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Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing

M. Braun* and **C. Limbach**

Gravitationsbiologie, Institut für Molekulare Physiologie und Biotechnologie der Pflanzen, Universität Bonn, Bonn

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Summary. Gravitropically tip-growing rhizoids and protonemata of characean algae are well-established unicellular plant model systems for research on gravitropism. In recent years, considerable progress has been made in the understanding of the cellular and molecular mechanisms underlying gravity sensing and gravity-oriented growth. While in higher-plant statocytes the role of cytoskeletal elements, especially the actin cytoskeleton, in the mechanisms of gravity sensing is still enigmatic, there is clear evidence that in the characean cells actin is intimately involved in polarized growth, gravity sensing, and the gravitropic response mechanisms. The multiple functions of actin are orchestrated by a variety of actin-binding proteins which control actin polymerisation, regulate the dynamic remodelling of the actin filament architecture, and mediate the transport of vesicles and organelles. Actin and a steep gradient of cytoplasmic free calcium are crucial components of a feedback mechanism that controls polarized growth. Experiments performed in microgravity provided evidence that actomyosin is a key player for gravity sensing: it coordinates the position of statoliths and, upon a change in the cell's orientation, directs sedimenting statoliths to specific areas of the plasma membrane, where contact with membrane-bound gravisensor molecules elicits short gravitropic pathways. In rhizoids, gravitropic signalling leads to a local reduction of cytoplasmic free calcium and results in differential growth of the opposite subapical cell flanks. The negative gravitropic response of protonemata involves actin-dependent relocation of the calcium gradient and displacement of the centre of maximal growth towards the upper flank. On the basis of the results obtained from the gravitropic model cells, a similar fine-tuning function of the actomyosin system is discussed for the early steps of gravity sensing in higher-plant statocytes.

Keywords: Actin; Actin-binding protein; *Chara* spp.; Gravity sensing; Protonema; Rhizoid; Tip growth.

Abbreviations: ADF actin-depolymerizing factor; ER endoplasmic reticulum.

* Correspondence and reprints: Gravitationsbiologie, Institut für Molekulare Physiologie und Biotechnologie der Pflanzen, Universität Bonn, Kirschallee 1, 53115 Bonn, Federal Republic of Germany.
E-mail: mbraun@uni-bonn.de

Introduction

Gravity is one of the most important environmental stimuli plants use to adapt to in order to cope with their environment in a most beneficial way and to optimize exploitation of resources. The early processes of gravity sensing in higher plants have been attributed to specialised cells, so-called statocytes, which, in contrast to all other cell types, contain sedimentable starch-filled amyloplasts that function as statoliths. Whereas our knowledge on hormone-dependent gravitropic response mechanisms is rapidly increasing (Blancaflor 2002, Ottenschläger et al. 2003, Aloni et al. 2004, Blilou et al. 2005), the cellular and molecular basis of the decisive early phases of gravity sensing is unknown. So far, there are only controversially discussed hypotheses trying to explain how the vectorial information of a physical displacement of statoliths is perceived by cellular components which elicit a gravitropic signalling pathway. Several studies have proposed that elements of the cytoskeleton, highly dynamic filamentous networks of microtubules and especially actin microfilaments, might be involved in gravity sensing, but their role in the gravitropic signalling pathways is unclear, since findings are contradictory (Sievers et al. 1991a, 2002; Kiss 2000; Blancaflor 2002; Hou et al. 2004).

The green algae *Chara* spp. provide two well established model cell types, rhizoids and protonemata, which are increasingly used to study specific aspects of gravitropism (Sievers et al. 1996, Braun 1997, Braun and Wasteneys 2000). The tubelike cells with diameters of up to 30 μm are more easily accessible for experimental approaches than gravity-sensing cells in higher plants. The complete gravitropic signal transduction and response pathways are appar-

