immune receptor MLA10 reveals its cell compartment specific activity in cell death and disease resistance. PLoS Pathogens 8:e1002752

Bailey BA, Dean JF, Anderson JD (1990) An Ethylene biosynthesis-inducing endoxylanase elicits electrolyte leakage and necrosis in nicotiana Tabacum cv Xanthi leaves. Plant Physiol 94:1849–1854

Bailey BA, Korcak RF, Anderson JD (1993) Sensitivity to an ethylene biosynthesis-inducing endoxylanase in nicotiana tabacum L. cv. Xanthi is controlled by a single dominant gene. Plant Physiol 101:1081–1088

Bar M, Avni A (2009) EHD2 inhibits ligand-induced endocytosis and signaling of the leucine-rich repeat receptor-like protein LeEix2. Plant J 59:600–611

Bar M, Sharfman M, Schuster S, Avni A (2009) The coiled-coil domain of EHD2 mediates inhibition of LeEix2 endocytosis and signaling. PLoS ONE 4:e7973

Bartels S, Lori M, Mbengue M, van Verk M, Klauser D, Hander T, Böni R, Robatzek S, Boller T (2013) The family of peps and their precursors in arabidopsis: Differential expression and localization but similar induction of pattern-Triggered immune responses. J Exp Bot 64:5309–5321

Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. J Exp Bot 65:1229–1240

Belfanti E, Silfverberg-Dilworth E, Tartarini S, Patocchi A, Barbieri M, Zhu J, Vinatzer BA, Gianfranceschi L, Gessler C, Sansavini S (2004) The HcrVf2 gene from a wild apple confers scab resistance to a transgenic cultivated variety. Proc Natl Acad Sci USA 101:886–890

Bender CL, Alarcón-Chaidez F, Gross DC (1999) Pseudomonas syringae phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol Rev 63:266–292

Bigeard J, Colcombet J, Hirt H (2015) Signaling mechanisms in pattern-triggered immunity (PTI). Mol Plant 8:521–539

Birkenbihl RP, Liu S, Somssich IE (2017) Transcriptional events defining plant immune responses. Curr Opin Plant Biol 38:1–9

Böhm H, Albert I, Oome S, Raaymakers TM, Van den Ackerveken G, Nürnberger T (2014) A conserved peptide pattern from a widespread microbial virulence factor triggers pattern-induced immunity in arabidopsis. PLoS Pathogens 10:e1004491

Bonardi V, Cherkis K, Nishimura MT, Dangl JL (2012) A new eye on NLR proteins: focused on clarity or diffused by complexity? Curr Opin Immunol 24:41–50

Bonaventure G, Baldwin IT (2010) Transduction of wound and herbivory signals in plastids. Commun Integr Biol 3:313–317
Bonaventure G, VanDoorn A, Baldwin IT (2011) Herbivore-associated elicitors: FAC signaling and metabolism. Trends Plant Sci 16:294–299
Boudsocq M, Sheen J (2013) CDPKs in immune and stress signaling. Trends Plant Sci 18:30–40
Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca(2+) sensor protein kinases. Nature 464:418–422
Boutrot F, Zipfel C (2017) Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu Rev Phytopathol 55:257–286
Bozhkov PV, Lam E (2011) Green death: revealing programmed cell death in plants. Cell Death Differ 18:1239–1240
Bozkurt TO, Mcgrann GRD, Maccormack R, Boyd LA, Akkaya MS (2010) Cellular and transcriptional responses of wheat during compatible and incompatible race-specific interactions with Puccinia striiformis f. sp. tritici. Mol Plant Pathol 11:625–640
Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proc Natl Acadf Sci 107:9452–9457
Burkhardt HJ, Maizel JV, Mitchell HK (1964) Avenacin, an antimicrobial substance Isolated from avena sativa II structure. Biochemistry 3:426–431
Caillaud MC, Asai S, Rallapalli G, Piquerez S, Fabro G, Jones JDG (2013) A downy mildew effector attenuates salicylic acid-triggered immunity in arabidopsis by interacting with the host mediator complex. PLoS Biol 11:e1001732
Camoni L, Visconti S, Aducci P, Marra M (2018) 14-3-3 proteins in plant hormone signaling: doing several things at once. Front Plant Sci 9:297
Cao H (1994) Characterization of an arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583–1592
Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. eLife 3:e03766
Carvalhais LC, Schenk PM, Dennis PG (2017) Jasmonic acid signalling and the plant holobiont. Curr Opin Microbiol 37:42–47
Cellini A, Buriani G, Rocchi L, Rondelli E, Savioli S, Rodriguez Estrada MT, Cristescu SM, Costa G, Spinelli F (2018) Biological relevance of volatile organic compounds emitted during the pathogenic interactions between apple plants and erwinia amylovora. Mol Plant Pathol 19:158–168
Champigny MJ, Cameron RK (2009) Action at a distance. Long-distance signals in induced resistance. Adv Bot Res 51:123–171, Academic Press
Chang IF, Curran A, Woolsey R, Quilici D, Cushman JC, Mittler R, Harmon A, Harper JF (2009) Proteomic profiling of tandem affinity purified 14-3-3 protein complexes in Arabidopsis thaliana. Proteomics 9:2967–2985
Chen Z, Zheng Z, Huang J, Lai Z, Fan B (2009) Biosynthesis of salicylic acid in plants. Plant Signal Behav 4:493–496
Chen X, Zuo S, Schwessinger B, Chern M, Canlas PE, Ruan D, Zhou X, Wang J, Daudi A, Petzold CJ, Heazlewood JL, Ronald PC (2014) An XA21-associated kinase (OsSERK2) regulates immunity mediated by the XA21 and XA3 immune receptors. Mol Plant 7:874–892
Cheng W, Munkvold KR, Gao H, Mathieu J, Schwizer S, Wang S, YBin Y, Wang J, Martin GB, Chai J (2011) Structural analysis of pseudomonas syringae AvrPtoB bound to host BAK1 reveals two similar kinase-interacting domains in a type III effector. Cell Host Microbe 10:616–626
Cheng Z, Li JF, Niu Y, Zhang XC, Woody OZ, Xiong Y, Djonović S, Millet Y, Bush J, McConkey BJ, Sheen J, Ausubel FM (2015) Pathogen-secreted proteases activate a novel plant immune pathway. Nature 521:213–216
Chevalier D, Morris ER, Walker JC (2009) 14-3-3 and FHA domains mediate phosphoprotein interactions. Annu Rev Plant Biol 60:67–91

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JDG, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500

Choh Y, Takabayashi J (2010) Herbivore-induced plant volatiles prime two indirect defences in lima bean BT. In: Sabelis MW, Bruin J (eds) Trends in acarology. Springer, Dordrecht, pp 255–258

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee SY, Stacey G (2014) Identification of a plant receptor for extracellular ATP. Science 343:290–294

Choi HW, Manohar M, Manosalva P, Tian M, Moreau M, Klessig DF (2016) Activation of plant innate immunity by extracellular high mobility group box 3 and its inhibition by salicylic acid. PLoS Pathogens 12:e1005518

Chung EH, El-Kasmi F, He Y, Loehr A, Dangl JL (2014) A plant phosphoswitch platform repeatedly targeted by type III effector proteins regulates the output of both tiers of plant immune receptors. Cell Host Microbe 16:484–494

Coaker G, Falick A, Staskawicz B (2005) Activation of a phytopathogenic bacterial effector protein by a eukaryotic cyclophilin. Science 308:548–550

Cohn JR, Martin GB (2005) Pseudomonas syringae pv. tomato type III effectors AvrPto and AvrPtoB promote ethylene-dependent cell death in tomato. Plant J 44:139–154

Coll NS, Epple P, Dangl JL (2011) Programmed cell death in the plant immune system. Cell Death Differ 18:1247–1256

Collier SM, Moffett P (2009) NB-LRRs work a "bait and switch" on pathogens. Trends Plant Sci 14:521–529

Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16:537–552

Cui H, Tsuda K, Parker JE (2015) Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol 66:487–511

Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

DebRoy S, Thilmony R, Kwack Y-B, Nomura K, He SY (2004) A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants. Proc Natl Acad Sci 101:9927–9932

Decreux A, Messiaen J (2005) Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. Plant Cell Physiology 46:268–278

Decreux A, Thomas A, Spies B, Brasseur R, Van Cutsem P, Messiaen J (2006) In vitro characterization of the homogalacturonan-binding domain of the wall-associated kinase WAK1 using site-directed mutagenesis. Phytochemistry 67:1068–1079

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J (1994) A central role of salicylic acid in plant disease resistance. Science 266:1247–1250

Delteil A, Gobbato E, Cayrol B, Estevan J, Michel-Romiti C, Dievart A, Kroj T, Morel JB (2016) Several wall-associated kinases participate positively and negatively in basal defense against rice blast fungus. BMC Plant Biol 16:17

Desaki Y, Kouzai Y, Ninomiya Y, Iwase R, Shimizu Y, Seko K, Molinaro A, Minami E, Shibuya N, Kaku H, Nishizawa Y (2017) OsCERK1 plays a crucial role in the lipopolysaccharide-induced immune response of rice. New Phytol 217:1042–1049

Desclos-Theveniau M, Arnaud D, Huang TY, Lin GJC, Chen WY, Lin YC, Zimmerli L (2012) The Arabidopsis lectin receptor kinase LecRK-V.5 represses stomatal immunity induced by Pseudomonas syringae pv. tomato DC3000. PLoS Pathogens 8:e1002513

Dewdney J, Lynne Reuber T, Wildermuth MC, Devoto A, Cui J, Stutius LM, Drummond EP, Ausubel FM (2000) Three unique mutants of Arabidopsis identify eds loci required for limiting growth of a biotrophic fungal pathogen. Plant J 24:205–218

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548

Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Mol Plant 8:506–520

Dong J, Xiao F, Fan F, Gu L, Cang H, Martin GB, Chai J (2009) Crystal structure of the complex between pseudomonas effector AvrPtoB and the tomato Pto kinase reveals both a shared and a unique interface compared with AvrPto-Pto. Plant Cell 21:1846–1859
Duan G, Christian N, Schwachtje J, Walther D, Ebenhöh O (2013) The metabolic interplay between plants and phytopathogens. Metabolites 3:1–23
Durian G, Rahikainen M, Alegre S, Brosché M, Kangasjärvi S (2016) Protein phosphatase 2A in the regulatory network underlying biotic stress resistance in plants. Front Plant Sci 7:812
Engelsdorf T, Gigli-Bisceglia N, Veerabagu M, McKenna JF, Augstein F, van der Does D, Zipfel C, Hamann T (2017) Pattern-triggered immunity and cell wall integrity maintenance jointly modulate plant stress responses. Biorxiv 130013
Epple P, Apel K, Bohlmann H (1995) An Arabidopsis thaliana thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins. Plant Physiol 109:813–820
Erbs G, Molinaro A, Dow JM, Newman M-A (2010) Lipopolysaccharides and plant innate immunity. Subcell Biochem 53:387–403
Faizal A, Geelen D (2013) Saponins and their role in biological processes in plants. Phytochem Rev 12:877–893
Feng F, Yang F, Rong W, Wu X, Zhang J, Chen S, He C, Zhou JM (2012) A Xanthomonas uridine 5′-monophosphate transferase inhibits plant immune kinases. Nature 485:114–118
Ferrari S (2013) Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. Front Plant Sci 4:49
Fiers M, Lognay G, Fauconnier ML, Jijakli MH (2013) Volatile compound-mediated interactions between barley and pathogenic fungi in the soil. PLoS ONE 8: e66805
Frost CJ, Appel HM, Carlson JE, De Moraes CM, Mescher MC, Schultz JC (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. Ecol Lett 10:490–498
Fuchs Y, Saxena A, Gamble HR, Anderson JD (1989) Ethylene biosynthesis-inducing protein from cellulysin is an endoxylanase. Plant Physiol 89:138–143
Fuchs S, Grill E, Meskiene I, Schweighofer A (2013) Type 2C protein phosphatases in plants. FEBS J 280:681–693
Furukawa T, Inagaki H, Takai R, Hirai H, Che F-S (2014) Two distinct EF-Tu epitopes induce immune responses in rice and arabidopsis. Mol Plant Microbe Interactions 27:113–124
Garcia AV, Al-Yousif M, Hirt H (2012) Role of AGC kinases in plant growth and stress responses. Cell Mol Life Sci 69:3259–3267
García-Olmedo F, Molina A, Segura A, Moreno M (1995) The defensive role of nonspecific lipid-transfer proteins in plants. Trends Microbiol 3:72–74
Gimenez-Ibanez S, Hann DR, Ntoukakis V, Petutschnig E, Lipka V, Rathjen JP (2009) AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol 19:423–429
Gimenez-Ibanez S, Boter M, Fernández-Barbero G, Chini A, Rathjen JP, Solano R (2014) The bacterial effector HopX1 targets JAZ transcriptional repressors to activate jasmonate signaling and promote infection in arabidopsis. PLoS Biol 12:e1001792
Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B (2013) Two distinct secretion systems facilitate tissue invasion by the rice blast fungus Magnaporthe oryzae. Nat Commun 4:1996
Glazebrook J, Rogers EE, Ausubel FM (1996) Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. Genetics 143:973–982
Göhre V, Spallek T, Häweker H, Mersmann S, Mentzel T, Boller T, de Torres M, Mansfield JW, Robatzek S (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Curr Biol 18:1824–1832
Gómez-Gómez L, Boller T (2000) Fls2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 5:1003–1011
Gottig N, Garavaglia BS, Daurelio LD, Valentine A, Gehring C, Orellano EG, Ottado J (2008) Xanthomonas axonopodis pv. citri uses a plant natriuretic peptide-like protein to modify host homeostasis. Proc Natl Acad Sci 105:18631–18636

Goverse A, Smant G (2014) The activation and suppression of plant innate immunity by parasitic nematodes. Annu Rev Phytopathol 52:243–265
Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175:776–777
Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, Powell TK, Lindow S, Kaiser M, Dudler R (2008) A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. Nature 452:755–758
Gudesblat GE, Torres PS, Vojnov AA (2008) Xanthomonas campestris overcomes arabidopsis stomatal innate immunity through a DSF cell-to-cell signal-regulated virulence factor. Plant Physiol 149:1017–1027
Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Götz F, Glawischnig E, Lee J, Felix G, Nürnberger T (2007) Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J Biol Chem 282:32338–32348
Gust AA, Willmann R, Desaki Y, Grabherr HM, Nürnberger T (2012) Plant LysM proteins: modules mediating symbiosis and immunity. Trends Plant Sci 17:495–502
Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333
Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K, Molinaro A, Kaku H, Shibuya N (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. Proc Natl Acad Sci 111:E404–E413
Heil M (2008) Indirect defence via tritrophic interactions. New Phytol 178:41–61
Heil M, Silva Bueno JC (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. Proc Natl Acad Sci 104:5467–5472
Hind SR, Strickler SR, Boyle PC, Dunham DM, Bao Z, O'Doherty IM, Baccile JA, Hoki JS, Viox EG, Clarke CR, Vinatzer BA, Schroeder FC, Martin GB (2016) Tomato receptor FLAGELLIN-SENSING 3 binds flgII-28 and activates the plant immune system. Nat Plants 2:16128
Hirsch AM, Bauer WD, Bird DM, Cullimore J, Tyler B, Yoder JI (2003) Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. Ecology 84:858–868
Hogenhout SA, Bos JIB (2011) Effector proteins that modulate plant-insect interactions. Curr Opin Plant Biol 14:422–428
Holbein J, Grundler FMW, Siddique S (2016) Plant basal resistance to nematodes: an update. J Exp Bot 67:2049–2061
Holton N, Nekrasov V, Ronald PC, Zipfel C (2015) The phylogenetically-related pattern recognition receptors EFR and XA21 recruit similar immune signaling components in monocots and dicots. PLoS Pathogens 11:e1004602
Hu K, Cao J, Zhang J, Xia F, Ke Y, Zhang H, Xie W, Liu H, Cui Y, Cao Y, Sun X, Xiao J, Li X, Zhang Q, Wang S (2017) Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. Nat Plants 3:17009
Hurley B, Lee D, Mott A, Wilton M, Liu J, Liu YC, Angers S, Coaker G, Guttman DS, Desveaux D (2014) The Pseudomonas syringae type III effector HopF2 suppresses arabidopsis stomatal immunity. PLoS ONE 9:e114921
Ishida H, Vogel H (2006) Protein-peptide interaction studies demonstrate the versatility of calmodulin target protein binding. Protein Pept Lett 13:455–465
Jalmi SK, Sinha AK (2016) Functional involvement of a mitogen activated protein kinase module, OsMKK3-OsMPK7-OsWRK30 in mediating resistance against Xanthomonas oryzae in rice. Sci Rep 6:37974
Jeworutzki E, Roelfsema MRG, Anschütz U, Krol E, Elzenga JTM, Felix G, Boller T, Hedrich R, Becker D (2010) Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca2+-associated opening of plasma membrane anion channels. Plant J 62:367–378
Jha G, Rajeshwari R, Sonti RV (2005) Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens. Mol Plant Microbe Interact 18:891–898

Jiang S, Yao J, Ma KW, Zhou H, Song J, He SY, Ma W (2013) Bacterial effector activates jasmonate signaling by directly targeting JAZ transcriptional repressors. PLoS Pathogens 9:e1003715

Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the Sinorhizobium – Medicago model. Nat Rev Microbiol 5:619–633

Kadota Y, Goh T, Tomatsu H, Tamauchi R, Higashi K, Muto S, Kuchitsu K (2004) Cryptogein-induced initial events in tobacco BY-2 cells: pharmacological characterization of molecular relationship among cytosolic Ca2+ transients, anion efflux and production of reactive oxygen species. Plant Cell Physiol 45:160–170

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci 103:11086–11091

Kang S, Yang F, Li L, Chen H, Chen S, Zhang J (2015) The Arabidopsis transcription factor BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 is a direct substrate of MITOGEN-ACTIVATED PROTEIN KINASE6 and regulates immunity. Plant Physiol 167:1076–1086

Kärkönen A, Kuchitsu K (2015) Reactive oxygen species in cell wall metabolism and development in plants. Phytochemistry 112:22–32

Kesarwani M, Yoo J, Dong X (2007) Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. Plant Physiol 144:336–346

Kim H-S, Desveaux D, Singer AU, Patel P, Sondek J, Dangl JL (2005) The Pseudomonas syringae effector AvrRpt2 cleaves its C-terminally acylated target, RIN4, from Arabidopsis membranes to block RPM1 activation. Proc Natl Acad Sci 102:6496–6501

Kim JG, Stork W, Mudgett MB (2013) Xanthomonas type III effector XopD desumoylates tomato transcription factor SlERF4 to suppress ethylene responses and promote pathogen growth. Cell Host Microbe 13:143–154

Kinkema M, Fan W, Dong X (2000) Nuclear localization of NPR1 is required for activation of PR gene expression. Plant Cell 12:2339

Klauser D, Desurmont GA, Glauser GDS, Vallat A, Flury P, Boller T, Turlings TCJ, Bartels S (2015) The Arabidopsis Pep-PEPR system is induced by herbivore feeding and contributes to JA-mediated plant defence against herbivory. J Exp Bot 66:5327–5336

Klessig DF, Durner J, Noad R, Navarre DA, Wendehenne D, Kumar D, Zhou JM, Shah J, Zhang S, Kachroo P, Trifa Y, Pontier D, Lam E, Silva H (2000) Nitric oxide and salicylic acid signaling in plant defense. Proc of the Natl Acad Sci 97:8849–8855

Kourelis J, van der Hoorn RAL (2018) Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. Plant Cell 30:285–299

Kouzai Y, Nakajima K, Hayafune M, Ozawa K, Kaku H, Shibuya N, Minami E, Nishizawa Y (2014) CEBiP is the major chitin oligomer-binding protein in rice and plays a main role in the perception of chitin oligomers. Plant Mol Biol 84:519–528

Kovacs I, Durner J, Lindermayr C (2015) Crosstalk between nitric oxide and glutathione is required for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent defense signaling in Arabidopsis thaliana. New Phytol 208:860–872

Krasileva KV, Dahlbeck D, Staskawicz BJ (2010) Activation of an Arabidopsis resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector. Plant Cell 22:2444–2458

Krol E, Mentzel T, Chinchilla D, Boller T, Felix G, Kemmerling B, Postel S, Arents M, Jeworutzki E, Al-Rasheid KAS, Becker D, Hedrich R (2010) Perception of the Arabidopsis danger signal peptide 1 involves the pattern recognition receptor AtPEPR1 and its close homologue AtPEPR2. J Biol Chem 285:13471–13479

Kunze G (2004) The N Terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. Plant Cell 16:3496–3507

Kurusu T, Hamada H, Sugiyama Y, Yagala T, Kadota Y, Furuichi T, Hayashi T, Umemura K, Komatsu S, Miyao A, Hirochika H, Kuchitsu K (2011) Negative feedback regulation of

microbe-associated molecular pattern-induced cytosolic Ca2+ transients by protein phosphorylation. J Plant Res 124:415–424
Larkan NJ, Lydiate DJ, Parkin IAP, Nelson MN, Epp DJ, Cowling WA, Rimmer SR, Borhan MH (2013) The Brassica napus blackleg resistance gene LepR3 encodes a receptor-like protein triggered by the Leptosphaeria maculans effector AVRLM1. New Phytol 197:595–605
Lawton KA, Potter SL, Uknes S, Ryals J (1994) Acquired resistance signal transduction in Arabidopsis is Ethylene independent. Plant Cell 6:581–588
Lawton K, Weymann K, Friedrich L, Vernooij B, Uknes S, Ryals J (1995) Systemic acquired resistance in Arabidopsis requires salicylic acid but not ethylene. Mol Plant Microbe Interact 8:863–870
Lecourieux D, Ranjeva R, Pugin A (2006) Calcium in plant defence-signalling pathways: Tansley review. New Phytol 171:249–269
Lee J, Manning AJ, Wolfgeher D, Jelenska J, Cavanaugh KA, Xu H, Fernandez SM, Michelmore RW, Kron SJ, Greenberg JT (2015) Acetylation of an NB-LRR plant immune-effector complex suppresses immunity. Cell Rep 13:1670–1682
Li B, Meng X, Shan L, He P (2016) Transcriptional regulation of pattern-triggered immunity in plants. Cell Host Microbe 19:641–650
Liu B, Li J-F, Ao Y, Qu J, Li Z, Su J, Zhang Y, Liu J, Feng D, Qi K, He Y, Wang J, Wang H-B (2012) Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. Plant Cell 24:3406–3419
Liu B, Li JF, Ao Y, Li Z, Liu J, Feng D, Qi K, He Y, Zeng L, Wang J, Wang HB (2013a) OsLYP4 and OsLYP6 play critical roles in defense signal transduction. Plant Signal Behav 8:e22980
Liu J, Ding P, Sun T, Nitta Y, Dong O, Huang X, Yang W, Li X, Botella JR, Zhang Y (2013b) Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. Plant Physiol 161:2146–2158
Liu T, Song T, Zhang X, Yuan H, Su L, Li W, Xu J, Liu S, Chen L, Chen T, Zhang M, Gu L, Zhang B, Dou D (2014) Unconventionally secreted effectors of two filamentous pathogens target plant salicylate biosynthesis. Nat Commun 5:4686
Lorrain S, Vailleau F, Balagué C, Roby D (2003) Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? Trends Plant Sci 8:263–271
Lozano-Durán R, Bourdais G, He SY, Robatzek S (2014) The bacterial effector HopM1 suppresses PAMP-triggered oxidative burst and stomatal immunity. New Phytol 202:259–269
Lozano-Durán R, Robatzek S, Lozano-dur R (2015) 14-3-3 proteins in plant-pathogen interactions. Mol Plant Microbe Interact 28:511–518
Lu D, Lin W, Gao X, Wu S, Cheng C, Avila J, Heese A, Devarenne TP, He P, Shan L (2011) Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. Science 332:1439–1442
Luna E, Pastor V, Robert J, Flors V, Mauch-Mani B, Ton J (2011) Callose deposition: a multifaceted plant defense response. Mol Plant Microbe Interact 24:183–193
Ma X, Xu G, He P, Shan L (2016) SERKing coreceptors for receptors. Trends Plant Sci 21:1017–1033
Maffei ME, Mithöfer A, Boland W (2007) Before gene expression: early events in plant-insect interaction. Trends Plant Sci 12:310–316
Malamy J, Carr JP, Klessig DF, Raskin I (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. Science 250:1002–1004
Malinovsky FG, Fangel JU, Willats WGT (2014) The role of the cell wall in plant immunity. Front Plant Sci 5:178
Manosalva P, Manohar M, Von Reuss SH, Chen S, Koch A, Kaplan F, Choe A, Micikas RJ, Wang X, Kogel KH, Sternberg PW, Williamson VM, Schroeder FC, Klessig DF (2015) Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. Nat Commun 6:7795
Maruta N, Trusov Y, Brenya E, Parekh U, Botella JR (2015) Membrane-localized extra-large G proteins and Gβγ of the heterotrimeric G proteins form functional complexes engaged in plant immunity in Arabidopsis. Plant Physiol 167:1004–1016

Mathieu J, Schwizer S, Martin GB (2014) Pto kinase binds two domains of AvrPtoB and its proximity to the effector E3 ligase determines if it evades degradation and activates plant immunity. PLoS Pathogens 10:e1004227

McLusky SR, Bennett MH, Beale MH, Lewis MJ, Gaskin P, Mansfield JW (1999) Cell wall alterations and localized accumulation of feruloyl-3′-methoxytyramine in onion epidermis at sites of attempted penetration by Botrytis allii are associated with actin polarisation, peroxidase activity and suppression of flavonoid biosynthesis. Plant J 17:523–534

Meindl T (2000) The bacterial elicitor flagellin activates its receptor in tomato cells according to the address-message concept. Plant Cell 12:1783–1794

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in innate immunity against bacterial invasion. Cell 126:969–980

Melotto M, Zhang L, Oblessuc PR, He SY (2017) Stomatal defense a decade later. Plant Physiol 174:561–571

Ménard R, De Ruffray P, Fritig B, Yvin JC, Kauffmann S (2005) Defense and resistance-inducing activities in tobacco of the sulfated β-1,3 glucan PS3 and its synergistic activities with the unsulfated molecule. Plant Cell Physiol 46:1964–1972

Mendy B, Wang'ombe MW, Radakovic ZS, Holbein J, Ilyas M, Chopra D, Holton N, Zipfel C, Grundler FMW, Siddique S (2017) Arabidopsis leucine-rich repeat receptor–like kinase NILR1 is required for induction of innate immunity to parasitic nematodes. PLoS Pathogens 13:e1006284

Meng X, Zhang S (2013) MAPK cascades in plant disease resistance signaling. Annu Rev Phytopathol 51:245–266

Miedes E, Vanholme R, Boerjan W, Molina A (2014) The role of the secondary cell wall in plant resistance to pathogens. Front Plant Sci 5:358

Mithofer A, Boland W (2008) Recognition of herbivory-associated molecular patterns. Plant Physiol 146:825–831

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc Natl Acad Sci 104:19613–19618

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance Regulate NPR1 function through redox changes. Cell 113:935–944

Mukhtar MS, McCormack ME, Argueso CT, Pajerowska-Mukhtar KM (2016) Pathogen tactics to manipulate plant cell death. Curr Biol 26:R608–R619

Murata Y, Mori IC, Munemasa S (2015) Diverse stomatal signaling and the signal integration mechanism. Annu Rev Plant Biol 66:369–392

Nitta Y, Ding P, Zhang Y (2015) Heterotrimeric G proteins in plant defense against pathogens and ABA signaling. Environ Exp Bot 114:153–158

Ntoukakis V, Balmuth AL, Mucyn TS, Gutierrez JR, Jones AME, Rathjen JP (2013) The tomato Prf complex is a molecular trap for bacterial effectors based on Pto transphosphorylation. PLoS Pathogens 9:e1003123

Nühse TS, Bottrill AR, Jones AME, Peck SC (2007) Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J 51:931–940

Oh CS, Martin GB (2011) Tomato 14-3-3 protein TFT7 interacts with a MAP kinase kinase to regulate immunity-associated programmed cell death mediated by diverse disease resistance proteins. J Biol Chem 286:14129–14136

Oh C-S, Pedley KF, Martin GB (2010) Tomato 14-3-3 protein 7 positively regulates immunity-associated programmed cell death by enhancing protein abundance and signaling ability of MAPKKK. Plant Cell 22:260–272

Okazaki Y, Saito K (2014) Roles of lipids as signaling molecules and mitigators during stress response in plants. Plant J 79:584–596

Oldroyd GED (2013) Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Oome S, Van den Ackerveken G (2014) Comparative and functional analysis of the widely occurring family of Nep1-like proteins. Mol Plant Microbe Interact 27:1081–1094

Oome S, Raaymakers TM, Cabral A, Samwel S, Böhm H, Albert I, Nürnberger T, Van den Ackerveken G (2014) Nep1-like proteins from three kingdoms of life act as a microbe-associated molecular pattern in Arabidopsis. Proc Natl Acad Sci 111:16955–16960

Ortiz D, de Guillen K, Cesari S, Chalvon V, Gracy J, Padilla A, Kroj T (2017) Recognition of the magnaporthe oryzae effector AVR-Pia by the decoy domain of the rice NLR immune receptor RGA5. Plant Cell 29:156–168

Papadopoulou K, Melton RE, Leggett M, Daniels MJ, Osbourn AE (1999) Compromised disease resistance in saponin-deficient plants. Proc Natl Acad Sci 96:12923–12928

Park CJ, Peng Y, Chen X, Dardick C, Ruan D, Bart R, Canlas PE, Ronald PC (2008) Rice XB15, a protein phosphatase 2C, negatively regulates cell death and XA21-mediated innate immunity. PLoS Biol 6:e231

Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, Pérez AC, Chico JM, Bossche RV, Sewell J, Gil E, García-Casado G, Witters E, Inzé D, Long JA, De Jaeger G, Solano R, Goossens A (2010) NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature 464:788–791

Pennell RI (1997) Programmed cell death in plants. Plant Cell 9:1157–1168

Penninckx IAMA, Thomma BPHJ, Buchala A, Métraux J-P, Broekaert WF (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell 10:2103–2113

Petutschnig EK, Jones AME, Serazetdinova L, Lipka U, Lipka V (2010) The Lysin Motif Receptor-like Kinase (LysM-RLK) CERK1 is a major chitin-binding protein in Arabidopsis thaliana and subject to chitin-induced phosphorylation. J Biol Chem 285:28902–28911

Piasecka A, Jedrzejczak-Rey N, Bednarek P (2015) Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. New Phytol 206:948–964

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Postma J, Liebrand TWH, Bi G, Evrard A, Bye RR, Mbengue M, Kuhn H, Joosten MHAJ, Robatzek S (2016) Avr4 promotes Cf-4 receptor-like protein association with the BAK1/SERK3 receptor-like kinase to initiate receptor endocytosis and plant immunity. New Phytol 210:627–642

Pruitt RN, Schwessinger B, Joe A, Thomas N, Liu F, Albert M, Robinson MR, Chan LJG, Luu DD, Chen H, Bahar O, Daudi A, De Vleesschauwer D, Caddell D, Zhang W, Zhao X, Li X, Heazlewood JL, Ruan D, Majumder D, Chern M, Kalbacher H, Midha S, Patil PB, Sonti RV, Petzold CJ, Liu CC, Brodbelt JS, Felix G, Ronald PC (2015) The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. Sci Adv 1:e1500245

Quintana-Rodriguez E, Morales-Vargas AT, Molina-Torres J, Adame-Alvarez RM, Acosta-Gallegos JA, Heil M (2015) Plant volatiles cause direct, induced and associational resistance in common bean to the fungal pathogen Colletotrichum lindemuthianum. J Ecol 103:250–260

Ranf S, Eschen-Lippold L, Pecher P, Lee J, Scheel D (2011) Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage-associated molecular patterns. Plant J 68:100–113

Ranf S, Gisch N, Schäffer M, Illig T, Westphal L, Knirel YA, Sánchez-Carballo PM, Zähringer U, Hückelhoven R, Lee J, Scheel D (2015) A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in Arabidopsis thaliana. Nat Immunol 16:426–433

Rasmussen MW, Roux M, Petersen M, Mundy J (2012) MAP kinase cascades in Arabidopsis innate immunity. Front Plant Sci 3:169

Ravensdale M, Bernoux M, Ve T, Kobe B, Thrall PH, Ellis JG, Dodds PN (2012) Intramolecular interaction influences binding of the Flax L5 and L6 resistance proteins to their AvrL567 LIgands. PLoS Pathogens 8:e1003004

Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone crosstalk in plant disease and defense: more than just JASMONATE-SALICYLATE antagonism. Ann Rev Phytopathol 49:317–343

Ron M, Kantety R, Martin GB, Avidan N, Eshed Y, Zamir D, Avni A (2000) High-resolution linkage analysis and physical characterization of the EIX-responding locus in tomato. Theor Appl Genet 100:184–189

Ronald PC, Salmeron JM, Carland FM, Staskawicz BJ (1992) The cloned avirulence gene avrPto induces disease resistance in tomato cultivars containing the Pto resistance gene. J Bacteriol 174:1604–1611

Rooney HCE, Van't Klooster JW, van der Hoorn RAL, Joosten MHAJ, Jones JDG, de Wit PJGM (2005) Cladosporium Avr2 inhibits tomato Rcr3 protease required for Cf-2-dependent disease resistance. Science 308:1783–1786

Rotblat B, Enshell-Seijffers D, Gershoni JM, Schuster S, Avni A (2002) Identification of an essential component of the elicitation active site of the EIX protein elicitor. Plant J 32:1049–1055

Rouxel T, Balesdent M-H (2013) From model to crop plant-pathogen interactions: cloning of the first resistance gene to Leptosphaeria maculans in Brassica napus. New Phytol 197:356–358

