How Can We Interpret the Large Number and Diversity of ABA Transporters?

Joohyun Kang, Youngsook Lee, and Enrico Martinoia

Contents

Abstract Abscisic acid (ABA) is generally known as the plant stress hormone. Functioning in a wide range of environmental responses, ABA plays a major role in drought tolerance. In addition to inducing stomatal closure during drought stress, ABA promotes suberization of the exodermis and endodermis, which reduces water loss from the root. Furthermore, ABA increases freezing tolerance and has a complex, but not completely understood, role in plant–pathogen interactions. ABA also functions in plant development; for example, ABA is a central player in maintaining seed dormancy. Whereas the enzymatic steps of ABA biosynthesis have been known for some time, our knowledge of ABA receptors and transporters is quite recent. This is due, at least partially, to redundancy among members of both the ABA receptor and transporter families. Many transporters from different

J. Kang (✉) and E. Martinoia
Division of Integrative Bioscience and Biotechnology, POSTECH, Pohang, Republic of Korea

Institute of Plant and Microbial Biology, University Zurich, Zurich, Switzerland
e-mail: azka@postech.ac.kr

Y. Lee
Division of Integrative Bioscience and Biotechnology, POSTECH, Pohang, Republic of Korea

Progress in Botany (2021) 82: 233–258, https://doi.org/10.1007/124_2020_43,
Published online: 28 December 2020

transporter families cooperate to transport ABA. The weak but distinct phenotypes described for the different loss-of-function mutants indicate that each of these transporters plays a specific role and, at least under a given condition or in a specific tissue, they are not completely redundant. However, for each function described so far, delivery of ABA at the target site requires the activity of several different ABA transporters. This strategy may ensure that ABA is transported to the correct target even if one of the transporters is nonfunctional or that plants can transport ABA under a given condition via several routes.

Keywords Abscisic acid, Drought, Freezing tolerance, Plant pathogen, Seed germination, Transport

Abbreviations

ABA	**Ab**scisic **a**cid
ABA-GE	ABA **g**lucose **e**ster
ABC	**A**TP-**b**inding **c**assette
AIT	**A**BA-**i**mporting **t**ransporters
AtBG1	Arabidopsis thaliana β-glucosidase
AWPM-19	**A**BA-induced **w**heat **p**lasma **m**embrane polypeptide-**19**
Cvi	**C**ape **V**erde **i**slands
DTX/MATE	**D**etoxification **e**fflux **c**arriers/**m**ultidrug and **t**oxic **c**ompound **e**xtrusion
GUS	β-Glucosidase
LATD/NIP	**Lat**eral root **d**efective/**n**umerous **i**nfection threads, **p**olyphenolics
Lr34res	The resistant Lr34 allele
Lr34sus	The susceptible Lr34 allele
NBF	**N**ucleotide **b**inding **f**old
nced3	**N**ine-**c**is-**e**poxycarotenoid **d**ioxygenase 3
NPF	**N**itrate transporter1/**p**eptide transporter **f**amily
OE	**O**ver**e**xpression
ost1	**O**pen **st**omata 1
PM1	**P**lasma **m**embrane protein**1**
PP2C	**P**hos**p**hatase **2C**
PYR/PYL/RCAR	**Py**rabactin **r**esistance1/**PY**R1-**l**ike/**r**egulatory **c**omponents of **A**BA **r**eceptor
TMD	**T**ransme**m**brane **d**omain

1 Introduction

The phytohormone abscisic acid (ABA), a sesquiterpenoid with a 15-carbon ring, has a critical role in a large range of physiological functions in a variety of plant tissues over the entire life span of plants. During seed development, ABA prevents vivipary and contributes to dormancy and desiccation tolerance (Robertson 1955; Meurs et al. 1992; Schwartz et al. 1997; Gubler et al. 2005). ABA also inhibits seedling development (Lopez-Molina et al. 2001). In Arabidopsis the hormone influences root architecture by preserving primary root growth and inhibiting the development of lateral roots at the meristem activation checkpoint (Zhang et al. 2010). In the shoot, ABA's major role is to regulate stomatal aperture and thereby modulate the hydraulic conductance of plants (Kim et al. 2010; Munemasa et al. 2015; Merilo et al. 2018). ABA also plays a role in reproductive organ development, with possible functions in male sterility and fruit development (Peng et al. 2006; Oliver et al. 2007).

Furthermore, ABA is a key mediator of physiological responses to multiple environmental stresses, such as drought, cold, and pathogen attack. In general, the earliest adaptive responses of plants to various stresses are induced by an increase in transcript levels of major ABA biosynthetic genes, which in turn leads to accumulation of cellular ABA and triggers the expression of multiple stress-responsive genes (Inuchi et al. 2001; Tan et al. 2003). These molecular processes have been widely studied in the model plant *Arabidopsis thaliana* (L.) Heynh., where most of the ABA signaling mechanisms have been elucidated.

Abiotic stress conditions, such as water stress, increase ABA levels and thus initiate signaling pathways that induce multiple cascades of molecular and cellular responses, including, in the case of water stress, expression of stress-related genes, cellular ROS accumulation, and stomatal closure. Stomatal closure and ROS accumulation also serve as a mechanism for pathogen defense (Melotto et al. 2006; Desikan et al. 2008), thereby providing a platform for crosstalk between biotic and abiotic stress responses. However, ABA can also directly activate protein kinases, as it does in the activation of SLAC, a guard cell anion channel, leading to stomatal closure (Raghavendra et al. 2010).

For the past several decades, ABA studies have focused on the hormone's biosynthesis and signaling pathways. Almost every ABA biosynthetic enzyme was identified quite some time ago (Nambara and Marion-Poll 2005). However, ABA's receptors (Park et al. 2009; Ma et al. 2009) and transporters (references below) were identified only recently. One of the main reasons for this delay is that, unlike the ABA biosynthetic enzymes, the protein families involved in perception and transport are highly redundant.

Over ten different proteins of the PYR/PYL/RCAR (**py**rabactin **r**esistance1/**PY**R1-**l**ike/**r**egulatory **c**omponents of **A**BA **r**eceptor) family act as ABA receptors in different tissues and at various developmental stages (Raghavendra et al. 2010; Gonzalez-Guzman et al. 2012). The ABA transporters that have been identified to date belong to several different protein families – the ABC (**A**TP-**b**inding **c**assette)

superfamily, the NPF (**n**itrate transporter1/**p**eptide transporter **f**amily) family, and the DTX/MATE (**d**etoxification **e**fflux **c**arriers/**m**ultidrug and **t**oxic **c**ompound **e**xtrusion) family. ABA transporter mutants often exhibit no apparent phenotypes under normal growth conditions, but show distinct phenotypes under a specific stress condition (e.g., pathogen exposure or drought) or during a specific developmental stage. Thus, ABA transporters may have specific functions and may act in concert with other ABA transporters but also be partially redundant with them.

Why did plants evolve to have such diverse ABA transporters belonging to so many different protein families? Are the physiological functions of these ABA transporters highly redundant or not? The answers to these questions hinge on further studies of mutants with knockouts of multiple ABA transporters. Here, we review the history of ABA transporter studies, the properties of the protein families involved in ABA transport, and the functions of ABA transporters in biological processes. We also propose some hypotheses to explain why plants have evolved so many ABA transporters.

2 Where Does ABA Come from, and Where Is It Going?

In general, ABA meets the definition of a hormone, in that it is synthesized in one place and acts at other places within the plant. However, ABA is synthesized in several places, not just one (Nambara and Marion-Poll 2005), and, as will be described below, there is at least one cell type that relies on cell intrinsic ABA biosynthesis. Furthermore, ABA, like other plant hormones, is often present in conjugated forms. Some of these conjugates can be hydrolyzed under specific conditions (Lee et al. 2006; Xu et al. 2012), releasing active ABA and triggering a signaling cascade within the cell.

Studies of ABA transport and signaling historically focused on how ABA reaches guard cells and induces stomatal closure. Early findings suggested that roots, leaves, and even guard cells can synthesize ABA (Zeevaart and Creelman 1988). Initial attempts to identify the ABA receptor suggested that the receptor was located in the plasma membrane. This implicated that ABA would be perceived at the apoplastic face of the guard cells and had not to be transported into guard cells (Zeevaart and Creelman 1988). Subsequent work revealed that the ABA receptor is a member of the PYR protein family (Park et al. 2009; Ma et al. 2009). These receptors are localized in the cytosol. Therefore this discovery changed the view where ABA is perceived and pointed out that ABA has to cross the plasma membrane to reach its receptor.