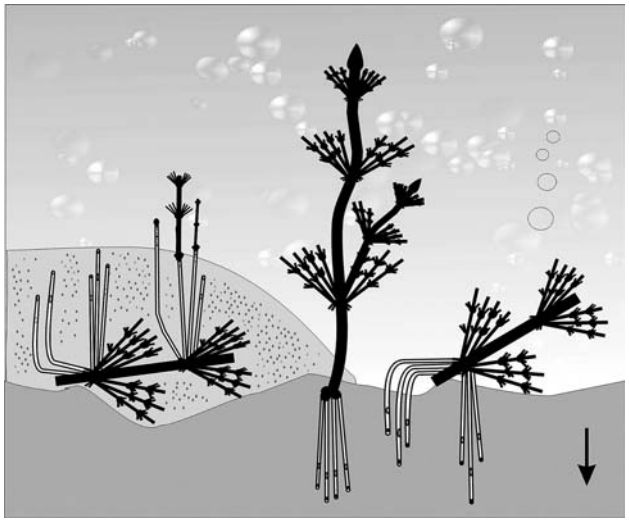

Fig. 1. Rhizoids and protonemata of characean algae are tubelike cells which originate from nodal cells of the green thallus. Rhizoids grow in the direction of gravity (positive gravitropism) to anchor the thallus in the sediment. Protonemata are produced in the absence of blue light when the thallus was accidentally buried and grow upward against the direction of gravity (negative gravitropism) back into the light, where they terminate tip growth, divide, and regenerate the complex thallus. The arrow denotes the direction of gravity

ently short and limited to the apical region of a single cell. Positively gravitropic (downward growing) rhizoids have a rootlike function and anchor the algal thallus in the sediment (Fig. 1). From the morphological point of view, protonemata are very similar cells; however, they respond negatively gravitropic (upward growing). They are produced by nodal cells in the absence of light, e.g., when the thallus was accidentally buried in the sediment (Fig. 1). As soon as protonemata have penetrated the substrate and reach the light, tip growth is arrested and a complex series of cell divisions is initiated that leads to the regeneration of the green thallus (Braun and Wasteneys 1998a).

The role of cytoskeletal elements in both characean cell types has been investigated in great detail. Microtubules maintain the prominent polar cytoplasmic zonation and the subapical organelle distribution, but they are not present in the apex and are not involved in the primary steps of gravitropic signalling (Braun and Sievers 1994, Braun and Wasteneys 1998b). The actin cytoskeleton, however, plays an essential role in the mechanisms of gravity sensing and gravity-oriented polarized growth (see Figs. 2 and 3). The multiple functions of the actin microfilament system are controlled by numerous actin-binding proteins (Braun et al. 2004). By interacting with myosins, actin microfilaments regulate the positioning of the $BaSO_4$-crystal-filled vesicles which serve as statoliths (Hejnowicz and Sievers 1981; Sievers et al. 1991b; Braun and Sievers 1993; Buchen et al. 1993; Braun 1996a, 2002; Cai et al. 1997). Upon gravistimulation, the actomyosin system directs sedimenting statoliths to the gravisensitive region of the plasma membrane, where the gravitropic signalling cascade is elicited that results in the reorientation of the growth direction (see Fig. 4) (Hodick 1994, Sievers et al. 1996, Braun 2002).

In this review, we summarize the results that have been collected over the last decades providing breakthroughs in the understanding of the processes of gravity susception and gravity perception as well as of the mechanisms that lead to the gravitropic response in the form of the reorientation of the growth direction in characean rhizoids and protonemata. We discuss the role of actomyosin forces, actin-binding proteins and calcium as major key players in gravitropic signalling pathways.

Cytoskeletal basis for gravity-oriented polarized growth

"Spitzenkörper", "apical body", or "clear zone" designate a vesicle-rich region in the apex of tip-growing cell types such as pollen tubes, root hairs, fungal hyphae, moss chloronemata and caulonemata, protonemata of ferns and algae, and rhizoids of mosses, ferns, and algae. The tip region is characterized by an accumulation of secretory vesicles and the exclusion of other organelles like dictyosomes, mitochondria, and in most cases also ER (endoplasmic reticulum) cisternae (Geitmann and Emons 2000, Hepler et al. 2001, Lovy-Wheeler et al. 2005). F-actin (filamentous actin) has not been labelled or seems to be scarce in the extreme tip (Geitmann and Emons 2000). In contrast to these actin patterns, in characean rhizoids and protonemata extensive arrays of distinct actin microfilaments penetrate all cytoplasmic regions including the extreme apex which accommodates the tip growth machinery (Braun and Wasteneys 1998b). The actin cytoarchitecture is similar in both cells and reflects their polar cytoplasmic organization. Fine actin bundles focus in a unique spherical actin area in the center of the Spitzenkörper (Braun and Wasteneys 1998b). This area contains a dense aggregate of ER membranes (Bartnik and Sievers 1988) and is surrounded by an accumulation of secretory vesicles which contain cell wall material (Fig. 2). The position of the Spitzenkörper defines the center of growth, the plasma membrane area where incorporation of vesicles is maximal (Hejnowicz et al. 1977, Sievers et al. 1979, Braun 1996b). Myosins mediate the transport of secretory vesicles along actin microfilaments towards the tip (Fig. 2C), where they accumulate, perform shuttlelike movements,