Russell AR, Ashfield T, Innes R (2015) Pseudomonas syringae effector AvrPphB suppresses AvrB-induced activation of RPM1, but not AvrRpm1-induced activation. Mol Plant Microbe Interact 28:727–735

Sandrock RW, VanEtten HD (1998) Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. Phytopathology 88:137–143

Santino A, Taurino M, De Domenico S, Bonsegna S, Poltronieri P, Pastor V, Flors V (2013) Jasmonate signaling in plant development and defense response to multiple (a)biotic stresses. Plant Cell Rep 32:1085–1098

Saur IML, Kadota Y, Sklenar J, Holton NJ, Smakowska E, Belkhadir Y, Zipfel C, Rathjen JP (2016) NbCSPR underlies age-dependent immune responses to bacterial cold shock protein in Nicotiana benthamiana. Proc Natl Acad Sci 113:3389–3394

Schreiber KJ, Baudin M, Hassan JA, Lewis JD (2016) Die another day: molecular mechanisms of effector-triggered immunity elicited by type III secreted effector proteins. Semin Cell Dev Biol 56:124–133

Schulz P, Herde M, Romeis T (2013) Calcium-dependent protein kinases: hubs in plant stress signaling and development. Plant Physiol 163:523–530

Segarra G, Santpere G, Elena G, Trillas I (2013) Enhanced botrytis cinerea resistance of arabidopsis plants grown in compost may be explained by increased expression of defense-related genes, as revealed by microarray analysis. PLoS ONE 8:e56075

Selote D, Kachroo A (2010) RIN4-like proteins mediate resistance protein-derived soybean defense against Pseudomonas syringae. Plant Signal Behav 5:1543–1546

Sels J, Mathys J, De Coninck BMA, Cammue BPA, De Bolle MFC (2008) Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol Biochem 46:941–950

Seo S, Katou S, Seto H, Gomi K, Ohashi Y (2007) The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants. Plant J 49:899–909

Seybold H, Trempel F, Ranf S, Scheel D, Romeis T, Lee J (2014) Ca2+ signalling in plant immune response: From pattern recognition receptors to Ca2+ decoding mechanisms. New Phytol 204:782–790

Seybold H, Boudsocq M, Romeis T (2017) CDPK activation in PRR signaling. Methods in Molecular Biology Humana Press, New York, pp 173–183

Shah J (2005) Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. Annu Rev Phytopathol 43:229–260

Shan L, He P, Li J, Heese A, Peck SC, Nürnberger T, Martin GB, Sheen J (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe 4:17–27

Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu F-F, Sharon M, Browse J, He SY, Rizo J, Howe GA, Zheng N (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405

Shetty NP, Jørgensen HJL, Jensen JD, Collinge DB, Shetty HS (2008) Roles of reactive oxygen species in interactions between plants and pathogens. Eur J Plant Pathol 121:267–280

Shigenaga AM, Argueso CT (2016) No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. Semin Cell Dev Biol 56:174–189
Song Y, Chen D, Lu K, Sun Z, Zeng R (2015) Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. Front Plant Sci 6:786
Souza CA, Li S, Lin AZ, Boutrot F, Grossmann G, Zipfel C, Somerville SC (2017) Cellulose-derived oligomers act as damage-associated molecular patterns and trigger defense-like responses. Plant Physiol 173:2383–2398
Staswick PE (2004) The oxylipin signal Jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell 16:2117–2127
Stergiopoulos I, de Wit PJGM (2009) Fungal effector proteins. Annu Rev Phytopathol 47:233–263
Stotz HU, Mitrousia GK, de Wit PJGM, Fitt BDL (2014) Effector-triggered defence against apoplastic fungal pathogens. Trends Plant Sci 19:491–500
Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J (2013) Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. Science 342:624–628
Tamogami S, Rakwal R, Agrawal GK (2008) Interplant communication: airborne methyl jasmonate is essentially converted into JA and JA-Ile activating jasmonate signaling pathway and VOCs emission. Biochem Biophys ResCommun 376:723–727
Terras F (1995) Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell 7:573–588
Thaler JS, Humphrey PT, Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 17:260–270
Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature 448:661–665
Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci 95:15107–15111
Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB (1997) Subcellular localization of H2O2 in plants. H2O2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. Plant J 11:1187–1194
Toyomasu T, Usui M, Sugawara C, Otomo K, Hirose Y, Miyao A, Hirochika H, Okada K, Shimizu T, Koga J, Hasegawa M, Chuba M, Kawana Y, Kuroda M, Minami E, Mitsuhashi W, Yamane H (2014) Reverse-genetic approach to verify physiological roles of rice phytoalexins: characterization of a knockdown mutant of OsCPS4 phytoalexin biosynthetic gene in rice. Physiol Plant 150:55–62
Urano D, Jones AM (2014) Heterotrimeric G protein-coupled signaling in plants. Annu Rev Plant Biol 65:365–384
van der Hoorn RAL, Kamoun S (2008) From guard to decoy: a new model for perception of plant pathogen effectors. Plant Cell 20:2009–2017
van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol 44:135–162
VanEtten HD (1994) Two classes of plant antibiotics: phytoalexins versus “phytoanticipins”. Plant Cell 6:1191–1192
Voigt CA (2016) Cellulose/callose glucan networks: the key to powdery mildew resistance in plants? New Phytol 212:303–305
Wan J, Zhang X-C, Neece D, Ramonell KM, Clough S, S-y K, Stacey MG, Stacey G (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell 20:471–481
Wan J, Tanaka K, Zhang X-C, Son GH, Brechenmacher L, Nguyen THN, Stacey G (2012) LYK4, a lysin motif receptor-like kinase, is important for chitin signaling and plant innate immunity in Arabidopsis. Plant Physiol 160:396–406
Wang X (2004) Lipid signaling. Curr Opin Plant Biol 7:329–336

Wang YS, Pi LY, Chen X, Chakrabarty PK, Jiang J, De Leon AL, Liu GZ, Li L, Benny U, Oard J, Ronald PC, Song WY (2006) Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. Plant Cell 18:3635–3646

Wang Y, Li J, Hou S, Wang X, Li Y, Ren D, Chen S, Tang X, Zhou J-M (2010) A Pseudomonas syringae ADP-Ribosyltransferase inhibits Arabidopsis mitogen-activated protein kinase kinases. Plant Cell 22:2033–2044

Wang S, Sun J, Fan F, Tan Z, Zou Y, Lu D (2016a) A Xanthomonas oryzae pv. oryzae effector, XopR, associates with receptor-like cytoplasmic kinases and suppresses PAMP-triggered stomatal closure. Sci China Life Sci 59:897–905

Wang WM, Liu PQ, Xu YJ, Xiao S (2016b) Protein trafficking during plant innate immunity. J Integr Plant Biol 58:284–298

Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in annals of botany. Ann Bot 111:1021–1058

Wasternack C, Kombrink E (2010) Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem Biol 5:63–77

Weerasinghe RR, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in Lotus japonicus. Proc Natl Acad Sci 102:3147–3152

Weerasinghe RR, Swanson SJ, Okada SF, Garrett MB, Kim SY, Stacey G, Boucher RC, Gilroy S, Jones AM (2009) Touch induces ATP release in Arabidopsis roots that is modulated by the heterotrimeric G-protein complex. FEBS Lett 583:2521–2526

Wernimont AK, Artz JD, Finerty P, Lin YH, Amani M, Allali-Hassani A, Senisterra G, Vedadi M, Tempel W, MacKenzie F, Chau I, Lourido S, Sibley LD, Hui R (2010) Structures of apicomplexan calcium-dependent protein kinases reveal mechanism of activation by calcium. Nat Struct Mol Biol 17:596–601

Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562–565

Williams CE, Wang B, Holsten TE, Scambray J, De Assis Goes Da Silva F, Ronald PC (1996) Markers for selection of the rice Xa21 disease resistance gene. Theor Appl Genet 93:1119–1122

Willmann R, Lajunen HM, Erbs G, Newman M-A, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono J-J, Cullimore JV, Jehle AK, Gotz F, Kulik A, Molinaro A, Lipka V, Gust AA, Nurnberger T (2011) Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. Proc Natl Acad Sci 108:19824–19829

Wilton M, Subramaniam R, Elmore J, Felsensteiner C, Coaker G, Desveaux D (2010) The type III effector HopF2 Pto targets Arabidopsis RIN4 protein to promote Pseudomonas syringae virulence. Proc Natl Acad Sci 107:2349–2354

Wu J, Hettenhausen C, Meldau S, Baldwin IT (2007) Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of nicotiana attenuata. Plant Cell 19:1096–1122

Wu S-J, Liu Y-S, Wu J-Y (2008) The signaling role of extracellular ATP and its dependence on Ca2+ flux in elicitation of salvia miltiorrhiza hairy root cultures. Plant Cell Physiol 49:617–624

Xiang T, Zong N, Zou Y, Wu Y, Zhang J, Xing W, Li Y, Tang X, Zhu L, Chai J, Zhou JM (2008) Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. Curr Biol 18:74–80

Xu M, Galhano R, Wiemann P, Bueno E, Tiernan M, Wu W, Chung IM, Gershenzon J, Tudzynski B, Sesma A, Peters RJ (2012) Genetic evidence for natural product-mediated plant-plant allelopathy in rice (Oryza sativa). New Phytol 193:570–575

Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, Wang Z, Xie D (2009) The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell 21:2220–2236

Zeng L, Velásquez AC, Munkvold KR, Zhang J, Martin GB (2012) A tomato LysM receptor-like kinase promotes immunity and its kinase activity is inhibited by AvrPtoB. Plant J 69:92–103

Zhang L, Kars I, Essenstam B, Liebrand TWH, Wagemakers L, Elberse J, Tagkalaki P, Tjoitang D, van den Ackerveken G, van Kan JAL (2014) Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the Arabidopsis receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1. Plant Physiol 164:352–364

Zheng XY, Spivey NW, Zeng W, Liu PP, Fu ZQ, Klessig DF, He SY, Dong X (2012) Coronatine promotes pseudomonas syringae virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 11:587–596

Zhou J, Wu S, Chen X, Liu C, Sheen J, Shan L, He P (2014) The Pseudomonas syringae effector HopF2 suppresses Arabidopsis immunity by targeting BAK1. Plant J 77:235–245

Zhou Z, Wu Y, Yang Y, Du M, Zhang X, Guo Y, Li C, Zhou J-M (2015) An Arabidopsis plasma membrane proton ATPase modulates JA signaling and is exploited by the pseudomonas syringae effector protein AvrB for stomatal invasion. Plant Cell 27:2032–2041

Zhu Z, An F, Feng Y, Li P, Xue LAM, Jiang Z, Kim J-M, To TK, Li W, Zhang X, Yu Q, Dong Z, Chen W-Q, Seki M, Zhou J-M, Guo H (2011) Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in Arabidopsis. Proc Natl Acad Sci 108:12539–12544

Zipfel C (2014) Plant pattern-recognition receptors. Trends Immunol 35:345–351

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts agrobacterium-mediated transformation. Cell 125:749–760

Zuo W, Chao Q, Zhang N, Ye J, Tan G, Li B, Xing Y, Zhang B, Liu H, Fengler KA, Zhao J, Zhao X, Chen Y, Lai J, Yan J, Xu M (2015) A maize wall-associated kinase confers quantitative resistance to head smut. Nat Genet 47:151–157

Srayan Ghosh is currently pursuing Ph.D. under the supervision of Dr. Jha.

Kamal Kumar Malukani is a Ph.D. student at CSIR-CCMB, Hyderabad, working under the supervision of Dr. Sonti.

Ravindra Kumar Chandan is a project SRF working with Dr. Jha.

Ramesh V. Sonti obtained his Ph.D. from the University of Utah, USA, and postdoctoral research at the Massachusetts Institute of Technology, USA. Since 1993, he has worked at the CSIR-Centre for Cellular and Molecular Biology in Hyderabad where he was a Chief Scientist before joining as Director, NIPGR. His research interests are in understanding the mechanisms that underlie the processes of attack and defense in plant-pathogen interactions using the interaction between rice and the bacterial pathogen *Xanthomonas oryzae* pv. oryzae as a model system. He is also keenly interested in the application of marker-assisted selection in rice improvement.

Gopaljee Jha obtained his Ph.D. in Life Sciences from Cellular and Molecular Biology, Hyderabad. Before joining NIPGR as staff scientist, he worked at the CSIR-Institute of Himalayan Bioresource Technology, Palampur, as Scientist. His research interest is to understand and develop disease tolerance against sheath blight disease in rice. He is keenly interested in understanding the molecular basis of fungal eating property of a novel rice endophytic bacterium, *Burkholderia gladioli* strain NGJ1. He is also exploring the interaction among rice-associated microbes to identify novel antimicrobial compounds.

Authors have known the Editor who is the Chairman of the Scientific Advisory Committee of NIPGR.

Integration of Multiple Signaling Cues

21

Priya Gambhir, Diksha Bhola, Shweta Sharma, Yashwanti Mudgil, and Arun Kumar Sharma

Abstract

Plants and other eukaryotes are quite complex organisms. They have highly specialized tissues carrying out various tasks. The activities of all these tissues is to be coordinated for normal function of plants. For example, when there are enough resources that are available for uptake by roots, aerial parts should be geared up for increased biosynthetic activity. They would need some communication to be ready for this enhanced biosynthetic activity. When conditions are not favorable, then plants would like to shut off or slow down biosynthetic activity to be in survival mode and wait for unfavorable conditions to go away. These unfavorable conditions are mostly sensed at the membrane level, and the biosynthetic activities are controlled at the nuclear level by genes and transcription factors regulating genes. The environmental conditions affecting plants can be varied like heat stress, cold stress, drought stress, or infection by some pathogen. These may be sensed in different ways but the effect may be a common effect, like decreasing or increasing the growth. This suggests that different signals might converge and crosstalk to achieve the desirable responses of plants in response to various developmental or environmental cues. We have identified some of the candidates which are involved in signal integration. Role of these integrators like Della proteins, calcium, phytochrome-interacting factors (PIFs), constitutive photomorphogenic 1 (COP1), ubiquitin ligases, mitogen-activated kinases, WRKY proteins, and mediator complex has been discussed. All these integrators mediate responses of plants to more than one environmental factor. These signal integrators have been found to also interact with each other. The complexity of the signal integration can be highlighted by one fascinating

P. Gambhir · S. Sharma · A. K. Sharma (✉)
Department of Plant Molecular Biology, University of Delhi, New Delhi, India
e-mail: arun@genomeindia.org

D. Bhola · Y. Mudgil
Department of Botany, University of Delhi, Delhi, India

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_21

example of signal integration involving Della proteins, which were initially identified as repressor of gibberellin responses. C-repeat binding factor (CBF1), which mediates responses to cold/desiccation stresses and PIFs, which were initially found to mediate light responses, stimulate expression of genes encoding Della proteins. Della proteins on the other hand are involved in mediating responses of several other hormones, including auxin, abscisic acid, and brassinosteroid at various levels.

Keywords
Brassinosteroids · Constitutive photomorphogenic 1 · DELLA proteins · Gibberellic acid · Mediator · Mitogen-activated protein kinase · Phytochrome-interacting factors · Signaling · Ubiquitin-proteasome system · WRKY proteins

21.1 Introduction

All living organisms, including plants, encounter environmental stresses that can be either biotic or abiotic. To survive, plants must adapt with adverse circumstances by enabling themselves to detect and perceive the environmental stimuli and trigger the appropriate response. The perception of the signal and downstream signaling involves several elements. Plants, being sessile, need to effectively integrate multiple signaling inputs in order to adapt and survive in adverse environmental conditions along with maintaining their growth and development. There are several signals and signaling mechanism by which the major pathways of growth and development operate. However, the events triggered by a particular signal are not always unique to the pathway triggered by that signal. For example, crosstalk between growth and immune signaling is a basic necessity for plants to balance the growth in adverse conditions in an efficient and timely manner. Though several intracellular signaling components have been identified using molecular and genetic studies in the recent past, the understanding of how these multiple signals are integrated to regulate growth and development under different environmental stresses is not very clear. We human beings do some adjustments when our financial condition is not good. In such situation, we tend to conserve our available resources and tend to tide over that situation by lying low and not investing much into our growth. Plants also tend to tide over various stresses by keeping their growth low under the period of various stresses. Positive factors like light, good supply of water, and nutrients affect growth in a positive manner while negative factors like water or temperature stress tend to inhibit growth. When both positive and negative factors affect growth in one way or another, it is logical to think that they would share some signaling component which regulates growth and responses to various stresses. Since hormones are key factors in regulation of growth and development, it is likely that various positive and negative factors would affect signal transduction components of hormone signaling at one level or another. Although each signaling pathway has some unique components, they also share some common elements. Recent reports suggest that there is a signal integration from multiple molecules or protein which

results into a common biological effect to maintain the growth and development of the plants, either by alterations in gene expression involving numerous genes such as *DELLA* genes or by quick cellular responses such as changes in calcium concentration in the cell.

21.2 Della Proteins and Signal Integration

DELLA proteins are transcriptional regulators and were initially recognized as key regulators of gibberellin (GA) signaling pathway. They have been identified as inhibitor of cell proliferation and expansion of plant growth throughout the life cycle of higher plants, mainly in response to the phytohormone gibberellin (GA) (Peng et al. 1997; Silverstone et al. 1998; Dill and Sun 2001; King et al. 2001). Only one DELLA protein was identified in rice (SLENDER RICE 1 [SLR1]) though *Arabidopsis* genome encodes five DELLAs which are named GA-INSENSITIVE (GAI), REPRESSOR OF GA1-3 (RGA), RGA-LIKE 1 (RGL1), RGL2, and RGL3 (Peng et al. 1997; Ikeda et al. 2001; Silverstone et al. 2001; Lee et al. 2002; Wen and Chang 2002). Previous reports have shown diverse but overlapping functions of DELLA proteins in plant development growth in response to environmental stresses (Lee et al. 2002; Cheng et al. 2004; Tyler et al. 2004; Achard et al. 2006).

21.2.1 Role of DELLA Proteins in Seed Germination and Floral Development

Seeds are major source of nutrition and also help in plant propagation and dispersal. At the molecular level, GA hormone induces GA signal transduction by triggering proteasomal degradation of DELLA repressors of GA responses. There are various DELLA proteins involved in seed germination with partly overlapping functions, for example, RGA (for REPRESSOR OF GA1), GAI (for GA-INSENSITIVE), RGL1 (for RGA-LIKE1), RGL2, and RGL3 (Cheng et al. 2004; Tyler et al. 2004; Cao et al. 2005). Out of these, RGL2 is the main repressor of seed germination, although RGA, GAI, and RGL3 also contribute to some extent (Lee et al. 2002; Tyler et al. 2004; Ariizumi and Steber 2007). There are several reports demonstrating the role of different DELLA proteins in various functions: RGA and GAI repress stem elongation (Dill and Sun 2001; King et al. 2001), RGL2 inhibits seed germination (Lee et al. 2002), and RGA, RGL1, and RGL2 together regulate floral development (Cheng et al. 2004; Tyler et al. 2004; Yu et al. 2004).

Further studies on RGL2 demonstrated its role in seed germination where it was shown that only RGL2, not RGL1, affected the seed germination in *Arabidopsis* (Lee et al. 2002). It has also been reported that *Arabidopsis* DELLA proteins RGA and RGL2 jointly repress petal, stamen, and anther development in GA-deficient plants, and this function is enhanced by RGL1 activity (Cheng et al. 2004). Cao and his group supported this data one step further by showing that RGL2 is an important repressor of seed germination in *Arabidopsis*, whereas other DELLA genes such as GAI, RGA, and RGL1 add to the better performance of RGL2 (Cao et al. 2005). It

was also reported that ga1-3 mutants, which lacks GAI, RGL, and RGL2, could germinate both in light and darkness, suggesting the role of DELLA proteins in seed germination in response to light (Cao et al. 2005).

21.2.2 Role of Della Proteins in Response to Stress

Studies have shown diverse and overlapping functions of DELLA proteins in plant development as well as in responses to environmental stresses (Lee et al. 2002; Cheng et al. 2004; Tyler et al. 2004; Achard et al. 2006). It has been reported that the growth of mutant plants lacking four of the five DELLAs (GAI, RGA, RGL1, and RGL2) is less inhibited by salt stress as compared to the wild-type plants (Achard et al. 2006, 2008). Role of DELLA has also been indicated in response to cold/freezing stress where C-repeat binding factor (CBF1) regulates RGL3 gene expression, which in turn increases DELLA accumulation and restrains plant growth. Moreover, the CBF1-induced DELLA accumulation also contributes in a synergistic manner with the CBF1-induced cold-regulated (COR) pathway to promote cold adaptation (Achard et al. 2008).

21.2.3 Role of DELLA in Trichome Development in *Arabidopsis*

Trichomes are unicellular epidermal structures of plants, where GA plays a major role for the development of trichomes. As discussed, there are five genes encoding DELLA proteins in *Arabidopsis*: *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR* of *ga1*-3 (RGA), and three *RGA-LIKE* genes (*RGL1*, *RGL2*, and *RGL3*; Peng et al. 1997; Silverstone et al. 1998; Lee et al. 2002; Tyler et al. 2004), which are known as repressor of growth and development in plants. Out of these, *RGA* and *GAI* are known to repress trichome formation on leaves because loss-of-function mutations in *RGA* and *GAI* can rescue leaf trichome initiation in *ga1-3* mutants (Dill and Sun 2001). Further, it was also found that the different repressors act synergistically in the control of trichome development, but specific DELLA proteins play predominant roles in the control of either initiation or branching (Gan et al. 2007).

21.2.4 Role of DELLA Proteins in Regulating Multiple Hormone Signaling

Growth and development of a plant is only possible if there is a functional machinery which is controlled precisely by coordination between different signal molecules. Plant hormones, involved in the intrinsic development, are the best examples of signal integration where most of the hormones are either directly or indirectly connected to perform during growth of plants (Santner and Estelle 2009). Nine plant hormones are well characterized till date. These include gibberellins (GAs), auxin, cytokinins (CKs), abscisic acid (ABA), jasmonate (JA), brassinosteroids (BR),

ethylene, nitric oxide, and strigolactones. These phytohormones play dual role in plants by governing and coordinating growth and developmental processes along with responding and conveying environmental stimuli to initiate adaptive responses.

GA and ABA are the hormones controlling seed germination where both of these act antagonistically. Whereas ABA is responsible for seed dormancy, GA enhances seed germination (Koornneef et al. 2002). Under favorable conditions, ABA levels drop down and GA synthesis begins, which initiates seed germination by promoting the degradation of RGL2, a member of DELLA protein family (Lee et al. 2002; Piskurewicz et al. 2008). Recently, it has been reported that DELLA interacts with ABI3 and ABI5 to form a protein complex which activates the transcription of target genes, negatively controlling the seed germination (Park et al. 2011; Lim et al. 2013). Another phytohormone, brassinosteroids (BR), plays a crucial role in the regulation of seedling growth in response to light and temperature. Mutant studies have shown that DELLA interacts with BRASSINAZOLE-RESISTANT 1 (BZR1), a transcription factor, and also with PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) and inhibits their DNA binding ability in response to light and temperature (Li and Chory 1997; Vert et al. 2005; Wang et al. 2012). Auxin is a phytohormone involved in hypocotyl elongation and performs its function by ubiquitination and degradation of AUX family proteins which allows auxin-responsive factor (ARFs) to bind promoters of auxin-responsive genes (Chapman and Estelle 2009). ARF6 co-operates with BZR1 and PIF4 to affect genes which are common targets of these factors. DELLAs inhibit this co-operative interaction among ARF6, BZR1, and PIF4 (Wang et al. 2014). DELLA proteins also function in plant immunity by influencing salicylic acid and jasmonic acid signaling in plants (Chini et al. 2007; Hou et al. 2010, 2013; Fernández-Calvo et al. 2011; Wild et al. 2012).

It can be inferred from the preceding discussion that DELLA proteins are involved in a high degree of interaction with other genes/proteins, resulting into a variety of functions (See Fig. 21.1). DELLAs are reported to be involved in almost all the hormone pathways (Oh et al. 2014), suggesting their role in regulation of plant growth and development. Positive growth responses involve degradation of DELLA proteins, whereas under stress, DELLAs inhibit binding of transcription factors which retards growth. Studies on DELLAs till date also provide the understanding of how these proteins are involved in crosstalk between various signaling pathways in order to maintain the developmental growth of plants.

21.3 Calcium as an Integrator

As highlighted in our previous section, there are several proteins which are involved in perceiving signals or act downstream in signaling pathways. However, there are some nonproteinaceous molecules which are involved in conveying signals to the plant machinery. Calcium (Ca^{2+}) is one of the most important molecules in this category, as calcium concentration is altered in response to almost all the signals perceived by cells as compared to other messengers (Knight 2002; Reddy et al. 2002). About four major classes of Ca^{2+} sensor families are identified till date in *Arabidopsis* including calcium-dependent protein kinase, calmodulin (CaM), calmodulin-like

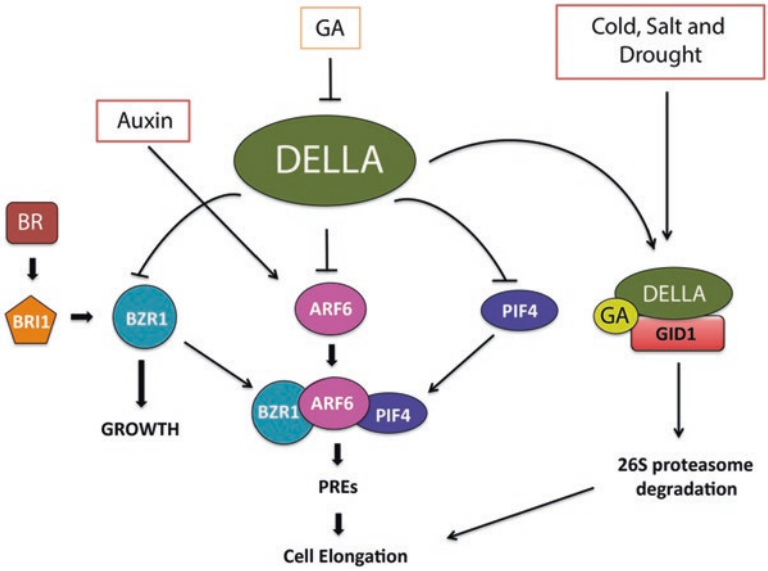

Fig. 21.1 DELLA as integrators. Schematic diagram of DELLA-mediated crosstalk between the different signaling pathways. DELLAs interact with PIFs and inhibit the activity of many transcription factors like BZR1 and ARF6 of the BR and auxin pathways, respectively, finally targeting photomorphogenesis. The ability of DELLAs to modulate DNA binding and transcriptional activities of many transcription factors allows GA to effectively control diverse developmental processes

(CML), and calcineurin B-like (CBL) proteins (Luan et al. 2002, 2009; Yang and Poovaiah 2003; Harper et al. 2004).

The calcium ion (Ca^{2+}) is a ubiquitous intracellular second messenger used extensively in plants, animals and microorganisms to couple extracellular stimuli to their characteristic intracellular responses and to coordinate a wide range of endogenous processes. Calcium plays important roles in plant development and in responses of plants to biotic and abiotic stresses. Details about the role of calcium are covered in another chapter in this book dedicated on this aspect.

21.4 PIFs as Integrators

Light is considered as one of the most important environmental factors influencing development, growth, and physiology of plants. This signal is perceived by a group of photoreceptors known as phytochromes, which exist in red light absorbing form (Pr) and far-red light absorbing form (Pfr) and help in monitoring the light quality and in adjusting to the different light conditions by transducing these light signals to downstream regulatory components of light signaling pathway. The transduction process involves the light-mediated conversion to active form of these receptors (Pfr) and their translocation in nucleus. Once inside the nucleus, Pfr is known to

interact with a small family of basic helix-loop-helix transcriptional regulators, i.e., PIFs or phytochrome-interacting factors. PIFs belong to a subfamily 15 *Arabidopsis* bHLH superfamily members. These are the key players involved in photomorphogenic responses. Upon interaction with the Pfr, PIFs are degraded, thereby leading to seed germination and shade avoidance response. PIFs bind specifically to a core DNA G-box motif (CACGTG) (Khanna et al. 2004). Return of the plants to darkness degrades the Pfr, thereby restoring PIF levels rapidly. PIFs are gaining popularity as integrators of signal transduction process, thus effectively establishing a link between light signaling and various other processes in plants (See Fig. 21.2).

21.4.1 Role of PIFs in Seed Germination

Light and cold treatment are the two primary requisites for breaking the seed dormancy in *Arabidopsis.* These factors work in concert with the phytohormone and gibberellin. PIFs integrate the light and gibberellic acid signal transduction pathways at multiple levels. During dark period, PIFs activate the expression of two

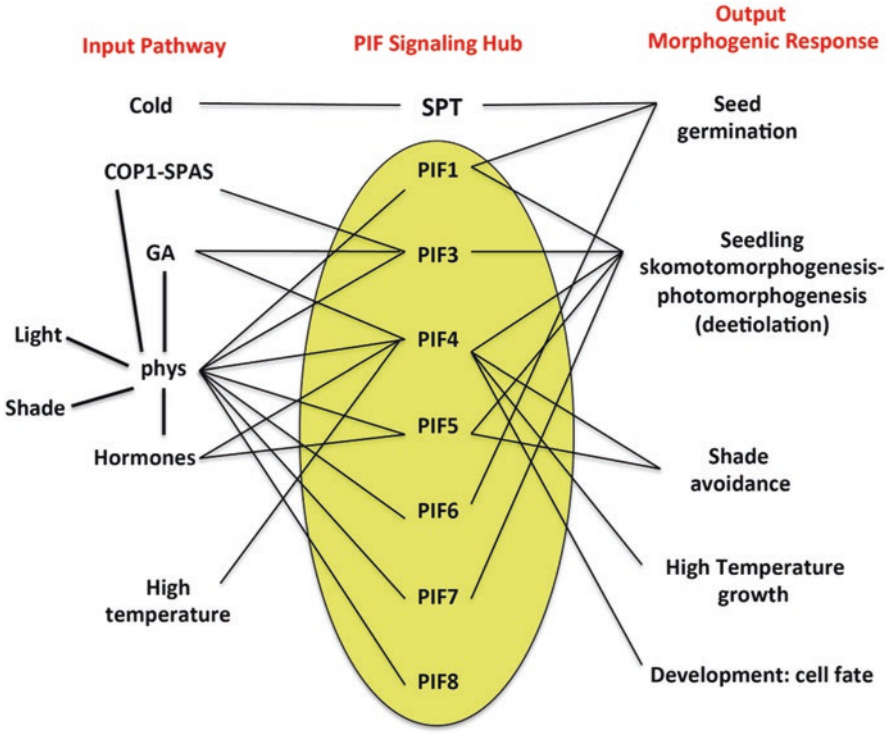

Fig. 21.2 PIFs as integrators. PIFs function redundantly and differentially in a cellular signaling hub at the convergence of multiple pathways by integrating responses to both environmental and endogenous signals

DELLA genes: *RGA1* (*Repressor of GA1-3*) and *GAI* (*Gibberellic Acid Insensitive*) by binding to their promoter regions, thereby repressing the GA signal transduction pathway (Oh et al. 2007). PIFs also regulate the biosynthetic pathway of gibberellins by upregulating the catabolic genes such as *GA2ox2* (GIBBERELLIN 2-OXIDASE 2). *DAG1* (*DOF AFFECTING GERMINATION 1*) and *SOM* (*SOMNUS*) genes known as the repressors of key biosynthetic genes *GA3ox1* and *GA3ox2* are also the direct targets of PIFs (Kim et al. 2008a, b).

Light-mediated degradation of PIFs restricts the process of germination in light. Upon degradation, the regulatory effects of PIFs are no longer in force, and hence, the GA biosynthetic and signaling pathway genes are expressed at high levels finally resulting in seed germination.

21.4.2 PIFs as Regulators of Circadian Clock

PIFs govern both input and output pathways of circadian clock influencing the growth and development in a diurnal manner. Light again comes into picture as an environmental information to the clock. The rhythmic expression of PIF4 and PIF5 during diurnal cycle brings about the maximum transcript accumulation either at dawn or in early morning in short day or long day conditions, respectively (Nozue et al. 2007). Recent reports have shown that the promoters of CCA1/LHY contain G-box element necessary for the binding of PIFs (Oh et al. 2012). Moreover, PIFs are required for metabolic signaling to the clock by binding to the promoters of CCA1/LHY promoters in response to sugars. The evening complex (EC) constituting ELF3, ELF4, and LUX downregulates the expression of PIF4/5, restricting the growth to dawn. Timing of CAB Expression 1 (TOC1), a component of circadian clock, interacts with PIF1, and this physical interaction between the two gene products leads to concurrent binding to the promoters of the dawn-phased genes, with CCA1 regulating the growth in the early morning (Martín et al. 2016).

21.4.3 Role of PIFs in Thermomorphogenesis

With light comes high temperature, which again acts as an environmental cue causing elongated hypocotyls, narrowing of leaves, and accelerated flowering, characteristic of thermal-induced morphogenesis. Of all the PIFs, only PIF4 has been associated to thermomorphogenesis. Recent insights into the light signal transduction pathway have revealed that the phytochrome receptors are prone to high temperature-mediated thermal reversion to inactive form, contributing to high levels of PIFs stability (Jung et al. 2016; Legris et al. 2017). Cryptochrome, receptors for blue light, also regulate the activity of PIFs by inhibiting their transcription (Maa et al. 2016). FCA (FLOWERING TIME CONTROL PROTEIN) also keeps a check on PIF4 activity during high temperature growth (Lee et al. 2014). When the plant encounters high temperature stress during daytime, PIF4 is stabilized due to the

degradation of photoreceptors. Once stabilized, PIF4 interacts with the genes involved in auxin biosynthesis (*YUCCA8/TAA1*) resulting in hypocotyl elongation.

