

Chapter 6

Gravitropism in Higher Plants: Cellular Aspects



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Abstract Due to their sessile life style, an important ability of plants is to adjust their growth towards or away from environmental stimuli. Plant responses that involve directed movements are called tropisms. Among the best-known tropisms are phototropism, the response to light, and gravitropism, the response to gravity. Gravity is one of the major factors that govern root growth in plants. Since the emergence of land plants, gravitropism allowed plants to adjust root growth to maximize access to water and nutrients, and shoots to explore and exploit space on and above the surface of the Earth. In this chapter we discuss current knowledge and point out open questions like the nature of the gravireceptor, the role of secondary messengers, hormones and the cytoskeleton. We review the history of plant gravitropism research, from early experiments performed by naturalists like Charles Darwin to the utilization of clinostats, centrifuges and experimentation in the almost stimulus-free environment of microgravity provided by drop towers, parabolic flights of aircrafts and rockets, satellites and low earth orbit space stations, which are increasingly contributing to our understanding of plant gravity sensing and orientation.

Keywords Auxin · Clinostat · Gravitropism · Gravity · Microgravity · Roots · Statolith

6.1 Introduction

All organisms on Earth are subject to the continuous influence of gravity. In a process called gravitropism, plants perceive the direction of gravity and can adjust their growth accordingly (Fig 6.1). Most plant organs are actively positioned at various defined angles from the gravity vector, the gravitational set point angle (GSA; Digby and Firn 1995).

This allowed plants to leave the water and conquer land, enabling them to explore and exploit space below, on and above the surface of the Earth in a most beneficial way and, thus, to provide food and resources for all animal and human life on



Fig. 6.1 Higher plant organs make use of the only constant environmental cue, the gravity vector of the Earth, for precisely orienting their organs. By orienting positive gravitropic roots they grow towards the center of gravity to anchor themselves in the soil and take up water and nutrients. Shoots grow negatively gravitropically away from the center of the Earth towards light. Gravitropism allows plants to arrange their different organs in a most beneficial way to exploit the space below, on and above the surface of the Earth

Earth. An organ like the primary root that is maintained vertically and grows downwards towards the gravity vector has a GSA of 0° , an extreme called positive gravitropism. An organ like the shoot that is maintained vertically but grows upwards against the gravity vector has a GSA of 180° , an extreme termed negative gravitropism (Frank 1868). Other organs do not show strict positive or negative gravitropism but have a GSA between these two extremes (plagiotropism;

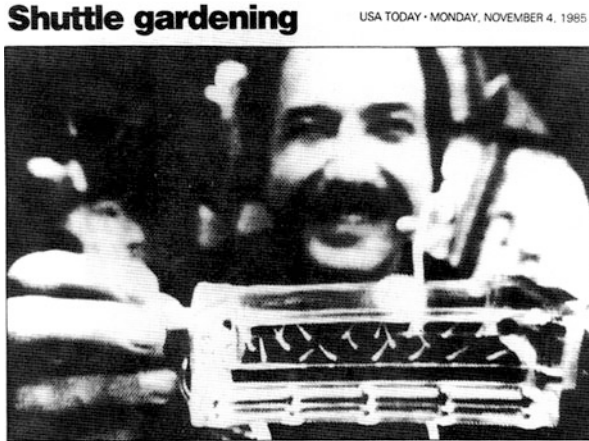


Fig. 6.2 Dutch astronaut Wubbo Ockels displays garden cress sprouts growing inside Spacelab aboard the space shuttle Challenger

cf. Fig. 1.2). One example is the gravitropic behavior of lateral roots in *Arabidopsis thaliana*. These bud from the main root orthogonal to the gravitational vector (diatropism) and upon reorientation to either a more upright or downward facing direction, the tip of the lateral root will display a rapid curvature that brings it back towards the original orientation (Mullen and Hangarter 2003; Guyomarc'h et al. 2012).

Research on gravity sensing and gravitropic responses has greatly benefitted from the advancement in molecular and cellular methods as well as from experiment platforms providing the almost stimulus-free environment of microgravity. During the German Spacelab-mission D1, US newspapers published a photograph of the Dutch astronaut Wubbo Ockels showing roots of the garden cress, which germinated and grew on board the Space Shuttle Challenger (see Fig. 6.2). In the absence of gravity, the roots grew straight in the direction given by the tip of the radicle in the seed, thus, confirming that Wilhelm Pfeffer's predicted "automorphose" really exists at the organ and the cellular level (Pfeffer 1904). It was found that the architecture and function of gravity perceiving cells, the statocytes, are genetically determined and not dependent on the presence of gravity or other factors (Volkman et al. 1986). Since then, numerous microgravity experiments performed in drop towers, on parabolic plane flights, on research rockets, on Russian and Chinese satellites and on US Space shuttles as well as on the International Space Station have opened new perspectives on the molecular, cellular and physiological mechanisms underlying gravity sensing and graviorientation in higher plants.