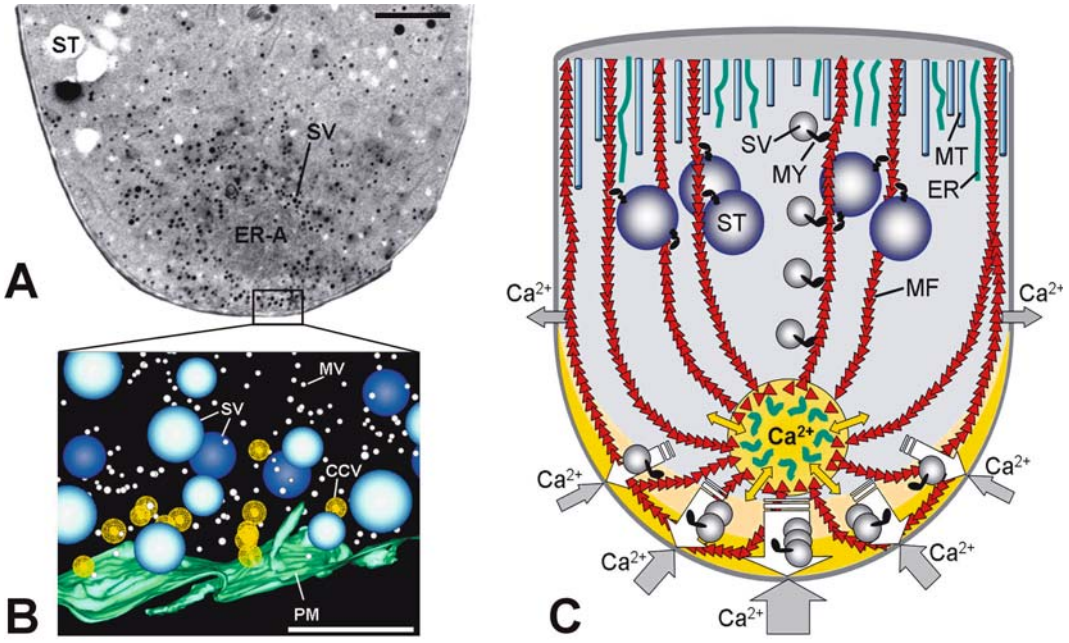

Fig. 2. **A** Ultrathin-section electron micrograph of the apical region of a high-pressure frozen and freeze-substituted characean rhizoid. A dense aggregation of ER cisternae (*ER-A*) in the center of the growth-organizing Spitzenkörper is surrounded by an accumulation of secretory vesicles (*SV*), which are destined for incorporating cell wall material in the apical plasma membrane. *ST* Statolith. Bar: 5 μm. **B** Three-dimensional tomographic model of the outermost apical region of a high-pressure frozen and freeze-substituted characean rhizoid (position of the modelled region is indicated by the box in **A**). Two different types of secretory vesicles (*SV*), modelled in blue and in light blue, and microvesicles (*MV*) are evenly distributed in the apical cytoplasm. Clathrin-coated vesicles (*CCV*) are confined to an approximately 500 nm broad region along the apical plasma membrane (*PM*). The apical plasma membrane exhibits tubelike intrusions into the cytoplasm as well as extrusions into the cell wall. Bar: 500 nm. **C** Schematic illustration of the apical region of a characean rhizoid displaying the polar distribution of organelles. Actin microfilaments (*MF*) originate from the center of the Spitzenkörper (yellow circle) with opposite polarities providing tracks for the myosin (*MY*)-driven acropetal transport of secretory vesicles (*SV*) which accumulate in the tip and incorporate cell wall material along the steep tip-high gradient of cytoplasmic free Ca^{2+} (yellow semilunar area). In normal vertical orientation, statoliths (*ST*) are kept in a dynamically stable position at a certain distance from the tip by myosins which interact with actin microfilaments to compensate the apically directed gravity force. In addition to actin, the center of the Spitzenkörper also contains a dense accumulation of ER membranes that might function as a storage compartment for calcium and might help to regulate the steepness of the calcium gradient. White arrows indicate the exocytosis rate, grey arrows indicate calcium fluxes at the apical plasma membrane. *MT* Microtubule; *ER* endoplasmic reticulum

and eventually incorporate into the plasma membrane, releasing new cell wall material (Braun 1996a).