21.4.4 Role of PIFs in Auxin Signaling

Plant growth and development is dependent largely on a class of phytohormones known as auxins. Auxins work by modulating cell division and cell elongation. Under shade, *TAA1 (Tryptophan aminotransferase1) and CYP79B2*, genes of auxin biosynthetic pathway, are the direct targets of PIF4 causing hypocotyl elongation in *Arabidopsis* (Franklin et al. 2011; Li et al. 2012; Sun et al. 2012). Recent studies illustrate the formation of a complex between ARF6 (Auxin Response Factor 6) and PIF4 to activate the genes involved in light and auxin signal transduction pathway (Oh et al. 2014). PIFs alternatively target either auxin sensitivity or its biosynthesis under low red/far-red ratio (R/FR) of different PAR. Plants under high R/FR PAR show increased levels of several auxin biosynthetic genes; however, under low R/FR, plants are more sensitive toward auxin due to increased expression of AFB1 (auxin co-receptor) (Hersch et al. 2014)

21.4.5 PIFs as Integrators of Light and Brassinosteroid Signaling Pathways

Brassinosteroids belong to a class of steroidal plant hormones that are important regulators of plant growth. The crosstalk between the light and BR signaling pathway occurs via the interaction of PIFs and BZR1 (BRASSINAZOLE RESISTANT 1) (Oh et al. 2012). This BZR1 belongs to a family of transcription factors that selectively binds to BR-responsive elements thus regulating their activity. The shared targets include the *PACLOBUTRAZOL RESISTANCE* (*PRE*) family of factors that induce hypocotyl elongation in response to hormonal and environmental signals (Bernardo-Garcia et al. 2014). PIF4-BZR1 heterodimer functions as transcriptional regulator of both light- and brassinosteroid-responsive genes, activating the ones involved in cell elongation. Another example of the interaction is observed at the level of BIN2 (BRASSINOSTEROID INSENSITIVE 2), a glycogen kinase that phosphorylates PIF4, sequestering it for its degradation via the ubiquitin-mediated pathway, maintaining the growth of hypocotyls. As far as the biosynthesis of brassinosteroid is concerned, PIF4 and PIF5 bind to promoter regions of *DWF4* (*DWARF4*) and *BR6ox2* (*BRASSINOSTEROID-6-OXIDASE 2*), genes encoding two key enzymes involved in BR biosynthesis (Wei et al. 2017).

21.4.6 PIFs Mediate Light and Ethylene Signaling

Triple response is extensively reviewed as characteristic phenomena, specific to gaseous phytohormone ethylene. This response includes elongation and thickening

of hypocotyl. Several lines of evidences suggest that integration of light and ethylene signaling pathways regulates many developmental processes. In *Arabidopsis*, overexpression lines of *PIF5* show an increase in ethylene levels in etiolated seedlings (Khanna et al. 2007), which could be attributed to PIF5 binding to the promoter of *ACS* gene (Gallego-Bartolome et al. 2011; Oh et al. 2012). Inhibition of photobleaching via interaction between PIF3 and EIN3/EIL1, leading to downregulation of protochlorophyllide biosynthetic genes and activation of the expression of *POR* genes, is another example of the crosstalk between the two pathways (Zhong et al. 2014). Increase in hypocotyl length in dark conditions and not in light conditions occurs due to an increased mRNA accumulation of PIF3. Thus, phytochrome and ethylene signaling pathways converge at the promoters of genes simultaneously targeted by PIFs and EIN3.

21.4.7 Role of PIFs in ABA Signaling

Abscisic acid is known as a leaf abscission and seed dormancy promoting class of phytohormone. ABA shows antagonistic affects with gibberellins, and the interaction of PIFs with ABA signaling genes at the molecular level is relatively less complex as compared to interactions with GA signaling genes. *ABI3* (*ABSCISIC ACID INSENSITIVE 3*) and *ABI5* are the two direct targets of PIF1 in imbibed seed incubated in dark. PIF1 activates the transcription of these genes by directly binding to their promoter regions. These genes are also known to repress GA signaling and finally inhibit seed germination (Oh et al. 2009; Park et al. 2011). The heterodimer formed between ABI3 and PIF1 coregulates the expression of *SOM*, a negative regulator of seed germination that further inhibits seed germination (Park et al. 2011). Thus, PIFs modulate the seedling establishment via the interactions with ABA signaling genes.

21.4.8 Role of PIFs in Immunity

Plants encounter multiple environmental stresses during their lifetime that affects their growth and development. There is a trade-off between growth and defense, i.e., defense is mounted at an expense of growth and vice versa. A classic example of this trade-off occurs during a pathogenic attack. Pathogens would alter the plant signal transduction mechanisms so as to favor growth while plants would suppress growth and promote defense by downregulation of several genes such as PIFs (Windram et al. 2012). Jasmonic acid (JA) plays an important role in transducing the activation of plant defense systems against pathogen attacks via the degradation of JAZ (JASMONATE-ZIM DOMAIN) proteins. JAZ9 is known to inhibit the interaction between PIF3 and RGA (DELLA repressor protein) (Campos et al. 2016). JAZ9 is a competitive inhibitor of PIF3 for the binding sites of RGA. When the plants are growing under normal conditions, the levels of JAZ9 is high and therefore forms a complex with RGA. PIF3 is free and thus promotes growth over immunity.

Activation of PACLOBUTRAZOL by PIF4-BZR1 complex upregulates HBI1 (HOMOLOG OF BEE2 INTERACTING WITH IBH1) that again favors growth over defense (Lozano-Duran and Zipfel 2015).

21.5 COP1 (Constitutive Photomorphogenic 1) Proteins as Integrator

COP1 is a ring-finger-type ubiquitin E3 ligase that represses photomorphogenic genes during dark phase. The COP1 protein consists of three defined domains—a RING-finger motif, a coiled domain, and a WD40 repeat. These domains help in establishing interaction of COP1 with different proteins and their own dimerization. The RING-finger and coiled domain at the N-terminal alone are capable of sustaining the function of COP1 protein. The C-terminal of the protein contains seven WD40 repeat domain. These N- and C-terminal domains, when introduced together, rescue the loss of function *cop1* allele (Stacey et al. 2000). Further COP1 has signals for nuclear import as well as export, and their localization is controlled by light (Yi et al. 2002; Bianchi et al. 2003). In dark phase, COP1 acts on light-responsive factors such as Long After Red Light (LAF1), Elongated Hypocotyl 5 (HY5), as well as Phytochrome-Interacting Factor 3 (PIF3) carrying out their proteolytic degradation. Proteasome-mediated proteolysis of the proteins requires an E3 ubiquitin ligase which recruits a ubiquitin-attached enzyme onto the RING-finger motif and the target substrate on other protein interaction domains. Further, light negatively regulates localization of COP1 in nucleus, thereby inhibiting degradation of these transcription factors which further induce photomorphogenic responses

21.5.1 Role of COP1 in Light Switch

Upon perception of light, the photoreceptors are stimulated and initiate various developmental processes, namely, seedling development, phototropism, as well as metabolic changes such as production of anthocyanins. This is carried out by the activation of partially overlapping multiple signal transduction cascades. Extensive genetic approaches have identified various constituents of the signaling cascades. Among these, the COP1 (Constitutive Photomorphogenic 1) protein acts as a central switch (Deng et al. 1991, 1992; Ma et al. 2002). The *COP1* mutants having recessive mutations in the *cop1* locus depicted photomorphogenic developments even in the absence of light while *cop1* null alleles did not thrive. These findings established the role of COP1 as a negative inducer of light-mediated plant development. Another important component of the light signaling cascade is HY5. Both COP1 and HY5 act antagonistically during the development of seedlings of *Arabidopsis thaliana*. HY5 is the first known target of COP1 and positively regulates photomorphogenesis by influencing the expression of downstream regulators in light signaling cascades (Chattopadhyay et al. 1998; Lee et al. 2007).

During the dark phase, COP1 is majorly localized in nucleus where it targets transcriptional factors for ubiquitination and degradation. The COP1 protein acts as E3 ubiquitin ligase for proteolytic degradation of light signaling regulators such as LAF1, HY5, HYH, HFR1, cry1, cry2, phyA, and phyB. Through ubiquitin-mediated degradation of transcription factors, COP1 represses the expression of photomorphogenic genes. HY5 is a bZIP transcription factor that on perceiving light stimulates light-responsive genes to promote photomorphogenic development. During dark-phase degradation of HY5 and HY5 homolog, HYH is carried out. The COP1 accumulated in nucleus abolishes photomorphogenic responses. LAF1, a myb transcription factor which positively regulates PHY A-mediated far-red signaling, is also a target of COP1-mediated ubiquitination. Further, HFR1 which is a bHLH transcription factor and is involved with far-red and blue light signaling is also degraded by COP1. Thus, COP1 during dark phase acts centrally in abolishing photomorphogenic responses by degrading transcription factors that stimulate light-responsive genes.

Further upon exposure to light there is a drastic decrease in the nuclear levels of COP1, thereby reducing the COP1-mediated degradation of transcription factors. Thus there is increase in the levels of transcription factors which in turn induce the expression of photomorphogenic genes. However the reduction in the nuclear COP1 levels is relatively slow suggesting alternate mechanisms for COP1 reduction. One such process is the cryptochrome facilitated reduction in COP1 activity in response to light.

21.5.2 COP1 and Transcription Factors

COP1 has also been observed to regulate signal transduction through ways other than protein degradation. In darkness, bHLH transcription factors such as PIF3 require COP1 for its nuclear accumulation rather than degradation. In contrast to other regulators of photomorphogenesis such as HY5/HYH or LAF1, PIF3 was observed to accumulate effectively in nuclei of dark grown seedlings, and on illumination, it turned over rapidly. On the basis of these observations, the role of COP1-mediated accumulation of PIF3 was further explored. Mutants of COP1 were studied. Dark grown *cop1-4* mutants which have a weak mutation resulting in a truncated COP1 product showed significantly lower accumulation of PIF3 in comparison to wild type. Also, degradation of PIF3 on induction of red or far-red light was similar in *cop1-4* as well as wild type. These findings strengthen the ground that COP1 is essentially required for accumulation of PIF3 in nuclei. Another COP1 mutant, *eid-6*, was also isolated which carries a single mutation at the conserved histidine site within its RING finger which disrupts the RING structure. In comparison to *cop1-4*, *eid-6* does not display photomorphogenic phenotype. However, it shows PhyB-mediated hypersensitivity toward light (Dieterle et al. 2003). Dark grown *eid-6* seedlings showed inhibited PIF3 accumulation as compared to the wild-type seedlings, whereas light-dependent degradation of PIF3 was unaffected. This suggests the involvement of COP1 in accumulation of PIF3 rather than their light-dependent turnover.

21.6 Plant Ubiquitin Ligases

The first ubiquitin polypeptide was isolated from calf thymus as "ubiquitous immunopoietic polypeptide" in 1975. Its ortholog was then characterized in plants like celery and carrot. Ubiquitin ligases are virtually involved in every aspect of plant growth and development. No variation in the fundamental role of this polypeptide has been observed in plants or in animals till date. The plant ubiquitin-proteasome system (UPS) includes three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) (Hershko and Ciechanover 1998). The basic process of ubiquitination either targets the substrate protein for destruction by 26S proteasome or alters their biochemical properties and subcellular localization. One of the very well-established E3 ubiquitin ligases in plants is the SCF complex. This complex consists of Skp1, Cul1/Cdc53, Roc1, and an F-box-containing protein that confers substrate specificity (Hua et al. 2011) (see Fig. 21.3).

21.6.1 Role of Ubiquitin-Proteasome System in Photomorphogenesis

In abundant light, plant seedlings grow, and this growth is referred to as photomorphogenesis. Extensive studies related to this phenomenon have revealed a group of

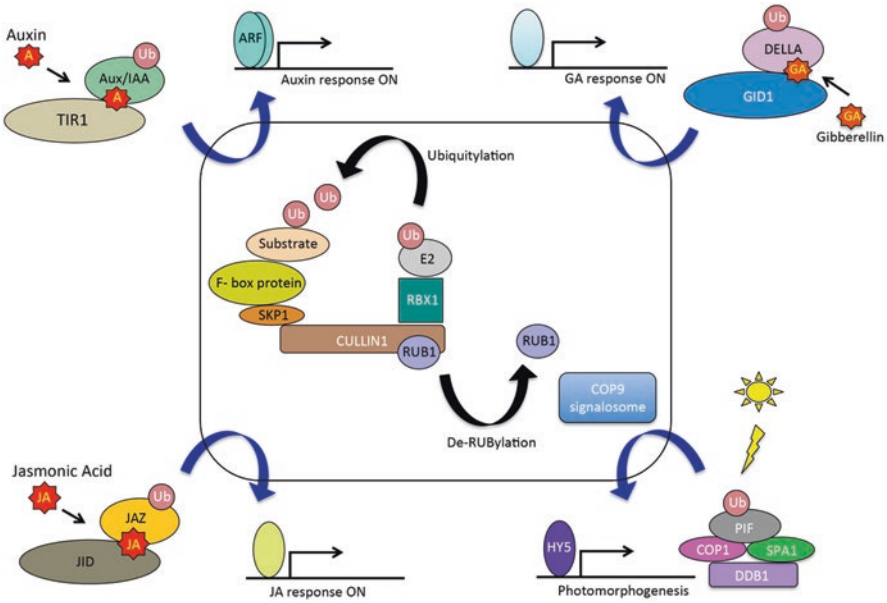

Fig. 21.3 Plant ubiquitin ligases as integrators. Schematic representation of the general modular architecture of SCF E3 ligase complex and the respective mechanisms for hormone-dependent substrate recognition

genes whose defects leads to constitutive light grown phenotypes in darkness (Serino and Deng 2003). Interestingly, almost all the genes are linked to UPS. COP1 encodes a RING-type E3, and COP10 encodes an E2 variant lacking the active site cysteine. DET1, part of the SCF complex, is also identified as a photomorphogenic repressor. Another F-box protein, MAX2, positively regulates facets of photomorphogenic development in response to light (Shen et al. 2012). MAX2 regulates GA and ABA biosynthesis in opposite manners to optimize seed germination.

21.6.2 Role of UPS as Auxin Receptor

Auxin essentially regulates every aspect of growth and development, leading to cell elongation and differentiation. Of all the auxin-resistant mutants available, the first cloned gene, AXR1, immediately pointed to UPS. AXR1 protein shares high sequence homology to E1. Further investigation of the auxin mutants leads to the discovery of another F-box protein TIRI which is a major part of the SCF complex. Later, TIR1 was designated as an auxin receptor. In the presence of auxin, SCF^{TIR1} E3 promotes the ubiquitination and degradation of a family of transcriptional repressors, AUX/IAA, thereby activating auxin-responsive genes.

21.6.3 Role of UPS as JA Receptor

COI1, another F-box protein, was identified as receptor for JA-isoleucine and other JA conjugates (Xie et al. 1998). Similar to auxin, JA-Ile enables SCF^{COI1} to catalyze the ubiquitination and degradation of the JAZ family of transcriptional repressors to trigger the expression of the JA-responsive genes.

21.6.4 Role of UPS as GA Receptor

SCF complex also mediates the signal transduction of another phytohormone, GA. In response to GA, the receptor GIDI binds to a DELLA family of transcriptional repressors. Once linked, this GIDI-GA-DELLA complex is targeted to the SCF^{GID2} E3 ligase resulting in ubiquitination and degradation of DELLA proteins.

Apart from SCFs, cullin-RING ligases (CRLs) have been extensively studied in plants for performing a variety of functions. The cullin proteins (CUL1, CUL3, and CUL4) act as elongated scaffold, holding the E2-docking catalytic subunit RBX1 at one end and substrate receptor subunit at another end. CRL3s are known to play important roles in phototropism, abscisic acid, and ethylene signaling (Hua et al. 2011). The receptors for salicylic acid, a plant immune signal generated in response to pathogenic attack, have been recently identified as $CRL3^{NPR3}$ and $CRL3^{NPR4}$ (Fu et al. 2012).

21.7 MAP Kinases as Integrators

Mitogen-activated protein kinases (MAPKs) act as a link between external stimuli and their corresponding cellular responses. MAPKs are known to regulate several cellular processes like cell cycle, cell differentiation, and stress responses. MAPK cascades consist of three kinase subfamilies, MAPKKK, MAPKK, and MAPK, affecting a wide range of downstream targets. Receptor-mediated activation of MAPKKKs can occur through physical interaction and/or phosphorylation by the receptor itself. MAPKKKs are serine/threonine kinases and activate MAPKKs through the phosphorylation of two serine/threonine residues in a conserved S/T--$X_{3–5}$-S/T motif. MAPKK on the other hand are dual kinases that phosphorylate MAPKs on tyrosine and threonine residues in the TXY motif (Claudia et al. 2002). Lastly, MAPKs are serine/threonine kinases that phosphorylate a variety of substrate proteins like transcriptional factors and protein kinases and are involved in majority of cellular processes. The existence of a large number of putative MAPKKKs suggests that these might be acting as convergence points but plant MAPKKKs can also act as divergent points in MAPK signaling as one MAPKKK can activate several MAPKKs targeting several pathways at once (see Fig. 21.4).

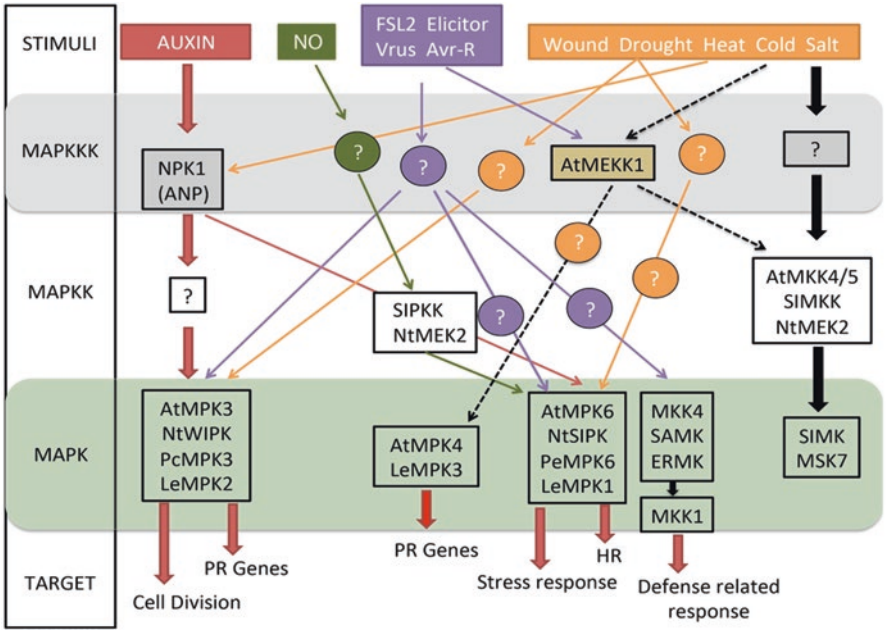

Fig. 21.4 MAPKs as integrators. Schematic representation of crosstalk between various plant MAP kinase signaling pathways. The scheme of general signal transduction pathway is shown on the left. The homologs in *Arabidopsis* (At), tobacco (Nt), parsley (Pc), and tomato (Le) are shown. "?" indicates unidentified MAP kinase components. FLS2 is the putative receptor for flagellin peptide elicitor. JA stands for jasmonic acid, SA stands for salicylic acid, PR stands for pathogenesis responsive, HR stands for hypersensitive response

21.7.1 Role of MAPKs in Pathogenic Response

In response to pathogenic attacks, plants activate several defense mechanisms, including rapid production of ROS, strengthening of cell wall, hypersensitive response, and also the production of pathogen-related proteins (PR proteins). In Alfalfa, four MAPKs, which include SIMK, MMK3, MMK2, and SAMK, are activated in response to fungal infection (Cardinale et al. 2000). SIMK and SAMK are also activated by various abiotic stresses. WIPK and SIPK are two MAPKs in tobacco activated in response to stress ranging from wounding responses to several abiotic stress responses, thereby acting as integrators of cellular pathways (Zhang and Klessig 2001). WIPK is also involved in crosstalk between SA and JA pathways in tobacco. Activation of elicitor-responsive MAPK (ERMK), by a fungal elicitor, results in the translocation of MAPK into the nucleus. These results suggested that ERMK might phosphorylate transcription factors that are involved in the plant defense responses.

21.7.2 Role of MAPKs in Osmotic Stress

Production of osmolytes is the response of cells encountering salt tolerance. MAPKs are rapidly activated by osmotic stress and help in cell survival and cell volume regulation. Several protein kinases are known to be involved in MAPK pathway during salt stress. AtMEKK1, an MAPKKK class member, plays an essential role during cold, hyperosmotic stress, touch, etc. A histidine kinase, AtHK1, is transcribed at very high levels during salt stress (Urao et al. 1999). SIPK and WIPK involved in pathogenic responses have shown a positive correlation with salt stress in tobacco suspension cells. SIPK is activated by both hyper- and hypoosmotic responses, whereas WIPK is expressed only under hypoosmotic conditions. The complexity of these kinases in different signaling pathways is attributed to the fact that these MAPKs are involved in salt stress as well as pathogenic responses. Another very well-known kinase associated with salt stress belongs to the family of SNF1 (SUCROSE NON-FERMENTING 1) protein kinases, playing a central role in hyperosmotic stress (Munnik et al. 1999).

21.7.3 Role of MAPKs in Hormone Signaling

Elucidation of signal transduction pathways of phytohormones has revealed the common points of interactions between them in different phases of growth and development. Several MAPKS have been associated with the transduction machinery of these hormones. In the presence of auxin, a MAPK-like kinase is activated in *Arabidopsis* root that is otherwise inhibited in *auxin-resistant 4* (*axr4*) mutants (Mockaitis and Howell 2000). Plants experiencing oxidative stress block the auxin-responsive genes and induce the expression of only stress-responsive genes, suggesting a crosstalk between these two pathways. CTR1, a negative regulator of ethylene signal transduction pathway, belongs to a family of MAPK protein kinases

functioning downstream of ethylene receptor ETR1 (ETHYLENE-RESISTANT 1) (Chang et al. 1993).

21.7.4 Role of MAPK in Cytokinesis

Cyclin-dependent kinases play a critical role in cell division during the segregation of the spindle fibers in complex with mitotic B-cyclins. These CDKs are in cytokinetic structures and cell plate in plants (Weingartner et al. 2001). MMK3 and Ntf6 are known to be involved in cytokinesis in Alfalfa and tobacco cells, respectively. Ntf6 is activated through the phosphorylation of NPK1, another MAPKKK, specifically activated during cytokinesis (Calderini et al. 1998).

MAPK signaling components can perform various different functions in different pathways, thus connecting them at various time points. The MAPK cascade is regulated by various posttranslational modifications. Overlapping roles of these kinases have been identified controlling diverse functions such as cell division, hormone signaling, and development and in response to abiotic stresses.

21.8 WRKY Proteins and Signal Integration

WRKY proteins are a family of transcriptional factors named as such due to the presence of conserved N-terminus WRKY domain. N-terminus of the protein contains 60 amino acid regions with conserved WRKYGQK amino acids together with the C2H2- or C2HC-type zinc-finger motifs which aid in the DNA-binding properties of WRKY proteins. The conserved nature of WRKY proteins can be attributed to its target site. These proteins bind to a highly conserved W-box (TTGACC/T) motif of DNA. However, in certain cases such as OsWRKY13 proteins or barley WRKY transcription factors, these proteins bind to PRE4 or SURE, the targets other than W-box (Cai et al. 2008; Sun et al. 2003). Although conserved in majority of WRKY proteins, the WRKY amino acid sequences in some proteins are replaced with WRRY, WSKY, WKRY, WVKY, or WKKY (Yamasaki et al. 2005; Xie et al. 2005). These proteins were essentially classified into three groups on the basis of the structure and number of zinc-finger motifs. Reportedly, 74 WRKY proteins are encoded in the genome of *Arabidopsis thaliana* (Eulgem et al. 2000; Dong et al. 2003). WRKY proteins act as a central switch in regulating various cellular processes such as seed germination, root development, plant growth, seed development, and senescence. Apart from the cellular processes, WRKY proteins play a major role in biotic and abiotic stress responses.

21.8.1 Role of WRKY Proteins in Biotic and Abiotic Stresses

Attacks caused by several pathogens lead to a series of plant defense responses. WRKY proteins play a role in central stage during such responses. The proteins carry out their functions in varied ways such as protein-protein interactions,

cross-regulation, as well as autoregulation. WRKY transcription factors are known to regulate various responses against stress through modulating phytohormone signaling pathways such as SA, jasmonic acid, and ethylene pathways. In some cases, overexpression of WRKY genes leads to regulation of resistance responses against pathogens. One example of such a case is overexpression of *Capsicum annum* WRKY protein, CaWRKY27 in tobacco. The overexpressed gene provided resistance against *Ralstonia solanacearum* (Dang et al. 2014; Shi et al. 2014; Wang et al. 2014). WRKY proteins are also involved in the responses induced by wounds. Studies have showed that WRKY8 in *Arabidopsis thaliana* modulates its susceptibility against pathogens like *Pseudomonas syringae*. Further, WRKY8 also regulates crosstalks between ABA and ethylene phytohormone pathways providing resistance against pathogens (Chen et al. 2013). Many WRKY transcription factors demonstrate roles as a positive regulator of resistance responses. However, majority of WRKY transcription factors are known to have a negative regulatory role. *AtWRKY38* and *AtWRKY62* are two structurally identical type III WRKY transcription factors of *Arabidopsis thaliana* and demonstrate negative regulation of defense against bacterial pathogen *Pseudomonas syringae.* Overexpression of these genes reduced resistance against disease (Kim et al. 2008a, b). In situations where WRKY genes positively regulate resistance, they modulate expression of resistance genes directly by binding to the W-box in the resistance genes.

Further, WRKY proteins have a major role in combat against abiotic stresses. On perception of any abiotic stress stimuli, various WRKY proteins are induced which function together to confer resistance against abiotic stress. Microarray profiling in *Arabidopsis thaliana* indicated upregulation of 18 WRKY genes in response to salt stress (Jiang and Deyholos 2006). Thus, it indicates a sharp increase in expression of WRKY gene on perception of stress signals. The accumulated WRKY proteins specifically bind to the *cis*-acting response elements in target genes, thereby modulating transcription. The protein AtWRKY6 also has a role in plant senescence and low-Pi stress response (Chi et al. 2013). Further, WRKY38 and WRKY62 of *Arabidopsis thaliana* interact with histone deacetylase 19 (HDA19) and regulate basal defense responses of plants against abiotic stress. This is done by maintaining the levels of acetyl groups on histone tails (Kim et al. 2008a, b).

21.8.2 WRKY-Dependent Signaling Pathways

Being centrally involved in critical stress responses, there is extensive regulation of signaling pathways by WRKY proteins. On perceiving a stress stimulus, WRKY proteins bind to W-Box and trigger the expression of target genes. This induction of target genes is mostly autoregulated via WRKY proteins or cross-regulated by different WRKY transcription factors. In *Arabidopsis thaliana*, three WRKY proteins belonging to group IIa (*AtWRKY18*, *AtWRKY40*, and *AtWRKY60*) possess a leucine zipper motif at the N-terminal through which they interact with each other (Xu et al. 2006). In Parsley, PcWRKY1 has affinity to the promoter of another

WRKY, PcWRKY43 (Turck et al. 2004). The expression of WRKY33 is induced by MAPK3/6. WRKY33 also autoregulates its expression through a positive feedback loop by binding to its own promoter (Mao et al. 2011). It is reported that cross-regulation among WRKY25, WRKY26, as well as WRKY33 is essentially important in withstanding high temperatures (Li et al. 2011). However, AtWRKY18, AtWRKY40, and AtWRKY60 negatively regulate expression patterns by binding to their own promoters (Li 2014). Thus, cross-regulation and autoregulation are essential in maintaining the balance of WRKY transcription factors in the cell. WRKY proteins belonging to group IId in *Arabidopsis thaliana* possess a short amino acid sequence called a C-motif (DxxVxKFKxVISLLxxxR) (Chi et al. 2013), which is a CaM binding site, indicating that these WRKY proteins might be regulated by CaM and Ca^{2+}. In *Arabidopsis thaliana*, WRKY proteins are targets of 14-3-3 proteins (Ishida et al. 2004). The 14-3-3 proteins are highly conserved regulatory proteins which interact with other proteins in a phosphorylation-dependent manner. The 14-3-3 proteins dimerize and bring both phosphorylated and unphosphorylated ligands together through interactions with the dimer. In case the WRKY proteins have phosphorylated binding sites, they indirectly interact with other proteins to form complexes thereby consequently participating in many cellular events.

21.8.3 Interaction of WRKY Proteins in Control of Plant Immunity by MAPKs

Mitogen-activated protein kinase (MAPK) signaling cascade is an important component downstream in the signaling of ABA-dependent defensive responses in the plants. These MAPKs are involved in the regulation of growth and development as well as in responses to various stresses via multiple phosphorylation events (Fiil et al. 2009; Ishihama and Yoshioka 2012). WRKY TFs containing a conserved motif in the N-terminal region are stimulated by MAPK-dependent phosphorylation, highlighting their significance in plant immunity (Ishihama and Yoshioka 2012). The WRKY33 transcription factor of *Arabidopsis thaliana* forms a complex with MAP kinase 4 (MPK4), when there is no pathogen infection. Upon infection, MPK4 is activated and phosphorylates its substrate MKS1 which disrupts the MPK4-MKS1-WRKY33 complex, leading to the release of AtWRKY33. The released AtWRKY33 then induces the expression of target genes for defense responses (Qiu et al. 2008). Further, AtWRKY22 and AtWRKY29 are essential components in MAPK-mediated resistance against bacterial and fungal pathogens. In *Arabidopsis thaliana*, transitory expression of AtWRKY29 provides resistance to pathogens (Asai et al. 2002). In rice, the OsWRKY30 of rice increases the resistance against drought through MAPK phosphorylation cascade (Danquah et al. 2014). Additionally, the MAPK and WRKY interaction pathway is also essential for burst of reactive oxygen species, produced by activation of RBOHB and NADPH oxidase (Adachi et al. 2015, Jiang et al. 2017).

21.8.4 Role of WRKY Proteins in Phytohormone Signaling

WRKY transcription factors play a vital role in salicylic acid (SA)- and abscisic acid (ABA)-mediated signaling pathways. In response to high temperature, SA, or methyl jasmonic acid (MeJA) treatment, there is induction of AtWRKY39 transcription factor which participates in the regulation of SA and JA signaling pathways (Li et al. 2010). Genes encoding AtWRKY38 or AtWRKY62, when overexpressed, negatively control resistance of plants against pathogens by inhibiting the SA-induced expression of the defensive gene Pathogenesis-Related 1 (*AtPR1*) (Kim et al. 2008a, b). In rice, *OsWRKY45* plays an important role in SA-mediated defensive responses. Its inhibition leads to impaired SA-mediated resistance while its overexpression significantly boosts resistance (Shimono et al. 2007). ABA has a major role in integrating various stress signaling pathways. WRKY TFs are also involved in ABA-mediated signaling pathways which control stress tolerance. In *Larrea tridentata*, WRKY21 regulates the promoter of *HVA22*, an ABA inducible gene by upregulating its expression through collective interactions with ABA and transcriptional activators such as VP1 and ABI5 (Zou et al. 2004). ChIP assays indicate direct binding of WRKY57 with W-box of Responsive to Desiccation 29A (*RD29A*) and promoter of gene encoding 9-cis-epoxycarotenoid dioxygenase 3 (*NCED3*), thereby initiating the gene expression (Jiang et al. 2012). Similarly, AtWRKY40 binds to the W-box of several genes induced by ABA such as *AtABF4*, *AtABI4*, *AtABI5*, *AtDREB1A*, *AtMYB2*, and *AtRAB18,* resulting in inhibition of their expression (Shang et al. 2010). A cucumber WRKY gene, *CsWRKY46*, is reported to be upregulated during cold stress and exogenous treatment of ABA (Zhang et al. 2016). Overexpression of *CsWRKY46* in transgenic *Arabidopsis thaliana* leads to higher seedling survival rates on very low temperatures, enhanced proline accumulation, less leakage of electrolyte, and much lower malondialdehyde (MDA) levels as well as hypersensitivity to ABA during germination of seeds (Zhang et al. 2016). *CmWRKY1* isolated from *Chrysanthemum morifolium* plays a vital role in the response to drought stress by an ABA-mediated pathway (Fan et al. 2016). Transgenic lines overexpressing this gene exhibit increased dehydration tolerance in response to polyethylene glycol (PEG) treatment (Fan et al. 2016). Further, the transgenic plants also exhibit reduced expression levels of genes negatively regulated by ABA (Jiang et al. 2017).

21.9 Mediator Complex as an Integrator

Mediator complex (MED) is a multi-protein complex that acts as a cofactor in regulation of basic transcription mechanism resulting in increase or decrease of transcription rate. This complex was originally discovered in yeast. It has been discovered in majority of eukaryotes (Boube et al. 2002; Bourbon 2008; Bourbon et al. 2004). Sequence homology studies hinted to the presence of mediator complex in plants, and these complexes have been purified from several plants (Boube

et al. 2002; Gonzalez et al. 2007; Backstrom et al. 2007). Analysis of these complexes, purified from plants, indicated that twenty-one subunits were conserved in all eukaryotes, while other six subunits were plant specific. MED complex enhances RNA polymerase II attachment to coding genes and stabilizes the machinery for transcription. Mediator complex is regarded as one of the major converging hubs for different signaling networks, responsive to various developmental and environmental changes. Various hormonal pathways converge and regulate MED subunit genes.

21.9.1 Hormonal Regulation of MED Complex

Various hormones have different effects on varied subunits of MED complex in *Arabidopsis thaliana.* Transcription of MED genes is significantly stimulated by Brassinosteroid and ABA. In comparison to these hormones, auxin and jasmonic acid affect the transcription of MED complex genes in a different manner. BR treatment upregulates plant-specific *AtMED37* mediator subunit as well as *AtMED12* (Gillmor et al. 2010). JA increases transcription levels of *AtMED18* by twofold (Zheng et al. 2013; Lai et al. 2014). Auxin downregulates the transcription levels of *AtMED15*, *AtMED5*, as well as *AtMED14* belonging to the tail module of the complex. Different environmental factors such as light, dark, cold, as well as high salinity also stimulate expression levels of mediator subunits (Samanta and Thakur 2015). Upregulation of mediator subunits by different environmental cues makes them an integrative hub where different signaling pathways merge.