6.2 Gravity Perception

The gravitropic response can be subdivided into three distinct and sequential events: signal perception, signal transduction and gravitropic response. The first step in signal perception is the conversion of the gravitational stimulus into a detectable physical change within the cell (Baldwin et al. 2013; Toyota and Gilroy 2013; Schüler et al. 2015). Various models have been proposed of how the physical stimulus of statolith sedimentation is transduced into a biochemical signaling event. The leading hypotheses are the statolith-dependent starch-statolith hypothesis and the tensegrity model (Yoder 2001; Zheng and Staehelin 2001) as well as the statolith-independent gravitational pressure or protoplast pressure models (Wayne and Staves 1996; Palmieri and Kiss 2007).

6.2.1 Tissue Localization of Gravitropism

All plant cells experience the gravitational stimulus equally. However, one must differentiate between a specific gravity sensing mechanism that is localized in specific tissues, and general unspecific physical and physiological or metabolic reactions that might be observable in all cells. For a gravitropic response, gravity must be perceived by specialized tissues (Fig. 6.3). Gravitropism in the shoot occurs mainly in the starch sheath in the endodermis. In *Arabidopsis thaliana*, a mutation of the gene *PHOSPHOGLUCOMUTASE* (*PGM*) prevents formation of the starch sheath, phenotypically observable by an agravitropic behavior of the shoot. This connection between defect and absent endodermis and gravitropism was further confirmed by mutant screens. The mutant *shoot gravitropism 1* (*sgr1*) has a mutation in the gene *SCARECROW* (*SCR*), the mutant *sgr7* has a mutation in gene *SHORT ROOT* (*SHR*). In both mutants, the endodermis is not formed in the shoot and consequently, in both mutants shoot gravitropism is no longer observable (Fukaki et al. 1998). All *shoot gravitropism* mutants, although likewise defective in root endodermis, still show normal root gravitropism which is easily explained because in the root, gravity is sensed in the columella cells in the root cap.

Charles Darwin was one of the first naturalists and biologists who had shown that the removal or damaging of the root tip leads to agravitropic growth and that these roots regain the ability to grow downward again after regeneration of the root cap (Darwin 1880). In particular the manipulation or removal of the columella cells impairs gravitropic behavior (Juniper et al. 1966; Konings 1968; Tsugeki and Fedoroff 1999). There are typically 4 layers of columella cells at the root tip, S1-S4 (Fig. 6.3). By selective removal of individual columella cells using laser ablation, it was shown that the innermost layers S1 and S2 are most important for gravitropism (Blancaflor et al. 1998).