As water stress is initially perceived in the root, the ABA required for stomatal closure was hypothesized to be produced in roots and translocated into shoots before being delivered to the guard cells (Zeevaart and Creelman 1988; Jackson 1993). To test this hypothesis, Blackman and Davies (1985) performed split root experiments with maize (*Zea mays* L.). One part of the root system was well watered, while the other was subjected to a reduced water potential. This experimental design allows

the shoot to remain unstressed while half of the root is exposed to water stress. The authors reported that plants subjected to this treatment had smaller stomatal apertures than control plants with both parts of their root systems well-watered. However, the authors could not detect any difference in ABA concentrations in the shoot between the treatment and control plants. Further experiments led to the hypothesis that a continuous supply of cytokinins might be the factor allowing maximal stomatal opening.

Other evidence challenges the hypothesis that root-to-shoot transport of ABA or cytokinins is required to control stomata opening in response to drought. For example, such a mechanism cannot account for the fast stomatal reaction observed in tall trees, and an alternative mechanism involving a hydraulic signal has been postulated several times (Christmann et al. 2007). At least for the plants investigated so far, there is no evidence that a chemical signal has to be transported from the root to the shoot to induce stomatal closure. Evidence to the contrary is mainly provided by grafting experiments. For example, Holbrook et al. (2002) grafted tomato (*Solanum lycopersicum* L.) shoots able to produce ABA onto mutant roots unable to do so and showed that stomatal conductance corresponded to the shoot genotype. This result was further confirmed by grafting experiments using split roots or a pressure chamber.

In a detailed study, Christmann et al. (2007) took advantage of a series of Arabidopsis mutants to gain insight into the question of the source of ABA involved in water stress responses in the shoot. These authors also confirmed that ABA is not transported from the root to the shoot; instead, shoot-derived ABA is the signal leading to stomatal closure. They also showed that a sudden change in root water availability results in a rapid change in shoot turgor pressure, which induces rapid synthesis of ABA. These experiments support the hypothesis that a hydraulic signal is the primary cause for the increase in ABA content and the resulting stomatal closure in the shoot.

Stomata close in response not only to water shortage in the soil but also to changes in atmospheric humidity. As mentioned above, guard cell intrinsic ABA synthesis has been postulated for a long time. More recently, Bauer et al. (2013) confirmed this hypothesis and showed that guard cell intrinsic ABA synthesis is important for protecting plants against changes in atmospheric water potential. Although wild-type Arabidopsis plants did not wilt when exposed to dry air, the ABA biosynthesis mutant *aba3-1* did. Rescuing ABA biosynthesis specifically in guard cells of this mutant restored the capacity to close stomata in dry air and the plants did not wilt.

A recent, more comprehensive study compared the corresponding mutants of Arabidopsis, pea (*Pisum sativum*), and tomato and also examined the relative importance of guard cell autonomous and shoot-dependent processes (Merilo et al. 2018). Besides highlighting several important regulatory mechanisms, the authors showed that the pools of ABA synthesized by guard cells and by phloem companion cells are redundant and that their relative importance may be plant specific. Therefore, under certain circumstances, transport of ABA from the leaf tissue may also function in stomatal closure in response to dry air.

Less information is available for other cases that may involve ABA transport. This may be either because the sites of ABA synthesis and action are too close together or because classical tools have not been able to determine whether ABA must be transported or not. One such case concerns seeds and seed germination. Most plants, with the exception of those growing in tropical climates, tightly control seed germination to avoid germination under unfavorable conditions. The mechanisms regulating this process have been studied extensively, and it is now generally accepted that ABA, which represses germination, and gibberellins, which promote germination, act antagonistically to regulate the process (Holdsworth et al. 2008). Dormant seeds typically contain high ABA concentrations, while seeds with impaired ABA production germinate precociously (Robertson 1955). Seed germination is a complex process that varies at least slightly among plants. In Arabidopsis, the embryo is surrounded by an active endosperm and the testa, a dead outer layer of maternal origin. In Arabidopsis, the endosperm is responsible for most or all of the germination-repressive activity (Bethke et al. 2007). Using a seed coat bedding assay, Lee et al. (2010) demonstrated that ABA is released from the endosperm of dormant seeds and is the factor inhibiting embryo development. Hence, export of ABA from the endosperm and uptake of this hormone into the embryo must occur during seed dormancy.

Transport of ABA is likely or may occur in other ABA-dependent processes, but the routes of ABA movement remain largely unknown. For example, under water stress, more suberin is also deposited in the endodermis in an ABA-dependent manner, and lateral root primordia are repressed, but it is not known whether the ABA that regulates these processes is de novo synthesized in situ or transported from elsewhere (Deak and Malamy 2005). ABA also decreases the number of hypodermal passage cells, non-suberized cells of the exodermis (Liu et al. 2019). Finally, in the development of nitrogen-fixing nodules in the Fabaceae, ABA regulates nodule production and probably also bacteroid number (Ding et al. 2008). An ABA transporter involved in this process has been recently identified; however, we still do not have much insight into the source of the ABA delivered to the nodule meristem.

3 Which Proteins Are Involved in ABA Transport?

ABA transport is catalyzed by a large number of transporters belonging to several families (Kuromori et al. 2018). The first class of transporters implicated in ABA transport was the ABC protein family. Subsequently it was demonstrated that some members of the NPF and MATE families are also able to transport ABA. Here, we present some characteristics of the transporter families involved in ABA transport. We have only limited knowledge about the AWPM-19 (**A**BA-induced **w**heat **p**lasma **m**embrane polypeptide-**19**) family; therefore we do not discuss this membrane protein family here.

3.1 The ABC Superfamily

ABC transporters are among the oldest transport proteins and are present in all living organisms from bacteria to humans. These transporters are classified as pumps, since they are directly activated by ATP hydrolysis (Kang et al. 2011; Hwang et al. 2016). The Arabidopsis genome encodes 131 ABC transporters, and a similar or higher number has been reported for all plants analyzed so far. This number is far higher than that for humans (49) and most other organisms.

A functional ABC transporter contains two cytosolic (**n**ucleotide **b**inding **f**old, NBF) and two transmembrane (**t**ransme**m**brane **d**omain, TMD) domains. These domains can be part of one large protein, a so-called full-size ABC transporter, or part of a half-size transporter, which contains only one NBF and TMD, or exist as single proteins corresponding to NBFs and TMDs (bacterial-like). In all cases, however, the proteins have to be assembled to contain two NBFs and two TMDs to act as a functional transporter.

All ABC transporters contain an ABC signature flanked by Walker A and Walker B domains, which are responsible for ATP binding on each NBF. In plants, ABC transporters have been shown to transport a plethora of compounds, such as lipids, secondary compounds, heavy metals, and many hormones. In most cases, a single ABC transporter is able to transport several to many chemically unrelated compounds. All the ABC transporters that have been described as being involved in ABA transport are full- and half-size ABC proteins of the ABCG class. This class has a so-called reverse arrangement, in which the first TMD is located at the N-terminus of the protein, followed by an NBD. So far, AtABCG25, AtABCG30, AtABCG31, and AtABCG40 in Arabidopsis, Lr34 in wheat (*Triticum aestivum* L.), and MtABCG20 in Medicago have been shown to transport ABA (Table 1).

3.2 NPFs (NRT1/PTR Family)

The name NPF (**n**itrate **p**eptide **f**amily) indicates that proteins of this family were originally identified as nitrate transporters (NRTs) and peptide transporters (PTRs) (Longo et al. 2018). The first member of this protein family was isolated in Arabidopsis in a screen for resistance to chlorate, a toxic nitrate analogue. This transporter was subsequently shown to be a nitrate uptake transporter (Tsay et al. 1993). Most NPFs are proton/substrate symporters, but some are facilitators, bidirectional transporters, or proton-coupled potassium antiporters.

This class of transporters has 12 transmembrane α-helices and is part of the large major facilitator superfamily (Léran et al. 2014; Longo et al. 2018). The N-termini and C-termini are both located in the cytosol. Like ABC transporters, NPFs have been identified in all living organisms, from bacteria to fungi to humans, and, also like ABC transporters, the number of NPFs is much higher in plants than in most other organisms. Plant NPFs have been divided into eight subclasses (Léran et al.