21.9.2 Role of MED Complex in Abiotic Stress Signaling

Mediator complex plays a vital role in integrating signaling pathways in response to various abiotic stresses. Two MED subunits that essentially integrate these responses are MED25 and MED16. MED25 subunit regulates salinity as well as drought stress. Seeds of *AtMED25* mutant of *Arabidopsis thaliana* exhibit increased sensitivity to salt stress during seed germination. The role of MED25 in responses to high salinity has been established across many plant species (Elfving et al. 2011). MED25 associates with stress-responsive transcription factors such as DREB2A, ZFHD1, as well as MYB and communicates with transcriptional machinery to bring about salt-responsive alterations in plants. In comparison to salt stress, MED25 negatively controls tolerance against drought in plants (Elfving et al. 2011). Expression levels of drought-responsive genes drastically increase in *Atmed25* mutants. Further, MED16 is involved in tolerance against cold responses (Knight et al. 1999, 2008; Wathugala et al. 2011). In mutants of *MED16*, expression of cold-responsive genes such as *LT178*, *COR15A*, and *KIN1/2* is not stimulated resulting in lack of tolerance against freezing temperature.

21.9.3 Role of MED Complex in Biotic Stress Signaling

Emerging studies have established the key role of MED complex in signaling against biotic stresses (An and Mou 2013). *Arabidopsis thaliana* subunit AtMED25 was the first subunit to be reportedly involved in defense responses (Kidd et al. 2009). MED25 provides protection against *Alternaria brassicicola* as well as *Botrytis cinerea* by affecting the expression of jasmonic acid-dependent genes. AtMED25 associates with a group of transcription factors such as BHLH, bZIP, MYB, AP2/ERF, as well as WRKY, some of which are known to be involved in JA signaling pathway (Çevik et al. 2012; Chen et al. 2012). MED21 has a probable suggested role during defense signaling (Dhawan et al. 2009). In *Arabidopsis thaliana*, the head module interacts with the effector of the fungus *Hyaloperonospora arabidopsidis*. This interaction results in proteasomic degradation of MED subunit which causes disbalance in regulation pathways, thereby weakening plant immunity against pathogen attack. Similarly, AtMED18 is known to exhibit a positive role toward fungal infection (Lai et al. 2014). Apart from these, three subunits from the tail region of the complex also participate in defense signaling (Canet et al. 2012; Zhang et al. 2012a, b, 2013). Further, AtCDK8, a kinase module component, interacts with AtMED25 and positively regulates disease response (Samanta and Thakur 2015).

21.10 Concluding Remarks

Plants are sessile organisms; however, their diversity and resilience to survive hostile conditions suggest that plants have developed ways to adjust and thrive in diverse conditions. Plants can sense perturbations in environmental conditions and adjust their growth and metabolism to survive those conditions. In fact, different mechanisms have been adopted by plants to sense different stresses, with these stress response pathways converging at some or the other control points in the signaling cascade. As growth is altered under stresses and considering the fact that light plays an important role in regulating plant growth, and that alterations in growth are executed by various hormones and their downstream components, it is highly likely that these response pathways share common components which are involved in responses to light as well as different stresses such as Della proteins, PIFs, MAPKs, calcium, COP proteins, ubiquitin-proteasome components, and WRKY transcription factors. These components do the job of mounting an integrated response to various signals. Hence, perception of stress and elicitation of growth alterations are indeed a multicomponent response needing integration of various cellular signals at each step.

Acknowledgments AKS acknowledges the continuous financial support of DBT as project grants numbered BT/PR6983/PBD/16/1007/2012 and BT/COE/34/SP15209/2015, infrastructure support of UGC in the form of SAP to the department, financial support of DST in the form of Purse grant, and in the form of infrastructure support in FIST programme to the department. The work in the laboratory of YM is funded by grant from DBT (project No. BT/BPA/118/206/2016), DST (EMR/2016/002780), and DU-DST Purse.

References

Achard P, Cheng H, Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 331:91–94

Achard P, Renou JP, Berthome R, Harberd NP, Genschik P (2008) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol 18:656–660

Adachi H, Nakano T, Miyagawa N, Ishihama N, Yoshioka M, Katou Y, Yaeno T, Shirasu K, Yoshioka H (2015) WRKY transcription factors phosphorylated by MAPK regulate a plant immune NADPH oxidase in Nicotiana benthamiana. Plant Cell 27:2645–2663

An C, Mou Z (2013) The function of the Mediator complex in plant immunity. Plant Signal Behav 8:e23182

Ariizumi T, Steber CM (2007) Seed germination of GA-insensitive sleepy1 mutants does not require RGL2 protein disappearance in Arabidopsis. Plant Cell 19:791–804

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. Nature 415:977–983

Backstrom S, Elfving N, Nilsson R, Wingsle G, Bjorklund S (2007) Purification of a plant mediator from Arabidopsis thaliana identifies PFT1 as the Med25 subunit. Mol Cell 26:717–729

Bernardo-Garcia S, de Lucas M, Martınez C, Espinosa-Ruiz A, Daviere JM, Prat S (2014) BR-dependent phosphorylation modulates PIF4 transcriptional activity and shapes diurnal hypocotyl growth. Genes Dev 28:1681–1694

Bianchi E et al (2003) Characterization of human constitutivephotomorphogenesis protein 1, a RING finger ubiquitin ligase thatinteracts with Jun transcription factors and modulates theirtranscriptional activity. J Biol Chem 278:19682–19690

Boube M, Joulia L, Cribbs DL, Bourbon H-M (2002) Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell 110:143–151

Bourbon HM (2008) Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex. Nucleic Acids Res 36:3993–4008

Bourbon H-M, Aguilera A, Ansari AZ, Asturias FJ, Berk AJ, Bjorklund S, Blackwell TK, Borggrefe T, Carey M, Carlson M, Conaway JW, Conaway RC, Emmons SW, Fondell JD, Freedman LP, Fukasawa T, Gustafsson CM, Han M, He X, Herman PK, Hinnebusch AG, Holmberg S, Holstege FCP, Jaehning JA, Kim YJ, Kuras L, Leutz A, Lis JT, Meisterernest M, Naar AM, Nasmyth K, Parvin JD, Ptashne M, Reinberg D, Ronne H, Sadowski I, Sakurai H, Sipiczki M, Sternberg PW, Stillman DJ, Strich R, Struhl K, Svejstrup JQ, Tuck S, Winston F, Roeder RG, Kornberg RD (2004) A unified nomenclature for protein subunits of mediator complexes linking transcriptional regulators to RNA polymerase II. Mol Cell 14:553–557

Cai M et al (2008) Identification of novel pathogen-responsive cis-elements and their binding proteins in the promoter of OsWRKY13, a gene regulating rice disease resistance. Plant Cell Environ 31:86–96

Calderini O, Bögre L, Vicente O, Binarova P, Heberle-Bors E, Wilson C (1998) A cell cycle regulated MAP kinase with a possible role in cytokinesis in tobacco cells. J Cell Sci 111:3091–3100

Campos ML, Yoshida Y, Major IT, de Oliveira FD, Weraduwage SM, Froehlich JE, Johnson BF, Kramer DM, Jander G, Sharkey TD (2016) Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. Nat Commun 7:12570

Canet JV, Dobón A, Pablo Tornero P (2012) Non-recognition-of-BTH4, an Arabidopsis mediator subunit homolog, is necessary for development and response to salicylic acid. Plant Cell 24:4220–4235

Cao D, Hussain A, Cheng H, Peng J (2005) Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. Planta 223:105–113

Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H (2000) Differential activation of four specific MAPK pathways by distinct elicitors. J Biol Chem 275:36734–36740

Çevik V, Kidd BN, Zhang P, Hill C, Kiddle S, Denby KJ (2012) MEDIATOR 25 acts as an integrative hub for the regulation of jasmonate- responsive gene expression in Arabidopsis. Plant Physiol 160:541–555

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two-component regulators. Science 262:539–544

Chapman EJ, Estelle M (2009) Mechanism of auxin - regulated gene expression in plants. Annu Rev Genetics 43:265–285

Chattopadhyay S, Ang LH, Puente P, Deng XW, Wei N (1998) *Arabidopsis thaliana* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. Plant Cell 10:673–683

Chen R, Jiang H, Li L, Zhai Q, Qi L, Zhou W et al (2012) The Arabidopsis Mediator subunit MED 25 differentially regulates jasmonate and abscisic acid Signalling through interacting with the MYC2 and ABI5 transcription factors. Plant Cell 24:2898–2916

Chen L, Zhanga L, Li D, Wanga F, Yu D (2013) WRKY8 transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in Arabidopsis. Proc Natl Acad Sci E1963–E1971

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131:1055–1064

Chi Y, Yang Y, Zhou Y, Zhou J, Fan B, Yu JQ, Chen Z (2013) Protein-protein interactions in the regulation of WRKY transcription factors. Mol Plant 6:287–300

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448:666–673

Claudia J, Lászlo Ö, Lászlo B, Heribert H (2002) Complexity, cross talk and integration of plant MAP kinase Signalling. Curr Opin Plant Biol 5:415–424

Dang F, Wanga Y, Shea J, Leia Y, Liua Z, Eulgemd T, Laia Y, Lina J, Yua L, Leia D, Guanb D, Lia X, Yuana Q, He S (2014) Overexpression of CaWRKY27, a subgroup IIe WRKY transcription factor of *Capsicum annuum*, positively regulates tobacco resistance to Ralstonia solanacearum infection. Physiol Plant 150:39–411

Danquah A, de Zelicourt A, Colcombet J, Hirt H (2014) The role of ABA and MAPK signaling pathways in plant abiotic stress responses. Biotech Adv 32:40–52

Deng X-W, Caspar T, Quail PH (1991) Copl: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. Genes Dev 5:1172–1182

Deng X-W, Matsui M, Wei N, DorisWagner D, Chu AM, Feldmann KA, Quail PH (1992) COP1, an arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gβhomologous domain. Cell 71:791–801

Dhawan R, Luo H, Foerster AM, Abuqamar S, Du H-N, Briggs SD et al (2009) HISTONE MONOUBIQUITINATION1 Interacts with a subunit of the mediator complex and regulates defense against necrotrophic fungal pathogens in Arabidopsis. Plant Cell 21:1000–1019

Dieterle M, Buche C, Schafer E, Kretsch T (2003) Characterization of a novel non-constitutive photomorphogenic cop1 allele. Plant Physiol 133:1557–1564

Dill A, Sun T-p (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. Genetics 159:777–785

Dong J, Chen C, Chen Z (2003) Expression profilesof the Arabidopsis *WRKY* gene superfamily during plant defence response, Plant Mol. Biol 51:21–37

Elfving N, Davoine C, Benlloch R, Blomberg J, Brännström K, Muller D et al (2011) The Arabidopsis thaliana Med25 Mediator subunit integrates environmental cues to control plant development. Proc Natl Acad Sci U S A 108:8245–8250

Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcriptionfactors. Trends Plant Sci 5:199–206

Fan Q, Song A, Jiang J, Zhang T, Sun H, Wang Y, Chen S, Chen F (2016) CmWRKY1 enhances the dehydration tolerance of chrysanthemum through the regulation of ABA-associated genes. PLoS ONE 11:e0150572

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico J-M, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, Pauwels L, Witters E, Puga MI, Paz-Ares J, Goossens A, Reymond P, De Jaeger G, Solano R (2011) The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell 23:701–715

Fiil BK, Petersen K, Petersen M, Mundy J (2009) Gene regulation by MAP kinase cascades. Curr Opin Plant Biol 12:615–621

Franklin KA, Lee SH, Patel D, Kumar SV, Spartz AK, Gu C, Ye S, Yu P, Breen G, Cohen JD, Wigge PA, Gray WM (2011) PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. Proc Natl Acad Sci U S A 108:20231–20235

Fu ZQ, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N, Dong X (2012) NRP3 and NRP4 are receptors for the immune signal salicylic acis in plants. Nature 486:228–232

Gan Y, Yu H, Peng J, Pierre Broun P (2007) Genetic and molecular regulation by DELLA proteins of Trichome development in Arabidopsis. Plant Physiol 145:1031–1042

Gallego-Bartolome J, Alabadi D, Blázquez M (2011) DELLA-induced early transcriptional changes during etiolated development in *Arabidopsis thaliana*. PLoS One 6:e23918

Gillmor CS, Park MY, Smith MR, Pepitone R, Kerstetter RA, Poethig RS (2010) The MED12-MED13 module of Mediator regulates the timing of embryo patterning in *Arabidopsis thaliana*. Development 137:113–122

Gonzalez D, Bowen AJ, Carroll TS, Conlan RS (2007) The transcription corepressor LEUNIG interacts with the histone deacetylase HDA19 and mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. Mol Cell Biol 27:5306–5315

Harper JF, Breton G, Harmon A (2004) Decoding Ca2+ signals through plant protein kinases. Annu Rev Plant Biol 55:263–288

Hersch M, Lorrain S, de Wit M, Trevisan M, Ljung K, Bergmann S, Fankhauser C (2014) Light intensity modulates the regulatory network of the shade avoidance response in *Arabidopsis*. Proc Natl Acad Sci U S A 111:6515–6520

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479

Hou X, Lee LYC, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell 19:884–894

Hou X, Lihua Ding L, Yu H (2013) Crosstalk between GA and JA signaling mediates plant growth and defense. Plant Cell Rep 32:1067–1074

Hua Z, Zou C, Shiu SH, Vierstra RD (2011) Phylogenetic comparison of F-Box (FBX) gene superfamily within the plant kingdom reveals divergent evolutionary histories indicative of genomic drift. PLoS ONE 6:e16219

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. Plant Cell 13:999–1010

Ishida S, Fukazawa J, Yuasa T, Takahashi Y (2004) Involvement of 14-3-3 signalling protein binding in the functional regulation of the transcriptional activator REPRESSION OF SHOOT GROWTH by gibberellins. Plant Cell 16:2641–2651

Ishihama, Yoshioka H (2012) Post-translational regulation of WRKY transcription factors in plant immunity. Curr Opin Plant Biol 15:431–437

Jiang YQ, Deyholos MK (2006) Comprehensive transcriptional profiling of NaCl-stressed *Arabidopsis thaliana* roots reveals novel classes of responsive genes. BMC Plant Biol 6:article25

Jiang Y, Liang G, Yu D (2012) Activated expression of WRKY57confers drought tolerance in Arabidopsis. Mol Plant 5:1375–1388

Jiang J, Ma S, Ye N, Jiang M, Cao J, Zhang J (2017) WRKY transcription factors in plant responses to stresses. J Integr Plant Biol 59:86–101

Jung JH, Domijan M, Klose C, Biswas S, Ezer D, Gao M, Khattak AK, Box MS, Charoensawan V, Cortijo S (2016) Phytochromes function as thermosensors in *Arabidopsis*. Science 354:886–889

Khanna R, Huq E, Kikis EA, Al-Sady B, Lanzatella C, Quail PH (2004) A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. Plant Cell 16:3033–3044

Khanna R, Shen Y, Marion CM, Tsuchisaka A, Theologis A, Schafer E, Quail PH (2007) The basic helix-loop-helix transcription factor PIF5 acts on ethylene biosynthesis and phytochrome signaling by distinct mechanisms. Plant Cell 19:3915–3929

Kidd BN, Edgar CI, Kumar KK, Aitken EA, Schenk PM, Manners JM et al (2009) The Mediator complex subunit PFT1 is a key regulator of jasmonate-dependent defence in Arabidopsis. Plant Cell 21:2237–2252

Kim DH, Yamaguch S, Lim S, Oh E, Park J, Hanada A, Kamiya Y, Choi G (2008a) SOMNUS, a CCCH-type zinc finger protein in *Arabidopsis*, negatively regulates light-dependent seed germination downstream of PIL5. Plant Cell 20:1260–1277

Kim KC, Lai Z, Fan B, Chen Z (2008b) Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defence. Plant Cell 20:2357–2371

King K, Moritz T, Harberd NP (2001) Gibberellins are not required for normal stem growth in Arabidopsis thaliana in the absence of GAI and RGA. Genetics 159:767–776

Knight H (2002) Calcium signalling during abiotic stress in plants. Int Rev Cytol 195:269–324

Knight H, Veale EL, Warren GJ, Knight MR (1999) The sfr6 mutation in Arabidopsis suppresses low-temperature induction of genes dependent on the CRT/DRE sequence motif. Plant Cell 11:875–886

Knight H, Thomson AJW, McWatters HG (2008) Sensitive to freezing6 integrates cellular and environmental inputs to the plant circadian clock. Plant Physiol 148:293–303

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol 5:33–36

Lai Z, Schluttenhofer CM, Bhide K, Shreve J, Thimmapuram J, Lee SY et al (2014) MED18 interaction with distinct transcription factors regulates multiple plant functions. Nat Commun 5:3064

Lee S, Cheng H, King KE, Wang W, Husssain A, Lo J, Harberd NP, Peng J (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is upregulated following imbibition. Genes Dev 16:646–658

Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao H, Lee I, Deng X-W (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 19:731–749

Lee HJ, Jung JH, Llorca LC, Kim SG, Lee S, Baldwin IT, Park CM (2014) FCA mediates thermal adaptation of stem growth by attenuating auxin action in *Arabidopsis*. Nat Commun 5:5473

Legris M, Nieto C, Sellaro R, Prat S, Casal JJ (2017) Perception and signalling of light and temperature cues in plants. Plant J 90:683–697

Li J (2014) Role of WRKY transcription factors in Arabidopsis development and stress responses. Helsinki University Printing House, Helsinki

Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 90:929–938

Li S, Zhou X, Chen L, Huang W, Yu D (2010) Functional characterization of Arabidopsis thaliana WRKY39 in heat stress. Mol Cells 29:475–483

Li SJ, Fu QT, Chen LG, Huang WD, Yu DQ (2011) *Arabidopsis thalianathaliana* WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. *Planta* 233:1237–1252

Li L, Ljung K, Breton G, Schmitz RJ, Pruneda-Paz J, Cowing-Zitron C, Cole BJ, Ivans LJ, Pedmale UV, Jung HS, Ecker JR, Kay SA, Chory J (2012) Linking photoreceptor excitation to changes in plant architecture. Genes Dev 26:785–790

Lim S, Park J, Lee N, Jeong J, Toh S, Watanabe A, Kim J, Kang H, Kim DH, Kawakami N (2013) ABA-insensitive3, ABA-insensitive5, and DELLAs interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in Arabidopsis. Plant Cell 25:4863–4878

Lozano-Duran R, Zipfel C (2015) Trade-off between growth and immunity: role of brassinosteroids. Trends Plant Sci 20:12–19

Luan S, Kudla J, Rodriguez-Concepcion M, Yalovsky S, Wilhelm Gruissem (2002) Calmodulins and Calcineurin B–like proteins: calcium sensors for specific signal response coupling in plants. Plant Cell S389–S400

Luan S, Lan W, Lee SC (2009) Potassium nutrition, sodium toxicity, and calcium signaling: connections through the CBL–CIPK network. Curr Opin Plant Biol 12:339–346

Ma L, Gao Y, Qu L, Chen Z, Li J, Zhao H et al (2002) Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in Arabidopsis. Plant Cell 14:2383–2398

Maa D, Li X, Guob Y, Chuc J, Fangc S, Yanc C, Noel JP, Liu H (2016) Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proc Natl Acad Sci 113:224–229

Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S (2011) Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis thaliana*. Plant Cell 23:1639–1653

Martín G, Soy J, Monte E (2016) Genomic analysis reveals contrasting PIFq contribution to diurnal rhythmic gene expression in PIF-induced and –repressed genes. Front Plant Sci 7:962

Mockaitis K, Howell SH (2000) Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of *Arabidopsis* seedlings. Plant J 24:785–796

Munnik T, Ligterink W, Meskiene I, Calderini O, Beyerly J (1999) Musgrave A and Hirt H (1999) Distinct osmo-sensing protein kinase pathways are involved in signalling moderate and severe hyper-osmotic stress. Plant J 20:381–388

Nozue K, Covington MF, Duek PD, Lorrain AA, Fankhauser C, Harmer SL, Maloof JN (2007) Rhythmic growth explained by coincidence between internal and external cues. Nature 448:358–361

Oh E, Yamaguchi S, Huc J, Yusukeb J, Jung B, Paik I, Leed HS, Sun TP, Kamiya Y, Choi G (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by directly binding to the GAI and RGA promoters in *Arabidopsis* seeds. Plant Cell 19:1192–1208

Oh E, Kang H, Yamaguchi S, Park J, Lee D, Kamiya Y, Choi G (2009) Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in *Arabidopsis*. Plant Cell 21:403–419

Oh E, Zhu JY, Wang ZY (2012) Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol 14:802–809

Oh E, Zhu JY, Bai MY, Arenhart RA, Sun Y, Wang ZY (2014) Cell elongation is regulated through a central circuit of interacting transcription factors in the *Arabidopsis* hypocotyl. elife 3:e03031

Park J, Lee N, Kim W, Lim S, Choi G (2011) ABI3 and PIL5 collaboratively activate the expression of SOMNUS by directly binding to its promoter in imbibed *Arabidopsis* seeds. Plant Cell 23:1404–1415

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev 11:3194–3205

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell 20:2729–2745

Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, Palma K, Suarez-Rodriguez MC, Sandbech-Clausen S, Lichota J et al (2008) Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J 27:2214–2221

Reddy VS, Ali GS, Reddy ASN (2002) Genes encoding calmodulin-binding proteins in the Arabidopsis genome. J Biol Chem 277:9840–9852

Samanta S, Thakur JK (2015) Importance of Mediator complex in the regulation and integration of diversesignaling pathways in plants. Front Plant Sci 6(6):757

Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signalling. Nature 459:1071–1078

Serino G, Deng XW (2003) The COP9 signalosome: regulating plant development through the control of proteolysis. Annu Rev Plant Biol 54:165–182

Shang Y, Yan L, Liu ZQ, Cao Z, Mei C, Xin Q, Wu FQ, Wang XF, Du SY, Jiang T, Zhang XF, Zhao R, Sun HL, Liu R, Yu YT, Zhang DP (2010) The Mg-chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. Plant Cell 22:1909–1935

Shen H, Zhu L, Bu Y and Huq E (2012) MAX2 Affects Multiple Hormones to Promote Photo morphogenesis Mol Plant 5: 750–762

Shi H, Wang X, Cheng F (2014) The Cys2/His2-type zinc finger transcription factor ZAT6 modulates biotic and abiotic stress responses by activating salicylic acid-related genes and CBFs in Arabidopsis. Plant Physiol 165:1367–1379

Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. Plant Cell 19:2064–2076

Silverstone AL, Ciampaglio CN, T-p S (1998) The Arabidopsis RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. Plant Cell 10:155–169

Silverstone AL, Jung HS, Dill A, Kawaide H, Kamiya Y, T-p S (2001) Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. Plant Cell 13:1555–1566

Stacey MG et al (2000) Modular domain structure of Arabidopsis COP1. Reconstitution of activity by fragment complementation andmutational analysis of a nuclear localization signal in planta. Plant Physiol 124:979–990

Sun C, Palmqvist S, Olsson H, Borén M, Ahlandsberg S, Jansson C (2003) A novel WRKY transcription factor, SUSIBA2, participates in sugar signalling in barley by binding to the sugar responsive elements of the iso1 promoter. Plant Cell 15:2076–2092

Sun J, Qi L, Li Y, Chu J, Li C (2012) PIF4-mediated activation of YUCCA8 expression integrates temperature into the auxin pathway in regulating *Arabidopsis* hypocotyl growth. PLoS Genet 8:e1002594

Turck F, Zhou A, Somssich IE (2004) Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to its native promoter and the defense-related gene *PcPR1-1* in Parsley. *Plant Cell* 16:2573–2585

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol 135:1008–1019

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. Plant Cell 11:1743–1754

Vert G, Nemhauser JL, Geldner N, Hong F, Chory J (2005) Molecular mechanisms of steroid hormone signaling in plants. Annu Rev Cell Dev Biol 21:177–201

Wang ZY, Bai MY, Oh E, Zhu JY (2012) Brassinosteroid signaling network and regulation of photomorphogenesis. Annu Rev Genet 46:701–724

Wang W, Bai M-Y, Wang Z-Y (2014) The brassinosteroid signaling network — a paradigm of signal integration. Curr Opin Plant Biol 21:147–153

Wathugala DL, Richards SA, Knight H, Knight MR (2011) OsSFR6 is a functional rice orthologue of SENSITIVE TO FREEZING-6 and can act as a regulator of COR gene expression, osmotic stress and freezing tolerance in Arabidopsis. New Phytol 191:984–995

Wei Z, Yuan T, Tarkowska D, Kim J, Nam HG, Nova O, He K, Gou X, Li J (2017) Brassinosteroid biosynthesis is modulated via a transcription factor cascade of COG1, PIF4, and PIF5. Plant Physiol 174:1260–1273

Weingartner M, Binarova P, Drykova D, Schweighofer A, David JP, Heberle-Bors E, Doonan J, Bogre L (2001) Dynamic recruitment of Cdc2 to specific microtubule structures during mitosis. Plant Cell 13:1929–1943

Wen CK, Chang C (2002) Arabidopsis RGL1 encodes a negative regulator of gibberellin responses. Plant Cell 14:87–100

Wild M, Davière JM, Cheminant S, Regnault T, Baumberger N, Heintz D, Baltz R, Genschik P, Achard P (2012) The Arabidopsis DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell 24:3307–3319

Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penfold CA, Baxter L, Breeze E (2012) *Arabidopsis* defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell 24:3530–3557

Xie DX, Feys BF, James S, Nieto-Rostro M and Turner JG (1998) COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility Science 280, 1091–1094

Xie Z, Zhang ZL, Zou X, Huang J, Ruas P, Thompson D, Shen QJ (2005) Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signalling in aleurone cells. Plant Physiol 137:176–189

Xu X, Chen C, Fan B, Chen Z (2006) Physical and functional interactions between pathogen-induced *Arabidopsis thaliana WRKY18, WRKY40*, and *WRKY60* transcription factors. Plant Cell 18:1310–1326

Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, Aoki M, Seki E, Matsuda T, Tomo Y et al (2005) Solution structure of an Arabidopsis WRKY DNA binding domain. Plant Cell 17:944–956

Yang T, Poovaiah BW (2003) Calcium/calmodulin-mediated signal network in plants. Trends Plant Sci 8:505–512

Yi C et al (2002) An initial biochemical and cell biological characterization of the mammalian homologue of a central plant developmental switch, COP1. BMC Cell Biol 3:30

Yu H, Ito T, Zhao Y, Peng J, Kumar P, Elliot M, Meyerowitz EM (2004) Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci U S A 101:7827–7832

Zhang S, Klessig DF (2001) MAPK cascades in plant defense signaling. Trends Plant Sci 6:520–527

Zhang H, Li D, Wang M, Liu J, Teng W, Cheng B, Huang Q, Wang M, Song W, Dong S, Zheng X, Zhang Z (2012a) The Nicotiana benthamiana mitogen-activated protein kinase cascade and WRKY transcription factor participate in Nep1(Mo)-triggered plant responses. Mol Plant Microbe Interact 25:1639–1653

Zhang X, Wang C, Zhang Y, Sun Y, Mou Z (2012b) The Arabidopsis Mediator complex subunit16 positively regulates salicylate-mediated systemic acquired resistance and jasmonate/ethylene-induced defense pathways. Plant Cell 24:4294–4309

Zhang X, Yao J, Zhang Y, Sun Y, Mou Z (2013) The Arabidopsis mediator complex subunits MED14/SWP and MED16/SFR6/IEN1 differentially regulate defence gene expression in plant immune responses. Plant J 75:484–497

Zhang Y, Yu H, Yang X, Li Q, Ling J, Wang H, Gu X, Huang S, Jiang W (2016) CsWRKY46, a WRKY transcription factor from cucumber, confers cold resistance in transgenic-plant by regulating a set of cold-stress responsive genes in an ABA-dependent manner. Plant Physiol Biochem 108:478–487

Zheng Z, Guan H, Leal F, Grey PH, Oppenheimer DG (2013) Mediator subunit18 controls flowering time and floral organ identity in Arabidopsis. PLoS ONE 8:e53924

Zhong S, Shi H, Xue C, Wei N, Guo H, Deng XW (2014) Ethylene-orchestrated circuitry coordinates a seedling's response to soil cover and etiolated growth. Proc Natl Acad Sci U S A 111:3913–3920

Zou X, Seemann JR, Neuman D, Shen QJ (2004) A WRKY gene from creosote bush encodes an activator of the abscisic acid signaling pathway. J Biol Chem 279:55770–55779

Priya Gambhir is currently pursuing her doctoral studies with Prof. Arun Kumar Sharma. She is working on characterization of ethylene response factors (ERFs) involved in tomato fruit ripening. She is characterizing some of the ERFs which show altered expression in *rin* mutant, which is impaired in fruit ripening in tomato.

Diksha Bhola obtained her BSc in Botany from the University of Delhi and MSc in Microbial Technology from the Amity University of Noida. Currently, she is working as a Junior Research Fellow (JRF) in a DST-SERB-funded project at the laboratory of Dr. Yashwanti Mudgil.

Shweta Sharma worked at ICGEB for her Ph.D. with the Editor where she worked on characterization and functional validation of abiotic stress-responsive genes in rice. She is currently working as a Kothari Postdoctoral Fellow at the Department of Plant Molecular Biology, University of Delhi, South Campus, and her work focuses on the identification of RNA-binding domain (RBD) containing proteins and their functional validation in tomato in response to various stresses.

Yashwanti Mudgil obtained her Ph.D. with the Editor and Prof. K.C. Upadhyay from JNU, New Delhi, on "Cloning and characterization enzyme topoisomerase." After that she had her postdoctoral training from the University of Toronto and University of North Carolina at Chapel Hill, USA. During her postdoc, she was involved with the discovery of U-Box E3 ubiquitin ligase family in *Arabidopsis* and discovery and characterization of novel G-protein interacting signaling component: NDL1, *Arabidopsis* N-MYC DOWN-REGULATED-LIKE1, a novel sugar-regulated downstream effector of Gβγ-mediated auxin transport in the root. She had been part of the G-protein interactome initiative and discovered NDL interactome. She is currently working as an Assistant Professor at the University of Delhi, North Campus. She has been a Visiting Faculty to Prof. Alan Jones laboratory at the University of North Carolina on DBT CREST award. Her current research interests include heterotrimeric G-protein beta gamma (Gβγ) subunit-mediated signaling pathways, specifically studying downstream signaling protein effectors to further dissect out the complete molecular mechanism involved in the AGB1-NDL1-mediated processes.

Arun Kumar Sharma received Ph.D. degree from JNU, New Delhi, for his work on "Phytochrome regulation of nitrate reductase and nitrite reductase in maize" with the Editor. He did postdoctoral work at School of Medicine, Yale University, New Haven, USA, and at School of Medicine, Wayne State University, Detroit, USA, with Dr. G. Kumar in the area of DNA-protein interaction. Afterwards, he did postdoctoral work at JNU with Editor and at the Department of Plant Molecular Biology, UDSC, New Delhi, with Prof. Akhilesh Tyagi in the field of signal transduction. Currently he is a Professor at the Department of Plant Molecular Biology, UDSC. His current areas of interests are improving nutritional quality and shelf life of tomato and study of role of methylated DNA-binding proteins in gene regulation and manipulation of epigenetic changes to regulate gene expression for applications in plant biotechnology.

Part IV

Death and Perspectives on Plant Life

"For life and death are one, even as the river and sea are one"

Khalil Gibran

"I never see what has been done, I only see what remains to be done"

Buddha

"The flower which is single need not envy the thorns that are numerous"

Rabindra Nath Tagore

Plant Death: Short and Long Life Span to Immortality

22

Shiv Shanker Pandey, Rohit Bhatt, and Budhi Sagar Tiwari

Abstract

Death is a universal physiological process that occurs in all living beings and results in termination of normal cellular activities required for life. In animals, loss of function of vital organs such as the liver, heart, or brain becomes a cause of death; however, in plants, death of a whole plant body is a cumulative effect of activities of all the cells associated with different organs such as stem, leaves, and roots. Therefore, in the case of plants, it becomes important to understand the plant cell death that will help to understand plant death. Cells of a plant tend toward death by two modes: controlled mode which is called programmed cell death (PCD) and uncontrolled mode called necrosis mediated by external factors such as infection and injury. PCD is generally mediated through apoptosis and autophagy. Programmed cell death (PCD) is a genetically regulated phenomenon of selective elimination of target cells that are either under pathological conditions or unwanted for the organism's normal growth and development. PCD renders some hallmarks like blebs in the cell membrane, lobe formation in the nuclear membrane, DNA nicks resulting to DNA ladder of 200 bp, and downstream activation of caspases. Here, we described importance of programmed cell death and other modes of death adopted by plants during their developmental process and to cope with the unfavorable changing environmental perturbations.

Keywords

Calcium signaling · Cell death mimicry · Immortality · Phytohormones · Programmed cell death (PCD) · Reactive oxygen species (ROS)

S. S. Pandey
Crop Protection Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

R. Bhatt · B. S. Tiwari (✉)
Plant Cell Biology and Biotechnology, Institute of Advanced Research Gandhinagar, Gandhinagar, India
e-mail: bstiwari@iar.ac.in

© Springer Nature Singapore Pte Ltd. 2019

S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_22

22.1 Introduction

Every living organism, either unicellular or multicellular, faces sequential death of cells ultimately leading to an end of the organism. Scientifically, death is defined as a point of irreversible seizure of all physio-biochemical processes. Culver and Gert (1982) defined death as "the permanent cessation of functioning of the organism as a whole." The term "organism as a whole" used by Culver and Gert (1982) leads to a confusion when death of a part of some component or vital subsystems like organs of an organism is considered. Under such circumstances, cell death becomes a cause of survival of the organism in totality, and this type of cell death is referred to as programmed cell death (PCD) or very commonly apoptosis (Green 2011).