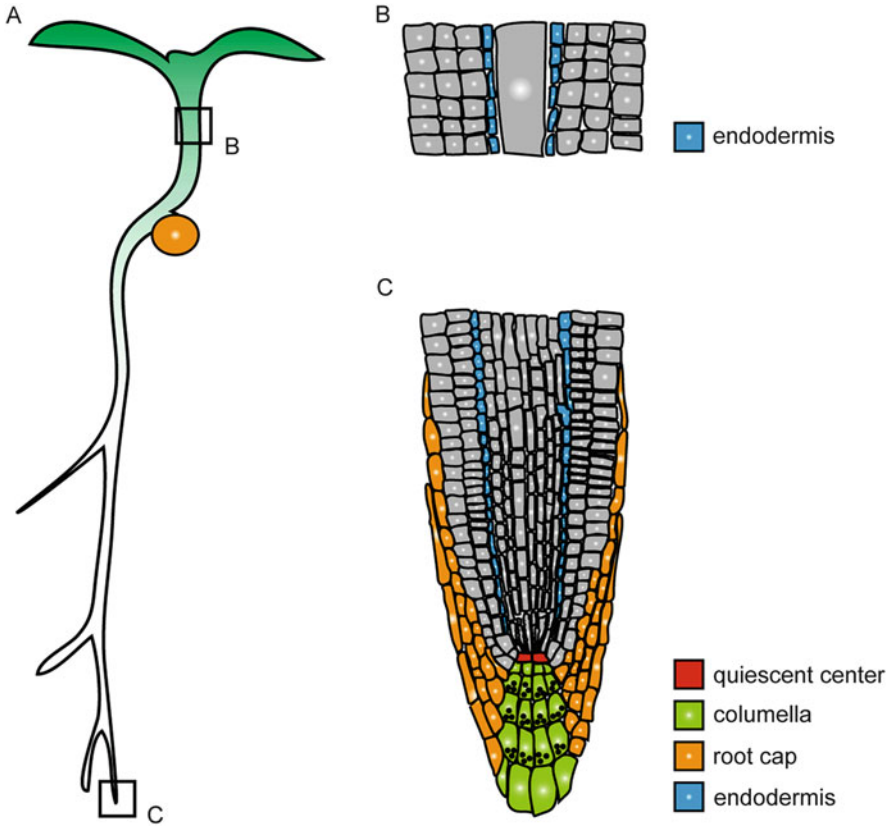


Fig. 6.3 Tissues of graviperception. The endodermis in the shoot and the columella cells in the root tip form gravity sensing statocytes

6.2.2 *Starch-Statolith Hypothesis*

The starch-statolith hypothesis for plant gravity sensing is widely accepted by most researchers today. Statocytes are highly specialized gravity-sensing cells of higher plants and are characterized by a polar architecture (first described in detail by Sievers and Volkmann 1971, 1977) and the presence of starch-filled amyloplasts, the so-called statoliths (Figs. 6.3 and 6.4). Statocytes in roots have small vacuoles, a nucleus that is positioned in the upper part of the cell by cytoskeletal elements, and a specific architecture of the endoplasmic reticulum (ER). The ER in statocytes is organized in two forms. Most of the ER is tubular and covers the periphery of the cell, the so-called cortical ER. In the lower part of most statocytes, ER cisternae form a cushion-like structure onto which statoliths seem to be already sedimented in statocytes in a normal vertically growing root. The starch-filled amyloplast-statoliths are the only organelles free to move and to sediment in the cell upon gravistimulation,

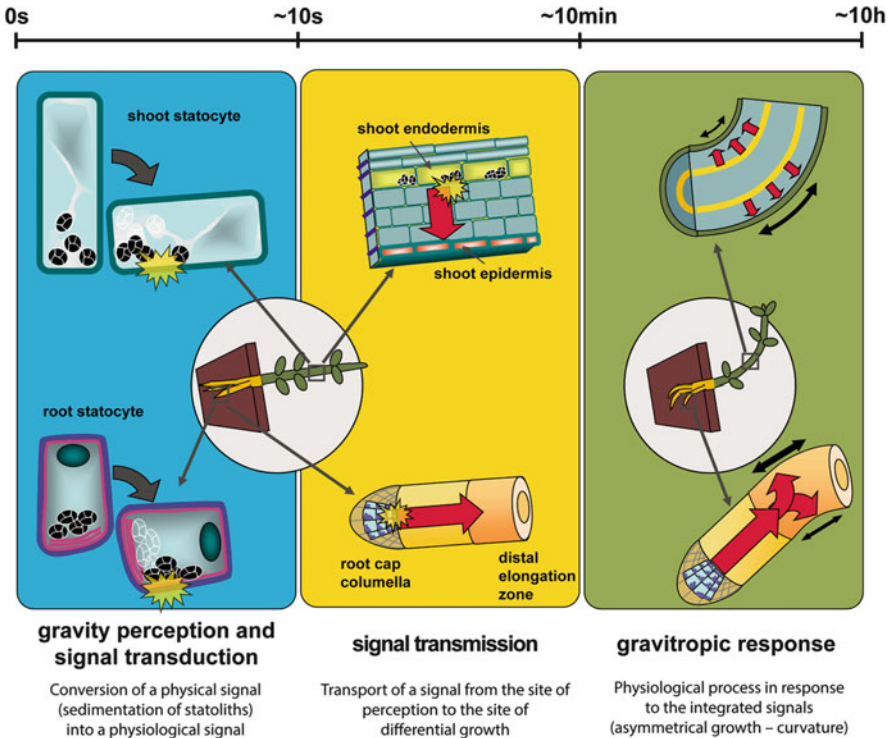


Fig. 6.4 Phases of gravitropism in higher plants. When the orientation of a plant organ changes relative to the vector of gravity, sedimentation of amyloplast statoliths in statocytes initiates signal perception—the conversion of a physical signal of statolith sedimentation into a physiological signal. This signal is then transmitted to the responding target cells. The nominal orientation is resumed by differential growth of the opposite flanks of the organs

probably favored by the absence of prominent actin microfilament bundles, extensive microtubule arrangements (Baluška et al. 1997) and ER cisternae in the central area as well as the proximal position of the nucleus and the starch content of the amyloplast-statoliths. All other organelles in statocytes as well as the organelles in all other cell types are precisely fixed in their position by cytoskeletal elements.