Table 1 List and properties of ABA transporters described in the text

Common name	Protein family	Species	Verification of transport activity	Vector	Main function	Major expression tissues	Reference
AtABCG25	ABC protein family	*Arabidopsis thaliana*	Sf9 insect cells	Exporter	ABA accumulation in the apoplastic area around guard cells by exporting biosynthetic ABA from vasculature	Vasculature	Kuromori et al. (2010), Kang et al. (2015)
AtABCG30	ABC protein family	*Arabidopsis thaliana*	Using knockout mutants	Importer	Embryo germination inhibition (dormancy maintenance) by ABA uptake in embryo	Embryo	Kang et al. (2015)
AtABCG31	ABC protein family	*Arabidopsis thaliana*	Using knockout mutants	Exporter	Embryo germination inhibition (dormancy maintenance) by ABA efflux from endosperm	Endosperm	Kang et al. (2015)
AtABCG40	ABC protein family	*Arabidopsis thaliana*	Yeast BY2 cells Arabidopsis protoplast	Importer	Regulation of stomatal aperture under drought condition Embryo germination inhibition (dormancy maintenance) by ABA uptake in embryo	Guard cells Embryo	Kang et al. (2010, 2015)
Lr34	ABC protein family	*Triticum aestivum*	Yeast	Importer	Contribution to fungal resistance	Unknown	Krattinger et al. (2019)
MtABCG20	ABC protein family	*Medicago truncatula*	BY2 cells BY2 cell-derived vesicles	Exporter	Positive regulation of lateral root primordium formation negative effect on the development of nodule primordia Facilitating germination	Vascular bundles and at the sites of lateral root primordium formation Hypocotyl–radicle transition zone of embryos	Pawela et al. (2019)
AIT1/ AtNRT1.2/ AtNPF4.6	NPFs (NRT1/ PTR)	*Arabidopsis*	Yeast	Importer	Regulation of stomatal aperture in inflorescence stems	Vascular tissues in inflorescence stems, leaves, and roots	Kanno et al. (2012)

AtDTX50	MATE	*Arabidopsis*	*E. coli*, Xenopus oocytes, Arabidopsis protoplast	Exporter	Negative regulation of drought tolerance	Guard cells Vascular tissues of leaves, roots, and germinating seeds	Zhang et al. (2014)
OsPM1 (plasma membrane protein1)	AWPM-19 like family	*Oryza sativa*	Yeast	Importer	Regulation of drought tolerance and seed germination	Vasculature, guard cells, root tip, mature embryo	Yao et al. (2018)

2014), but assignment of an NPF to a particular subclass does not necessarily predict its substrate(s). Like ABC transporters, NPFs transport a plethora of substrates such as nitrate, peptides, glucosinolates, auxin, jasmonic acid, and ABA. The ABA importer AIT (**A**BA-**i**mporting **t**ransporters) was the first and so far only member characterized in detail (Kanno et al. 2012). However, ABA transport activity has been demonstrated or postulated for an additional 11 NPFs (Chiba et al. 2015).

3.3 MATEs

MATE transporters were originally identified as multidrug and toxic compound extrusion proteins conferring resistance against drugs in bacteria (Morita et al. 1998). In Arabidopsis, these transporters are also called DTX proteins. As in the case of ABC transporters and NPFs, they have been identified in most living cells, and plants contain a far higher number of these transporters compared to other organisms. For Arabidopsis, 56 MATEs have been reported, whereas humans have only 2 (Takanashi et al. 2014; Upadhyay et al. 2019).

Most MATEs contain 12 α-helices, with 2 long extensions at the N-terminus and C-terminus, but there are a few exceptions where more or fewer α-helices have been predicted. Furthermore, most MATEs act as proton antiporters, extruding compounds from the cytosol into either the apoplast or the vacuole. MATEs transport a wide variety of compounds, such as toxic abiotic compounds, a large range of plant secondary metabolites (e.g., flavonoids and alkaloids), citrate, salicylic acid, auxin, and ABA. It is also likely that a given MATE can transport multiple chemically unrelated compounds. Recently it was shown that two MATEs act as chloride channels and have a strong impact on stomatal movement (Zhang et al. 2017). AtDTX50 is so far the sole ABA transporter identified in this family (Zhang et al. 2014).

4 What Is the Role of ABA Transporters in Plants?

4.1 Modulation of Hydraulic Conductance in Sessile Plants

Our views on ABA transport have been changed by the results showing that during water stress ABA is not transported from the root to the shoot and subsequently to guard cells but rather is redistributed within a leaf (Christmann et al. 2007). However, even in this case, ABA has to move from the phloem parenchyma cells, the site of biosynthesis, to the guard cells. Since guard cells are not connected to mesophyll or phloem parenchyma cells via plasmodesmata, ABA must cross membranes to make this journey. Older work postulated that an ABA importer on the guard cells is not required, because apoplastic pH was reported to be 5.5 to 6.0. At this pH, a substantial proportion of the ABA (pK_a 4.75) is protonated and can diffuse across

membranes. However, it has now been shown that under water stress conditions the pH of the apoplastic space increases to 7.2, and consequently the proportion of protonated, freely diffusible ABA decreases. Under these conditions, only a small fraction of ABA can enter guard cells by simple diffusion, implying that guard cells need a transporter to import ABA from the apoplast to the cytosol. By contrast, for ABA export from the cytosol to the apoplast, a transporter has always been assumed to exist, since at a cytosolic pH of 7.5 only negligible amounts of uncharged, freely diffusible ABA is present.

Two plasma membrane-localized transporters of the ABC protein family were the first ABA transporters to be identified. One, AtABCG25, catalyzes ABA export from the cytosol to the apoplast (Kuromori et al. 2010); the other, AtABCG40 (Kang et al. 2010), catalyzes the uptake of ABA from the apoplast into the cytosol. Both transporters are expressed in roots and shoots. While in shoots AtABCG25 is localized predominantly to the vasculature of leaves, AtABCG40 is mainly expressed in guard cells. ABA transport by these proteins has been studied using membrane vesicles isolated from insect cells overexpressing AtABCG25 and yeast and BY2 cells expressing AtABCG40. Transport was strictly ATP-dependent, and both transporters exhibited high and specific affinity for ABA, with K_Ms of 0.26 and 1 μM for AtABCG25 and AtABCG40, respectively.

Thermal imaging of Arabidopsis plants overexpressing AtABCG25 revealed that these plants were warmer than the wild type, in line with increased ABA release and decreased stomatal aperture. In the case of AtABCG40, thermal imaging revealed that *atabcg40* mutant plants were cooler than the corresponding wild-type plants under osmotic stress conditions or when exogenous ABA was supplied. This result indicated that the stomata of the mutant could not close efficiently in response to elevated ABA. Indeed, measurement of stomatal apertures revealed that *atabcg40* mutant plants were less sensitive to ABA-induced closure. Transformation of the mutant plants with AtABCG40 completely complemented the stomatal defects. These results demonstrate that AtABCG25 acts as an ABA exporter and AtABCG40 as an ABA importer. However, the phenotypes of *atabcg40* mutant plants were quite mild and not nearly as strong as those described for ABA biosynthesis mutants. This raised the question of whether diffusion could play a role or additional ABA transporters are present.

To identify additional ABA transporters, Kanno et al. (2012) devised an elegant screening method. The authors took advantage of the fact that some of the PYR/PYL/RCAR ABA receptors interact with a group of PP2C-type protein phosphatases in the presence of ABA. Using a modified yeast two-hybrid system, they transformed a yeast strain with these two proteins, ABA receptor, PYR1 and ABI1 or HAB1, PP2C-type phosphatase (Park et al. 2009; Ma et al. 2009). In the presence of 0.1 μM ABA, there was no interaction between the partners because the ABA could not enter the yeast cells. The rationale of the screen was that a transporter would allow efficient uptake of ABA into the yeast cells, leading to an interaction between the receptor and the phosphatase. A screen of two cDNA libraries led to the identification of four members of the NRT1/PTR (NPF) family that facilitated interaction between the partners. One of these candidates had previously been

characterized as the low-affinity nitrate transporter NRT1.2 (Huang et al. 1999) and exhibited the most pronounced ABA transport activity when expressed in yeast and insect vesicles. This transporter, also called AIT1 by the authors, was characterized in more detail. The protein is localized in the plasma membrane and catalyzes the uptake of ABA with a K_M of 5 μM.

Interestingly, although this transporter is mainly localized to the vasculature of leaves and inflorescences and not in guard cells, thermal imaging of *ait1* mutants showed that inflorescence stems but not leaves were cooler than in the wild type. This result indicates that AIT1 regulates stomatal closing in inflorescence stems but not in leaves. Changes in thermal imaging in line with increased ABA uptake activity were observed in leaves only when AIT1 was overexpressed. The authors hypothesize that AIT1 acts mainly as importer at guard cells in inflorescence stems and in the cells of the vasculature resembles the localization of ABA biosynthetic enzymes to avoid loss of ABA from these cells.

The three other AITs identified in the modified yeast two-hybrid screen were not characterized in detail. In a follow-up paper, the same laboratory looked systemically for NPFs able to transport ABA and extended the method to search for transporters of other hormones, such as gibberellins and jasmonic acid-isoleucine (Chiba et al. 2015). Eleven additional NPFs emerged as candidate ABA transporters from this study, but their physiological roles merit further examination.