The word apoptosis has its plant origin meaning fall of leaves from the plant. Back during the eighteenth century, Roman physician and naturalist Galen noticed that the autumnal defoliation is an innately instituted phenomenon by plants to protect the plant from being broken by snow in the winter. The term apoptosis was originally used in the seminal work of Kerr et al., (1972) who defined "programmed cell necrosis" in the animal cell undergoing cell death process during physical trauma (Kerr et al. 1972). Although having its origin in plants, a detailed account of the PCD process in plants is still a gray box.

Cell death in plants can be observed during different developmental stages, under moderate biotic and abiotic stress conditions and physical trauma. Cell death during different developmental conditions as well as moderate biotic and abiotic perturbations provides a sufficient time for the target cell to take "death decision," and under such situation, sacrificing cells do not affect their neighboring cell's physiology. However, during cell death due to physical trauma, cells undergo a spontaneous death process affecting their neighboring cells due to rupture of their cell membrane and splashing out of their cellular content. The former process has been grouped in PCD, while the latter process is termed as necrosis.

22.2 Plants and Their Death Pathway

Plant is a multicellular organism, and being sessile in nature, it is inclined to get exposed with various environmental conditions during its life cycle starting from seed germination to maturation/seed setting stage. During the whole life cycle or under various stress conditions, plants adopt via expressing various developmental programs including elimination of unwanted cells, organs, and parts in a very finely tuned and controlled manner that includes PCD and uncontrolled mode of death called necrosis. PCD is a genetically programmed physiological process involved in the selective elimination of unwanted cells in a multicellular organism, having highly organized physiological structure. PCD is a *survival mechanism* for an organism that strictly controls the cells' number via maintaining the homeostasis between natality and mortality of the cells. PCD take place during developmental stages including differentiation of tracheary elements, embryo formation, abscission of floral organs, shaping the morphology of certain leaves, cells, tissues, and

organs, control of cell populations, and defense against invading microbes and during exposure to unfavorable environmental conditions and during hypoxia and senescence (Gadjev et al. 2008). Figure 22.1 summarizes the regions where PCD occurs. PCD is well discriminated to necrosis as it involves specific molecular hallmarks such as DNA laddering, cytochrome c release, caspase involvement, ATP depletion, cytoplasmic swelling and loss of membrane integrity, and involvement of specific proteases (Pennell and Lamb 1997).

In contrast to apoptosis that involves the death of target cell without hampering neighbouring cells, necrosis involve the demise of group of cells in unorganised and uncontrolled fashion. Necrosis follows overwhelming stress, where swelling of the cell occurs because of cell losing its ability to osmoregulate, resulting in water and ion flooding into the cell. In short, cells that die as a result of injury and in response of it typically swell and burst and they spill their content all over the neighbors. Necrosis is passive and cause irreversible injury.

PCD is very important and highly regulated multistep process; it requires tight signaling within and between organelles in plant cells for regulating it. Reactive oxygen species (ROS) are a by-product of aerobic metabolism with strictly controlled cellular level. ROS also function as signaling molecule in many biological processes and it became identified as important modulators of plant PCD. The generation of ROS is triggered by different abiotic and biotic stress conditions. Plants and other living organisms have innate intrinsic machinary to produce many antioxidants and other molecules that scavenge ROS. Any imbalance between these normal reactions in the cell results to oxidative stress through a high rate of ROS production that in turn lead to photo-oxidative damage of DNA, proteins, and lipids and finally cell death. Chloroplast and mitochondria are the major sources of ROS generation.

22.2.1 Programmed Cell Death in Plant Development

In plants, PCD is involved in vegetative and reproductive development of plants and during response to environmental stresses both biotic and abiotic. PCD varies with different developmental stages and plant cell types (Beers 1997). PCD occurs as an inherent final differentiation step of particular cell types, e.g., anther tapetum, xylem, or root cap cells (Fig. 22.1). Some cell types, however, can initiate PCD in a facultative fashion, for instance, as a result of cell-to-cell signaling during self-incompatibility responses or on the basis of positional information during aerenchyma formation or leaf perforation. Finally, age-induced PCD occurs in all cell types of organs or even in the entire organism as the end point of plant senescence.

During the development of embryo suspensor and xylem elements, the process of vacuolar cell death in plants occurs in which the content of the dying cell is gradually engulfed by growing lytic vacuoles without loss of protoplast turgor and culminating in vacuolar collapse.

Some cells in order to fulfill their specific function are destined to undergo PCD. One example of these types of cells is root cap cells, a group of cells that

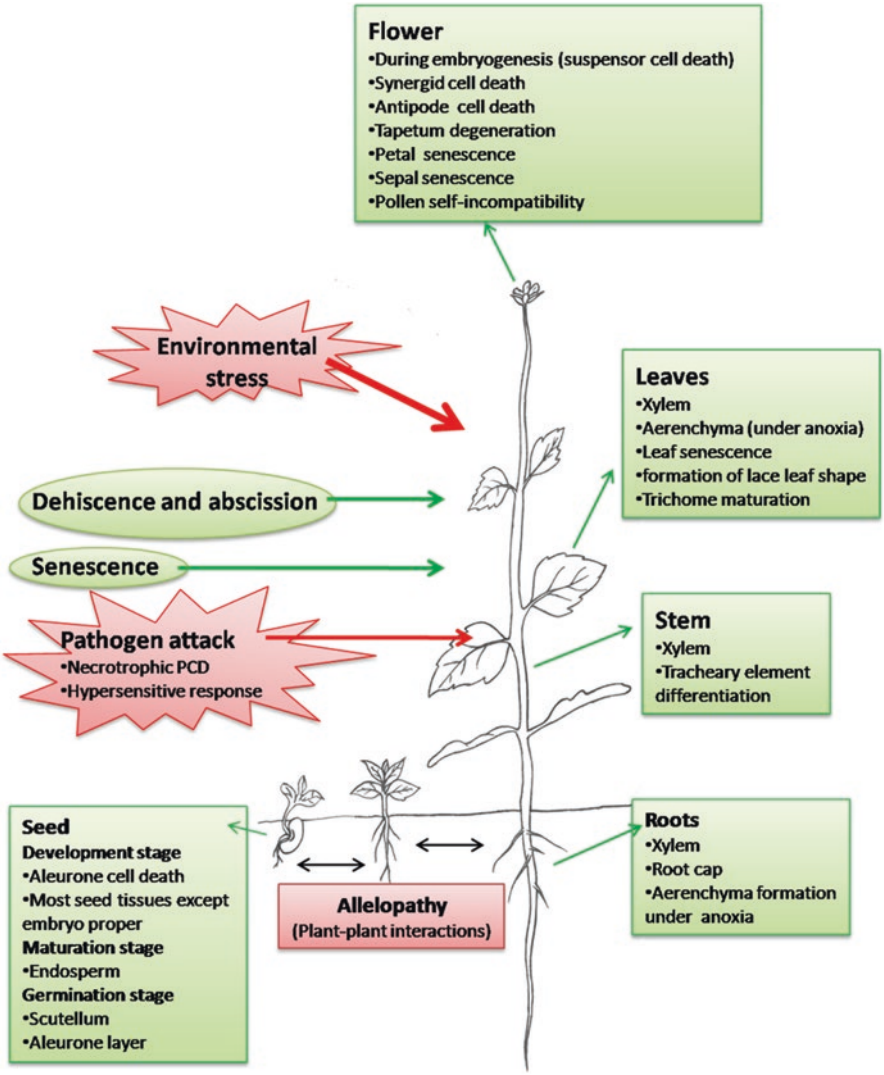

Fig. 22.1 Involvement of cell death during different life stages of a plant
Plant life starts from the germination of a seed which is the end product of the plant life. The development and germination of seeds involve growth and differentiation of new tissues involving regulated disappearance of cells mediated via programmed cell death (PCD). During the developmental stage of the seed, PCD occurs in the nucellus, pericarp, and nucellar projections resulting in remobilization of their cellular components to make available nourishment to the embryo and endosperm. At the stage of seed maturation, endosperm undergoes PCD. At the seed germination stage, scutellum and aleurone cells undertake PCD, and their cellular contents are utilized to support the growth of the germinated embryo. Cell death is also involved during the different developmental processes of a plant. Developmentally controlled cell death occurs in the xylem of roots, stems, and leaves. Cell death is involved in the root cap of some species and during aerenchyma formation (under anoxia condition). PCD occurs during embryogenesis (suspensor elimination), tapetum degeneration, pollen self-incompatibility, formation of lace leaf shape, synergid and antipode cell

protects the root apical meristem during seed germination and seedling growth stages. Root cap cells are formed in the meristem as initial cells and continuously displaced to the root periphery and eventually die and get replaced by new cells. PCD is an integral part of the normal development of root cap cells (Laux and Jürgens 1997; Schiefelbein et al. 1997). Charles Darwin concluded in his book *The Power of Movement in Plants* that growing root must be determined by its tip and root tip functions not only as sensory organ, but its role is rather vast for growth and development of the entire plant. The tip of root radicle is actually controlled and functioned by the particular plant organ that unsheathes the root tip called the root cap. At the root tip, the root caps follow the diverse developmental pattern throughout the plant development. Organ growth generally follows two opposing developmental principles—determinate and indeterminate growth. Roots follow indeterminate growth of meristems producing new cells, continuously increasing the organ size. Determinate growth is found in lateral organs such as flower and leaves and produced by groups of cells with limited proliferation leading to predetermined size of organs (Tsukaya 2003). However, root cap follows neither of these principles. When the root cap cell is continuously produced by root cap stem cells in an indeterminate fashion, the cell number and size are in a determine manner, and root cap maintain the size and number of the cells by disposal of old cells and adding new cells (Barlow 2003). For the intermediate root growth, plant root tips contain a stem cell pool. Root cap also gives protection during soil penetration. The root cap can be divided in two parts, the central columella root cap (CRC) and the lateral root cap (LRC) (Arnaud et al. 2010). As plant cells are connected to their neighboring cells by a common cell wall and therefore cannot migrate, for the coordination with stem cells, root cap cells have to continuously create new root cap cells. In contrast, root epidermal cells persist after expansion and maturation, and hence, the root cap cells have to be disposed to avoid the extension of the root cap beyond the meristematic regions. For this problem, different plant species come up with different solutions. Pea, cucumber, and cereals are some species that dissolve the cell wall connections of root cap cells with their neighbors, resulting in the release of border cells into the rhizosphere (Driouich et al. 2007). In *Arabidopsis*, LRC cells undergo cell death and rapid autolysis on the root surface as soon as they reach the edge of the elongation zone (Fendrych et al. 2014). The death process occurs cell by cell toward the more proximally located root cap cells, until it reaches the LRC cells that are close to the COL cells. There is the zone of gradual transition in which packets of dead, dying, and living are found between LRC and CRC. Rapid and stepwise succession of cellular events occurs during the loss of vital functions of the cell. The first event preceding cell death consists of acidification of the cytoplasm, followed by plasma membrane disintegration, and finally collapse of the large central vacuole (Fendrych et al. 2014).

Fig. 22.1 (continued) death in the female gametophyte, tracheary element differentiation, and some types of trichome maturation. Cell death also occurs during senescence of plant organs such as petals, sepals, and leaves. Dehiscence and abscission processes might also involve cell death events. Cell death occurs during allelopathic interactions. Cell death is also involved during plant pathogen attack leading to necrotrophic or hypersensitive response (HR). Responses of plants toward environmental fluctuations (biotic or abiotic factors) also involve PCD

Sexuality in the plant kingdom is of two types: monoecious species bearing flowers having both sexes in the same plant and dioecious species having unisexual flowers on different individuals. In the flower of a monoecious species, sex determination involves the discriminating abortion of either male or female organ primordia within the bisexual floral meristem (Cheng et al. 1983). One example of the monoecious species is maize in that ear and tassel flowers are bisexual; the arrest and abortion of one of the organ primordia either the pistil primordia in the tassel or the stamen primordia in the ear are marked to transition from bisexual to unisexual state (Dellaporta and Calderon-Urrea 1994). Through a PCD, elimination of the pistil primordia in the tassel involves cellular vacuolation and degradation of organelles, while adjacent stamen initials continue to divide and differentiate until they reach sexual maturity. In female flowers, the same process of PCD occurs, which is initiated near the apex of the primordium and propagated basipetally (Cheng et al. 1983; Dellaporta and Calderon-Urrea 1994; Calderon-Urrea and Dellaporta 1999). Therefore, PCD is essential for transition of bisexual flowers into sexual flowers. PCD also occurs during embryogenesis in plants. For normal development of the embryo, cell death is necessary, and this includes the death of scutellar cells surrounding the developing radicle, death of suspensor, and death of nucleus from which the egg cell originates.

PCD also occurs during germination of seeds in the storage tissues. In monocot seeds, *aleurone cells* form a secretory tissue that releases hydrolysis for digestion of the endosperm and nourishes the embryo. For postembryonic development, aleurone cells are unnecessary and die after completion of the germination process (Kuo et al. 1996).

Under certain conditions, targetted cells die to take over their function. Normaly such cells are located on a special location like conducting vessel and root cap. One of the best examples of these types of cells are xylem *tracheary elements* (TEs). These are found in a vascular plant for transport of water in columns of dead cells. The most significant feature of these types of cells is that they all start their function after their death. TE differentiation involves cell elongation, deposition of cell wall components such as lignin, and then autolysis (Fukuda 1997), which indicates significant changes in the cell wall, which is another feature in these types of cells.

22.2.2 Programmed Cell Death and Stress Conditions

Plants in their life face mainly two types of stresses, biotic and abiotic, and both stresses can lead to faster cell and plant death. A vast array of bacteria, fungi, and viruses attack on plants at their various developmental stages of life cycle, and severity of biotic stress can also be altered by different abiotic stresses which include temperature, salinity, high concentration of heavy metals and UV rays, water logging, etc. Therefore, PCD induced by stress can significantly affect plant yield fundamentally important for productivity of the agriculture (Bostock et al. 2014; Mittler and Blumwald 2010).

During the pathogen attack, the plant cells exposed to pathogen go under PCD which is triggered by activation of specific signals for protein synthesis and specific

metabolic pathway activation (He et al. 1994; Greenberg 1996, 1997). This aspect is covered in detail in Chap. 20. When plant-pathogen interaction occurs, two major types of PCD processes are activated for inhibiting the spreading pathogens to nearby tissues, and this response is called hypersensitive response (HR), and when this process becomes a failure, the disease appears. PCD is thus a strategy of plants to prevent spreading of pathogen by sacrificing an infected cell. HR response is a result of the activation of a PCD pathway (Mittler et al. 1997). In HR cell death, accumulation of ROS, especially O^{2-} and H_2O_2 is triggered in different cellular compartments, leading to elevation in the cytosolic Ca^{+2} and triggering a protein kinase-mediated cell death processes (Mehdy 1994; Levine et al. 1996). It was also found that plant mitogen-activated protein kinases (MAPKs) are converging nodes after perception of pathogens and elicitors followed by activation of Ca^{+2}-dependent kinase pathways that at the end, switch on the mode of cell death.

In response to waterlogging, cell death occurs in the cortex of the root and stem base. In aerenchyma, cell death generated internal air spaces that facilitate more efficient transfer of O_2 from aerial organs to waterlogged stem bases and roots (Armstrong 1979). Aerenchyma cells that are aerated tissues containing gas spaces are mainly present in the roots of wetland species and also found in dryland species under unfavorable condition. Basically, two types of aerenchyma are generally found, lysigenous and schizogenous. Lysigenous aerenchyma is composed of previously dead cells within the tissue that creates gas spaces. Another type, schizogenous aerenchyma, is formed when intracellular gas spaces develop within tissues without death of cells. Under *hypoxia* condition, ethylene is implicated in the death of the cell, and induction of aerenchyma formation takes place by ethylene, produced endogenously (Jackson et al. 1985).

Senescence is the endogenously controlled end phase of development in the organ, tissue, or cell, where nutrients are remobilized from the senescing parts to the other parts of the plant, which is mediated through PCD and induced by unknown age factors (Guo and Gan 2005). Other factors that influence senescence are biotic and abiotic stresses. Senescence occurs in individual cells or in a co-coordinately regulated manner in tissues, organs, or whole organisms. In all the cells and tissues of one individual organ, senescence does not occur synchronously (Thomas and Donnison 2000). One example of this process is leaf senescence which is accompanied by an organ-wide operation of PCD. In older leaves, senescence occurs after new leaves develop at the top, and a single leaf can undergo senescence due to exposure to environmental stress conditions. In several plant species, SAGs (senescence-associated genes) are upregulated during stress conditions. Enzymes coded by SAGs are mainly involved in cell degradation and mobilization of nutrients. It has been shown that during senescence, autophagy is also upregulated which is required for nutrient reallocation (see Marshall and Vierestra 2018). Both aging senescence and stress-induced senescence are multifunctional processes involving regulation of several genes at many stages. Generally senescence induced by ethylene requires nuclear function and accelerated level of cellular O^{2-} and H_2O_2, the well-known ROS signaling molecules involved in PCD. Reports on senescence suggested that the parts of a plant that are under senescence show similar hallmarks of

PCD process. In senescing organs, PCD also helps to prevent infection and spreading of disease in plants (Pennell and Lamb 1997).

22.2.3 How Cells Decide to Die

As mentioned above, ROS are main signaling molecules found in PCD. A detailed role of ROS is covered in Chap. 14.

Life under aerobic conditions is intimately linked with ROS production. Demonstration of the involvement of ROS in PCD was based on spatiotemporal correlations between increased level of ROS and cell death. However, ROS in normal plants is generated as a by-product of energy-generating processes in the mitochondria as respiration and in the chloroplast as photosynthesis (Foyer and Noctor 2005). Antioxidant machinery is present in these organelles for regulating optimum cellular ROS level. When ROS level in a cell remains relatively small, the housekeeping antioxidant machinery is sufficient to maintain cell homeostasis. On the other hand, when optimum growth conditions are disrupted, i.e., during biotic and abiotic stress conditions, then a transient oxidative burst occurs, and redox environment in the cell can lead to an uncontrolled ROS level (Polle 2001; Mittler et al. 2004). Different abiotic cues either directly or indirectly (through the action of other signals and hormones) lead to the production of ROS. In turn, ROS may influence a variety of signal transduction systems, thus providing positive or negative feedback control mechanisms. The function of the antioxidant machinery is to prevent dangerous elevations of ROS levels. The outcome of ROS signaling depends mainly on the ROS concentration, but other factors like the site of ROS synthesis, previous stress exposure, developmental stage, and interaction with other signals like reactive nitrogen species (RNS) and Ca^{2+} are also integrated into the response. In general, relatively weak stressors cause only a slight rise in ROS quantities which leads to adaptation. At more intensive abiotic stimuli, the price for adaptation may be impaired growth and development of the plant. Severe stress usually causes massive accumulation of ROS and the initiation of PCD or in extreme cases even necrosis of the tissue. (Petrov et al. 2015).

As described above, mitochondria and chloroplast are the main organelles for the production of ROS in the cells and both are connected with the nucleus. Therefore, the connection and cross talk of the nucleus with other organelles decide the future fate of the cell.

22.3 Regulatory Mechanisms in Plant PCD

22.3.1 Mediators of PCD Signaling

22.3.1.1 Reactive Oxygen Species (ROS)

ROS play an important role in the maintenance of cellular homeostasis. A brief description about the role of ROS in the cell death process has been discussed in the previous section. In this section, we describe the role of ROS in modulation of

signaling. At optimum conditions, cellular ROS level is very low (optimum level) and acts as a signaling molecule for several cellular processes; however, under unfavorable conditions, its production increases and becomes toxic, and if not detoxified through cellular antioxidative defense mechanism, it can cause damage to the cell, tissue, DNA, and lipid membrane leading to cell death. Mitochondria are the main organelles and produce a large amount of ROS, that is why it actively participates in the PCD process. While mitochondria detect a stress signal by extrinsic or intrinsic factor, it produces ROS. Mainly two harmful ROS are formed by mitochondria, H_2O_2 and $O^{\cdot-2}$. In mitochondrial electron transport chain, two compartments are responsible for ROS production, complex I (NADH dehydrogenase) and complex III (Møller et al. 2007; Noctor et al. 2007). Flavoprotein region in complex I of mitochondria reduces O_2 to $O^{\cdot-2}$. In complex I, ROS production is more enhanced; when reverse electron flow occurs from complex III to complex I due to lack of NAD^+-linked substrate, the electron flow is controlled by ATP hydrolysis. In complex III, complete reduction of ubiquinone donates an electron to cytochrome c1 leaving behind unstable ubiquinone semi-radicle, which favors leakage of electron to O^2 and formation of $O^{\cdot-2}$ (Murphy 2009). The role of ROS in modulation of cell death is an established fact (Tiwari et al. 2002; Van Breusegem and Datt 2006; Zhao et al. 2018) and mitochondria has been shown to play a pivotal role in PCD modulation (Kroemer and Reed 2000; Tiwari et al. 2002).

PCD signals produced by mitochondria are based on the following process: Permeability of the mitochondria is based on permeability transition pore (PTP), and opening of PTP requires Ca^{+2} that results in swelling and release of intramembrane space protein such as cytochrome c (Tiwari et al. 2002). ROS formation causes change in mitochondrial membrane potential and leads to initiation of PCD. Following the release of cytochrome c, DNA fragmentation occurs through caspase-like proteins. Caspases are the enzymes found to be specific for a protein substrate in animals and are the key players for degradation of proteins and execution of PCD. Caspase-mediated protein degradation eventually leads to dismantling of cells. In plants, no homologue of caspase gene has been found, but there is a caspase-like protein having similar activity with caspases called metacaspase. There are some proteases that act like caspases. These are metacaspases which are cysteine-dependent proteases having caspase-like activity and show some structure similarity with caspases (Fig. 22.2).

In context to ROS-mediated apoptosis, particularly in plants, along with mitochondria (Bras et al. 2005; Petrov et al. 2015), existence of chloroplast that contributes significantly in cellular ROS has recently been studied for its role in the PCD modulation (Ambastha et al. 2015; Doyle et al. 2009; Ambastha et al. 2017). Initial evidence of participation of chloroplast during stress-induced cell death was recorded by Samuilov's group in the Russian Academy of Sciences. Through a series of elegant studies in epidermal peel of leaf, the group has shown an apoptosis-enhancing effect of illumination on chloroplast-containing guard cells, but not on chloroplast-less epidermal cells (Samuilov et al. 2003). Following this study, scattered reports on the involvement of chloroplasts in the modulation of PCD came. The first significant report on the direct participation of components of

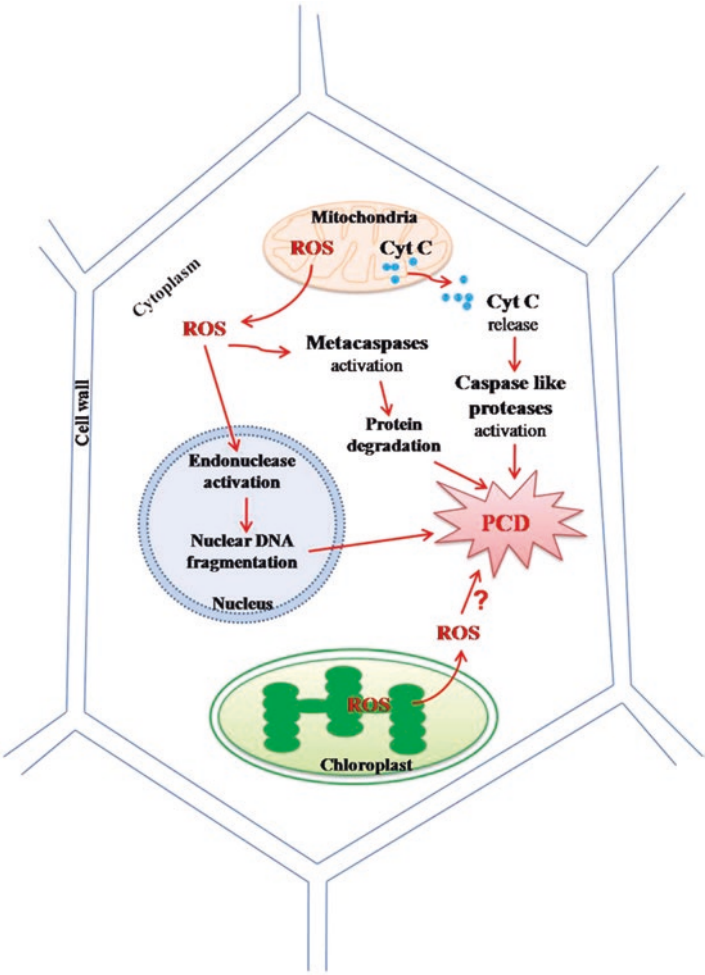

Fig. 22.2 Mitochondria-mediated programmed cell death in plants
Perturbations arisen due to environmental cues amplifies cellular ROS in mitochondria. Amplified ROS facilitates release of cytochrome c through mitochondrial transition pore and activation of caspase-like protease that eventually leads to PCD. Amplified ROS also activates metacaspases that in turn drives target cell to PCD. ROS also activates specific endonucleases (Zen endonucleases) in the nucleus that results in DNA fragmentation. Along with mitochondria, chloroplasts are also a site for ROS amplification. Although accumulating evidences suggest their role in the execution of PCD, a stepwise sequence of events is not known

photochemical reactions of photosynthesis through the release of cytochrome f was made by Peters and Chin (2005). They presented evidence for the involvement of cytochrome f as was shown in eggplants subjected to palmitoleic acid-mediated cell death (Peters and Chin 2005). In another report, cytochrome f release was noted in the green alga *Chlorella* during heat shock. Cytochrome f release was also related

with hallmarks of programmed cell death (Zuppini et al. 2009). Until 2009, both available reports advocate the release of cytochrome f during abiotic stress-induced PCD as well as PCD mediated by fatty acids. Only recently, another study depicts mechanistic details of cytochrome f during leaf senescence. In this study, authors have shown that during dark-induced senescence in rice leaf, cytochrome f is released from chloroplasts followed by the activation of caspase-3-like proteases and subsequent interaction with the proteasome system (Wang et al. 2014). Although this report has provided a beacon of light in sketching out sequence of events involved in chloroplast-mediated regulation of PCD, more aspects of regulation are needed to be touched upon to understand involvement of chloroplast in abiotic stress-induced cell death.

22.3.1.2 Calcium Signaling

Involvement of Ca^{2+} during cell death is well established. Exposure of plants to different abiotic and biotic stresses leads to generation of various ROS molecules in which H_2O_2 represents as a major ROS molecule. Rise in H_2O_2 level under stress conditions acts as a signal transmitted through the alteration in Ca^{2+} fluxes that finally modulate the cellular redox state. This whole process is dependent on a specific calcium signature that regulates several downstream signaling events and processes that finally end with escape/survival mechanism or protection/tolerance or cell death. Downstream signaling events regulated with calcium signature include numerous Ca^{2+}-interacting proteins such as calmodulins, calcium-dependent protein kinases, and a huge network of MAPKs (Gadjev et al. 2008). The role of calcium in cell signaling is covered in Chap. 11.

22.3.1.3 Phytohormone

Phytohormones significantly influence the stress response induced by ROS and regulate PCD. Ethylene and SA are positive regulators of several types of H_2O_2-induced cell death (Gadjev et al. 2008). In general, almost all types of biotic and abiotic stresses led to oxidative stress that stimulates ethylene biosynthesis and its accumulation. In addition, the amplified levels of both SA and ethylene can overamplify the H_2O_2 signal (Wang et al. 2002). Involvement of GA in the stimulation of H_2O_2 burst through inhibition of antioxidant enzymes to trigger H_2O_2-dependent cell death in the aleurone layer of monocots has also been demonstrated (Fath et al. 2001). Small polypeptide hormones such as systemin and AtPep1 stimulate H_2O_2 production and activate expression of defense genes in *Arabidopsis*. The study on AtPep1, PROPEP1, and PROPEP2 showed involvement of phytohormone and H_2O_2 in cell death (Gadjev et al. 2008).

Salicylic acid (SA) is found to be involved in pathogen defense response which is mediated through PCD (Brodersen et al. 2005). Induction of effector-triggered immunity occurs at the site of pathogen infection in the plant which results in programmed cell death (PCD); however, systemic acquired resistance (SAR) is initiated in other parts of the plant. SAR is regulated by SA, and its production is maximum at the site of infection and gradually decreases with increasing distance from the infection site. SA controls the nuclear translocation of NPR1 (nonexpresser

of PR genes 1) which is the transcriptional cofactor required for SAR. NPR1 accumulation induces the expression of genes involved in SAR response; however, its degradation induced PCD. Therefore, NPR1 acts as a molecular switch between SAR and PCD.

22.3.1.4 Lipid Signaling

Lipid signaling plays diverse roles in various cellular and physiological processes. Lipid messengers are found to be involved in ROS-mediated cell death (Gechev and Hille 2005). At low concentration of $^1O_2$, it acts as a signaling molecule which is mediated by lipid-derived molecules called lipid messengers. Specificity of a cellular response to particular environmental conditions (specifically biotic or abiotic stresses) depends on the cellular content of specific ROS and its sites of generation which is proportional to the levels of stress. Changes in cellular ROS level resulted to specific signaling events which are regulated by plant developmental stage, prestress encounters, phytohormones, and lipid messengers. Higher accumulation of cellular ROS results in lipid peroxidation causing accumulation of oxidized lipids that trigger PCD. Lipid-derived messengers such as sphingolipids, sphingoid bases, oxylipins, and phospholipids interplay with cellular ROS level and modulate PCD (Gadjev et al. 2008).

22.4 Activators and Core Regulators of Cell Death

Animal PCD involves caspases, having cysteine-dependent aspartyl protease activity (De Pinto et al. 2012; Thornberry and Lazebnik 1998). True caspases have not yet been described in plant yeasts and protozoans (Lord and Gunawardena 2012; Lam and Zhang 2012). Nevertheless, involvement of caspase-like proteases including cysteine endopeptidases and serine endopeptidases in plant PCD has been well established (Rojo et al. 2004; Coffeen and Wolpert 2004).

Cysteine endopeptidases are further divided into two groups, vacuolar processing enzymes and metacaspases. Metacaspase is a family of cysteine proteases that belongs to the C14 family and contains a caspase-specific catalytic dyad of histidine and cysteine, as well as a conserved caspase-like secondary structure found in plants, fungi, and protists based on homology with caspase-like domains. Plant metacaspases are classified into type I and type II based on overall structure and the level of sequence similarity. Type I metacaspases exhibit an N-terminus extension that usually contains a zinc-finger motif as well as a proline-rich stretch and may or may not contain a glutamine-rich region (Lam and Zhang 2012). Type II metacaspases lack such a prodomain but have a linker region of 160–180 amino acids between the putative large (p20) and small (p10) caspase-like subunits. Unlike aspartate-specific caspases, metacaspases possess arginine/lysine substrate cleavage specificity. Type II metacaspases have only been identified in plants.

Metacaspases are different from caspases in specificity of active sites. Metacaspases prefer R or K at the cleavage site, instead of cleaving substrate with D residue at P1 position (referred to as the N-terminus direction from the cleaved

bond) in the case of caspases in animals (Vercammen et al. 2007; Watanabe and Lam 2005). Together with the eukaryotic caspases, legumains, separases, paracaspases, and the bacterial gingipains and clostripains, they belong to the clan CD of cysteine proteases. Clan CD includes organisms that utilize a catalytic His-Cys dyad for their activity (Vercammen et al. 2006).

It has been suggested that type I metacaspases represent the ancient form of the metacaspase family and that the evolution of type II had occurred before the emergence of multicellular plants from their photosynthetic, unicellular ancestors. It is speculated that eukaryotic metacaspases originate possibly from a horizontal gene transfer between the mitochondrial endosymbionts (α-proteobacteria) and the early eukaryotes. Moreover, metacaspase-like proteins are present not exclusively in α-proteobacteria but also in all *Bacterial* groups, such as cyanobacteria, the known ancestors of plant chloroplasts.

The distribution of the caspase-like protease family demonstrated that while caspases and paracaspases are, so far, limited to metazoans and *Dictyostelium*, respectively, metacaspases are highly conserved in plants and fungi. This distribution suggests that metacaspases are likely the most closely representative of the eukaryote ancestral protease (Uren et al. 2000).

22.5 Cell Death from Life Span to Immortality

There is a wide diversity of life forms in the plant kingdom ranging from a unicellular phytoplankton to a large sequoia tree. This also reflects the range of their life span. For example, a bloom may exist for weeks, while the average life of a sequoia ranges between 1700 and 3200 years. Similarly, some clonal plants such as *Lomatia tasmanica* can survive in an order of magnitude of 3600 years, while a nonclonal pine (*Pinus tasmanica*) plant may survive for 5062 years (Munné-Bosch 2014) Thus, clonal growth has been considered as a major factor in determining the life span of a plant. It provides a reflection that bypassing sexual reproduction provides longevity to a plant. While considering and correlating cell death with life span of the plant, considering developmental requirement remains to be a prerequisite. In short life spanned plants, major cell death may be observed in certain developmental niche such as leaf cells or developing flower and root while in long lived trees where major mass is wood, a very high rate of xylogenesis may be observed throughout the life that goes along with organellar cell death (Reape et al. 2008).