Upon reorientation statoliths sediment along the gravitational vector. Already at the beginning of the twentieth century, this was considered a trigger for gravitropic responses (Haberlandt 1900; Nemeč 1900). Convincing evidence in favor of the statoliths-based sensing mechanism comes from magnetophoretic experiments with barley coleoptiles and flax roots as well as in moss protonemata (*Ceratodon purpureus*) showing clearly that a lateral displacement of statoliths without tilting the plant organs or the protonemata from the vertical growth direction is sufficient to trigger typical gravitropic responses independent of the gravitational vector (Kuznetsov and Hasenstein 1996, 1997; Kuznetsov et al. 1999). Cold treatment, starvation and destarching amyloplast-statoliths with gibberellic acid or kinetin

reversibly reduced or even completely abolished graviresponsiveness in cress roots (Audus 1979; Busch and Sievers 1990). Moreover, mutant analyses also confirmed a connection between starch content of amyloplasts and gravitropic responses. Mutants of *PHOSPHOGLUCOMUTASE* (*PGM*) in *A. thaliana* and *Nicotiana sylvestris* that form starchless plastids in columella cells are impaired in gravitropism, highlighted by a delayed and weak gravitropic response (Kiss and Sack 1989; Kiss et al. 1989). It was assumed that a reduction in the mass of the amyloplasts led to a delayed sedimentation and thereby to a delayed graviperception (Kiss and Sack 1989). This was confirmed by experiments with the *Arabidopsis thaliana* mutant *starch excess 1* (*sex1*) which has amyloplasts with higher starch content in the hypocotyl and requires a shorter presentation time before bending can be observed (Vitha et al. 2007).

Auxin redistribution in response to plant reorientation is also severely disrupted in the *pgm 1* mutant as shown by the auxin sensor DII-Venus (Band et al. 2012). The process of statolith sedimentation therefore appears to be fundamental for the perception of gravity.

The starch-statolith hypothesis includes all perception mechanisms that are driven by the sedimentation of statoliths. It is based on mechanisms in which the sedimentation of statoliths ultimately leads to a contact with or a movement along the ER or plasma membrane and thereby to perception of the biophysical stimulus. This can be achieved by the opening of mechanosensitive channels or via protein-protein interaction, which can both trigger a biochemical signaling cascade. Statolith sedimentation exerts pressure on the ER or plasma membrane. A proportion of amyloplasts is usually already in close proximity to the distal ER and moves along the ER upon gravistimulation. Both movement and pressure can cause membrane deformation after sedimentation (Behrens et al. 1985; Leitz et al. 2009), which yields the potential to open membrane-localized mechanosensitive ion channels (Hamilton et al. 2015). The resulting ion current can trigger further signaling cascades (Toyota and Gilroy 2013). Calcium ions are suspected to be the ions that are transported, as the ER is a prominent calcium store (Meldolesi and Pozzan 1998). Other ions that can pass mechanosensitive channels are potassium and chloride (Toyota and Gilroy 2013). The identity of the suspected mechanosensitive channels has not been revealed yet (reviewed by Baldwin et al. 2013). A binding of receptor (in the ER membrane) and ligand (in the statolith membrane) could also trigger gravitropic signaling cascades. Experiments with *Chara* rhizoids offer results that could in part also be true for higher plants (see Chap. 4). A transfer of results obtained with *Chara* to higher plants, however, must consider that statocytes have a different architecture. *Chara* rhizoids do not have a peripheral positioned ER. Contact between statolith and membrane in *Chara* takes place with the plasma membrane, in higher plants with the ER. If protein-protein interactions play a role in gravitropism in higher plants, an evolutionary relocalization of proteins or new receptor ligand complexes would have to be assumed.