A MATE protein has also been identified as an ABA exporter. In an effort to understand the function of MATE/DTX proteins, the laboratory of Professor Luan produced homozygous mutants for a large number of Arabidopsis *MATE* genes. They observed that the *dtx50* mutant was smaller and yellower than the wild type when grown in soil (Zhang et al. 2014). To obtain clues about the substrate(s) of DTX50, the authors screened a multitude of compounds to determine if any would affect the phenotype of the *dtx50* mutant. They observed that the growth of *dtx50* mutants was more severely inhibited than that of the wild type and other mutants in the presence of ABA. This result suggested that *dtx50* mutants might accumulate more ABA and struggle to release it. Indeed, the *dtx50* mutants had increased ABA and increased expression of ABA marker genes.

Using *E. coli* and Xenopus as heterologous expression systems, as well as wild-type and *dtx50* Arabidopsis protoplasts, Zhang et al. (2014) convincingly showed that AtDTX50 is an ABA exporter exhibiting high specificity for the physiological enantiomer of ABA. As expected from the *dtx50* phenotype, DTX50 is localized to the plasma membrane. Expression of DTX50 was mainly observed in veins and guard cells. The guard cell localization was quite surprising, but is consistent with the observation that *dtx50* plants were more drought tolerant and that their stomata closed faster. Apparently, release of ABA from guard cells is important for fine-tuning the regulation of stomatal aperture.

All the work we have described so far used Arabidopsis as a model plant. Much less is known about stomatal regulation in monocotyledons. In a screen for drought-induced genes in rice, Yao et al. (2018) identified OsPM1 (**p**lasma **m**embrane protein**1**), a membrane protein belonging to the AWPM-19 family. It was already known that genes of this small family are strongly induced by abiotic stress (Koike

et al. 1997; Rerksiri et al. 2013; Chen et al. 2015). OsPM1 is mainly expressed in mature embryos, vasculature, and guard cells and is highly induced by ABA. By measuring fluorescence resonance energy transfer in yeast expressing OsPM1, the authors showed that OsPM1 exhibits ABA uptake activity. Physiological experiments revealed that OsPM1 plays an important role in tolerating drought stress, since *OsPM1*-RNAi plants had a lower survival rate and OsPM1-overexpression (OE) plants had a higher survival rate compared to the wild type. Measurements of water loss and stomatal aperture (which is very difficult in rice) were in line with the survival rates observed: RNAi plants lost more water, while OE plants retained more water. Furthermore, no differences in stomatal apertures could be detected under control conditions, but significantly more stomata were closed in OE plants compared to the wild type in the presence of ABA. By contrast, RNAi plants had significantly more open stomata. All these results indicate that OsPM1 is an ABA transporter required to regulate stomatal aperture and hence drought resistance in rice.

A study on AtABCG22 is worth mentioning in this context (Kuromori et al. 2011). AtABCG22 is highly expressed in guard cells and is required for stomatal regulation. Mutant plants lacking this transporter lose much more water and are more susceptible to drought. However, AtABCG22 had no detectable ABA transport activity, and furthermore, double mutants of *atabcg22* combined with the biosynthesis mutant *nced3* (**n**ine-**c**is-**e**poxycarotenoid **d**ioxygenase 3) or with *ost1* (**o**pen **st**omata 1) had enhanced ABA-related phenotypes. These results, together with other physiological observations presented in this work, preclude that AtABCG22 acts as an ABA transporter. Instead, the protein may either transport an unidentified factor that regulates stomatal aperture or be involved in an ABA-independent pathway of stomatal regulation.

Merilo et al. (2015) used gas exchange assays to study the roles of different ABA-related genes and ABA transporters in stomatal regulation. Under reduced air humidity, the only mutant that displayed a gas exchange phenotype was *atabcg22*, which, as mentioned above, lacks an ABC protein not involved in ABA transport. In response to exogenous ABA, *atabcg40* and *ait1* had slightly, but not significantly, longer ABA response half-times and a slower ABA-induced decrease in stomatal conductance.

These results can be interpreted in several ways. The large number of ABA importers and exporters and the relatively subtle phenotypes of loss-of-function ABA transporter mutants indicate that there is considerable redundancy among these proteins. Hence, multiple-knockout mutants should be produced to get a clearer picture. Another reason Merilo et al. (2015) might not have detected strong phenotypes is that their experiments were relatively short-term. As shown for the stomatal reaction to changes in relative humidity, the internal ABA stores of guard cells may be sufficient for the initial response. A third point is that, in our experience, plant growth conditions have a strong effect on the reactivity of guard cells. Therefore, different results may be obtained in different laboratories as a function of their growth conditions. This may also explain why some phenotypes have not been reproduced in other laboratories.

4.2 Pathogen Resistance

Numerous reports have dealt with the role of ABA in plant–pathogen interactions (e.g., Cao et al. 2011). Intriguingly, ABA can have opposite effects depending on the situation: it can either promote or inhibit the interaction between the pathogen and the plant. The effect of ABA may depend on the nature of the pathogen, the type of affected tissue, or the developmental stage of the plant, but many questions remain. One of the few genes conferring sustainable pathogen resistance in wheat is the full-size ABCG transporter Lr34res (the resistant Lr34 allele). This allele evolved from the ancestral, susceptible allele (Lr34sus) by two gain-of-function mutations after wheat domestication (Krattinger et al. 2013).

To learn more about the mechanism underlying this important trait, Krattinger et al. (2019) analyzed the transcriptomes of plants expressing the resistant and susceptible forms of the Lr34 transporter. They observed that many ABA-related genes were highly upregulated in plants expressing Lr34res, mimicking to a large extent ABA-dependent stress reactions. To verify that this deregulated expression of ABA-dependent genes has a physiological impact, Krattinger et al. (2019) performed drought stress and stomatal aperture experiments and established that Lr34res plants lost less water and had a reduced stomatal conductance compared to Lr34sus plants. Krattinger et al. (2019) also investigated whether Lr34res could act as an ABA transporter.

Indeed, Lr34res and also, surprisingly, Lr34sus were able to transport ABA in Lr34-expressing yeast cells. However, although immunoblots clearly showed the presence of the Lr34res protein, no Lr34sus protein could be detected. Furthermore, ABA accumulation assays using Lr34-expressing rice seedlings showed that Lr34res-expressing seedlings accumulated more ABA than wild-type and Lr34sus-expressing seedlings. Therefore, although both forms can transport ABA in a heterologous system (yeast), Lr34res is probably the only form that performs this function in plants because it is the only one present in detectable amounts. Nevertheless, exactly how Lr34res-mediated ABA transport confers resistance is an open question. The tissue distribution of Lr34 is still unknown, and the ABA levels in leaves of Lr34res-expressing plants are not changed. Hence, it is tempting to speculate that it is ABA redistribution, not increased levels of the hormone, that leads to pathogen resistance. The ability to visualize ABA redistribution may clarify why ABA renders plants more susceptible to pathogens in some cases and more resistant in others.

Phylogenetic analyses have revealed that there are many homologs of the ABA transporter AtABCG40 in Arabidopsis and other organisms (Hwang et al. 2016). So far, these homologs have been shown to transport of two categories of chemicals: ABA and defense molecules. AtABCG34 and AtABCG36 transport chemicals important in defense against pathogens (Khare et al. 2017; Stein et al. 2006; Lu et al. 2015; Matern et al. 2019), AtABCG30 transports ABA (Kang et al. 2015), and AtABCG31 transports ABA (Kang et al. 2015) and possibly some pathogen defense chemicals (Cho et al. 2019). Indeed, Cho et al. (2019) suggested that many of the

full-size ABCGs in plants have co-evolved with the ABCGs of their oomycete pathogens in a long-term arms race.

We can imagine that some of the many AtABCG40 homologs in plants are involved in pathogen defense indirectly via ABA transport, while others evolved to directly transport different substrates for defense against specific pathogens. Future research on the identity of the substrates of these transporters will clarify the many questions on the role of ABA and ABA transporters in plant pathogen defense.

4.3 Regulation of Seed Development and Germination

As mentioned above, seed dormancy depends on a continuous supply of ABA from the endosperm to the embryo. Furthermore, some reports suggest that ABA must also be exported from the embryo to allow seed germination (Holdsworth et al. 2008). All of the studies we described earlier in the section on drought stress and guard cells have also addressed the issue of whether seed germination is affected, but with the exceptions detailed below, none has analyzed this aspect in further detail.