Roots generally represent an organ for anchorage and supplying water and nutrient to the plants. Apart from providing a base for the support, roots are site for hormone production that in turn is involved in a number of metabolic activities. More importantly, roots bear meristematic cells that in combination with shoot meristematic tissue and vascular tissue form the "essential core of life." For a plant to be considered as dead, its aboveground and underground meristems have to be dead. The importance of roots in perenniality and determination of life span is very elegantly described by Munné-Bosch (2014) wherein the author pointed out some key traits in determining life span in a perennial plant. It has been observed that most of

perennials are relatively resistant to stress conditions compared to annuals. When it comes to longevity of a plant, modular growth provides expression of the plant's opportunistic response to environmental variations in resource availability and thus plays a key role in adaptation of plants to various biotic and abiotic stresses (Halle 1986). Along the line of modular growth, dormancy of aerial and underground meristem has been studied in a perennial orchardgrass (*Dactylis glomerata*) and a tall fescue (*Lolium arundinaceum*). The performance of cultivars and population indicate that dormancy is an important trait for stress tolerance in perennial plants (Nie and Norton 2005). Similarly, aging-related cell division has been shown to be another factor in determining life span of a plant. In an elegant communication, Munné-Bosch (2018) has emphasized that growth and longevity of a plant or a tall tree are affected not only by biotic or abiotic stress they encounter during their life span but also by age-related structural manifestations like hydraulic limitation for water transport and vascular discontinuities. Further, he advocates that continuous growth along with plastic branching in a tree is key for longevity; however, immortality can only be achieved either through clonal production or germ line.

22.6 Life Span Through Mimicking Cell Death

Survival of an organism is directly proportional to the availability of water. Water is unambiguously known as the universal solvent, and in a biological system, it acts as a medium for almost all metabolic reactions. In lower plants like algae and early land plants, water is the medium for fertilization. Against the background of the critical importance of water, there are numerous organisms that survive in extreme scarcity of water. To survive under such state of desiccation, some organisms undergo an extraordinary state of dryness called anhydrobiosis. This state is characterized by almost zero level of detectable metabolism (0.01% of normal) collectively called cryptobiosis (Crowe and Cooper 1971). It is not only desiccation; cryptobiosis has been observed during lack of oxygen (anoxybiosis) and extreme cooling (cryobiosis) as well. It has been observed that some anhydrobiotes are not restricted only to survive extreme desiccation, but they exhibit their survival during extremes of temperature and ionizing radiation as well suggesting a possibility of having common regulatory mechanisms in these extraordinary abilities.

To survive under extreme inhospitable state of water availability, anhydrobiotic organisms opt a number of strategies. Their first line of adaptation is structural and morphological changes. To survive such situation, some bryophytes and lichens institute an ability to dehydrate slowly and rehydrate quickly. The ability to dehydrate slowly has been suggested as one of the mechanisms to reduce desiccation-induced damage by minimal generation of reactive oxygen species and oxidative burst (Cruz de Carvalho et al. 2012; Singh et al. 2015). To minimize water loss and reducing dependency on water for sexual reproduction and dispersal of propagules, some structural adaptations like development of conducting vessels with thick cell walls and development of cuticle layer on epidermis were evolved in bryophytes and mosses (Gaff and Oliver 2013; Singh et al. 2015).

Removal of cellular water directly affects structural integrity of cell membrane and associated macromolecules within a cell. As a second line of adaptation to survive desiccation, anhydrobiotes undergo metabolic changes particularly related to sugar metabolism. One of the strategies widely observed in a number of anhydrobiotic systems is overproduction of osmolytes, particularly sucrose, trehalose, myo-inositol, proline, and quaternary ammonium compounds (glycine betaine). All these compounds protect desiccation-induced damage by targeting different touch points in the cell. For example, polyols (sorbitol, mannitol, pinitol, etc.) act on cell membrane and macromolecules during desiccation (Hincha and Hagemann 2004), while trehalose acts on cell membrane and macromolecules to replace structural water reversibly to maintain structural integrity (Crowe 2007). Under very extreme desiccation, vitrification—formation of glossy layer by osmolytes that is biologically inert—has been reported. Vitrification acts as a protective matrix for the cell against desiccation (Buitink and Leprince 2008). At molecular level, production of inherently disordered hydrophilic proteins (IDPs) like late embryogenesis abundant (LEA) proteins has been shown to confer desiccation tolerance in a number of plants as their absence has been reported to make that plant osmo-sensitive (Goyal et al. 2005).

At physiological level, anhydrobiotes suspend their metabolic activities in a very programmed and reversible manner beginning with suspension of energy-consuming reactions followed by energy-generating reactions. A subaerial cyanobacterium, *Scytonema geitleri* has been studied for sequence of suspension and revival of some key metabolic activities during serial desiccation and rewetting process, and observation suggested that nitrogen fixation was the first reaction to be stopped at mild drying; further, intensification of water removal resulted to a drop in CO_2 fixation activity. Photochemical reactions of photosynthesis became untraceable at very high level of desiccation. Interestingly, revival of life in this cyanobacterium upon regulated rewetting followed a reverse trend, i.e., light reactions of photosynthesis appeared first following CO_2 fixation activity. Nitrogenase activity appeared when cyanobacterial mats were completely rehydrated (Tiwari and Tripathi 1998). These evidences suggest, along with targeted cell that is very popular in higher plants, mimicking death via reversible seizure of metabolic is another opted strategy to prolong life in lower plants.

22.7 Conclusion

Cell death is an integral event in the life of all living creatures. It is observed at different developmental niches and nodes at different time points during the developmental processes in a plant. During the process of somatic development, cell death has been observed as a process of survival that is attributed through the process of embryo suspensor cell death, leaf shaping, xylogenesis, etc. However, cell death, as observed during reproductive phase, although essential for proper functioning and execution of sexual reproduction, is more important to complete the life cycle of the plant. In higher plants, regulated cell death also acts as one of the parameters

determining the life span of a plant. Interestingly, some lower plants that are a blend of unicellular and multicellular systems exhibit suspension of their metabolic activities during very adverse conditions and behave like a dormant propagule. With the onset of favorable conditions, these organisms revive their metabolic processes to become alive again. Thus, mimicking death could be another strategy to attain longer life.

References

Ambastha V, Tripathy BC, Tiwari BS (2015) Programmed cell death in plants: A chloroplastic connection. Plant Signal Behav 10:e989752

Ambastha V, Sopory SK, Tiwari BS, Tripathy BC (2017) Photo-modulation of programmed cell death in rice leaves triggered by salinity. Apoptosis 22:41–56

Armstrong W (1979) Aeration in higher plants. Adv Bot Res 7:225–332

Arnaud C, Bonnot C, Desnos T, Nussaume L (2010) The root cap at the fore front. C R Biol 333:335–343

Barlow PW (2003) The root cap: cell dynamics, cell differentiation and cap function. J Plant Growth Regul 21:261–286

Beers EP (1997) Programmed cell death during plant growth and development. Cell Death Differ 4:649–661

Bostock RM, Pye MF, Roubtsova TV (2014) Predisposition in plant disease: exploiting the nexus in abiotic and biotic stress perception and response. Annu Rev Phytopathol 52:517–549

Bras M, Queenan B, Susin SA (2005) Programmed cell death via mitochondria: different modes of dying. Biochemistry (Mosc) 70:231–239

Brodersen P, Malinovsky FG, Hématy K, Newman MA, Mundy J (2005) The role of salicylic acid in the induction of cell death in *Arabidopsis* acd11. Plant Physiol 138:1037–1045

Buitink J, Leprince O (2008) Intracellular glasses and seed survival in dry state. CR Rev 331:788–795

Calderon-Urrea A, Dellaporta S (1999) Cell death and cellprotection genes determine the fate of pistils in maize. Development 126:435–441

Cheng PC, Greyson RI, Walden DB (1983) Organ initiation and the development of unisexual flowers in the tassel and ear of *Zea mays*. Am J Bot 70:450–462

Coffeen WC, Wolpert TJ (2004) Purification and characterization of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in *Avena sativa*. Plant Cell 16:857–873

Crowe JH (2007) Trehalose as a chemical chaperone: fact and fantasy. Adv Exp Med Biol 594:143–158

Crowe JH, Cooper AF (1971) Cryptobiosis. Sci Am 225:30–36

Cruz de Carvalho R, Catala M, Marques da Silva J, Branquinho C, Barreno E (2012) The impact of dehydration rate on the production and cellular location of reactive oxygen species in an aquatic moss. Ann Bot 110:1007–1016

Culver CM, Gert B (1982) Philosophy in Medicine: Conceptual and ethical issues in medicine and psychiatry. Oxford University Press, Oxford

De Pinto MC, Locato V, De Gara L (2012) Redox regulation in plant programmed cell death. Plant Cell Environ 35:234–244

Dellaporta SL, Calderon-Urrea A (1994) The sex determination process in maize. Science 266:1501–1505

Doyle SM, Diamond M, McCabe PF (2009) Chloroplast and reactive oxygen species involvement in apoptotic-like programmed cell death in *Arabidopsis* suspension cultures. J Exp Bot 61:473–482

Driouich A, Durand C, Vicré-Gibouin M (2007) Formation and separation of root border cells. Trends Plant Sci 12:14–19
Fath A, Bethke PC, Jones RL (2001) Enzymes that scavenge reactive oxygen species are down-regulation prior to gibberellic acid-induced programmed cell death in barley aleurone. Plant Physiol 126:156–166
Fendrych M, Van Hautegem T, Van Durme M, Olvera-Carrillo Y, Huysmans M, Karimi M, Lippens S, Guérin CJ, Krebs M, Schumacher K, Nowack MK (2014) Programmed cell death controlled by ANAC033/SOMBRERO determines root cap organ size in *Arabidopsis*. Curr Biol 24:931–940
Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875
Fukuda H (1997) Tracheary element differentiation. Plant Cell 9:1147–1156
Gadjev I, Stone JM, Gechev TS (2008) Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. Int Rev Cell Mol Biol 270:87–144
Gaff DF, Oliver M (2013) The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. Funct Plant Biol:315–328
Gechev TS, Hille J (2005) Hydrogen peroxide as a signal controlling plant programmed cell death. J Cell Biol 168:17–20
Goyal K, Walton LJ, Tunnacliffe A (2005) LEA proteins prevent protein aggregation due to water stress. Biochem J 388:151–157
Green DR (2011) Means to an end: apoptosis and other cell death mechanisms. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. ISBN 978-0-87969-888-1
Greenberg J (1996) Programmed cell death: a way of life for plants. Proc Natl Acad Sci U S A 93:12094–12097
Greenberg JT (1997) Programmed cell death in plant-pathogen interactions. Annu Rev Plant Physiol Plant Mol Biol 48:525–545
Guo Y, Gan S (2005) Leaf senescence: signals, execution, and regulation. Curr Top Dev Biol 71:83–112
Halle F (1986) Modular growth in seed plants. Philos Trans R Soc Lond B 313:77–87
He SY, Bauer DW, Collmer A, Beer SV (1994) Hypersensitive response elicited by *Erwinia amylovora* harpin requires active plant methabolism. Mol Plant Microbe Interact 7:289–292
Hincha DK, Hagemann M (2004) Stabilization of model membranes during drying by compatible solutes involved in the stress tolerance of plants and microorganisms. Biochem J 383:277–283
Jackson MB, Fenning TM, Drew MC, Saker LR (1985) Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. Planta 165:486–492
Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 26:239–257
Kroemer G, Reed JC (2000) Mitochondrial control of cell death. Nat Med 6(5):513–519
Kuo A, Cappelluti S, Cervantes-Cervantes M, Rodriguez M, Bush DS (1996) Okadaic acid, a protein phosphataseinhibitor, blocks calcium changes, gene expression, and celldeath induced by gibberellin in wheat aleurone cells. Plant Cell 8:259–269
Lam E, Zhang Y (2012) Regulating the reapers: activating metacaspases for programmed cell death. Trends Plant Sci 17:487–494
Laux T, Jürgens G (1997) Embryogenesis: A new start in life. Plant Cell 9:989–1000
Levine A, Pennell RI, Alvarez ME, Palmer R, Lamb C (1996) Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Curr Biol (4):427–437
Lord CE, Gunawardena AH (2012) Programmed cell death in *C. elegans*, mammals and plants. Eur J Cell Biol 91:603–613
Marshall RS, Vierestra RD (2018) Autophagy: the master of bulk and selective recycling. Ann Rev Plant Biol 69:173–208
Mehdy MC (1994) Active oxygen species in plant defense against pathogens. Plant Physiol 105:467–472

Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenge sand perspectives. Annu Rev Plant Biol 61:443–462
Mittler R, Simon L, Lam E (1997) Pathogen-induced programmed cell death in tobacco. J Cell Sci 110(Pt 11):1333–1344
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) The reactive oxygen gene network in plants. Trends Plant Sci 9:490–498
Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481
Munné-Bosch S (2014) Perennial roots to immortality. Plant Physiol 166:720–725
Munné-Bosch S (2018) Limits to tree growth and longevity. Trends Plant Sci. pii: S1360-1385 (18):30167–30165
Murphy M (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13
Nie Z, Norton MR (2005) Stress tolerance and persistence of perennial grasses: the role of the summer dormancy trait in temperate Australia. Crop Sci 49:2405–2411
Noctor G, De Paepe R, Foyer CH (2007) Mitochondrial redox biology and homeostasis in plants. Trends Plant Sci 12:125–134
Pennell RI, Lamb C (1997) Programmed cell death in plants. Plant Cell 9:1157–1168
Peters JS, Chin C (2005) Evidence for cytochrome f involvement in eggplant cell death induced by palmitoleic acid. Cell Death Differ 12:405–407
Petrov V, Hille J, Mueller-Roeber B, Gechev TS (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. Front Plant Sci 6:69
Polle A (2001) Dissecting the superoxide dismutase-ascorbate-glutathione pathway in chloroplasts by metabolic modeling: computer simulations as a step towards flux analysis. Plant Physiol 126:445–462
Reape TJ, Molony EM, McCabe PF (2008) Programmed cell death in plants: distinguishing between different modes. J Exp Bot 59:435–444
Rojo E, Martín R, Carter C, Zouhar J, Pan S, Plotnikova J, Jin H, Paneque M, Sánchez-Serrano JJ, Baker B, Ausubel FM, Raikhel NV (2004) VPE gamma exhibits a caspase-like activity that contributes to defense against pathogens. Curr Biol 14:1897–1906
Samuilov VD, Lagunova EM, Kiselevsky DB, Dzyubinskaya EV, Makarova YV, Gusev MV (2003) Participation of chloroplasts in plant apoptosis. Biosci Rep 23:103–117
Schiefelbein JW, Masucci JD, Wang H (1997) Building a root: The control of patterning and morphogenesis during root development. Plant Cell 9:1089–1098
Singh S, Ambastha V, Levine A, Sopory SK, Yadava PK, Tripathy BC, Tiwari BS (2015) Anhydrobiosis and programmed cell death in plants: Commonalities and Differences. Curr Plant Biol 2:12–20
Thomas H, Donnison I (2000) Back from the brink: plant senescence and its reversibility. In: Bryant J, Hughes SG, Garland JM (eds) Programmed cell death in animals and plants. Bios, Oxford, pp 149–162
Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281:1312–1316
Tiwari BS, Tripathi SN (1998) Effect of hydration and dehydration on initiation and dynamics of some physiological reactions in desiccation tolerant cyanobacterium *Scytonema geitleri*. Indian J Biochem Biophys 35:172–178
Tiwari BS, Belenghi B, Levine A (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol 128:1271–1281
Tsukaya H (2003) Organ shape and size: a lesson from studies of leaf morphogenesis. Curr Opin Plant Biol 6:57–62
Uren AG, O'rourke KO, Aravind L, Pisabarro MT, Seshagiri S, Koonin EV, Dixit MV (2000) Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 6:961–967
Van Breusegem F, Datt JF (2006) Reactive oxygen species in plant cell death. Plant Physiol 141:384–390

Vercammen D, Belenghi B, Van de Cotte B, Beunens T, Gavigan JA, De Rycke R, Brackenier A, Inze D, Harris JL, Van Breusegem F (2006) Serpin 1 of *Arabidopsis thaliana* is a suicide inhibitor for metacaspase 9. J Mol Biol 364:625–636

Vercammen D, Declercq W, Vandenabeele P, Van Breusegem F (2007) Are metacaspases caspases? Cell Biol 179:375–380

Wang KLC, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:131–151

Wang H, Zhu X, Li H, Cui J, Liu C, Chen X, Zhang W (2014) Induction of caspase- 3-like activity in rice following release of cytochrome-f from the chloroplast and subsequent interaction with the ubiquitin-proteasome system. Sci Rep 4:5989

Watanabe N, Lam E (2005) Two Arabidopsis metacaspases AtMCP1b and AtMCP2b are arginine/lysine-specific cysteineproteases and activate apoptosis-like cell death in yeast. J Biol Chem 280:14691–14699

Zhao M, Luo L, Xu J, Xin P, Guo H, Wu J, Bai L, Wang G, Chu J, Zuo J, Yu H, Huang X, Li J (2018) Malate transported from chloroplast to mitochondrion triggers production of ROS and PCD in *Arabidopsis thaliana*. Cell Res 28:448–461

Zuppini A, Gerotto C, Moscatiello R, Bergantino E, Baldan B (2009) *Chlorella saccharophila* cytochrome f and its involvement in the heat shock response. J Exp Bot 60:4189–4200

Shiv Shanker Pandey obtained his MSc in Biochemistry from Lucknow University and Ph.D. from JNU. Subsequently, he has worked in the area of plant-microbes interaction for his postdoctoral research at CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India, where he is presently working in the Crop Protection Division.

Rohit Bhatt is a doctoral student with Dr. Tiwari. Her research interest involves genetic manipulation of sgt1 gene that is involved in α-Solanine production for the reduction of the level of α-Solanine levels in potato.

Budhi Sagar Tiwari obtained his master's and Ph.D. degree in Botany from Banaras Hindu University, India. His doctoral work was on deciphering the desiccation tolerance in subaerial cyanobacterial system. Following his doctoral degree, he underwent series of postdoctoral trainings in The Hebrew University of Jerusalem, Israel; Rutgers: The State University of New Jersey; University of Nebraska at Lincoln, USA; and Swedish University of Agricultural Sciences, Uppsala, Sweden. Before his return to JNU as a Ramalingaswami Fellow sponsored by DBT India, he spent a brief time at Virginia Tech, USA. While at JNU, he interacted with the Editor and has jointly published a few papers. His research interests are focused on deciphering anhydrobiosis in plants and involvement of chloroplast in the modulation of abiotic stress-induced programmed cell death in plants.

Sentient Nature of Plants: Memory and Awareness

23

Sudhir Sopory and Tanushri Kaul

Abstract

From the previous chapters in this volume, it is evident that plants have developed very subtle molecular mechanisms to perceive ever-changing environment and respond accordingly to ensure their proper development as engraved in their genome. Plants, thus able to sense and adaptively reciprocate to extraneous signals, anticipate inevitable threats and stresses via the elaborate intercellular systems especially the receptors, microtubules, organ-to-organ communications as well as communicating with both allies and enemies. The appropriate response by plants is needed not only for their own survival but also for reproduction, developing seeds and their dispersal for the continuation of the progeny. There are reports that some form of plant "memory" is used for rapid adaptability of plants to stress and strengthen their defence mechanisms. Exploration of this emerging avenue of research in plant sensory biology is becoming more ascribable with avant-garde breakthroughs via omics approach, high-throughput sequencing technologies and time-lapse as well as Kirlian photography. These new technological interventions would ensure unprecedented deciphering of the secret new-fangled mysteries of the plant world. In many ways, the sensory behaviour of plants seems to be similar to that noticed in the animal world. The question that we have also tried to discuss in this chapter is whether this "intelligent" response of plants falls into the domain of awareness or consciousness as has been proposed by some authors.

Keywords

Action potential · Anaesthetics · Carnivorous plants · Epigenetics · Memory · Mimosa · Neurotransmitters · Perception · Prion-like domain proteins · Stress · Touch · HKT1 transporter

S. Sopory (✉) · T. Kaul
International Centre for Genetic Engineering and Biotechnology, New Delhi, India
e-mail: sopory@icgeb.res.in

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_23

23.1 Introduction

Are plants conscious? Incredibly picturized in the epic movie "Avatar" wherein prior to the war, the proponent Jake interfaced with the Tree of Souls through a neuronal or transcendental link to negotiate on account of Na'vi, the blueish harmonious denizens of the moon. The take-home message is that humans have failed miserably in attempting to delve into the conscious nature of flora that encompasses us. There is a general debate whether consciousness exists outside the realms of the human domain. If yes, in which form or disposition and at what levels of configuration does it exist, and if not, then how during evolution this trait evolved in the *Homo sapiens*. Human beings contemplate time and consciousness exclusively in terms of human perspectives. Consciousness is perceived as the propensity of being alive, a combined affair of the body and the brain that confers the ability to think, learn, move or behave. However, it is rather elusive to comprehend how and why any of this should be considered as consciousness.

In this chapter, enduring beyond the boundaries of these questions, we would explore the nature of awareness in the vegetal world that is similar or different from the animal world. Without getting into the depth of the philosophical or psychological concepts and definitions, Wikipedia simply defines consciousness "as the state or quality of awareness or of being aware of an external object or something within oneself. It has been defined as sentience, awareness, subjectivity, the ability to experience to feel, wakefulness, having a sense of selfhood and the executive control system of the mind". Margulis and Sagan, in their book on what is life, explained consciousness as awareness of the outside world, which requires a network of sensory perception and integrating information. This present book has dealt with some aspects on perception and signalling in the earlier chapters. In line with the above, this chapter will, therefore, elaborate on awareness in plant life as per the views expressed in ancient belief systems, then present some scientific work on plant memory and finally discuss various views on conscious nature in plants.

23.2 Ancient Views on Plant Life

A commentary at the end of this book by Jaya Mehta has given an opinion on this from one perspective. We present some other ancient views to find out how they perceived the origin and nature of plant life. Citing from Bundahishn, a collection of Zoroastrian cosmogony and cosmology, as given in Wikipedia, the story goes that a demoness Jeh was sent to kill Gayomard—a gender neutral. Though successful, moon (mah) captured his seed before the animal died, which later became the progenitor of all animal life. From Gayomard's corpse grew a tree and its seeds, from which all the plant life originated. Taken together, it would mean that they considered commonality between plant and animal life. A similar concept is revealed in Norse mythology, according to which, Embla and Ask happened to be the first woman and man who originated from trees. Similarly, in Persian mythology, the ancient woman and man, Meshiane and Meshia, were also formed from trees. As

described in a translation of Mahabharata by Sri Kisari Mohan Ganguli, the dialogue between Bhrigu and Bharadwaj reveals our ancient concept of plant life. According to Bhrigu, the five elements, such as wind, sound, heat, water and earth, are represented in all life forms. Bharadwaj wonders if that be so, why trees do not appear to have heat, do not hear or see and are not capable of perception of scent and taste. Nor do they have perception of touch. However, in this book, we have shown that modern experiments revealed that these properties exist in plants. Incidentally, the answer given by Bhrigu as interpreted and translated by Ganguli is as quoted: "Without doubt, though possessed of density, trees have space within them. The putting forth of flowers and fruits is always taking place in them. They have heat within them, in consequence of which, leaf, bark, fruit and flower, are seen to droop. They sicken and dry up which shows they have perception of touch. Through sound of wind, fire and thunder, their fruits and flowers drop down. Sound is perceived through the ear. Trees have, therefore, ears and do hear. A creeper winds round a tree and goes about all its sides. An organism sans eyesight cannot independently find its way. For this reason, it is truly evident that trees have vision. Further, trees recover vigour and put forth flowers effusing different odours, good and bad, as that of the sacred perfume of diverse kinds of Dhupas (incense). It is crystal clear that trees have scent. They drink water via their roots. They catch diseases of diverse kinds. Those diseases again are cured by different operations. From this, it is evident that trees have perceptions of taste. As one can suck up water through a bent lotus-stalk, trees also, with the aid of wind, drink through their roots. Fire and wind cause the water thus, sucked up, to be digested. Again, according to the quantity of the water taken up, the tree advances in growth and becomes humid". They are susceptible to pleasure and pain, and grow when cut or lopped off. These characteristics verify that trees have life and they can't be categorized as inanimate.

Hinduism believes that consciousness exists in all life forms. It is probably exhibited at different levels. In plants, it could be in "sleep" mode whereas in humans it is most "alert". According to Buddhism, plants are life forms possessing one faculty (ekindriya jiva). These are classified based on whether they are propagated by roots, stems, joints, cuttings or seeds. Buddha envisioned that plants should not be unnecessarily damaged or destroyed. In Jainism, life forms, called Jiva, can also be divided into non-mobile (sthavar jiva) or mobile (trasa jiva). Under non-mobile, plants are put under vanaspratikaya or plant-bodied jiva. Though they also consider plants as single-sense beings, or ekindriya jiva, it is mentioned that plant life forms can have one or more souls. Plants have four of the six paryapatis, viz., ahar (food), sharir (body), indriya (senses), shwasochchhwas (respiration), lack bhasha (speech) and man (mind). It is also thought that ekindriya jiva have four pranas, viz., touch, respiration, body and longevity. Thus, according to Jainism, plants have the power of perception, and hence, injury to any kind of life is considered as himsa (violence). According to Hindu mythology, as mentioned in the Triyak Sarga, Lord Brahma created six different types of vegetation like trees, herbs, creepers, etc. The trees can also fulfil wishes of devotees, as it is commonly mentioned for the fig tree (***Ficus*** *benghalensis*) or Kalpavriksha. According to mythology, roots of this tree are the abode of Maha-Vishnu; in trunk resides

Keshava, on branches live Narayana, on leaves Srihari, on fruits Achyuta and in fact, the whole tree is God Maha-Vishnu himself. Silverstone in his books has mentioned that his son got cured by regularly touching a tree as he was advised to do, and something he never believed in. In Gita Chapter 10 verse 26, Lord Krishna says:

Asvattah sarva vrkasanam, devarsinam ca naradah
Gandharvanam chitraratha, sidhanam kapilo munih

Meaning: I am Ashwatha amongst all plants and trees; I am Narada amongst divine sages; Chitraratha amongst the Gandharvas; the muni Kapila amongst the siddhas. Where Krishna resides, that life form has to have some sort of consciousness? Incidentally, in a similar context, in verse 35 of Sambapancaska, as translated by Swami Laxman Joo, it says that the lord as referred to the divine sun has its presence in all plants. In an article in Times of India (edition, Feb 09, 2019) entitled "Beauty is an outcome of photosynthesis", in Speaking Tree column, Vir Singh writes that "light demonstrates its exquisiteness in nature by synthesizing all brilliant vivid and stunning pigments in plants. Not only green chlorophyll, all flamboyant pigments in nature are also synthesized by plants". The sun, being the source of light and plants and all existence on this planet, suggests that the cosmic energy is all pervading, including the plants. Thus, the universal consciousness, the consciousness of the creator, in one form or the other, is manifested in all the creations. In a different perspective Shanta (2016) has given overall perspective on life and consciousness and from a scientific query, Trewavas (2014, 2016) and Marder (2013a, b) have illustrated the intelligent and cognitive behaviour of plants. Some of these aspects are dealt within this chapter.

23.3 Plant Awareness via Sensory Perception and Communication

Michael Marder in his book "Plant thinking: a philosophy of vegetal life" has revealed that plants are smarter than all of us. An insight into the inner life of plants has been very lucidly portrayed by Daniel Chamovitz, a plant scientist from Israel, in his book "What a Plant Knows" (Chamovitz 2012). Since this topic is the main theme of the present book and has been covered in previous chapters, we will briefly touch upon this subject from the perspective of highlighting the nature of awareness in terms of communication with self and other systems in plants.

Plants can perceive light of different wavelengths to perform photosynthesis as well as for their development. Plants sense gravitropic signals and their roots forage in search of water through hydrotropic or even sound signals, as was shown by some elegant experiments by Gagliano et al. (2017). Baluska et al. (2004) has referred to roots as plant connect centres, in the same context as Darwin referred to roots as the brain of the plant. According to James Cahill, an experimental plant ecologist from University of Alberta, plants possess intricate feeding behaviours, above and below ground. Time-lapse cameras have displayed the movements and behaviour of foraging roots in seek of nutrients. Within a few days, their growth rate exacerbates as they find

a nutrient patch and they absorb the nutrients to their heart's fill. Roaming legs or elongating roots via dividing growth cells, the mechanism may vary in plants compared to animals but the foraging behaviour is akin. A huge body of evidence shows that Earth's electromagnetic field (EMF), which is a natural component of the environment, has an influence on biological processes and living systems, including plant growth and development. The effect on different plants would depend on the strength and direction of EMF (Maffer 2014). As has been described in earlier chapters, even touch and variations in temperature are perceived by plants. Plants communicate with other biological species as also with plants of the self-species or different species. This is mediated through chemical communication via volatiles in response to herbivory, pathogen interaction or even after touch (Markovic et al. 2019). There are reports suggesting that plants do show the kinship behaviour. An interesting study conducted by Monica Gagliano and a team from Australia showed that few plants like to grow in the neighbourhood of some specific plant species. Gagliano and Renton (2013) revealed an alternative signalling modality that functions as a local indicator of the presence of hetero-specifics, facilitating seeds to check and recognize a neighbour prior to interacting in a more finely tuned but potentially exorbitant response. They showed seed germination was positively influenced by the occurrence of a good neighbour, even though the known signalling methods were curtailed. This suggested light, touch or chemical stimuli may not be imperative for various plant species to perceive each other's existence. Gagliano found that chilli plants flourish in the presence of basil. How does this recognition happen? Chilli plants were isolated from basil in a way that plants did not touch each other and also no chemical or light was allowed to traverse. Despite all these barriers, the effect was still observed. Authors came to a conclusion that only sound could have reached chilli plants (see Chap. 6 for more details on sound signalling). They proposed this as a substitutive signalling technique that acted as a natural indicator of the existence of hetero-specifics, allowing seeds to ascertain a neighbour (Gagliano and Renton 2013). Gagliano stated that root-to-root alerts and signals could transform a forest into an organic switchboard. Plants ascertain the existence of their nearby residents and consequently modulate their development patterns. In their neighbourhood, plants are subjected to a series of mechanical stimuli, for instance, hyponastic movements of leaves, touching due to wind and circumambulations of their organs. Ample studies have revealed that over-the-ground mechanical stimuli affect below-ground plant-plant communications. Experiments conducted by Elhakeem et al. (2018) displayed that the primary roots of young maize seedlings rampantly extended towards growth solution with control or normal plants than towards solution that was touched with stressed plants. Further, their findings exhibited that roots could potentially differentiate between normal and stressed growth solutions. Incidentally, roots approached growth solutions of touched plants but eventually they reprogrammed their movement in the direction of the growth solution from control plants. However, the reverse was not observed. It was inferred that over-the-ground plant-plant interaction via a short span of touch may evoke reactions in the proximal untouched plants transmitted via underground communication.

Previously, a study conducted by Guerrieri et al. (2002) revealed that physiological alterations in infested plants by pea aphid can influence its non-infested neighbours via root-root interaction to be more alluring to the parasitoid *Aphidius ervi*.

Earlier, Falik et al. (2011) reported relay communication of stress cues. They were not only capable to sense, acclimatize and respond to extraneous cues, but simultaneously plants were able to prevision impending stresses and dangers. Unstressed plants responded to stress cues elicited from their abiotically stressed nearby residents and in turn, induced stress responses (e.g. stomatal closure) in other unstressed plants situated farther off from stressed plants. Novel means of communication have been reported by Prof. Olaf Kruse and his team (Blifernez-Klassen et al. 2012). Plants like algae, have channel alternative origins of energy from neighbouring plants. Plants affect each other in several ways and communicate via "nanomechanical oscillations" which are vibrations of the finest atomic or molecular scale, in turn being super close to telepathic communications.

Recently, it has been shown that the evolution of different floral colours is used by plants for visual signalling. Vanderkooi et al. (2019) studied anatomical and optical properties of four different plants and found that chromatic and achromatic contrast, hue, brightness, saturation, along with gloss, fluorescence, polarization, etc. brings about specific floral colour signals for interacting with animals. To further elucidate the intelligent behaviour of plants, it was enthralling to know how the "Daughter Vine" termed as the "Dracula" of the plant world, an obligate parasite, selects its host from several plants in its vicinity. They have no roots, incompetent to produce their food and live exclusively on a host plant. Prof. Consuelo de Moraes, an entomologist at Penn State University, monitored an acute pressure on these obligate parasites, with a mere 72 h time in their hand to choose their host plant or else perish. This vine prefers some plants over the others and as soon as it identifies its host, it develops miniature teeth like probes to penetrate into the victim's stem, depriving them of their vital sap. In a series of experiments, they grew the wheat and tomato plants together in a pot and placed the daughter vine between them and then recorded the activities via a time-lapse camera. As fascinating as it can be, the daughter vine seedling circumnavigates the air, like a slithery snake and opts for tomato plant in nine out of ten times. This predatory plant sniffed out its prey as several plants emit scented or aromatic chemicals efficaciously discerned by the daughter vine. Furthermore, the theory was ratified by replacing the real tomato plant with the captured scent of a tomato plant obtained by condensing the chemical odour released from the plant in a vial. Noteworthily, the release of chemical odour from tomato plants is equivalent to a "scream", which is triggered in response to the attack as a "cry for help" SOS signal to the ambience. Prof. Moraes interprets this "cry for help" in terms of pre-effectory reinforcements to invite insects which can eat those insects that eat these plants. Precisely, it is attributed to the sedentary habit of plants that eventually evolved multiplex strategies for self-defence, especially in deserts that pose as an ecological nightmare to plants.

Looking at the forest ecosystem, biologists, ecologists, foresters and naturalists progressively hash over, that trees speak, and we can learn to listen to this language. 'It leaves discomfiture in people who combat with this concept as they are unable to perceive that trees are interconnected', proposes biologist George David Haskell in his 2017 book *The Songs of Trees*. Connection in a network, Haskell says, necessitates communication and breeds languages; understanding that nature is a network, is the first step in hearing trees talk. In the forester Peter Wohlleben's 2016 book, *The Hidden Life of Trees*: What They Feel, How They Communicate – Discoveries

from a Secret World, the author suggests to comprehend the abilities of trees as social beings who depend on a network to communicate amongst themselves, quiet similar to any group of people or animals. Wohlleben revealed that the groups of trees he studied formed friendships, used electric signals to communicate and even kept their fallen comrades alive for several additional years, even centuries.