It is worth noting that there is a significant difference in timing between the duration of statolith sedimentation and first measurable responses. Complete sedimentation of statolith was reported to take about 5–10 min (Leitz et al. 2009;

Baldwin et al. 2013). However, with intermittent stimulation of cress roots on a clinostat, the perception time was calculated to be in a range of 0.5 s, which strongly indicates that a minor movement of statoliths already settled on ER-cisternae is sufficient for graviperception to occur (Heinowicz et al. 1998). Measurements of membrane potentials following a 5–15 s reorientation show membrane depolarizations already 8 s after reorientation (Behrens et al. 1985). An increase in cytoplasmic inositol-1,4,5-triphosphate (InsP₃) was observed in *Zea mays* 10 s after reorientation (Perera et al. 1999). Measurements of cytosolic calcium in parabolic flight experiments also suggest changes within seconds of the microgravity stimulus (Neef et al. 2016).

6.2.3 *Tensegrity Model and the Role of the Actin in Gravity Sensing*

Tensegrity models describe the connection between graviperception and the cytoskeleton of the cell. Tensegrity is a composite of the words tension and integrity and describes biologically the connection between mechanical force and mechanisms or functions of the cell (Ingber et al. 2014). Statoliths are surrounded by an only very delicate actin microfilament meshwork (Collings et al. 2001). First tensegrity models described that the actin cytoskeleton surrounding the statoliths is connected to statolith and ER cisternae or the plasma membrane. Sedimentation of a statolith would put tension on the cytoskeleton and open mechanosensitive channels in the plasma membrane (Sievers et al. 1989). One prediction from this model was that a disruption of the actin cytoskeleton would prevent graviperception. But in fact, experiments in which the actin filaments were disrupted by Latrunculin B did not lead to an agravitropic phenotype and application of Latrunculin B and Cytochalasin B, another actin depolymerizing drug, even increased statolith sedimentation velocity and gravitropic responses in cress roots (Sievers et al. 1989) and in *Zea mays* roots (Blancaflor 2002; Hou et al. 2003, 2004). Furthermore, the promoted graviresponse that was found in roots, which were gravistimulated for only a short time and then rotated on a clinostat, was attributed to the inability of fragmented actin microfilaments to terminate the curvature response (Hou et al. 2004). Thus, the actin cytoskeleton likely does not play a crucial role in the gravity-sensing process and it is tempting to speculate that actin is not essential for gravity sensing *per se* but is required for controlling and fine-tuning an appropriate and functional resting position as well as unimpeded sedimentation of statoliths. In fact, actomyosin forces may even increase the energetic noise level of the sensing mechanism by increasing the random movements of statoliths, making statocytes less susceptible for unfavorable fast responses to quickly changing or only transient gravistimulation, e.g. when the wind repeatedly bends crop stalks (Braun and Limbach 2006).

6.3 Signal Transduction and Transmission

It is obvious that a mode of communication exists between the root tip cells that sense gravity and the cells in the elongation zone that respond to the gravity signal by expanding differentially on the opposite root flanks which leads to the downward curvature. After conversion of the physical stimulus into a biochemical signal (signal transduction) in the columella cells, the signal of all statocytes must be integrated, enhanced, transmitted and received in the responding tissue.

6.3.1 Secondary Messengers

Signal transduction and transmission employs a plethora of secondary messengers including calcium ions, InsP₃ and protons (Fasano 2001; Perera 2006).

Calcium is an essential secondary messenger in many signaling processes in plants and has been considered as an important messenger in gravitropism. It was noted early on that radioactively labeled ⁴⁵Ca²⁺ is transported basally in *Zea mays* roots after gravistimulation (Lee and Evans 1985). One-sided application of calcium to the root leads to bending towards the source of calcium (Lee et al. 1983a). Gravitropic bending is severely impaired by application of calcium chelators or by inhibition of calmodulin or calcium-channels (Lee et al. 1983b; Vanneste and Friml 2013). These observations suggest that calcium plays a role in the apoplast of the root (Toyota and Gilroy 2013). Calmodulins, prominent calcium binding proteins, are highly expressed in the root tip (Stinemetz et al. 1987), which might suggest a role of calcium also in gravity perception.