Recently, the distribution of vesicles in the apical region was analyzed in high-pressure frozen and freeze-substituted rhizoids (to be published elsewhere). It is generally accepted that high-pressure freeze fixation yields much better results than chemical fixation with respect to ultrastructural preservation. By using the innovative technique of dual-axis electron tomography (Mastronarde 1997, Ladinsky et al. 1999) for high-resolution ultrastructural analysis, two different types of secretory vesicles as well as microvesicles were found to be evenly distributed in the apical region of rhizoids. In contrast, clathrin-coated vesicles were exclusively located in close vicinity of the apical plasma membrane (Fig. 2B). These vesicles are supposedly involved in endocytotic processes which mediate recycling of excessive membrane material and turnover of membrane-bound proteins like ion channels. When the concerted action of exocytotic and endocytotic processes was disturbed by inhibitor-induced disruption of the actin cytoskeleton, the tip-focussed distribution pattern of calcium channels and the steep, tip-high gradient of cytoplasmic free calcium dissipated and tip growth stopped (Braun and Richter 1999). The calcium gradient dictates the incorporation pattern of secretory vesicles and also spatiotemporally controls the activity of actin-binding proteins. These signalling mechanisms, which include actin and calcium, contribute to a complex feedback regulation pathway that controls tip growth, a special type of polarized growth (Fig. 3).

The multiple functions and the dynamic nature of the actin cytoskeleton in rhizoids and protonemata are coordinated by the concerted action of numerous actin-binding proteins. The actin-binding proteins identified in rhizoids and protonemata so far are mostly identical to those that have been shown to regulate cytoarchitecture and function of the actin microfilament system in other tip-growing cell

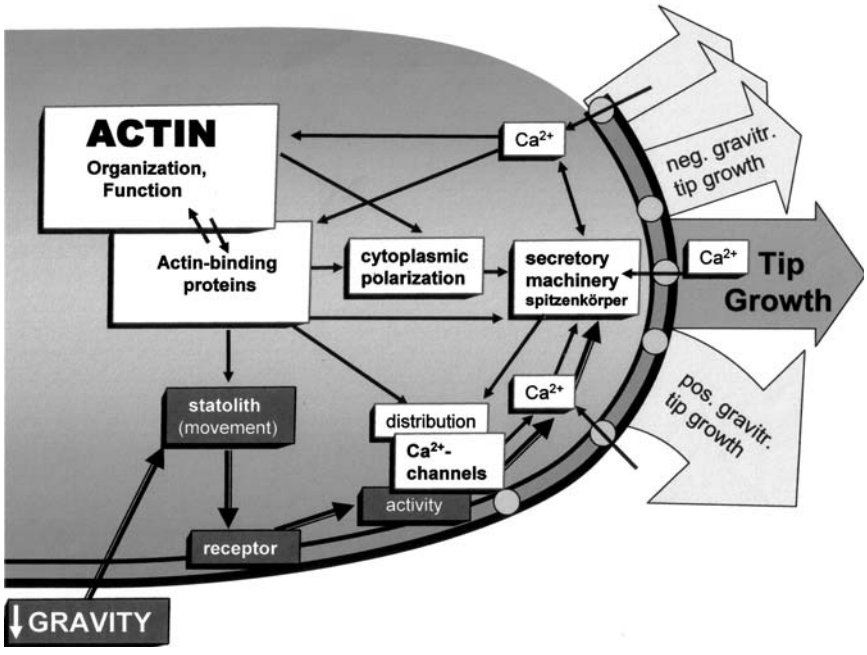

Fig. 3. Working model for the feedback mechanisms of gravitropic tip growth. Actin is a central key player that interacts with numerous actin-binding proteins to orchestrate actin polymerization and dynamic remodelling as well as to fulfil the multiple functions such as cytoplasmic streaming, interaction with microtubules, delivery of vesicles, spatial control of exocytosis, and regulation of statolith positioning and transport. Gravity modulates statolith positioning and can redirect statolith movements. Any deviation of the cell axis from the normal vertical orientation results in a lateral displacement of statoliths. Actomyosin forces are essentially involved in the gravisensing process by guiding sedimenting statoliths to specific gravisensitive plasma membrane areas where gravisensor molecules (receptors) transform the physical stimulus of statolith sedimentation into a physiological signal. This signal initiates a cascade of events that likely involve a local change of calcium channel activity. In rhizoids, a local decrease in the concentration of cytoplasmic free calcium at the lower cell flank leads to differential growth of the opposite subapical cell flanks in the form of a smooth bowing downward. In protonemata, a stepwise readjustment of the Spitzenkörper and the center of growth results in the reorientation of the cell tip upward