23.4 Do Plants Have Memory?

In one of his articles on plant thinking, Marder writes "Plant thinking attests to the existence of a non-conscious, involuntary memory in plants. Their memory is in Nietzsche's estimation, imageless and non-representational for instance in Mimosa, we may find memory but no consciousness. Memory of course, involves no image in the plant and has nothing to do with nerves or brain. It is primal quality". Leopold (2014) described three plant behaviours in his article on smart plant: memory and communication without brains. Memory means time keeping, chemical communication and interaction within and with outside world of insects, birds and animals. He says "the beneficial adaptable behaviour may be interpreted as some type of consciousness". Memory is an important attribute of being aware or consciousness in humans and animals.

In one of our experiments, we got a clue for plant "memory". The enzyme nitrate reductase (NR) requires both nitrate and light for its induction. Both these inducers were thought to work together for the expression of the genes encoding for NR. In our experiments, we initially treated the plants with light, and after a specific period of darkness, nitrate was given. We found that the previous light treatment was remembered by the plants to bring about the same effect, when the two treatments were given consecutively. However, on keeping a gap of 8 h between light and dark treatments, the effect was lost. It was inferred that the light memory in this particular case stayed for about 8 h (Sharma and Sopory 1984). In fact, Baluška et al. (2018) in their recent book have talked in-depth about the skills of plant behavior and on how these plants facilitate signaling between themselves and their environment during the process of learning and creating memories.

23.4.1 Experiments on Mimosa and Insectivorous Plants

As mentioned earlier about the touch-me-not plant Mimosa, when touched, the leaves collapse. Interesting, early experiments on this plant have been described in detail by Stephan Mancuso (2018). Lately, Gagliano and her colleagues grew this plant in a plastic pot, which was hooked onto a stand. The pot was allowed to fall. The leaves of the Mimosa plants collapsed; however, no harm accrued to the leaves. This experiment was repeated a few times. It was observed that after a few falls, the plants would not close the leaves. The experience taught them that no harm is coming to them and, hence, they need not close the leaves, as it normally happens, as a defence response (Gagliano et al. 2014, 2017, 2018). Charles Darwin, who analysed the Venus flytrap *Dionaea muscipula*, was enraptured by this plant's capability to perceive and grab animals to evade the constraints of its nutrient-deprived niches.

Touch, a mechanical stimulus, leads to an instantaneous upsurge in jasmonic acid (JA) biosynthesis as observed by Prof. Rainer Hedrich and his team, when they used a machine to simulate an insect touching Venus flytraps (Böhm et al. 2016). The machine discharged electric pulses to beguile the plants into contemplating that an insect had just descended. Researchers exhibited that each numbered pulse or touch was correlated to a specific response. On the first pulse, the plant's trap launches into a "ready to go" mode, sensing the stimulation. At pulse two, the trap embarks to wrap around the elicitor of the stimulation. On pulse three, the trap wraps compactly, and further at pulse four, the plant generates a hormone essentially required for feeding process. Finally, on pulse five, glands on the internal side of the trap release digestive juices and transporters that aid in nutrient uptake. Nonetheless, if the trigger was a real insect or any other victim, it would be dinner. Prof. Hedrich's team displayed that by counting and integrating the mechano-electric signals elicited by the trapped prey *Dionaea muscipula* triggers synthesis of the touch hormone JA, formation of lytic enzymes and ion channel-interceded uptake of prey nutrient-related sodium encumbrances (Scherzer et al. 2017). The more the insect or prey feels ambushed, the more the plant encompasses the victim. Professor Hedrich elucidated that the number of action potentials apprises and forewarns the plant with regard to the nutrient composition and size of the agitated prey (Escalante-Pérez et al. 2011). Taken in sync, results indicated that a mobile object is recognized as a panicked Na^+-rich animal attempting to escape the trap. Subsequently, touch-number-directed expression and generation of digestive juices is associated with an enhanced number of HKT1 transporters (Libiakova et al. 2014; Scherzer et al. 2017). Uptake and accumulation of sodium in the trap parenchyma of the carnivorous *Dionaea* is evocative of the salt distribution and regulation by the succulent-leaf-type halophytes. Hence, it has been proved that plants can learn in terms of counting and memorizing action potentials evoked due to mechanical stimuli, for instance, touch, and further interpreting these stimuli to effectuate gene expression in order to balance the cost and benefit of hunting (Böhm et al. 2015, 2016). Moreover, plants can effectuate learning in terms of counting the mechanical stimuli generated by their prey. The carnivorous *Dionaea muscipula* (Venus flytrap) captures and processes nutrient- and sodium-rich prey via recognition of mechanosensor stimulation. Mechano-electrical waves actuate (JA) signalling pathways that activate prey digestion. A number of stimulations regulate the generation of digesting enzymes and uptake elements. Similarly, application of jasmonates has been found to be sufficient to stimulate leaf bending, triggering the formation of an 'outer stomach' in the carnivorous sundew plants (Nakamura et al. 2013). As Charles Schultz quoted, "I think I've discovered the secret of life–you just hang around until you get used to it". Basically, it refers to "getting tuned to it" or reduced response to a stimulus post-recurrent exposure, which is termed as behavioural habituation. Nevertheless, it is an immensely adaptive trait of life; gratefully, organisms learn to focus on stimuli that are really meaningful in their ambience, whereas neglecting those that have proven insignificant. Gagliano et al. (2018) examined the most pertinent behavioural properties of habituation in relation to a broader ecological perspective—if Mimosa's capability to learn via the habituation of its defensive leaf-folding response was mediated by environmental cues, for instance, low and

high light. Hence, within this ecological context, Gagliano et al. (2018) verified that (a) a repeated stimulus (i.e. a vertical drop) caused a continuous reduction in the amplitude of the defensive response (i.e. the leaf-folding behaviour) and (b) habituation of the defensive response was specific to stimulus and (c) may be differentiated from sensory adaptation (loss of sensitivity) and fatigue (loss of leaf-folding motion, as the response system is drained).

23.4.2 Priming and Stress Memory

It has now been reported in many laboratories that when plants face stress, they undergo some changes which can lead to either the survival or death of plants, depending on whether the plant is tolerant or susceptible to stress. However, if following a mild stress treatment, like cold or high temperature, plants are allowed to recover, they remember to have undergone the stress conditions, since a subsequent spell of stress is not as harmful as the first stress even in sensitive plants. Plants sense and assimilate data from ambience to figure out the time at which crucial transitions happen in their lives. Plants and animals both take decisions in response to the environmental cues to augment their vigour and robustness. Plants use the property of dormancy in seeds to tide over time and space, and timing of transition to germination is affected by extraneous cues that include temperature. Temperature variability is coordinated via a spatially installed decision-making hub in the root tip of dormant seeds that shares a configuration with some systems inside the human brain instructing to break dormancy in seeds of plants, as has been reported in *Arabidopsis* by George W. Bassel and his team of co-workers at the University of Birmingham, UK. Crisp et al. (2016) revealed that pre-existent moderate stress exposure might inevitably prime a plant in the face of forthcoming stress or boost an acclimated state that prevails down to consequential exposure. In spite of the ability to be primed via epigenetic memory, in several situations these memories are not developed (Boyko and Kovalachuk 2011; Birhaum and Roudier 2017). There exists a huge void in our comprehension of the length of memory and the mechanics of memory loss or forgetfulness. Researchers emphasize that stress memory or stress priming may likely be attributed as an exception rather than the rule.

Stress memory in plants is a crucial component of "intelligent" demeanour that can be interrogated at primarily three states of complexity: (a) seed priming, to circumvent stress during germination via induction of cross-stress tolerance, (b) memory of plants at post-embryonic stage for survival during climatic variations and (c) transgenerational memory wherein the effects are transgressed to subsequent generations that may prove effective from the ecological perspective (Munne-Bosch and Alegre 2013). Numerous molecular mechanisms conferring plant memory have been illustrated to date. Firstly, persistent alterations in the levels of vital signalling metabolites, secondary messengers or transcription factors may create memories in plants, which in turn clarifies the mechanisms underlying altered and stabilized states of plant metabolism (Bruce et al. 2007; Conrath 2011; Walter et al. 2011). The role of calcium in memory is briefly described in Chap. 11. Post-stress consistent invocation of transcription factors (TFs) or signalling proteins, for instance,

constant expression of micro-RNAs (miRNAs) to modulate Squamosa Promoter-Binding Protein-Like (SPL) TFs, was crucial for heat shock memory responses (Stief et al. 2014). Excitation of secondary messengers and signalling components is essential for BABA-induced priming of salicylate-dependent defence (Ton et al. 2005). Secondly, epigenetics ascribes heritable arrangements of phenotypic variations that are not solely due to variance in DNA sequences, and histone modifications and DNA methylation are known to be inherited via mitosis or cell divisions (Eichten and Schmitz 2014). Variations in the chromatin states, for instance, histone tail modifications, DNA methylation or stalled RNA polymerase II (Pol II), may enact subsequent role in synchronized alterations in the gene expression patterns forming the basis of memory responses. Moreover, a diverse range of environmental stresses have revealed to alter chromatin and associated epigenetic signatures (Liu et al. 2016; Crisp et al. 2016; Avramova 2015, 2018; Kinoshita and Seki 2014; Eichten and Schmitz 2014; Paszkowski and Grossniklaus 2011). Another detailed mechanism is transgenerational stress priming via seed provisioning, whereby environmental stresses affect the resources that are packaged into seeds, crucial for germination and initial development of the seedling (Herman and Sultan 2011). Strategies for memory development may occur particularly in the course of stress recovery. Factually, in the case of FLC, even though during the cold span, repressive chromatin marks are engrained at the nucleation regions, it is not until reversing to warm conditions (recovery) that the Polycomb Repressive Complex 2 (PHD-PRC2) is demodulated across the entire FLC locus and H3K27me3 enhances significantly throughout the complete gene in order to effectuate epigenetic silencing. Therefore, span of recovery is crucial for vernalization and development of cold stress memories (Angel et al. 2011; Lucia et al. 2008).

Further, repetitive stress episodes can drastically impair plant's fitness; however, continual stress exposure capacitates "training" of a plant to confront the specific stress and, thereby, alleviating the fitness cost. The theory of "transcriptional memory" (Avramova 2015) suggests that stress-responsive genes can be "trained" by stress endurance and, thereby, exhibit exponential expression in antiphon to stress repetition. Consequently, plants can distinguish a single stress from repeated stress exposures and correspondingly transform the expression of the stress-responsive genes. Spans of recovery between the stress episodes might augment "training". Reports have analysed the comparative effects of pulsed UV-B exposure on plant metabolites levels that invoke UV-B protection with those of the uninterrupted UV-B exposure (Höll et al. 2019). Despite the span of pulsed and continuous UV-B exposure being the same, plants that endured the pulsed exposure accrued enhanced UV-B protective flavanols and had the opportunity to succinctly recoup from UV-B exposure. These "interruptions" led to an increased expression of genes encoding enzymes functioning in flavanol biosynthesis, thereby causing an increment in flavanol concentrations. Furthermore, Xu et al. (2018) demonstrated that strawberry leaves primed by UV-C exposure revealed improved salvation in the face of a subsequential infection by *Mycosphaerella fragariae*, the pathogenic fungus that causes leaf spot disease. Moreover, besides regulation of stress priming, memory, and signalling at the transcriptional and translational levels, post-translational

modifications foster stress priming. Protein kinases are involved in the induction of stress responses. For instance, role of CDPKs in stress priming and memory has been highlighted by Hake and Romeis (2018). CDPKs are crucial for the Ca^{2+}- and ROS-mediated initiation of stress signalling (Dubiella et al. 2013) and sustain the hormone-directed systemic signal proliferation during pathogen infection. Protein kinases may further control activities of enzymes involved in the biosynthesis of defensive secondary plant metabolites. Hake and Romeis (2018) proposed that CDPKs kick off a "primed conformation" post their first priming stimulus that promotes complete invigoration as a consequence of repeated stress exposures. Stress priming or conditioning the plant's own stress response system can bestow agricultural sustainability (Hilker and Schmülling 2019).

Incidentally, an animal hormone melatonin, which is also present in plants, may also be involved in plant stress memory (Arnao and Hernandez 2019). On exogenous application of melatonin to roots, it is absorbed and mobilized according to the flow of transpiration and subsequently gets accumulated in the leaves (Yoon et al. 2019). Extracellular melatonin enhances intracellular melatonin levels as deduced from the expression of a crucial regulatory enzyme encoded by *TaSNAT* transcript in the melatonin biosynthetic pathway. In addition, melatonin upregulated polyamine contents, by promoting the synthesis of polyamines from the precursor amino acids arginine and methionine, and also alleviated the degradation of salt-induced polyamines. Synergistically, results revealed that melatonin mitigates salt stress mainly through its regulation on polyamine metabolism of wheat seedlings (Ke et al. 2018). Melatonin is now being considered as a plant hormone although its multiple actions also point to it being an important master regulator of redox homeostasis in plants (Arnao and Hernandez 2019). Nevertheless, umpteen plants regenerate entirely or partly via vegetative propagation that notably involves mitotic memories. However, stress is usually ephemeral, and as stress adaptation is stabilized via circumvention of stress, memories are counteracted and equilibrized by recovery through reprogramming, when memories happen to be dysfunctional (Crisp et al. 2016). One alternative that has been proposed for resetting is a strategy similar to DNA damage checkpoint mechanisms (Gutzat et al. 2012). Screening for factors associated with expunction of epigenetic stress memory highlighted that decrease in DNA methylation (*DDM1*) and Morpheus' Molecule 1 (*MOM1*), play a crucial role in transgenerational memory (Iwasaki and Paszkowski 2014). As opposed to the huge body of evidences related to adaptation and memory, there exists a paucity of reports on stress recovery. Noteworthily, authors analysed that above-ground organs recovered completely within a day of drought stress reversal and majority of the stress-responsive genes reciprocated in the opposite manner (Zhang et al. 2014). Perrone et al. (2012) and Oono et al. (2003) revealed enrichment of genes associated with flavonoid biosynthetic pathway, aquaporins and rehydration-inducible genes. Nonetheless, umpteen open-ended questions involving physiological, molecular and ecological arenas of stress priming and memory still remain unanswered. For instance, paucity of information exists on whether the ability to get primed or "primability" is an incessant trait throughout the lifetime or if it relies on the stage of growth or development of a plant or if it is organ or tissue

specific (Engelberth and Engelberth 2019). Additionally, supplementary research focusing on the impact of diurnal and seasonal alterations on stress priming and memory would unravel neoteric insights. Moreover, it will be interesting to highlight the influence of a vigorously altering climate on plant's memory of a distinct stress episode in their life cycle. Employing high-precision genetic approaches would aid in deciphering the underlying mechanisms of stress priming and memory that would elucidate their spatio-temporal configurations and the costs and benefit of data storage and retrieval. Finally, as priming impacts plant performance, productivity and reproductive progress, it will further determine plant population and community architecture that needs to be explored.

23.4.3 Memory During Flower Transition

Furthermore, an intriguing mode of biochemical memory is offered by prions via sustainable changes in their protein conformation and function. These proteins have been identified in fungi, mammals and plants (Chakrabortee et al. 2016). Candidate prion domains (PrDs) in nearly 500 plant proteins were dissected utilizing computational modelling techniques. Strikingly, Luminidependens protein behaved as prion-like conformational switches that were evolutionarily conserved and may function in a range of divergent biological processes. An evolutionarily conserved prion conformation of the cytoplasmic polyadenylation element binding protein suffices as a "molecular memory" for the sustained stability of neuronal synapses in *Aplysia* and *Drosophila* (Si et al. 2003, 2010; Majumdar et al. 2012). Plant flowering is of huge interest with respect to biological memory, as its regulation implies memorizing and assimilating previously endured ambient conditions. The prion-developing ability of the three prion candidates associated with flowering were probed utilizing a yeast model, wherein prion characteristics were explicitly known. In yeast, prions absolutely alter protein functions by templating monomers into higher-order assemblies. In most yeast prions, the ability to transform into a prion dwells in a discrete prion domain. Eventually, novel prion-forming domains may be characterized by functional complementation of a known prion domain. The prion-like domains (PrDs) of all three of the tested proteins formed higher-order oligomers. It has been reported that Luminidependens, which are prion-like proteins, may be responsible for memory in plants, and as they keep changing their activity based on past events, they help plants to decide when to flower (Chakrabortee et al. 2016). If conditions were not conducive post cold stress, the flowering was delayed until the temperature and light conditions are fine, which suggested that they "remember" their exposure to cold.

It has been established that it is the leaves of the plants, which perceive environmental signals, like light, that regulate flowering behaviour. The hypothesis is that a chemical or a molecule moves from leaves to the shoot apex to initiate flowering at a specific time of plant development and in a particular season. However, one always wondered how some plants, like apple, flower in early spring season even before the leaves, which had dropped down before the onset of winter, have appeared on the branches of the tree. For such plants, exposure to cold also seems to be essential for

flowering. Hence, it seems plants may have stored memories in the form of chemicals before the leaf fall, which at the onset of favourable conditions signals the plants to bloom. Vernalization emerges as the explicitly understood environment-responsive epigenetic phenomenon, whereby FLC or the Flowering Locus C is transcriptionally suppressed by cold stress and repression is then epigenetically reinforced during subsequential growth in warmer temperatures, fostering a memory of the previous cold exposure (Crisp et al. 2016; Berry and Dean 2015; Woods et al. 2014; Eichten and Schmitz 2014).

23.5 Awareness in Plants

23.5.1 Experiments with Anaesthetic Chemicals

Sir Jagdish Chander Bose was one of the pioneers in conducting experiments that revealed plants can feel and respond to various stimuli. He measured cell membrane potentials using an equipment called "crescograph", assembled by him. As and when he would treat the telegraph plant *Desmodium* with chemicals like chloroform, which caused anaesthesia in animals, the plant's electrical signals would show a different pattern. Based on the analysis of nature of variations of the cell membrane potentials, he concluded that plants do have some sort of nervous mechanisms. He published his work in a few books like *The Nervous Mechanisms in Plants* and *Plant Autographs and Their Revelations*. Though many were sceptic about his work, he convincingly demonstrated his experiments at various international fora. Unfortunately, not many followed up on his work, not even in India. In fact, Charles Darwin, who had studied the carnivorous plant which closes its trap to capture insects, had indicated the presence of animal nerve-like communication system and, with the help of a medical physiologist, Burdon-Sanderson, tried to show electrical signalling. In the early nineteenth century, Claude Bernard had also predicted occurrence of fast reactions in plants. In one of our own study on Sorghum plants, done in collaboration with a neuroscientist, we showed that plants do transmit electric stimuli from root to shoot and this conduction is rather fast and may have consequences in relation to plant signalling and development (Sannan-Mishra et al. 2001). The details on electric signalling have been given in Chap. 19.

Yokawa et al. (2017) administered diethyl ether on four different plants, namely, Mimosa, Dionaea, Drosera and pea, to study the movement of tendrils. It was found that upon application of the anaesthetic compound for a period of time, the leaves of Mimosa or the insectivorous plants did not respond to touch, and even tendrils did not move to take hold of a nearby support. Moreover, these effects were reversible. Thereby, plants emerged as sensitive entities like animals and humans, revealing that anaesthetics administered at specific dosage stalled action potentials and deactivated organs by influencing action potentials, endocytic vesicle recycling and ROS homeostasis. Gremiaux et al. (2014) had also earlier argued that the effects of anaesthetics indicate that there are similarities between plants and animals, and this pinpoints the existence of "consciousness" in plants.

23.5.2 Role of Neurotransmitters

Plants, as in animals, have hormones and regulators whose concentrations are very well regulated like in animals and which control many plant processes, right from seed germination to senescence and plant death. Cellular signalling in the nervous system functions at specific nodes of contact termed as synapses via neurotransmitters. Pre- and postsynaptic cells coordinate and reshuffle into a complex convolution for swift and efficacious synaptic transmittance. Chemical compounds that enact a crucial role in peripheral and central neurotransmission of animals, for instance, biogenic monoamines (e.g. dopamine, noradrenaline, acetylcholine, adrenaline, serotonin or 5-hydroxytrptamine, gamma amino butyric acid or GABA) and acetylcholine, have even displayed their functionality in the plant kingdom, as reported by several researchers, and this has been covered in detail in Chap. 16. Earlier, a book entitled *Neurotransmitters in Plant Life* by Roshchina had been published, which examines the role of neurotransmitters and how plants respond to neuromediators. This book was originally published in Russian in 1991, and its English translation was brought out in 2001. We ourselves found that serotonin mediates light responses in plants through biochemical mechanisms similar to that operating in the human system (Chandok and Sopory 1994). Recently, the role of serotonin and consciousness has been discussed by Tonello et al. (2015), who believe that since serotonin is a tryptophan derivative, it may be involved in conversion of light to excitation energy, which in turn might orient leaves towards sunlight. Further, auxin drives root "arborescence" in soil, and simultaneously, serotonin presumably fosters enteric nervous system linkage within the gastrointestinal tract in humans. The aforesaid auxin/serotonin analogy implicates that root branch axis in plants might be an evolutionary forerunner or ancestor to the gastrointestinal-brain axis in human beings (Tonello et al. 2017). They hypothesised that light may enact as a crucial factor both in gastrointestinal dynamics and brain function. Finally, they deciphered a potential role for the interplay of light and serotonin in neuronal physiology that included both sympathetic and parasympathetic nervous systems. Even in animals, serotonin could participate in interactions with microtubules, which are being shown to be involved in proto-consciousness, as we will mention later. This action could be similar to those obtained using anaesthetics. Whether data obtained in the experiments of Bose and others using anaesthetics can be explained via serotonin-based mechanisms cannot be confirmed unless further direct experiments are done.

23.5.3 Views on Plant Consciousness

Are plants conscious of their status in the environment? Numerous books and review articles have been published to date that enumerate and elucidate novel experiments to reveal that plants are highly sensitive to varying environments, encompassing them and eventually operate accordingly for their growth and survival. Michio Kaku (2015), an American physicist, futurist and champion of science who professes theoretical physics in City College, New York, defines consciousness in his book titled *The Future of The Mind*, as the number of feedback loops required to create a model

of your position in space, with relationship to other organisms and finally their relationship to time. Are plants capable of doing that? For instance, a thermostat possesses one unit of consciousness as it perceives or senses the ambient temperature. He further states that a flower carries 10 units of consciousness as it is able to sense and comprehend the weather, temperature, humidity, etc. Michio Kaku suggested that sensing is the first line of consciousness in plants which are astonishingly more sensitive than animals. Incidentally, higher sensitivity may be attributed to the fact that a single plant is proficient to detect at least 20 disparate physico-chemical and biotic parameters, all through. These may range from electrical and magnetic gradients or fields, heavy metals, pathogens to herbivores, sniffing their preferential hosts and sensing vibrations to extending their roots towards the source of sound. The recent past has witnessed the evolution of an entirely intriguing genre of scientists, namely, "plant neurobiologists"—a term detested by fellow plant scientists, who in their recent findings highlighted that plants have extraordinary capabilities to perceive and reciprocate to the ambience. One in this league of scientists, Michael Pollan, who is the author of books as *The Omnivore's Dilemma* and *The Botany of Desire: A Plant's-Eye View of the World* and the writer of "The Intelligent Plant", reconnoitred some of the latest research, probing the occurrence and degree of plants' adeptness to make sense of their environment via strategies that are analogous to seeing, hearing and smelling. An unnerving volley of questions raised by plant scientists has been catered to in this book. For instance, do plants learn the way we comprehend the term, to learn? Can we truly state that plants are intelligent or conscious? Aren't these features reclusive for systems harbouring brains? As plants don't have them, so what does plant neurobiology signify? Analysing the nervous systems is the bottom line of neuroscience; thus, usage of the term plant neurobiology is tantamount to breaking a law, right?

Stephan Mancuso advocated the term "Plant Neurobiology" to reinforce the concept that plants coincidentally share biochemistry, cell biology and electrophysiology synergistically identical to the human brain (Baluška and Mancuso 2009). Eric D. Brenner, an American plant molecular biologist, Stefano Mancuso, an Italian plant physiologist; František Baluška, a Slovak cell biologist; and Elizabeth Van Volkenburgh, an American plant biologist have contradicted that the refined demeanour of plants may currently be inadequately interpreted by mundane mechanisms pertaining to genetics and biochemistry. They state that there is a resident brain-like data processing network in plants that integrates information from the ambience and unequivocally correlates it into a concerted response, displayed by them while reciprocating to variables like microbes, herbivores, light, water, gravity, temperature, soil structure, nutrients and toxins. Moreover, the authors exhibited that plants have been characterised by homologous electrical and chemical signalling systems to those demonstrated in animal nervous systems. Strikingly, the manifestation of umpteen neurotransmitters including dopamine, acetylcholine, glutamate and GABA, possessing an excitatory or inhibitory role in the mammalian cortex, has been surprisingly found in plants too. Should it imply that Aristotle's delineation between plants that are devoid of sensory traits, and animals that harbour them, may no more hold any significance?

Rene Descartes, a French philosopher in the seventeenth century had the notion that only the human body has a soul and other animals are like robots who cannot feel pain nor can reason. From this concept, we have come a long way, and an alternative belief has evolved that consciousness is ubiquitous in all living organisms. In 2012, at the first Francis Crick (who along with James Watson discovered the structure of DNA and received a Nobel Prize) Memorial Conference at Cambridge, declaration on consciousness in non-human animals was proclaimed. According to Lynn Margulis, "every organized living being is conscious. In the simplest terms, consciousness is awareness of the outside world" (Margulis and Sagan 2000). Trewavas and Baluska (2011) stated that "consciousness in its many forms could well be ubiquitous, even down to the simplest of organisms". Giulio Tononi has advanced a theory of consciousness called integrated information theory, which simply means that conscious experience means integrating a wide range of information from sensory systems and cognitive processes. One of the major questions that aroused was whether mind and consciousness are linked to any physical entity, like the brain? Many groups have been working to discover specific neurons or an area in the brain, which can be linked to awareness or consciousness. Notwithstanding the above, many organisms, even plants, as elaborated in this book and briefly described here, do fit in the integrated information theory of Tononi. In this context, Leopold, a famous plant biologist, had written a paper in 2014 entitled "Smart plants: Memory and communication without brains". Barbara McClintock, a Nobel Prize-winning plant biologist, had mentioned that each cell has the knowledge, which it uses in an intelligent way. Thus, cells and groups of interacting cells can form self-organized "thinking structure" to receive, integrate and propagate information. Charles Darwin in his book on "The power of movement in plants" (1880) wrote "It is hardly an exaggeration to say that the tip of the radicle (root) thus endowed, and having the power of directing the movements of the adjoining parts, acted like the brain of one of the lower animals; being seated within the anterior end of the body, receiving impressions of the sense-organs and directing several movements". And Allmann (1999) wrote "some of the most basic properties of brain such as sensory integration, memory, decision making and control of behaviour can be found in these simple organisms". It was in 1902 that Charles Minot said "a frank unbiased study of consciousness must convince every biologist that is one of the fundamental phenomena of at least all animal life, as is quite possible of all life" (Trewavas and Baluska 2011).

Peter Barlow, University of Bristol, has tried to analyse the question of plant consciousness by invoking the Hameroff-Penrose quantum physical Orch OR (Orchestered Objective Reduction) theory of universal consciousness. Briefly, it states that "when sufficient mass of tubulin protein molecules assembled into cytoskeleton microtubules within neuronal cells of the brain, they serve as sites of quantum computation and of quantum state reduction (OR) events resulting in moments of proto-consciousness" (Barlow 2015). As we know, plants and humans share a lot of genes coding for similar proteins. It has been found that plants do have tubulin proteins and these, like in animals and humans, polymerize to form microtubules which are similar to neuro-tubules and are involved in many plant functions. It is,

thus, possible that plant microtubules might be the site of quantum reduction events, as proposed by Barlow, and thus proto-consciousness.

Proprioception or sensing one's own shape has been shown to have significant functional inference in animal physiology that pertains to mobility and posture regulation. Intriguingly, proprioception brought in the concept of feedback in biology that states if the central nervous system induces mobility via the initiation of muscle contraction, this can in turn lead to muscle being able to sense a deformity and signal this information to the cerebellum. As growth may be understood in terms of deformities and dislocation, the concept of proprioception has been extended to developmental biology that has recently included plants in addition to animals (Hamant and Moulia 2016). Incidentally, shape-dependent diffusive gradients and shape-derived mechanical stress patterns have shown relevance in both forms of life, i.e., fauna and flora.

The earlier experiments of Bose and others have shown that animals and plants shared similarities in their responses to anaesthetic agents or chemicals (Gremiaux et al. 2014). These studies support the concept of primary consciousness state of plants as these anaesthetics effect plant responses by destabilizing microtubular structures, which are also crucial for electrical signalling between neurons in animals. Whether a similar phenomenon operates for plant electrical signalling is yet elusive. While there are others who have their reservations for the Hameroff-Penrose theory, it provides some explanation for plant-based sensory perceptions and memory residing in proto-consciousness state. John Gardiner from the University of Sydney also feels that "two major concomitants of consciousness in animals are microtubule functions and electrical gamma wave synchrony. Both these factors may also play role in plant consciousness". It is possible as suggested by Gardiner (2012) that electrical properties in the plant cells may substitute the role of gamma waves in promoting consciousness. He also suggested the importance of quasicrystal (fivefold symmetry crystals) in quantum mechanics and reported them in plants in the form of pentagonal arrays of ribosomes (site for protein synthesis in cells).

23.6 Conclusion

While we are still to get to terms on the scientific basis on the nature of consciousness in plant life, it is nevertheless obvious that plants have the ability to sense the environment, integrate information and have the "will" to survive and, thus, are aware of their surroundings. Definitely more thoughts, experiments and work will continue on this topic to understand the cognitive nature of plants and the existence of consciousness in biological species outside the human domain. Numerous studies and observations riveted us to think, if the world is what we see or understand or are we missing out on something. Or is it that we are under the awe of science and its description of the nature that we overlook or feel apprehensive of expressing our perspectives on issues that go beyond the theories and dimensions of the present-day scientific explanations, as has been dealt in the book entitled *Blinded by Science* by Silverstone. It can be said that nature is only slowly revealing its secrets of the

biological world. There is so much more to learn about the commonality and differences, as also the interactions amongst different life forms. Are the trinity skills, i.e., intelligence, memory and learning, anomalous or rather outlandish terms in plant science as these abilities are solely confined to organisms with neural systems, or is there something more to it, remarkable designing that makes plants appear smart? Incidentally, like members of an animal family, the plant scientists presume that intelligence implicates plausible physical mobility. However, the extensive evidence on awareness via sensory perception and communication, stress priming, memory and signalling, and the presence of neurotransmitters in plants, indicates the necessity for improved appreciation of their inherent intelligence. Plant intelligence obviates the use of brain and that the intricate communications, though minimally comprehended in plants, may be sufficient. Future probing must focus on signal assessment. The tenor of much of the plant research has concentrated on identifying signals, the positive feedbacks that initiate change. Perhaps the more crucial are the negative feedback interactions that indicate receipt of a signal and control its further expression, however, virtually nothing is known about them. Nervous systems evolved due to the need to move in order to find food, a specific pattern of living, however, only one of the few that we have in common. Intelligence presumably emerged consequentially in organisms that persistently face variable ambient factors, both plants and animals. Sans intelligence, competition and fitness would never have synergized evolutionary variations in a pre-set manner. In contrast to the primary controversial jumpstart in 2003, investigations on plant intelligence have branched into diverse themes of study, offering productive concepts that foster the comprehension of plant cognition and it continues to expand. Revelations about degrees of complexity in behaviours thought to be reclusive to domain of animals in the past, due to scientific testimonies over the last couple of decades, has robustly questioned the Aristotelian perspective which states that 'the apportioning between plants and animals is the lack of memory, learning and behaviour in the former and their presence in the latter' and solicits revisiting the definitions of memory, learning and behaviour to accommodate and embrace plants.

Acknowledgement SKS is thankful to SERB (Govt of India) for providing the Distinguished Fellowship award.