More recent experiments with the calcium sensor aequorin support a role for calcium during early gravitropic responses (Toyota et al. 2007). Aequorin fluorescence is dependent on the availability of intracellular calcium (Shimomura et al. 1962). After reorientation experiments, two calcium waves were measured in statocytes of the hypocotyl, the first after 4 s, the second after 1 min. The first calcium wave was considered an effect of the mechanical reorientation, the second as an effect of the gravitational response. It remains unclear whether calcium is part of the signal perception or part of the later signaling events. The second wave suggests an involvement in signal transduction and not signal perception. This is supported by physiological changes, like membrane depolarization, that appear earlier than 1 min (Behrens et al. 1985). More recently introduced calcium sensors, like Cameleon YC3.6, have been used to detect a calcium wave arising on the lower side of the root and moving towards the elongation zone like auxin (Monshausen et al. 2011). Calcium could play different roles in early as well as progressed signal transduction. Modern real-time measurements, e.g. in parabolic plane flights, promise more detailed results in the coming years (Neef et al. 2016).

Calcium intimately interplays with auxin. Auxin induces calcium levels and signaling (Toyota and Gilroy 2013). Increases in calcium regulate auxin transport

(Robert and Offringa 2008). Calcium elevations change cell wall pH which regulates elongation via acid growth (Monshausen et al. 2011). The proton concentration (pH) of a cellular compartment has a strong effect on all containing proteins and metabolites. A change in pH can have a modulating influence on various cellular activities. Using a pH sensor, a movement of protons from the cytoplasm to the apoplast was observed in the columella in *Arabidopsis thaliana* upon gravistimulation (Fasano 2001). Alkalization of the cytoplasm occurs within the first 2 min with a change from pH 7.2 to pH 7.6, while the pH in the apoplast changes from pH 5.5 to 4.5. Later pH changes in the cell wall in the elongation zone were attributed to tropic growth. Similar observations of pH changes were made in *Zea mays* pulvini in the statocytes-containing endodermis (Johannes et al. 2001). With the help of caged protons that were released upon UV irradiation, the beginning of tropic growth could be delayed by manual manipulation of the pH value (Fasano 2001).

Mutants defective in columella cell alkalization fail to relocate auxin transporters of the PIN family, thereby affecting auxin redistribution (Boonsirichai et al. 2003; Harrison and Masson 2008).

Phosphatidylinositol-4,5-bisphosphate (PIP₂) is cleaved via hydrolysis by phospholipase C into diacylglycerol and InsP₃. Both hydrolysis products play a role in downstream signaling cascades. Mutants of *Phosphatidylinositol-monophosphate-5-kinase (PIP5K)* are delayed in their gravitropic response and impaired in polar auxin transport (Mei et al. 2012). One of the products of PIP5K, InsP₃ modulates intracellular calcium signals in animal cells. InsP₃ opens ER-localized calcium channels which trigger downstream signaling cascades (Berridge 2009). InsP₃ could have a similar influence on calcium signaling in plants during gravitropism. A role for InsP₃ in gravitropism is supported by several experiments. A fivefold increase in InsP₃ concentration in the lower pulvinus, the area of bending growth in *Zea mays*, was measured 10 s after reorientation (Perera et al. 1999). The increase in InsP₃ correlates with membrane depolarization after 8 s (Behrens et al. 1985) and suggests that InsP₃ plays a role during early gravitropism signaling or even graviperception. The InsP₃ concentration is still increased after 2 hours, suggesting an involvement also in later signaling processes. Following the gravitropic response (8–10 h), the InsP₃ concentration reverts to its initial level. In plants overexpressing human INOSITOL-POLYPHOSPHATE-5-PHOSPHATASE, catalyzing constitutive hydrolysis of InsP₃, gravitropic responses were present but reduced (Perera 2006). Inhibition of InsP₃ synthesis by blocking PHOSPHOLIPASE C in *Arabidopsis thaliana* leads to reduced gravitropic responses in roots and shoot (Andreeva et al. 2010).

It is noteworthy that gravitropic responses in InsP₃-deficient plants were never abolished but always reduced. InsP₃ can carefully be considered modulating but not essential for gravitropism. InsP₃ may have to be considered in the context of other secondary messengers. A direct connection between InsP₃ and calcium and InsP₃-activated calcium channels is a point of future research.

While calcium, InsP₃, and pH play a role as secondary messengers in gravitropism signaling, the resulting hormone distribution leads to the final curvature responses.

6.3.2 *Asymmetric Hormone Distribution Leads to Directed Growth*

Cholodny and Went independently discovered a connection between phytohormones and tropisms in higher plants. Cholodny observed that a gravitational stimulus disturbs the even distribution of phytohormones in the root of higher plants. Went also observed that the phytohormone auxin specifically enriches on the bending side. Later experiments on the influence of light on hormone distribution led to similar results (Went and Thimann 1937). Based on these observations the Cholodny-Went hypothesis was postulated that claims that the bending growth typical for tropisms is a result of polar auxin distribution (Cholodny 1929).