types (Geitmann and Emons 2000, Hepler et al. 2001). However, the cytoskeletal arrangement in characean rhizoids and protonemata is unique and seems to be closely linked to the mechanisms of gravity sensing and gravity-oriented growth. Spectrin-like epitopes, actin-depolymerizing factor (ADF), and profilin specifically accumulate in the center of the Spitzenkörper (Braun et al. 2004). Spectrin-like proteins most likely participate in the structural integrity of the ER aggregate by forming cross-links between ER membranes and actin microfilaments (Braun 2001). Furthermore, spectrins are known to provide a mechanism for recruiting specific subsets of membrane proteins and to form functional microdomains in animal cells, and thus, they might help to create the particular physiological conditions for the mechanisms of gravity sensing and polarized growth (Braun 2001, and references therein). Molecular studies failed to identify spectrins in *Chara* sp. (our unpubl. results) and spectrins have not been identified in the Arabidopsis genome (for a review, see Drobak et al. 2004). However, immunolocalization of spectrin-like epitopes in *Chara globularis* and immunocytochemical analyses implicate the existence of an actin-binding protein that at least shares similar functional domains with spectrins and has a molecular mass almost identical with theirs (Braun 2001).

The accumulation of the actin-binding proteins ADF and profilin in the center of the Spitzenkörper indicates high actin turnover rates and an actin-polymerizing function of this central area (Braun et al. 2004). Strong evidence comes from cytochalasin D-induced disruption of the actin cytoskeleton, which causes a complete dissociation of the center of the Spitzenkörper. Immunolocalization of actin, ADF, profilin, and the ER aggregate dissipate and tip growth terminates. Removal of the inhibitor is followed by the reorganization of the actin cytoskeleton that starts with the reappearance of a dense actin array in the outermost tip (Braun et al. 2004). As soon as actin microfilaments radiate out, the actin array rounds up and is repositioned in the center of the apical dome. This process is accompanied by the reaccumulation of ER membranes and the reappearance of ADF and profilin and is followed by the resumption of tip growth

(Braun et al. 2004). The results suggest that the center of the Spitzenkörper functions as an amazingly localized apical actin polymerization site that has not been found in any other tip-growing cell type. It is tempting to speculate that the complexly coordinated, highly dynamic actin architecture in the rapidly extending tip is functionally related to the fundamental role of the actomyosin system in the different phases of the gravitropic signalling pathways and the gravity-oriented polarized growth.

The actin-crosslinking protein fimbrin has been localized in the apical and subapical region, suggesting that this actin-binding protein is involved in the formation of the mainly axially oriented, dense actin meshwork (Braun et al. 2004). Immunolocalization of the actin-bundling protein villin is restricted to the basal zone, where actin microfilaments are separated according to their polarities and two populations of thick actin cables are formed, generating the rotational cytoplasmic streaming around the large central vacuole (Braun et al. 2004).

Gravity-sensing apparatus: balance of actomyosin forces and gravity keeps statoliths in dynamically stable position

In tip-downward growing rhizoids, the statoliths are actively kept at a distance of 10–35 μm basal to the tip. By exerting net-basipetal forces the actomyosin system prevents statoliths from settling into the tip. In tip-upward growing protonemata, actomyosin prevents statoliths from sedimenting towards the cell base by acting net-acropetally (Hodick et al. 1998, Braun et al. 2002). Inhibitor studies have shown that disrupting the actin cytoskeleton in rhizoids and in protonemata not only stopped tip growth but also caused statoliths to fall into the tip or towards the nucleus, respectively, following the direction of gravity (Hejnowicz and Sievers 1981, Bartnik and Sievers 1988, Sievers et al. 1996). After removal of the drug, statoliths were readily repositioned and tip growth continued.

Microgravity and simulated weightlessness represent suitable conditions for unravelling the role of gravity and the role of actomyosin-based forces in the complex regulation of statolith positioning (Buchen et al. 1993, 1997; Cai et al. 1997; Hoson et al. 1997; Braun et al. 2002). When the influence of gravity was abolished during the microgravity phases of parabolic flights of Texus (Technologische Experimente unter Schwerelosigkeit) sounding rockets (Buchen et al. 1993) and during rotation on the three-dimensional and the fast-rotating clinostat (Hoson et al. 1997, Braun et al. 2002), actomyosin forces generated a displacement of statoliths against the former direction of gravity. This observation justified the conclusion that in normal, vertically oriented rhizoids and protonemata, the statoliths are kept in a dynamic equilibrium position by actomyosin forces which exactly compensate the effect of gravity on the statoliths (see Fig. 4). Interestingly, during long-term microgravity conditions of space shuttle missions IML-2 (Second International Microgravity Laboratory) and S/MM05 (Fifth Shuttle-to-Mir Mission), the absence of gravity did not result in a random distribution of statoliths in rhizoids. Instead, after an initial basipetal transport at the beginning of microgravity, the statoliths spread over the entire statolith region but never left this cell area (Braun et al. 2002).