In the case of AtABCG25 and DTX50, the seeds of loss-of-function mutant plants were more sensitive to ABA; their germination and early seed establishment were more severely limited by an exogenous application of ABA than were seeds from wild-type plants (Kuromori et al. 2010; Zhang et al. 2014). By contrast, AIT1 and ABCG40 loss-of-function mutants were less sensitive to exogenously applied ABA compared to the corresponding wild type (Kang et al. 2010; Kanno et al. 2012). These results demonstrate that at least some of the same ABA transporters are involved in drought stress prevention as well as in germination, but the results presented could not explain exactly how these transporters act.

To shed light on this question, Kang et al. (2015) sought to identify ABC transporters that are responsible for the export of ABA from the seed coat and uptake into the embryo. First, the authors analyzed the expression of all 43 ABCG genes from Arabidopsis and established that 10 are highly expressed in seeds. Screening the corresponding mutant lines revealed that four mutants exhibited an altered germination pattern. Second, to establish if these ABCG transporters are more likely involved in ABA release from the seed coat (testa and endosperm) or ABA uptake into the embryo, Kang et al. (2015) separated the seed coat from the embryo and performed a qPCR analysis. Concomitantly the authors generated transgenic plants expressing promoter GUS (β-glucuronidase) reporter gene, *uidA* constructs. The two methods demonstrated that AtABCG25 and AtABCG31 are localized mainly to the endosperm, while AtABCG30 and AtABCG40 are localized mainly to the embryo. These results suggest that AtABCG25, which had already been characterized as an ABA efflux transporter, and AtABCG31 release ABA from the endosperm, whereas AtABCG30 and AtABCG40 import ABA into the embryo (Kang et al. 2015).

To gain insight into the role of these transporters in seed germination, the authors used the seed coat bedding assay established by Lee et al. (2010). When isolated embryos of the *aba2-1* mutant were placed on seed coats of wild-type seeds, germination was completely inhibited. By contrast, when placed on *atabcg31* and *atabcg25* seed coats, *aba2-1* embryos germinated, indicating that AtAGCG31 and AtABCG25 release ABA, and consequently the seed coats of mutants lacking these proteins release less ABA and have an impaired embryo germination inhibitory effect.

To analyze the function of AtABCG30 and AtABCG40, the embryos of mutants lacking these proteins and wild-type embryos were incubated on Cvi (**C**ape **V**erde **i**slands) seed coats bedding. These seed coats release more ABA than those of the Columbia ecotype. In line with the expression analysis, germination of wild-type embryos was strongly inhibited, whereas the *atabcg30* and *atabcg40* embryos germinated, indicating that less ABA was taken up by these embryos. These results indicate that AtABCG30 and AtABCG40 import ABA into the embryo.

Finally, transport experiments confirmed that AtABCG25 and AtABCG31 catalyze ABA export from the seed coat (testa and endosperm), whereas AtABCG30 and AtABCG40 act as embryo-localized importers. However, and similar to ABA transport under drought stress, it can be estimated that for both ABA import and export the respective pairs of transporters are responsible for only about 50–60% of the total ABA transport activity. As the NPFs as well as the MATE DTX50 have been shown to also be localized to seeds, they may mediate at least part of the remaining transport activity. A small part of ABA import may also occur by diffusion.

A novel aspect of ABA transport and seed germination was recently reported by Pawela et al. (2019). The authors characterized in detail an ABC transporter, MtABCG20, from *Medicago truncatula* that was strongly induced when the plants were exposed to polyethylene glycol, which mimics water stress, and ABA. They showed that this transporter is localized to the plasma membrane, and, by introducing MtABCG20 into tobacco BY2 cells, they observed that ABA was released much faster by these cells than by those transformed with an empty vector. Furthermore, ABA transport could also be observed in vesicles isolated from these cells. These results indicate that MtABCG20 is an ABA exporter. Using promotor-GUS constructs, Pawela et al. (2019) demonstrated that MtABCG20 is localized to both roots and seeds. *MtABCG20* expression increased strongly after imbibition, and the expression level remained high for more than 3 days if the seeds were kept at 4°C, while the transcript level declined to less than 50% when the seeds were kept at 24°C.

Germination of *mtabcg20* was inhibited to a greater extent by ABA than was the wild type. To establish why this was so, the authors performed a detailed analysis on the site of expression. They observed that in this case the MtABCG20 ABCG transporter was localized to a region that had not previously been reported to contain a transporter, the hypocotyl–radicle transition zone. This is the zone where embryo elongates and is of central importance for germination. The exact mechanism by which embryo-localized ABA inhibits seed germination remains unclear, but

apparently reduction of this ABA pool at the specific hypocotyl/radicle elongation zone is a prerequisite for efficient germination. This is in line with the observation of the authors that the ABA concentration in the embryo axis of *mtabcg20* plants is more than double that in the wild type. This result is supported by the observation that much higher transcript levels of a highly ABA-induced PP2C gene (*HAI2*) were detected in *mtabcg20* plants compared to the wild type when ABA was supplied exogenously.

In conclusion, work published so far has shown that ABCG proteins are involved in three steps of seed dormancy and germination: the release of ABA from the endosperm, the uptake of ABA by the embryo, and the release of ABA from the embryo. The first two transport processes contribute to maintaining dormancy and preventing early seed germination. The third process concerns the release of ABA from the embryo and therefore promotes embryo elongation and seed germination. This last observation highlights the notion that the ABA content in the embryo is also an important factor in seed germination, at least in some plants.

4.4 Additional Roles for ABA Transporters

As mentioned in the introduction, ABA functions in many developmental and stress-related processes. While the role of ABA has been clearly demonstrated for most of these processes, it is not always known whether ABA is synthesized directly within the target cells or transported to these cells. For instance, it remains to be established whether ABA, serving as a signal that induces the formation of suberin, which reduces water loss from the roots under drought stress (Hose et al. 2001), is produced directly in the exodermis and endodermis or transported from the root phloem parenchyma cells to these cell layers.

4.4.1 Lateral Root Production

The role of ABA in regulating lateral root formation and elongation varies between plant species. In Arabidopsis, ABA inhibits lateral root development (De Smet et al. 2003). The lateral roots of mutant plants lacking AtABCG40 are smaller when they were exposed to exogenously applied ABA. However, since the detailed localization of AtABCG40 is not known, the function of this transporter in lateral root emergence and elongation remains to be determined (Kang et al. 2010).

Work on Fabaceae indicates that, in this plant family, ABA is involved in the pre-emergence development of lateral roots (Gonzalez et al. 2015). In contrast to Arabidopsis, in Fabaceae, low concentrations of ABA increase the number of lateral root primordia and also the number of total lateral roots (Harris 2015). In line with these results, Pawela et al. (2019) showed that the loss-of-function mutants of MtABCG20 described above produce fewer lateral roots, indicating that this transporter delivers ABA to the sites of lateral root primordia formation and is involved in

regulating lateral root number. In Medicago, an NPF (MtNRT1.3) has been shown to regulate lateral root formation and inhibit primary root growth (Pellizzaro et al. 2014). However, since these proteins are involved in nitrate uptake and some members also in ABA transport, it is challenging to distinguish between the effect of nitrate nutrition and ABA.

4.4.2 ABA Transporters in Nitrogen-Fixing Nodules

Most plants rely on soil nitrogen for their growth. However, some plants, such as Fabaceae and a few others, can establish a symbiosis with nitrogen-fixing bacteria, in the case of Fabaceae rhizobia. The symbiosis with nitrogen-fixing bacteria enables the plants to obtain nitrogen independently of the soil nitrogen status (Udvardi and Poole 2013). In a complex crosstalk, plants produce nodules, the organs hosting bacteria, which due to their special environment are also called bacteroids. Plants provide bacteroids mainly with carboxylates, while bacteroids release ammonia through the membrane separating the bacteria from the plant cell, the so-called peribacterial membrane, into the plant cytosol. To establish an equilibrium of these exchanges, the number of nodules has to be tightly controlled, and it has been shown that ABA inhibits nodule formation (Ding et al. 2008). ABA also affects the later stages of nodule formation. Exposure of infected roots to external ABA leads to a decreased amount of leghemoglobin, and hence it can be anticipated that the oxygen concentration will increase in these nodules and reduce nitrogen fixation (González et al. 2001).

Loss-of-function mutants for MtABCG20 produced fewer nodules compared to the wild type (Pawela et al. 2019). The rather small, but significant, changes in nodule number suggest that additional ABA transporters are involved in the regulation of nodule formation. LATD/NIP (**lat**eral root **d**efective/**n**umerous **i**nfection threads, **p**olyphenolics) could be such a candidate, since the expression of this transporter is regulated by ABA (Yendrek et al. 2010). In legumes, *LATD/NIP* function is required for the development of two kinds of lateral root organs, lateral roots and nitrogen-fixing root nodules. However, it remains to be established whether this protein is indeed an ABA transporter.