Later, the Cholodny-Went hypothesis was refined by the observation that besides auxin, the phytohormone jasmonic acid (JA) also shows a polar distribution. Inhibition of the JA gradient led to a delay of gravitropic responses. JA-deficient *Oryza sativa* mutants show delayed but observable gravitropic responses (Gutjahr et al. 2005). This suggests a modulating function of JA in gravitropism.

A central element of the gravitropic growth response is, therefore, the generation of hormone gradients within the growing organ. For auxin this is achieved via carrier-mediated asymmetric transport.

6.4 Gravitropic Growth

6.4.1 *Polar Hormone Distribution*

While the importance of auxin for tropic growth was known since the Cholodny-Went hypothesis, the mechanism of auxin transport was only identified later. The most important member of the group of auxins, free Indole-3-acetic acid can be protonated (IAAH) or deprotonated (IAA^-) depending on the pH. Only IAAH can freely diffuse through the plasma membrane. At pH 7 IAA^- prevails and requires active transport from cell to cell (Friml and Palme 2002).

Experiments with auxin transport inhibitors led to different results in Boston Ivy (*Parthenocissus tricuspidata*). Triiodobenzoic acid (TIBA) inhibits auxin efflux from the cytoplasm. Auxin accumulation in the cytoplasm was still observed besides TIBA application. 2,4-Dichlorophenoxyacetic acid inhibits auxin influx. Based on these observations, the chemiosmotic theory was postulated. The interplay of passive auxin transport with the activity of auxin efflux and influx carriers allows polar auxin transport in plants (Rubery and Shelldrake 1974).

Modern molecular biology methods allowed for the identification of the efflux and influx carriers. Mutant analyses identified *AUXIN RESISTANT 1* (*AUX1*) as the gene coding for the influx carrier (Bennett et al. 1996). Similar mutant experiments identified *PIN-FORMED 1* (*PIN1*) as a gene coding for an efflux carrier, with polar localization in the plasma membrane and thereby allowing for polar auxin transport

(Gälweiler et al. 1998). Further PIN proteins and their subcellular localization were identified. In general, expression and localization of PIN proteins is cell-type dependent (Michniewicz et al. 2007). Further efflux carriers encompass proteins of the *ABC-B/MULTI-DRUG RESISTANCE/P-GLYCOPROTEIN (ABC/MDR/PGP)* family. They are localized symmetrically in the plasma membrane and are involved in auxin homeostasis (Cho and Cho 2013).

The polar auxin transport must work more efficiently on one side of the gravistimulated organ after reorientation of the plant, so that according to Cholodny and Went an asymmetric auxin distribution can occur. As a result, stronger growth on one side will lead to bending of the plant. It was known that the cell polarity of PIN localization depends on cell type and developmental stage of the tissue and therefore allows directed auxin flow. PIN1 and PIN7 have different polarized localizations in pro-embryo and adult plant (Friml 2003). For PIN3 it was shown that gravistimulation has an influence on the localization of the efflux carrier. PIN3 relocation in columella cells always appears at the new physiological bottom (Friml et al. 2002). An accumulation of PIN3 at the new physiological bottom would favor auxin transport along the new lower side of the root and lead to root bending.

PIN proteins are localized via vesicular transport. An important component of these vesicular transport systems are ENDOSOMAL SORTING COMPLEXES REQUIRED FOR TRANSPORT (ESCRT) that are formed by various VESICULAR SORTING PROTEINS (VSPs). Mutations in the regulatory system of ESCRT lead to different localizations of PIN1, PIN2 and AUX1. A double mutant of CHARGED MULTIVESICULAR BODY PROTEIN/CHROMATIN MODIFYING PROTEIN1A (CHMP1A) and CHMP1B leads to accumulation of AUX1, PIN1 and PIN2 in late endosomes and only marginal localization at the plasma membrane (Spitzer et al. 2009).