Detailed analysis of the movements of statoliths in microgravity and of statoliths which were displaced in the different cell regions by optical laser tweezers (Braun 2002) or by centrifugation revealed the surprising complexity of the transport system by which actomyosin forces control statolith positioning (Braun et al. 2002). Individual acropetal and basipetal movements of statoliths were observed in both cell types, indicating that statoliths interact with the mainly axially oriented actin microfilaments with opposite polarities. When statoliths were centrifuged into the subapical region, a statolith transport back to the original position was observed that is not notably influenced by gravity (Sievers et al. 1991b, Braun and Sievers 1993). Active transport occurs along actin microfilaments and statoliths do not sediment onto the lower cell flank until they have reached the statolith region near the tip, where statolith sedimentation is not constrained by microtubules (Braun and Sievers 1994).

Taken together, our data reveal that in the cell regions basal and apical from the statolith region, that actomyosin component is always the strongest which points towards the statolith region. This ensures that statoliths are always kept in or are retransported to their original position (see Fig. 4). In the statolith region, however, gravity plays a critical role as an additional passive transport component that contributes to the positioning of statoliths. During vertical growth, both forces that act on statoliths, i.e., gravity and active actomyosin transport components, are precisely balanced so that the statoliths are kept in a dynamically stable position without any net-transport (Braun et al. 2002). Changes in the orientation of the cell with respect to the direction of gravity or changing the amount of the acceleration must inevitably result in a displacement of statoliths.

There are indications from experiments in microgravity that statoliths interact with actin also in higher-plant stato-

cytes. Statoliths move in the direction against the originally acting gravity force (Volkmann et al. 1991) until they reach a new nonrandom steady-state position (Driss-Ecole et al. 2000). Their sedimentation has also been reported to be modulated by actomyosin forces (Perbal et al. 2004). However, consistent evidence for the role of actin in graviperception is still missing.

Gravisusception: actomyosin-guided sedimentation of statoliths upon changes in cell's orientation relative to direction of gravity

The actomyosin forces described above, acting oppositely on statoliths in rhizoids and protonemata, have important implications on how fast and to where the statoliths sediment, thus, indicating the critical role of actin in the process of gravisensing (Fig. 4). Upon a change in the orientation of the cell with respect to the gravity vector, sedimenting statoliths are directed to distinct graviperception sites which are the only regions of the plasma membrane where gravitropic signalling can be triggered. The graviperception site is confined to a narrow beltlike region 10–35 μm behind the tip in rhizoids and to the plasma membrane of the apical dome (5–10 μm behind the tip) in protonemata. Forcing statoliths to sediment outside these areas by optical laser tweezers or centrifugation did not result in a gravitropic response (Braun 2002).

Microgravity experiments (Buchen et al. 1997) and optical laser tweezer experiments (Leitz et al. 1995) have shown that, in lateral direction, the statolith position is only weakly controlled by the actomyosin system in both cell types; the force needed to move statoliths towards the apex is greater than the force to move the statoliths towards the flank. Recently, the forces acting on statoliths in lateral direction were characterized in detail by microgravity experiments during two Maxus (enlarged version of Texus) sounding-rocket flights. It was demonstrated