4.4.3 Internal Transporters Important for ABA Homeostasis

ABA glucose ester (ABA-GE) is the major ABA conjugate and to our knowledge is present in various organs of all plant species analyzed so far (Piotrowska and Bajguz 2011). ABA-GE can be hydrolyzed and serves as a source of biologically active, free ABA. ABA-GE has been reported to be localized to vacuoles (Bray and Zeevaart 1985). However, the first report demonstrating the importance of ABA-GE hydrolysis was the identification of AtBG1, a specific ER-localized ABA-GE β-glucosidase (AtBG1) (Lee et al. 2006). Plants lacking this glucosidase activity exhibit pronounced ABA-deficiency phenotypes, including sensitivity to

dehydration, impaired stomatal closure, earlier germination, and lower ABA levels, demonstrating the importance of this conjugated ABA pool.

Two subsequent papers showed that the vacuolar ABA-GE pool also plays an important role in the ABA-related stress response, since two vacuolar β-glucosidases able to hydrolyze vacuolar ABA-GE exhibit a similar, although less pronounced, function as AtBG1 (Wang et al. 2011; Xu et al. 2012). The observation that the single mutants of the vacuolar β-glucosidases exhibit a weaker phenotype than those lacking AtBG1 may be due to a partial redundancy.

These results raise the question of which transporters mediate ABA-GE accumulation in the ER and vacuolar lumen and how free ABA hydrolyzed by glucosidases is exported from these two compartments. Burla et al. (2013) showed that vacuoles can take up ABA-GE via two transport mechanisms. One is directly activated by ATP and exhibits properties consistent with the action of an ABC transporter. Indeed, two vacuolar ABC transporters, AtABCC1 and AtABCC2, are able to transport ABA-GE into vesicles from yeast transgenically expressing these transporters. Vacuolar ABA-GE uptake was also shown to be driven by a proton antiporter. However, in this case the nature of the transporter remains to be elucidated.

The K_M for the uptake of ABA-GE is approximately 1 mM for both transport mechanisms and hence far higher than the cytosolic concentration of this conjugate. Thus, the vacuolar uptake of ABA-GE does not have a regulatory role in ABA-GE storage. Whether ABA released after hydrolysis diffuses across the vacuolar membrane or is delivered to the cytosol by a transporter is unknown. In the acidic vacuole, a large proportion of ABA is present in the protonated, diffusible form and would probably allow efficient delivery to the cytosol. However, the pH of the ER lumen is distinctly less acidic than that of the vacuole, and diffusion of ABA to the cytosol would probably be much less efficient (Martinière et al. 2013). Therefore, a transporter that has yet to be discovered likely mediates the export of ABA from the ER to the cytosol.

5 Concluding Remarks

ABA is involved in many different environmental responses and developmental processes. Not all functions are mentioned in the transport sections; for instance, there is evidence that ABA is also involved in bud dormancy (Cooke et al. 2012; Li et al. 2018), in root hydrotropism and xylem formation, and as a factor regulating the circadian clock and senescence (Dietrich et al. 2017; Ramachandran et al. 2018; Lee et al. 2016). The members of the ABA receptor complex are present as small gene families. In Arabidopsis, there are 14 RCAR/PYR/PYL receptor proteins and 9 clade A PP2Cs (Yoshida et al. 2019). This diversity in ABA receptors greatly expands the number of possible combinations, influencing where and how ABA can act. Indeed, it has been hypothesized that this diversity allows ABA to be perceived in a wide concentration range and in different cells embedded in different tissues (Yoshida

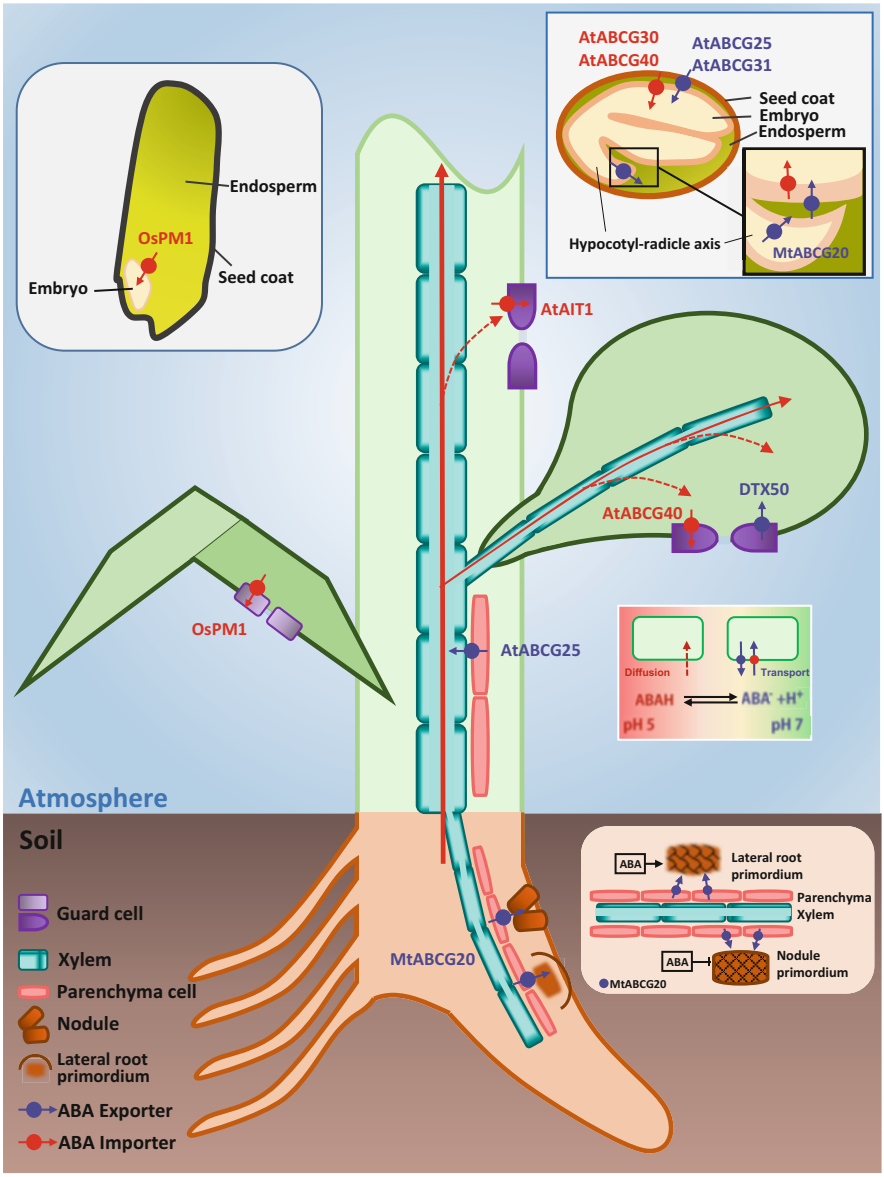

Fig. 1 ABA transporters described to date function in plant development and adaptation to changing environmental conditions. For details, see text

et al. 2019). The large number of potential ABA receptor combinations allows the plant to act in a versatile way to different demands during development and in response to environmental stimuli. Together, the large number of potential receptor

combinations implies that ABA transport must be versatile so that the plant can respond to various developmental stages and environmental conditions.

It is striking that the phenotypes of mutants with defects in ABA transporters described so far are rather moderate compared to those lacking enzymes involved in ABA biosynthesis. This suggests that, in all the responses described so far, more than one transporter is involved in delivering ABA and activating a given process. However, if there were complete redundancy among members of the ABA transporter families, mutants lacking an ABA transporter would not have an aberrant phenotype. Hence, more than one transporter is likely required for optimal ABA action.

It is likely that, depending on the growth conditions, the absence of a given ABA transporter leads to a more or less pronounced phenotype. We hypothesize that ABA transport is mediated by several transporters that have overlapping expression in a given tissue or cell type. The presence of several ABA transporters with a limited transport activity may help fine-tune the amount of ABA delivered to a specific site. This is important, since in many of the processes where ABA is involved, ABA is not the only player but participates in a complex phytohormone network and different internal ABA concentrations may have different effects. Furthermore, the presence of several transporters may act as security, since the absence of one transporter would not necessarily lead to plant death.