6.5 Microgravity Research and Modifying Gravitational Acceleration Changed Our Perspective on Gravitropism

Centrifugation has been widely used to alter the amount and the direction of mass acceleration that acts on the sedimentable masses in gravity sensing systems. In a pioneering experiment, Sir Thomas Andrew Knight used a water-driven horizontal wheel to show that plant roots neither grow in the direction of gravity nor in the direction of the centrifugal acceleration but grow in the direction of the resulting acceleration angle, thus, providing evidence that plants sense the direction of accelerations not gravity *per se* (Knight 1806). Centrifugation intensifies weak gravity responses (improved graviorientation in starchless *Arabidopsis* mutants), renders intracellular processes more clearly visible and was used to characterize gravisensitive membranes by moving statoliths (cf. Chap. 4). Very high acceleration

forces were even used to reversibly abolish graviresponsiveness by disturbing the polar cytoplasmic organization of statocytes (Wendt and Sievers 1986).

Since Julius Sachs made his first attempts to neutralize the unilateral effect of gravity by rotating plants around a horizontal axis (Sachs 1882), numerous types of clinostats have been developed in order to simulate the effects of weightlessness on Earth (cf. Chap. 2). Classical clinostats rotating with 1–10 rpm, fast-rotating clinostats rotating with 50–120 rpm as well as three-dimensional clinostats and random positioning machines rotating around one axis or two axes have shed light on the gravisensitivity, perception and presentation time of gravitropism in roots and shoots.

A new quality of research tools for studies on gravitropism became available with the advent of drop towers, parabolic flights of aircrafts and rockets, NASAs Space Shuttles, satellites and Space Stations in the low Earth orbit (cf. Chap. 2). With the completion of the International Space Station ISS with the commissioning of the Columbus module in 2008, the biggest microgravity lab that ever existed provides an almost stimulus-free environment of real microgravity for long-term experimentation. The microgravity quality in a range of 10^{-3} – 10^{-6} g is beyond the susceptibility of most biological sensory systems and is, therefore, regarded as functional weightlessness allowing biologists to address especially molecular and cellular mechanisms involved in plant gravity sensing and graviorientation. Only after cress seeds had been germinated on the Space Shuttle (Volkman et al. 1986) and on Russian Bion satellites (Laurinavicius et al. 1996), it became evident that the development of polarly organized statocytes and gravisensing mechanisms are neither induced nor affected by the absence of gravity. And although a reduced starch content was reported, microgravity-grown roots responded more strongly to only small acceleration doses in microgravity aboard Space Shuttles (Volkman and Tewinkel 1996; Perbal et al. 2004). The presentation time, the time a stimulus needs to be applied continuously to a sensing system in order to trigger a response, was found to be in the range of 20–30 s when stimulated with normal 1 g acceleration, whereas the presentation time of cress and lentil roots grown on a 1 g centrifuges was in a range of 50–60 s (Perbal and Driss-Ecole 1994; Volkman and Tewinkel 1996). By rotating roots on a clinostat and stopping it several times, a perception time of 1 s was determined for cress roots. The perception time defines the minimum time a stimulus is registered by sensing systems but must be given repeatedly in order to trigger a response (Heinowicz et al. 1998). In this short period, statoliths in gravistimulated roots are displaced only a fraction of a μm , which is good evidence that—taken into account that actin is not required for graviperception as was demonstrated by the uninhibited graviresponse of roots with disrupted actin microfilaments (Hou et al. 2003)—graviperception must occur very close to statoliths already sedimented on or in close contact with a gravisensitive endoplasmic reticulum membrane.

Clinorotating seedling has been used very often to successfully prevent static gravitropic stimulation of roots by randomizing the gravity vector and, consequently, roots continued to grow straight. However, clinorotation failed in most cases to eliminate also dynamic stimulation. There are studies reporting an overloading of the sensory system due to vibrations and shifting statoliths

continuously, which could even result in the complete disintegration of the polar structural organization of the statocytes after hours of clinorotation (Hensel and Sievers 1980; Hoson et al. 1997). Therefore, research focusing on specific molecular and cellular components of gravity sensing mechanisms and on the role of second messengers like cytoplasmic free calcium, InsP3, and pH in gravity signaling pathways are best performed under real-microgravity conditions in space. Recently, experiments on the ISS have provided evidence that the establishment of the auxin-gradient system, the prerequisite for the curvature response, is gravity independent. The cytokinin distribution, however, was different in space-grown and control roots suggesting that cytokinin-associated process involved in gravitropism might be affected (Ferl and Paul 2016).

Further research on the ISS and other microgravity platforms is currently planned to increase our knowledge on the hormone-associated components of gravitropic responses. In particular future research on mutants in the stimulus-free microgravity environment promises to further contribute to the unravelling of the fascinating processes of gravity sensing and gravitropic orientation in higher plants.

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