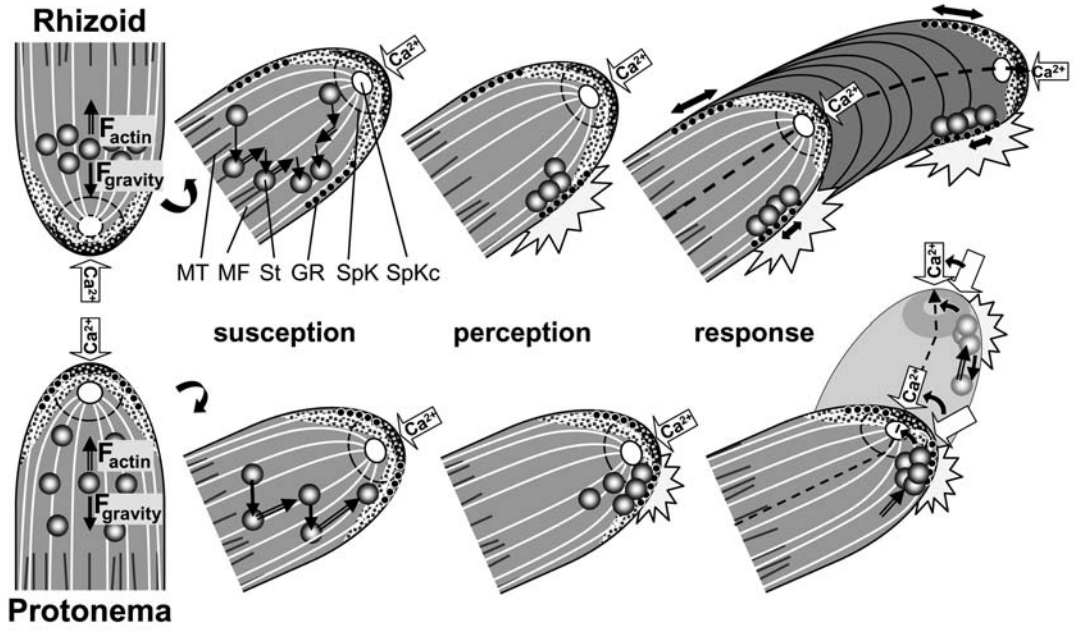

Fig. 4. Illustration of the gravity-sensing mechanisms in characean rhizoids and protonemata. In tip-downward growing rhizoids (upper row), the statolith (*St*) position results from net-basipetally acting actomyosin forces (F_{actin}) compensating gravity ($F_{gravity}$). Upon reorientation, statoliths sediment onto the lower cell flank. Net-acropetally acting actomyosin forces in the basal part of the statolith regions and in the subapical region prevent statoliths from leaving the apical region and transport the sedimenting statoliths onto membrane-bound gravireceptors (*GR*) which are restricted to a narrow, beltlike area of the plasma membrane 10–35 μm from the tip. The Spitzenkörper (*Spk*) remains arrested at the tip and the calcium gradient (indicated by darker and lighter grey dotted area) is always highest at the tip. Statolith sedimentation causes a local reduction of cytosolic Ca^{2+} that results in differential extension of the opposite cell flanks (double-headed arrows). In upward growing protonemata (lower row), the effect of gravity on statoliths is compensated by net-acropetally acting forces mediated by the actin microfilaments. Upon horizontal positioning, statoliths settle onto the gravireceptors which are located near the growth center at the tip by gravity-induced and acropetally directed, actomyosin-based movements. This causes a drastic shift of the calcium gradient and then of the Spitzenkörper towards the upper flank and the new outgrowth occurs at that site. Until the protonema reaches the upright position, statoliths frequently fall out of the apical dome following the gravity vector and are transported back onto the graviperception site by actomyosin forces. The white arrows point to the area of maximal calcium influx. *MF* Actin microfilament; *MT* microtubule; *SpKc* center of the Spitzenkörper

that in vertically downward growing rhizoids, lateral acceleration forces in a range of 0.1 **g** were sufficient to displace statoliths towards the membrane-bound gravireceptors. In conclusion, the molecular forces acting on a single statolith in lateral direction were determined to be in a range of 2×10^{-14} N (Limbach et al. 2005). When rhizoids are reoriented by 90°, the sedimenting statoliths mainly follow the gravity vector and settle onto the lower cell flank of the statolith region, where graviperception takes place and the graviresponse is initiated. However, when cells were rotated in angles different from 90°, statoliths did not simply follow the gravity vector (Fig. 4) (Hodick et al. 1998). Instead, even in inverted cells, statoliths were actively redirected against gravity and were guided to the confined graviperception site in the statolith region.

Gravistimulation of protonemata causes an actin-mediated acropetal displacement of sedimenting statoliths into the apical dome, where they sediment onto the gravisensitive plasma membrane area close to the tip (Fig. 4) (Hodick et al. 1998). During the upward bending of protonemata, the statoliths periodically sediment along the gravity vector and leave the graviperception site, which deactivates the gravireceptor and is reflected by phases of straight growth. Actomyosin-mediated transport of statoliths back to the gravisensitive membrane area reinitiates gravitropic bending until the vertical orientation is resumed (Fig. 4).