Acknowledgment The work on ABA transport done in the authors' laboratories was supported by the Basic Science Research Program NRF-2018R1A2A1A05018173 (to Y.L.) through the National Research Foundation of Korea funded by the Ministry of Science and ICT (Information and Communication Technology) and the Swiss National Foundation. J Kang was supported by Ambizione fellowship of the Swiss National Science Foundation (PZ00P3_168041) and Brain Pool Program through the NRF funded by the Ministry of Science and ICT (2019H1D3A2A02102582).

References

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

Bethke PC, Libourel IGL, Aoyama N, Chung Y-Y, Still DW, Jones RL (2007) The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiol 143:1173–1188

Blackman PG, Davies WJ (1985) Root to shoot communication in maize plants of the effects of soil drying. J Exp Bot 36:39–48

Bray EA, Zeevaart JAD (1985) The compartmentation of abscisic acid and β-d-glucopyranosyl abscisate in mesophyll cells. Plant Physiol 79:719–722

Burla B, Pfrunder S, Nagy R, Francisco RM, Lee Y, Martinoia E (2013) Vacuolar transport of abscisic acid glucosyl ester is mediated by ATP-binding cassette and proton-antiport mechanisms in Arabidopsis. Plant Physiol 163:1446–1458

Cao FY, Yshioka K, Desveaux D (2011) The roles of ABA in plant–pathogen interactions. J Plant Res 124:489–499

Chen H, Lan H, Huang P, Zhang Y, Yuan X, Huang X, Huang J, Zhang H (2015) Characterization of OsPM19L1 encoding an AWPM-19-like family protein that is dramatically induced by osmotic stress in rice. Genet Mol Res 14:11994–12005

Chiba Y, Shimizu T, Miyakawa S, Kanno Y, Koshiba T, Kamiya Y, Seo M (2015) Identification of Arabidopsis thaliana NRT1/PTR family (NPF) proteins capable of transporting plant hormones. J Plant Res 128:679–686

Cho CH, Jang S, Choi BY, Hong D, Choi DS, Choi S, Kim H, Han SK, Kim S, Kim MS, Palmgren M, Sohn KH, Yoon HS, Lee Y (2019) Phylogenetic analysis of ABCG subfamily proteins in plants: functional clustering and coevolution with ABCGs of pathogens. Physiol Plant. https://doi.org/10.1111/ppl.13052

Christmann A, Weiler EW, Steudle E, Grill E (2007) A hydraulic signal in root-to-shoot signalling of water shortage. Plant J 52:167–174

Cooke JE, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35:1707–1728

De Smet I, Signora L, Beeckman T, Inzé D, Foyer CH, Zhang H (2003) An Abscisic acid-sensitive checkpoint in lateral root development of Arabidopsis. Plant J 33:543–555

Deak KI, Malamy J (2005) Osmotic regulation of root system architecture. Plant J 43:17–28

Desikan R, Horák J, Chaban C, Mira-Rodado V, Witthöft J, Elgass K, Grefen C, Cheung MK, Meixner AJ, Hooley R, Neill SJ, Hancock JT, Hauter K (2008) The histidine kinase AHK5 integrates endogenous and environmental signals in Arabidopsis guard cells. PLoS One 3:e2491

Dietrich D, Pang L, Kobayashi A, Fozard JA, Boudolf V, Bhosale R, Antoni R, Nguyen T, Hiratsuka S, Fujii N, Miyazawa Y, Bae T-W, 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 3:17057

Ding Y, Kalo P, Yendrek C, Sun J, Liang Y, Marsh JF, Harris JM, Oldroyd GE (2008) Abscisic acid coordinates nod factor and cytokinin signaling during the regulation of nodulation in Medicago truncatula. Plant Cell 20:2681–2695

Gonzalez AA, Agbévénou K, Herrbach V, Gough C, Bensmihen S (2015) Abscisic acid promotes pre-emergence stages of lateral root development in Medicago truncatula. Plant Signal Behav 10:e977741

González EM, Gálvez L, Arrese-lgor C (2001) Abscisic acid induces a decline in nitrogen fixation that involves leghaemoglobin, but is independent of sucrose synthase activity. J Exp Bot 52:285–293. https://doi.org/10.1093/jexbot/52.355.285

Gonzalez-Guzman M, Pizzio GA, Antoni R, Vera-Sirera F, Merilo F, Bassel GW, Fernández MA, Holdsworth MJ, Perez-Amador MA, Kollist H, Rodriguez PL (2012) Arabidopsis PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to Abscisic acid. Plant Cell 24:2483–2496

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 8:183–187

Harris JM (2015) Abscisic acid: hidden architect of root system structure. Plants (Basel) 4:548–572

Holbrook NM, Shashidhar VR, James RA, Munns R (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J Exp Bot 53:1503–1514

Holdsworth MJ, Bentsink L, Soppe WJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 179:33–54

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W (2001) The exodermis: a variable Apoplastic barrier. J Exp Bot 52:2245–2264

Huang NC, Liu KH, Lo HJ, Tsay YF (1999) Cloning and functional characterization of an Arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake. Plant Cell 11:1381–1392

Hwang JU, Song W-Y, Hong D, Ko D, Yamaoka Y, Jang S, Yim S, Lee E, Khare D, Kim K, Palmgren M, Yoon HS, Martinoia E, Lee Y (2016) Plant ABC transporters enable many unique aspects of a terrestrial plant's lifestyle. Mol Plant 9:338–355

Inuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J 27:325–333

Jackson MB (1993) Are plant hormones involved in root to shoot communication? Adv Bot Res 19:103–187

Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E, Lee Y (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc Natl Acad Sci U S A 107:2355–2360

Kang J, Park J, Choi H, Burla B, Kretzschmar T, Lee Y, Martinoia E (2011) Plant ABC transporters. Arabidopsis Book 9:e0153

Kang J, Yim S, Choi H, Kim A, Lee KP, Lopez-Molina L, Martinoia E, Lee Y (2015) Abscisic acid transporters cooperate to control seed germination. Nat Commun 6:8113

Kanno Y, Hanada A, Chiba Y, Ichikawa T, Nakazawa M, Matsui M, Koshiba T, Kamiya Y, Seo M (2012) Identification of an abscisic acid transporter by functional screening using the receptor complex as a sensor. Proc Natl Acad Sci U S A 109:9653–9658

Khare D, Choi H, Huh SU, Bassin B, Kim J, Martinoia E, Sohn KH, Paek K-Y, Lee Y (2017) Arabidopsis ABCG34 contributes to defense against necrotrophic pathogens by mediating the secretion of camalexin. Proc Natl Acad Sci U S A 114:E5712–E5720. https://doi.org/10.1073/pnas.1702259114

Kim T-H, Böhmer M, Hu H, Nishimura N, Schroeder JI (2010) Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. Annu Rev Plant Biol 61:561–591

Koike M, Takezawa D, Arakawa K, Yoshica S (1997) Accumulation of 19-kDa plasma membrane polypeptide during induction of freezing tolerance in wheat suspension-culture cells by abscisic acid. Plant Cell Physiol 38:707–716

Krattinger SG, Jordan DR, Mace ES, Raghavan C, Luo MC, Keller B, Lagudah ES (2013) Recent emergence of the wheat Lr34 multi-pathogen resistance: insights from haplotype analysis in wheat, rice, sorghum and Aegilops tauschii. Theor Appl Genet 126:663–672

Krattinger SG, Kang J, Bräunlich S, Boni R, Chauhan H, Selter LL, Robinson MD, Schmid MW, Wiederhold E, Hensel G, Kumlehn J, Sucher J, Martinoia E, Keller B (2019) Abscisic acid is a substrate of the ABC transporter encoded by the durable wheat disease resistance gene Lr34. New Phytol 223:853–866

Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, Moriyama Y, Shinozaki K (2010) ABC transporter AtABCG25 is involved in abscisic acid transport and responses. Proc Natl Acad Sci U S A 107:2361–2366

Kuromori T, Sugimoto E, Shinozaki K (2011) Arabidopsis mutants of AtABCG22, an ABC transporter gene, increase water transpiration and drought susceptibility. Plant J 67:885–894

Kuromori T, Seo M, Shinozaki K (2018) ABA transport and plant water stress responses. Trends Plant Sci 23:513–522

Lee KH, Piao HL, Kim H-Y, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee I-J, Hwang I (2006) Activation of glucosidase via stress-induced polymerization rapidly increases active pools of Abscisic acid. Cell 126:1109–1120

Lee KP, Piskurewicz U, Turecková V, Strnad M, Lopez-Molina L (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. Proc Natl Acad Sci U S A 107:19108–19113

Lee HG, Mas P, Seo PJ (2016) MYB96 shapes the circadian gating of ABA signaling in Arabidopsis. Sci Rep 6:17754