References

Allman JM (1999) Evolving brains. Scientific American library. No.68. Scientific American Library, New York . ISBN 0–7167–5076-7

Angel A, Song J, Dean M, Howard C (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–108

Arnao MB, Hernandez RJ (2019) Melatonin: A new plant hormone and/or a plant master regulator? Trends Plant Sci 24:38–48

Avramova Z (2015) Transcriptional memory of a stress: Transient chromatin and memory (epigenetic) marks at stress-response genes. Plant J 83:149–159

Avramova Z (2018) Defense-related priming and responses to recurring drought: Two manifestations of plant transcriptional memory mediated by the ABA and JA signaling pathways. Plant Cell Environ 42:983–997

Baluska F, Gagliano M, Witzamy G (eds) (2018) Memory and learning in plants, Series on Signalling and communication in plants. Springer, p 222
Baluška F, Mancuso S (2009) Deep evolutionary origins of neurobiology: turning the essence of'neural'upside-down. Commun Integr Biol 2:60–65
Baluska F, Mancuso S, Volkmann D, Barlow PW (2004) Root apices as plant command centres: the unique brain- like status of the root apex transition zone. Biologia 59:9–14
Barlow PW (2015). The natural history of consciousness, and the question of whether plants are conscious in relation to the Hameroff-Penrose quantum –physical Orch OR theory for universal consciousness. Communicative and Integrative Biology 8:4 e 1041696
Berry S, Dean C (2015) Environmental perception and epigenetic memory: mechanistic insight through FLC. Plant J 83:133–148
Birhaum KD, Roudier F (2017) Epigenetic memory and cell fate reprogramming in plants. Regeneration 4:15–20
Blifernez-Klassen O, Klassen V, Doebbe A, Kersting K, Grimm P, Wobbe L, Kruse O (2012) Cellulose degradation and assimilation by the unicellular phototrophic eukaryote *Chlamydomonas reinhardtii*. Nat Commun 3:1214
Böhm J, Scherzer S, Shabala S, Krol E, Neher E, Mueller TD, Hedrich R (2015) Venus flytrap HKT1-type channel provides for prey sodium uptake into carnivorous plant without conflicting with electrical excitability. Mol Plant 9:428–436
Böhm J, Scherzer S, Krol E, Shabala S, Neher E, Hedrich R (2016) The Venus Flytrap *Dionaea muscipula* Counts Prey-Induced Action Potentials to Induce Sodium Uptake. Curr Biol 26:286–295
Boyko A, Kovalchuk I (2011) Genome instability and epigenetic modifications-Heritable responses to environmental stress? Curr. Opin. Plant Biol 14:260–266
Bruce TJA, Matthes MC, Napier JA, Pickett JA (2007) Stressful memories of plants: Evidence and possible mechanisms. Plant Sci 173:603–608
Chakrabortee S, Kayatekin C, Newby GA, Mendillo ML, Lancaster ML, Lindquist S (2016) Luminidependens (LD) is an Arabidopsis protein with prion behaviour. Proc Natl Acad Sci U S A 113:6065–6070
Chamovitz D (2012) What a plant knows. One World Publications, England, p 213. ISBN 978-1-85168-910-1
Chandok MR, Sopory SK (1994) 5-hydroxytryptoamine affects turnover of polyphosphoinositides in maize and stimulates nitrate reductase in the absence of light. FEBS Lett 356:39–42
Conrath U (2011) Molecular aspects of defnce priming. Trends Plant Sci 16:523–531
Crisp PA, Ganguly D, Eichten SR, Borevitz JO, Pogson BJ (2016) Reconsidering plant memory: intersections between stress recovery, RNA turnover and epigenetics. Sci Adv 2:e1501340
Dubiella U, Seybold H, Durian G, Komander E, Lassig R, Witte P, Romeis T (2013) Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. Proc Natl Acad Sci U S A 110:8744–8749
Eichten SR, Schmitz RJ (2014) Springer, Epigemnetics: beyond chromatin modifications and complex genetic regulation. Plant Physiol 165:933–947
Elhakeem A, Markovic D, Broberg A, Anten NP, Ninkovic V (2018) Aboveground mechanical stimuli affect belowground plant-plant communication. PLoS One 13:e0195646
Engelberth J, Engelberth M (2019) The costs of green leaf volatile induced priming. Plants 8:E23
Escalante-Pérez M, Krol E, Stange A, Geiger D, Al-Rasheid KAS, Hause B, Neher E, Hedrich R (2011) A special pair of phytohormones controls excitability, slow closure, and external stomach formation in the Venus flytrap. Proc Natl Acad Sci U S A 108:15492–15497
Falik O, Mordoch Y, Quansah L, Fait A, Novoplansky A (2011) Rumor has it…: relay communication of stress cues in plants. PLoS One 6:e23625
Gagliano M, Renton M (2013) Love thy neighbour: facilitation through an alternative signalling modality in plants. BMC Ecol 13:19
Gagliano M (2014) In a green frame of mind: perspectives on the behavioural ecology and cognitive nature of plants. AoB Plants 7 plu 075

Gagliano M, Grimonprez M (2015) Breaking the silence-language and making of meaning in plants. Ecopsychology 7:145–153
Gagliano M, Grimmonprez M, Depczynski M, Rent M (2014) Plants roots use sound to locate water. Oecologia 184:1521–1160
Gagliano M, Grimonprez M, Depczynski M, Renton M (2017) Tuned in: plant roots use sound to locate water. Oecologia 184:151–160
Gagliano M, Abramson CI, Depczynski M (2018) Plants can learn and remember-lets get used to it. Oecologia 186:9–31
Gardiner J (2012) Insights into plant consciousness from neuroscience, physics and mathematics: a role for quasicrystals? Plant Signal Behav 7:1045–1055
Gremiaux A, Yokawa K, Mancuso S, Baluska F (2014) Plant anaesthesia supports similarities between animals and plants. Plant Signal Behav 9:e27886
Guerrieri E, Poppy GM, Powell W, Rao R, Pennachio F (2002) Plant-to-Plant Communication Mediating In-Flight Orientation of *Aphidius ervi*. J Chem Ecol 28:1703–1715
Gutzat R, Borghi L, Gruissem W (2012) Emerging roles of RETINOBLASTOMA-RELATED proteins in evolution and plant development. Trends Plant Sci 17:139–148
Hake K, Romeis T (2018) Protein kinase-mediated signaling in priming: Immune signal initiation, propagation, and establishment of long term pathogen resistance in plants. Plant Cell Environ 42:904–917
Hamant O, Moulia B (2016) How do plants read their own shapes? New Phytol 212:333–337
Haskell DG (2017) The songs of trees: stories from nature's great connectors. Penguin Random House. ISBN: 9780525427520
Herman JJ, Sultan SE (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms and implications for natural populations. Front Plant Sci 2:102
Hilker M, Schmülling T (2019) Stress priming, memory and signaling in plants. Plant Cell Environ 42:753–761
Höll J, Lindner S, Walter H, Joshi D, Poschet G, Pfleger S, Ziegler T, Hell R, Bogs J, Rausch T (2019) Impact of pulsed UV-B stress exposure on plant performance: how recovery periods stimulate secondary metabolism while reducing adaptive growth attenuation. Plant Cell Environ 42:801–814
Iwasaki M, Paszkowski J (2014) Identification of genes preventing transgenerational transmission of stress-induced epigenetic states. Proc Natl Acad Sci U S A 111:8547–8552
Kaku M (2015) The future of the mind. Penguin Group, UK. ISBN:9780141975870.
Ke Q, Ye J, Wang B, Ren J, Yin L, Deng X, Wang S (2018) Melatonin mitigates salt stress in wheat seedlings by modulating polyamine metabolism. Front Plant Sci 9:914
Kinoshita T, Seki M (2014) Epigenetic memory for stress response and adaptation in plants. Plant Cell Physiol 55:1859–1863
Leopold AC (2014) Smart plants: memory and communication without brains. Plant Signal Behav 9(10):e972268
Libiaková M, Floková K, Novák O, Slováková L, Pavlovič A (2014) Abundance of cysteine endopeptidase dionain in digestive fluid of Venus flytrap (*Dionaea muscipula Ellis*) is regulated by different stimuli from prey through jasmonates. PLoS ONE 9:e104424
Liu N, Staswick PE, Avramova Z (2016) Memory responses of jasmonic acid-associated Arabidopsis genes to a repeated dehydration stress. Plant Cell Environ 39:2525–2529
Lucia D, Crevillen P, Jones ME, Greb T, Dean C (2008) A PHD-Polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. Proc Natl Acad Sci U S A 105:16831–16836
Maffer ME (2014) Magnetic effects on plant growth, development and evolution. Front Plant Sci 5(445):1–15
Majumdar A, Cesario WC, White-Grindley W, Jiang H, Ren F, Khan MR, Li L, Choi EM, Kannan K, Guo F, Unruh J, Slaughter B, Kausik S (2012) Critical role of amyloid-like oligomers of *Drosophila* Orb2 in the persistence of memory. Cell 148:515–529
Mancuso S (2018) The revolutionary genius of plants. Atria Books, ISBN 978-1-5011-8785-8.
Markovic D, Colzi I, Taiti C, Ray S, Scalone R, Gregory Ali J, Mancuso S, Ninkovic V (2019) Airborne signals synchronize the defenses of neighboring plants in response to touch. J Exp Bot 70:691-700.

Marder M (2013a) What is plant thinking? Philos Nat 25:124143
Marder M (2013b) Plant thinking: a philosophy of vegetal life. Columbia University Press
Margulis L, Sagan D (2000) What is life? Univ of California Press
Munne-Bosch S, Alegre L (2013) Cross-stress tolerance and stress "memory" in plants: An integrated view. Environ Exp Bot 94:1–2
Nakamura Y, Reichelt M, Mayer VE, Mithöfer A (2013) Jasmonates trigger prey-induced formation of 'outer stomach' in carnivorous sundew plants. Proc Biol Sci 280:20130228
Oono Y, Seki M, Nanjo T, Narusaka M, Fujita M, Satoh R, Satou M, Sakurai T, Ishida J, Akiyama K, Iida K (2003) Monitoring expression profiles of Arabidopsis gene expression during rehydration process after dehydration using ca. 7000 full-length cDNA microarray. Plant J 34:868–887
Paszkowski J, Grossniklaus U (2011) Selected aspects of transgenerational epigenetic inheritance and resetting in plants. Curr Opin Plant Biol 14:195–203
Perrone I, Gambino G, Chitarra W, Vitali M, Pagliarani C, Riccomagno N, Balestrini R, Kaldenhoff R, Uehlein N, Gribaudo I, Schubert A (2012) The grapevine root-specific aquaporin VvPIP2; 4N controls root hydraulic conductance and leaf gas exchange under well-watered conditions but not under water stress. Plant Physiol 160:965–977
Sannan-Mishra N, Mallick BN, Sopory SK (2001) Electrical signalling from root to shoot in *Sorgum bicolor*: induction of leaf opening and evidence for fast extracellular propagation. Plant Sci 160:237–275
Scherzer S, Shabala L, Hedrich B, Fromm J, Bauer H, Munz E, Jakob P, Al-Rascheid KAS, Kreuzer I, Becker D, Eiblmeier M, Rennenberg H, Shabala S, Bennett M, Neher E, Hedrich R (2017) Insect haptoelectrical stimulation of Venus flytrap triggers exocytosis in gland cells. Proc Natl Acad Sci U S A 114:4822–4827
Shanta BN (2016) Life and consciousness-the vedantic view. Commun Integ Biol 8(5):e1085138
Sharma AK, Sopory SK (1984) Independent effects of phytochrome and nitrate on nitrate reductase and nitrite reductase activity in maize. Photochem Photobiol 39:49–494
Si K, Giustetto M, Etkin A, Hsu R, Janisiewicz AM, Miniaci MC, Kim JH, Zhu H, Kandel ER (2003) A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in *Aplysia*. Cell 115:893–904
Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER (2010) *Aplysia* CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. Cell 140:421–435
Stief A, Altmann S, Hoffman K, Pant BD, Scheible BI (2014) Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. Plant Cell 26:1792–1807
Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, Maeder MN, Metraux J-P, Mauch-Mani B (2005) Dissecting the β-aminobutyric acid-induced priming phenomenon in Arabidopsis. Plant Cell 17:987–999
Tonello L, Cocchi M, Gabrielli F, Tuszynski JA (2015) On the possible quantum role of serotonin in consciousness. J Integr Neurosci 14:295–308
Tonello L, Gashi B, Scuotto A, Cappello G, Cocchi M, Gabrielli F, Tuszynski (2017) The gastrointestinal-brain axis in humans as an evolutionary advance of the root-leaf axis in plants: A hypothesis linking quantum effects of light on serotonin and auxin. J Integr Neurosci 1:1-11.
Trewavas A (2014) Plant behaviour and intelligence. Oxford University Press. ISBN: 9780199539543
Trewavas A (2016) Intelligence, cognition and language of green plants. Front Psychol 7:588
Trewavas AJ, Baluska F (2011) The ubiquity of consciousness. EMBO Rep 12:1221–1225
Vanderkooi CJ, Dyer AG, Kevan PG, Lunan K (2019) Functional significance of the optical properties of flowers for visual signalling.
Walter L, Nagy R, Hein R, Rascher U, Beierkuhnlein C, Willner E, Jentsch WA (2011) Do plants remember drought? Hints towards a drought memory in grasses. Environ Exp Bot 71:34–40
Wohlleben P (2016) The hidden life of trees: what they feel, how they communicate-discoveries from a secret World. Penguin Books, Penguin Random House India Private Limited, ISBN: 978-0670089345.

Woods DP, Ream TS, Amasino RM (2014) Memory of the vernalized state in plants including the model grass *Brachypodium distachyon*. Front Plant Sci 5:1–7

Xu Y, Charles MT, Luo Z, Mimee B, Tong Z, Veronneau PY, Rolland D (2018) UV-C priming of strawberry leaves against subsequent *Mycospaerella fragariae* infection involves the action of ROS, plant hormones and terpenes. Plant Cell Environ 42:815–831

Yokawa K, Kagenishi T, Pavlovic A, Gall S, Weiland M, Mancuso S, Baluška F (2017) Anaesthetics stop diverse plant organ movements, affect endocytic vesicle recycling and ROS homeostasis, and block action potentials in Venus flytraps. Ann Bot 22:747–756

Yoon YH, Kim M, Park WJ (2019) Foliar accumulation of melatonin applied to the roots of maize seedlings. Biomol Ther 9:26

Zhang JY, Cruz De Carvalho MH, Torres-Jerez IV, Kang YU, Allen SN, Huhman DV, Tang Y, Murray J, Sumner LW, Udvardi MK (2014) Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. Plant Cell Environ 37:2553–7256

Sudhir Sopory – See under Chapter 1 contributions.

Tanushri Kaul did her Ph.D. from the University of Delhi and postdoctoral work at ICGEB in Plant Molecular Biology group, where the Editor was the Group Leader. She has also served as Visiting Research Scientist in the Institute of Plant Sciences, Agricultural Research Organization, Volcani Centre, Bet Dagan in Israel. She is currently the Group Leader of the Nutritional Improvement of Crops group at ICGEB.

Bhumandala Sanrachana: The Indian Worldview of the Natural and Plant World

24

Jaya Mehta

As is a mighty tree, so indeed is a man
Brihadaranyaka Upanishad

Green to red, chlorophyll to blood.
Colours must ripen. Or they leave no progeny
How I became a Tree (Sumana Roy, 2017)

Abstract

The Indian visualization of the natural universe, as described in textual sources and Indian art, declares plants as living beings. This idea is beautifully illustrated through the concepts of rta, rasa and manas. Trees are also likened to having tremendous sattvic element, which makes them a repository of sattvic plant, karma. Forests of trees have been described as sacred plant groves with high fields of energy. These traditional Indian associations with plant life have helped to create an environment of ecological conservation in different parts of the country. From my experience as an artiste and a dancer, herein, I have tried to highlight the powerful role of art and personal association. In human life, art and cultural associations are often the powerful network, through which we tap into the inner life of the natural world, and engage in conversation with plants as sentient beings. These ideas from across disciplines are presented to bring about a wider and more holistic understanding of the perception of plant life, with specific reference to Indian culture and thought.

J. Mehta (✉)
Odissi Kala Ashram, New Delhi, India
URL: http://www.jayamehta.in/; http://www.thepoeticsaree.com/

© Springer Nature Singapore Pte Ltd. 2019
S. Sopory (ed.), *Sensory Biology of Plants*,
https://doi.org/10.1007/978-981-13-8922-1_24

Keywords
Life force · Rta (cosmic law) · Rasa (sap/juice) · Manas (mind) · Dravya-manas (material body and its mind) · Bhava-manas (biological information energy) · Trees as sattvic beings · Tapovan (sacred groves) · Ecological conservation · Art as association and inspiration · Method of inquiry

24.1 Introduction

In the Indian perception of the universe, all life is embraced and it is the same life current which flows from the sun to the rivers, rocks, trees, water, fire and clouds (1982). Human agency is highlighted through karma, and man is connected to all the elements through this agency.

Ancient Indian texts and Indian art illustrate a rich connection between man and his environment. Underlying the life of man is his connection with every aspect of existence. This entire existence and its "pulse" was understood as the divine energy that permeated the universe. What was man's connection with this divine energy? How did people in ancient India connect with this energy?

In my opinion, even today, it is impossible to study or connect with Indian studies of any kind and understand its traditional art forms without understanding this vital connection. In the modern times, as individuals, we face a great disconnect between our daily lives and our larger understanding of where we are on this planet.

However, ancient Indians had a different approach of great sensitivity towards their natural environment. It is this visualization that I seek to explore in this essay through textual sources, traditional Indian art and dance. I would also like to illustrate, how as a performing artiste in modern times, I continue to work with a traditional dance form, and find through it a tremendous connect with the natural and plant world.

24.2 Of *Rta, Rasa* and *Manas*: Indian Religious Traditions and Their Understanding of the Plant World

In order to grasp whether Indian religious traditions, like Hinduism, Jainism and Buddhism, believed that plants were alive, it is vital to first understand their view of the universe. Some of these ideas are surprisingly modern and environmentally sensitive. To others, they may seem almost poetic in their grasp of the essence of life on earth. As Ellison Banks Findly (2008) points in her book *Plant Lives – Borderline Beings in Indian Traditions*, 'in the Indian Vedic tradition the entire cosmos prevails on the operating power of *rta. Rta* is the cosmic order or law prevailing in nature. It is the truth belonging to each natural element that identifies it and defines its place in the natural world. The sun's *rta* for example, is to give light and warmth and to mark divisions of time and space; the river's *rta* is to flow water, the cow's *rta* is to

give milk and the *rta* of humans is to make the natural elements remain truthful and in place.'

With this respect to the self-perpetuating order in nature, man is reminded to understand his agency in the larger scheme of things. According to Vedic traditions, he has immense responsibility to let the *rta* of all beings, follow its own course and not be forced to be unnatural.

Within this natural world, there are further ideas that examine the life of plants. What does it mean to be alive as a plant? What are the markers of their aliveness? What do we understand through these texts of the life force and mental faculties of a plant? As Findly (2008) illustrates, the attribution of life is connected to the ideas of sap, growth and breath. She starts with the idea of sap or the unctuous quality of living things. This sap is referred to as '*rasa*' in ancient Indian texts. The medicinal treatise Sushruta Samhita talks about *rasa* in its chapter on blood, and associates this term with the sap or fluid that is continually flowing through and permeating every vital principle of an animated organism.

This *rasa* or life force is a shared common essence or life force that flows through all living things: humans, animals, trees or plants. The *rasa* concept is also of great connect to Indian dance/theatre artistes, who work extensively to create *rasa* in a performance. The *rasa* in an Indian dance or theatre performance is the aesthetic emotion that the viewer gets to 'taste', and it is the very life of the artwork. A performance without *rasa* is called '*neerasa*', only a sum total of its parts, and lacks the life which is experienced vividly in a brilliant performance. This experience of *rasa* is also said to move the viewer beyond themselves, to another time and place, to think and feel deeply rather than merely entertain.

This imagery of *rasa* as embodying the life of a dance, tree or human, is a very vivid picture of life force. A life force, which is fluid, flowing, growing and contains the very essence of things. In a human, it could be blood and other secretions, in a tree, it's sap and in a dance, it's inner life force.

The second quality other than the *rasa* is the quality of growth. Living organisms display the capacity for growth and death. In the Sushruta Samhita, there are references to plants and vegetables that sprout in the rainy season and mature by the early winter season or *Hemanta ritu*. And in the Vrkshayurveda of Surapala, he notes that just as animals can die of injury or disease, so can plants, whose roots succumb to damage bringing death and whose trunks, branches, leaves and flowers can shrivel and dry up.

The quality of breath is explained with a very different perspective in Hindu texts. The Atharva Veda assesses the place of life-breath (*prana-tattva*) as the supreme element of the cosmos. This life-breath (*prana*) pervades through the earth, atmosphere and heaven. The life-breath (*prana*) of the heaven covers the earth through the sunrays, the life-breath (*prana*) of the atmosphere reaches the earth through the rains and on the earth, the life prevails in the form of life-breath (*prana*). All beings have life upon the earth in the form of life-breath (*prana*).

The popular Hindu view as Findly (2008) points is also that trees purify the air, which implies the inhaling and exhaling of air by plants. In Vrshayurveda, the Hindu tree doctor Surapala states that plants breathe insofar as they inhale and

exhale water from their environment. Texts like the Parasara also consider plants as having *sira* or channels that enable them to circulate both water and air, which is considered the reason why they have an ability to be healed from injuries.

One of the most interesting understanding on the life of plants is in the tradition of Jainism. It goes beyond the idea of living beings as born with a *manas* (mind) or no mind. In the commentaries on the Tattvarth Sutra, there is the idea of *manas* being of two kinds: the physical (*dravya-manas*), which is the physical matter or brain of living beings, and the *bhava-manas*, which is of a more fine and subtle constituency, that is the 'biological information energy', which supports and gives rise to the physical brain. It happens that beings who are declared to be devoid of *manas* are actually only devoid of *dravya-manas,* the physical brain, but not the *bhava-manas* and therefore, still possess the potential for the creator of the full mental process. Plants are thus, recognised as possessing an information energy like other sentient beings, and having a mental process despite not having a brain like human beings. They are also acknowledged in various texts as having features of life, breath, growth and death.

24.3 Trees as *Sattvic* Beings and Hosts of Sacred Congregations

In the Hindu karma theory, living beings and humans possess the qualities of *tamas*, *rajas* and *sattva*. The *tamas guna* or quality represents darkness, delusion, lethargy and inactivity. The quality of *rajas* includes energy, motion, stimulation, envy, pride and dishonesty. *Sattva guna* or quality represents intelligence, reflection, purity, goodness, freedom from attachment, fear, anger and violence. Indian medical texts also describe *sattva* beings as life forms having the strength or stable mooring to stabilise themselves in the midst of others, as being patient, free from perturbance and equanimous to all.

Noting these qualities, Findly (2008) points to us that plants are remarkable in two ways: they are central to the renunciant dwelling at the root of trees, and the use of trees in metaphors for spiritual advancement. In traditional Indian literature, we find countless references to the forest as being cherished by sages, as a place of transcendence and more like a state of mind. Nanditha Krishna raises the point that in the Vedic period, all of nature was divine – part of an indivisible life force uniting the world of humans, animals and vegetables (Krishna and Amirthalingam 2014). Grasses and herbs were also considered sacred. She mentions that the Rig Veda states that the trees are the homes and the mansions of the gods. The *Aranyakas* or forest books of Vedic literature have arisen out of forest experiences of introspection, reflection and spiritual realization. They belong to conversations at '*aranyas*' or peaceful resorts where sages like Yajnavalkya lived and maintained their *ashramas*. Indian epics like the Ramayana and Mahabharata have an *aranyakanda* or forest section, in which the exile to the forest is an exile to a place of knowledge and illumination, a refuge of spiritual wisdom.

If these trees in the forest are deep repositories of the *sattvic* plant karma, displaying higher qualities of being, then the forest itself can be rightfully described as a sacred grove. Nanditha Krishna (2017) in 'Hinduism and Nature' talks of the sacred grove as the single most important ecological heritage of India. A sacred grove is a mini-biosphere reserve, which is an area of conservation as well as spiritual retreat. Sacred groves were the *tapovana* that once existed within the forests of ancient India, where the *ashramas* of *rishis*/sages were located. These forests were considered inviolate by urban and village settlers, and unlike other forests could not be touched for hunting or commercial purposes. These forests were also often attached to a settlement or community, and their biodiversity was preserved through their sacred association. Thousands of such bioreserves have survived, as local communities have dedicated part of these to local folk deities or ancestral spirits.

An interesting example of this is seen in the capital city of India. To the south of Delhi is a patch of forest known as 'Mangarbani'. Pradip Krishen (2006) in his book, 'Trees of Delhi', highlights the interesting fact that unlike most of Delhi's ridge area that has been deforested for human settlement, this small region has remained unspoiled by human beings and even their livestock. It is considered a sacred forest, consecrated in the memory of a holy man, Gudariya Baba, and protected by the superstition that anyone who breaks a branch or grazes his goats there, will suffer serious harm. Many tree specimens that have vanished from the rest of Delhi still survive in the Mangarbani forest. Similarly, many such small forest areas exist in India, in which local communities have refrained felling due to sacred associations. As an interesting comparison, Peter Wohlleben (2015) mentions in his book 'Hidden life of trees', a similar association created in the German village of Hummel, which has helped preserve their ancient forests. An entire old beech forest has been placed under protection in an innovative way: part of the forest is used as an arboreal mortuary, where the trees are leased out as living gravestones for urns buried under them. While the forest survives, the people get to be a part of the ancient forest after death.

Coming back to the Indian context, ecological conservation in many regions of India is closely tied with studying these cultural associations. These sacred groves in India can become a powerful space of study for scientists and researchers interested in the continuity of plant life and its behaviour. Indian culture and thought has been deeply engaged with plants and trees. India's very own medicinal system of Ayurveda is based on an incredible knowledge of plant and tree components, where each part of the tree yields a specific medicinal use. This medicinal knowledge of plant life along with ancient textual sources, which I referred to in the previous section, is a vast vista of how richly, trees were embodied into the human universe. While this exploration of ideas could cover an entire book, I wish to instead deviate to an artist's investigation of the natural world. In the process, I would also like to explore how Indian art and dance creates the worldview and perception that is intimately connected with nature.

24.4 The Mango Tree: Different Artistic Inspirations

As a student in school, I always gravitated towards painting trees and landscapes, than studying about them. The process of artistic creation was deeply moving to me, in the amount of details it showed me, of a tree. Its rough, lined trunk, its fine pointy leaves, the amount it moved in the wind and the month when it flowered and bore fruit. Each part of that flower, fruit or leaf was also special and unique in its shape, colour, smell and touch. But most important to me was what it made me feel and what it reminded me of. In the course of growing up, these associations were fed by a nature-filled Pusa institute campus life in New Delhi and the incredible number of artistes' works I saw in art books, from Manet's Lily Pond to the sculptures of *shalabhanjikas* on the Sanchi stupa in India. Sometimes, it was in the art books that I saw nature, visualised through art and began to mentally store some beautiful forms and associations.

As a painting student, I strived to 'see' and 'feel' even more than paint the details and forms of the natural world. A friend questioned me regarding the need to paint a flower when it could be photographed! I remember and cherish that idea that came as a reply. I believe that artistes can slow down and experience a plant or tree, with a different vision from that of a photograph. They observe something different in that particular tree, and do not capture it as a sum of its parts. Instead, they highlight what seems remarkable or its 'essence' to them. This process is also largely guided by how their persona is, and it guides their faculties of observation and representation. So quite naturally, it was while studying art and gazing at these images in books, that I found my own inner artistic landscape of trees and foliage.

When I became an Indian classical dancer, I had to deepen my association with trees further through my body. From visual arts to dance, the observation deepened due to a different kind of immersion. In Odissi dance, we represent through our bodies the sculpture motifs of ancient temples. Often these figures have a vivid imagery connected with trees. The *shalabhanjika* image, for example, is the sculpture of a woman grasping an Ashoka tree. Found in the iconic Khajuraho, Konark temples and the Sanchi stupa, this image is the iconic Indian image of women as a symbol of fertility and prosperity.

In recreating the *shalabhanjika* sculpture through our bodies in dance, we internalise the woman-tree association and many other plant images like the curving of creepers, the blossoming of the mango tree and even the shape of tree branches. This representation of the tree first as a painter, and then as a traditional dancer, has forever changed my ability to engage with trees. When I 'meet' trees, I am often finding very clear personalities and character. These trees have inspired poems to emerge and dances to grow more vivid and alive within me. In '*Saja saaja saraja*' (a dance inspired from regional Odiya poetry), I play the role of Radha's friend, urging her to go meet Krishna, who awaits her on the banks of river Yamuna in the grove of Mango trees. The entire dance revels in the Mango tree in bloom with a thousand flowers in the *Vasant ritu* (spring season). In Sanskrit poetry, Mango tree was exalted as the messenger of spring and a symbol of love. When spring came, the mango tree's raw mangoes would attract the koel bird (cuckoo) to come, sit on it and

sing in the fifth note, once its sour juices cleared her throat! Depicting these very trees in bloom, the koel singing and its ethereal landscape, in the human emotional universe has changed my way of looking at the Mango tree forever.

24.5 The Power of Art as an Associative Personal Connection

Through these personal experiences as a multi-disciplinary artiste, I have come to understand that art has a very pivotal role in connecting cultural associations between humans and nature. I believe that art creates *association*, instead of merely information. I observed in my engagement with college and school students' workshops on the plant world that a dance enactment of Tagore's poem, the 'Champa Tree', rather than preaching about trees and the environment, moved them deeply. In his connection of the Plumeria with a mother-child relationship, Tagore finds the playful, imaginative life force flowing between all creatures. It reminds us that what we '*feel*' is at the crux of our being. In human life, art is the powerful network that connects our emotional world, spiritual energy and the knowledge of the world. We do not ascribe human qualities to plant life, but instead begin to see the life force and conversation running through many forms of nature.

When we study trees as social beings and understand their habitat as living creatures, we also need to understand that trees are not just any subject of study. They are 'connected' to our *perception* of them. Our human agency and behaviour towards plant life is deeply influenced by our associations with them. In ancient India and even in village life to this date, the cultural ideas that are deeply embedded, create a powerful connection with the trees. In some cases, it prevented a sacred grove from destruction. In some villages, a Peepal and a Banyan tree would be married to each other like a man and a woman, as symbols of fertility, so that their roots and branches intermingle. The Gond tribe of central India believe that trees are central to life, and paint beautiful images of tree spirits in Gond art, to express their perceptions of trees.

Indian painting, sculpture, poetry, sacred literature, music and dance are teeming with magnificent associations of plants with the natural world. Equally impressive is the way this world has been systematically studied in ancient Indian texts and literature, and what they inform us of plant behaviour, ecological balance through social structures and art as a medium of connection.

As an artiste and a dancer, I have highlighted the role of nature in my dance-poems, as a means of spiritual engagement with the universe. Like the ancient Indians in the *aranyakas*, I believe that trees are in conversation. Not only do their high *sattvik* energy create a space for meditation, but they are themselves beings with subtle energy that is in conversation with each other. This non-human universe has its own energy in flow, and has inspired most of the Indian spiritual traditions. Buddha found his own spiritual growth under the Bodhi tree. We can also be sensitive to the conversation continually flowing around us. It is not on social media, but running parallel to it, as a large, vast sharing of energy. Sometimes we come 'in the way' of trees and their conversation within the context of 'their' social media. We find our scientific

discoveries and artistic ideas at that point, but actually it is our 'receptivity' that determines how well we can access this 'network' of conversation.

As an artiste, I have been enquiring these human and non-human connections and the 'silent life' of our supposedly inanimate world. In my dance-poems, titled 'the Poetic Saree', I engage with the Javakusum flower I meet on a walk, the Sauparnika river (in Karnataka) that inspires me to feel poetry as a river, a conversation with the ancient boulders of Hampi about their creation, the 'Two Moons' that I found reflected in the rice-paddy fields and the changing seasons of Delhi with its vividly changing treescape. The intuitive explosion of these poems was a result of the time spent with nature and a spiritual art form: an immersion, interaction and then conversation.

These poems from 'the Poetic Saree' book of poetry created an inter-disciplinary understanding of nature, Indian dance, art, poetry and the saree. The poem-dance videos, as we visualised them, mirrored back to me the many moods of the Nayika (heroine) in Odissi, how she finds a connection with so many aspects of nature: the sun, moon, birds, trees, growing leaves, blossoming flowers and the unseen but uplifting wind. Through Indian art and dance, I have been fortunate in deepening my awareness of these realms. *And when Art changes our perceptions of life, it is a precious, life-altering experience*. Art, through dance, is to me a *Sakhi*, a friend and inner guide that comforts, nourishes, confides and cajoles to experience a beautiful 'oneness with the universe'.

As an artiste and seeker, I have been fortunate to have found the fertile self, which is open to this tree network, the world of natural forms and its communication and so have many other foresters, spiritual sages of ancient India and village folk. Their connect with the natural world comes from a keenness to listen to this network, receive it and respond to its energy. The larger questions of conservation and scientific studies of the natural world will reach a more fruitful understanding when we involve these cultural and artistic associations. The role of plants in Indian art and culture is a beautiful, intuitive example of connections between man and his environment in various dimensions. What is the connection between man and the natural world? Indian art and thought have worked over centuries to develop this rich ideational universe, and as modern individuals we are greatly enriched to examine it further. It is a deep legacy of Indian culture and for scholars, artistes, educators and individuals to encourage this inter-disciplinary line of enquiry.

References

Findly EB (2008) Plant lives: borderline beings in Indian traditions. Motilal Banarsidass Publishers, New Delhi, pp 3–4, 83–87, 148–149, 247–254

In the Image of Man (1982) Festival of India catalogue, The Arts Council of Great Britain

Krishen P (2006) Trees of Delhi. Dorling Kindersley, (India) Pvt. Ltd, pp 24–25

Krishna N (2017) Hinduism and nature. Penguin, Gurgaon, pp 31–71

Krishna N, Amirthalingam M (2014) Sacred plants of India. Penguin, Gurgaon (India), pp 12–16

Roy S (2017) How I became a tree. Aleph Book Company, pp 122–123

Wohlleben P (2015) The hidden life of trees. Penguin, Gurgaon, pp 112–113

Jaya Mehta is a multimedia artiste with a master's in Ancient Indian History (Jawaharlal Nehru University) and a bachelor's in Fine Arts (College of Arts, New Delhi). She is an Indian classical dancer and poet whose writings on Indian art and culture have been published in national newspapers like *The Hindustan Times*, *Times of India*, and *Economic Times*. She has also published research articles like "Unravelling the Picturesque: Understanding the creation of a past," for the volume *Archaeology as History in Early South Asia*, published as ICHR monograph series, New Delhi (ed. H.P Ray and C.M Sinopoli), and "Ellora: Understanding the creation of a past," published in the University of Delhi history reader titled, *Cultural History of Early South Asia* (ed. by Shonaleeka Kaul).

Jaya Mehta has a keen interest in unraveling the Indian worldview of the natural world, which she has explored in her book of dance-poems titled, *The Poetic Saree*. As a dancer, poet, and writer, she seeks to investigate these nuances with an interdisciplinary approach. She was invited by the Editor and Dr. Neeti Sanan Mishra to demonstrate these interconnections at the ICGEB workshop on plant responses to stress, held in New Delhi. More information on the artiste is available at: www.jayamehta.in and www.thepoeticsaree.com