Léran S, Varala K, Boyer JC, Chiurazzi M, Crawford N, Daniel-Vedele F, David L, Dickstein R, Fernandez E, Forde B, Gassmann W, Geiger D, Gojon A, Gong JM, Halkier BA, Harris JM, Hedrich R, Limami AM, Rentsch D, Seo M, Tsay YF, Zhang M, Coruzzi G, Lacombe B (2014) A unified nomenclature of nitrate transporter 1/PEPTIDE transporter family members in plants. Trends Plant Sci 19:5–9

Li J, Xu Y, Niu Q, He L, Teng Y, Bai S (2018) Abscisic acid (ABA) promotes the induction and maintenance of pear (Pyrus pyrifolia White Pear Group) flower bud endodormancy. Int J Mol Sci 19:E310

Liu G, Stirnemann M, Gübeli C, Egloff S, Courty P-E, Aubry S, Vandenbussche M, Morel P, Reinhardt D, Martinoia E, Borghi L (2019) Strigolactones play an important role in shaping exodermal morphology via a KAI2-dependent pathway. iScience 17:144–154

Longo A, Miles NW, Dickstein R (2018) Genome mining of plant NPFs reveals varying conservation of signature motifs associated with the mechanism of transport. Front Plant Sci 9:1668

Lopez-Molina L, Mongrand S, Chua N-H (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc Natl Acad Sci U S A 98:4782–4787

Lu X, Dittgen J, Piślewska-Bednarek M, Molina A, Schneider B, Svatoš A, Doubskỳ J, Schneeberger K, Weigel D, Bednarek P, Schulze-Lefert P (2015) Mutant allele-specific uncoupling of PENETRATION3 functions reveals engagement of the ATP-binding cassette transporter in distinct tryptophan metabolic pathways. Plant Physiol 168:814–827

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324:1064–1068

Martinière A, Bassil E, Jublanc E, Alcon C, Reguera M, Sentenac H, Blumwald E, Paris N (2013) In vivo intracellular pH measurements in tobacco and Arabidopsis reveal an unexpected pH gradient in the endomembrane system. Plant Cell 25:4028–4043

Matern A, Böttcher C, Eschen-Lippold L, Westermann B, Smolka U, Döll S, Trempel F, Aryal B, Scheel D, Geisler M, Rosahl S (2019) A substrate of the ABC transporter PEN3 stimulates bacterial flagellin (flg22)-induced callose deposition in Arabidopsis thaliana. J Biol Chem 297:6857–6870

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in innate immunity against bacterial invasion. Plant Cell 126:969–980

Merilo E, Jalakas P, Kollist H, Brosché M (2015) The role of ABA recycling and transporter proteins in rapid stomatal responses to reduced air humidity, elevated CO_2 and exogenous ABA. Mol Plant 8:657–659

Merilo E, Yarmolinsky D, Jalakas P, Parik H, Tulva I, Rasulov B, Kilk K, Kollist H (2018) Stomatal VPD response: there is more to the story than ABA. Plant Physiol 176:851–864

Meurs C, Basra AS, Karssen CM, van Loon LC (1992) Role of abscisic acid in the induction of desiccation tolerance in developing seeds of Arabidopsis thaliana. Plant Physiol 98:1484–1493

Morita Y, Kodama K, Shiota S, Mine T, Kataoka A, Mizushima T, Tsuchiya T (1998) NorM, a putative multidrug efflux protein, of Vibrio parahaemolyticus and its homolog in Escherichia coli. Antimicrob Agents Chemother 42:1778–1782

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

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56:165–185

Oliver SN, Dennis ES, Dolferus R (2007) ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. Plant Cell Physiol 48:1319–1330

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu JK, Schroeder JI, Volkman BF, Cutler SR (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324:1068–1071

Pawela A, Banasiak J, Biała W, Martinoia E, Jasiński M (2019) MtABCG20 is an ABA exporter influencing root morphology and seed germination of Medicago truncatula. Plant J 98:511–523

Pellizzaro A, Clochard T, Cukier C, Bourdin C, Juchaux M, Montrichard F, Thany S, Raymond V, Planchet E, Limami AM, Morère-Le Paven M-C (2014) The nitrate transporter MtNPF6.8 (MtNRT1.3) transports abscisic acid and mediates nitrate regulation of primary root growth in Medicago truncatula. Plant Physiol 166:2152–2165

Peng Y-B, Zou C, Wang D-H, Gong H-Q, Xu Z-H, Bai S-N (2006) Preferential localization of abscisic acid in primordial and nursing cells of reproductive organs of Arabidopsis and cucumber. New Phytol 170:459–466

Piotrowska A, Bajguz A (2011) Conjugates of abscisic acid, brassinosteroids, ethylene, gibberellins, and jasmonates. Phytochemistry 72:2097–2112
Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signaling. Trends Plant Sci 15:395–401
Ramachandran P, Wang G, Augstein F, de Vries J, Carlsbecker A (2018) Continuous root xylem formation and vascular acclimation to water deficit involves endodermal ABA signalling via miR165. Development 145:dev159202
Rerksiri W, Zhang X, Xiong H, Chen X (2013) Expression and promoter analysis of six stress-induced genes in rice. Sci World J 2013:397401
Robertson DS (1955) The genetics of vivipary in maize. Genetics 40:745–760
Schwartz SH, Tan BC, Gage DA, Zeevaart JAD, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276:1872–1874
Stein M, Dittgen J, Sánchez-Rodríguez C, Hou B-H, Molina A, Schulze-Lefert P, Lipka V, Somerville S (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 18:731–746
Takanashi K, Shitan N, Yazaki K (2014) The multidrug and toxic compound extrusion (MATE) family in plants. Plant Biotech 31:417–430
Tan BC, Joseph LM, Deng WT, Liu L, Li QB, Cline K, McCarty DR (2003) Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J 34:44–56
Tsay YF, Schroeder JI, Feldmann KA, Crawford NM (1993) The herbicide sensitivity gene CHL1 of Arabidopsis encodes a nitrate-inducible nitrate transporter. Cell 72:705–713
Udvardi M, Poole PS (2013) Transport and metabolism in legume-rhizobia symbioses. Annu Rev Plant Biol 64:781–805
Upadhyay N, Kar D, Deepak Mahajan B, Nanda S, Rahiman R, Panchakshari N, Bhagavatula L, Datta S (2019) The multitasking abilities of MATE transporters in plants. J Exp Bot 70:4643–4656
Wang PT, Liu H, Hua HJ, Wang L, Song C-P (2011) A vacuole localized β-glucosidase contributes to drought tolerance in Arabidopsis. Chin Sci Bull 56:3538–3546
Xu Z-Y, Lee KH, Dong T, Jeong JC, Jin JB, Kanno Y, Kim DH, Kim SY, Seo M, Bressan RA, Yun DJ, Hwang I (2012) A vacuolar β-glucosidase homolog that possesses glucose-conjugated abscisic acid hydrolyzing activity plays an important role in osmotic stress responses in Arabidopsis. Plant Cell 24:2184–2199
Yao L, Cheng X, Gu Z, Huang W, Li S, Wang L, Wang Y-F, Xu P, Ma H, Ge X (2018) The AWPM-19 family protein OsPM1 mediates abscisic acid influx and drought response in Rice. Plant Cell 30:1258–1276
Yendrek CR, Lee YC, Morris V, Liang Y, Pislariu CI, Burkart G, Meckfessel MH, Salehin M, Kessler H, Wessler H, Lloyd M, Lutton H, Teillet A, Sherrier DJ, Journet EP, Harris JM, Dickstein R (2010) A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in Medicago truncatula. Plant J 62:100–112
Yoshida T, Christmann A, Yamaguchi-Shinozaki K, Grill E, Fernie AR (2019) Revisiting the basal role of ABA – Roles outside of stress. Trends Plant Sci 24:625–635. https://doi.org/10.1016/j.tplants.2019.04.008
Zeevaart JAD, Creelman RA (1988) Metabolism and physiology of abscisic acid. Annu Rev Plant Physiol Plant Mol Biol 39:439–473
Zhang H, Han W, De Smet I, Talboys P, Loya R, Hassan A, Rong H, Jürgens G, Paul Knox J, Wang MH (2010) ABA promotes quiescence of the quiescent centre and suppresses stem cell differentiation in the Arabidopsis primary root meristem. Plant J 64:764–774
Zhang H, Zhu H, Pan Y, Yu Y, Luan S, Li L (2014) A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in Arabidopsis. Mol Plant 7:1522–1532
Zhang H, Zhao F-G, Tang R-J, Yu Y, Song J, Wang Y, Legong L, Luan S (2017) Two tonoplast MATE proteins function as turgor-regulating chloride channels in Arabidopsis. Proc Natl Acad Sci U S A 114:E2036–E2045