Gravity perception: activation of membrane-bound gravireceptors

It was shown that statoliths have to be fully sedimented on yet unknown membrane-bound gravireceptors in order to trigger graviperception and to induce the gravitropic signalling cascade (Braun 2002). Lateral movements of statoliths that do not lead to a contact with the plasma membrane do not induce a curvature response. Most recently, experiments have been performed during parabolic flights aboard of the A300 Zero-G aircraft to elucidate the mode of gravireceptor activation in characean rhizoids (Limbach et al. 2005). Statoliths which were weightless but still in contact with the plasma membrane were able to activate the membrane-bound gravireceptor. In conclusion, it could be ruled out that the pressure exerted by the weight of statoliths is required for gravireceptor activation. This finding was supported by control experiments on ground which demonstrated that increasing the weight of sedimented statoliths by lateral centrifugation did not enhance the gravitropic response. However, graviperception was terminated within seconds when the contact of statoliths with the plasma membrane was interrupted by inverting gravistimulated cells. These results provide evidence that graviperception in characean rhizoids relies on direct contact allowing yet unknown components on the statoliths' surface to interact with membrane-bound receptors rather than on pressure or tension exerted by the weight of statoliths (Limbach et al. 2005). This parabolic-flight experiment unambiguously characterized the mode of gravireceptor activation. The nature of the receptor and the downstream physiological steps of graviperception, however, remain to be clarified.

Gravity responses: calcium-regulated processes reset nominal growth direction

The smooth downward curvature response of a rhizoid is best described as "bending by bowing", whereas the response of a protonema was described as "bending by bulging" (Braun 1996b) referring to the bulge that appears on the upper cell flank indicating the drastic upward shift of cell growth. The Spitzenkörper and, in consequence, also the center of maximal growth is displaced upon gravistimulation of protonemata by intruding statoliths (Fig. 4). Rhizoids can be forced to respond to some extent like protonemata, but only by pushing statoliths asymmetrically into the apical dome with optical tweezers or by centrifugal forces of >50 **g** (Braun 2002). There is evidence from centrifugation experiments (Braun 1996b, Hodick and Sievers 1998) and from attaching particles to the surface of gravitropically responding rhizoids (Sievers et al. 1979) that the position of the growth center at the cell tip is relatively stable and that in rhizoids the Spitzenkörper is anchored by cytoskeletal forces more tightly than it is in protonemata.

The idea that the specific properties of the actin cytoskeleton which are responsible for Spitzenkörper anchorage are depending on calcium is strongly supported by calcium imaging, demonstrating a drastic shift of the steep tip-high calcium gradient towards the upper flank during initiation of the graviresponse in protonemata, but not in rhizoids (Braun and Richter 1999). In accordance with this observation, dihydropyridine fluorescence indicating the tip-focussed distribution of putative calcium channels was also found to be displaced towards the upper flank in graviresponding protonemata (Braun and Richter 1999), which was also not found in rhizoids. The results suggest that the early asymmetric distribution of the calcium gradient in protonemata either results from statolith-induced repositioning of calcium channels or, more

likely, might be caused by differential activation and/or inhibition of apical calcium channels. This leads to an asymmetric influx of calcium and, thus, alters the exocytosis pattern and causes an asymmetric incorporation of calcium channels which then establishes the new polarity and the new growth direction. The asymmetric influx of calcium could also mediate the repositioning of the Spitzenkörper and the growth center by differentially regulating the actin anchorage or the activity of actin-associated proteins along the shifting calcium gradient (Braun and Richter 1999).

Support for the proposed gravitropic response mechanisms in protonemata comes from immunofluorescence labelling of spectrin-like proteins in the actin-rich area which contains the ER aggregate in the center of the Spitzenkörper. The labelling, which localizes to the median cell axis during vertical growth, is drastically displaced towards the upper flank, the site of future outgrowth, during initiation of the graviresponse in protonemata, clearly before curvature is recognisable (Braun 2001). In contrast, the same labelling in rhizoids remains symmetrically positioned in the apical dome throughout the graviresponse. The findings confirm that a repositioning of the Spitzenkörper is involved in the negative graviresponse of protonemata but not in the positive graviresponse of rhizoids (Fig. 4) (Braun 2001). The tendency of protonemata to reorient towards the former growth axis after only short gravistimulation indicates that the new growth axis induced by the upward shift of the Ca^{2+} gradient is rather labile and may require actin cytoskeletal anchorage to stabilize the new growth direction (Braun and Richter 1999, Braun 2001).

Recently, calcium imaging indicated that the impact of statolith sedimentation in rhizoids seems to be limited to a local decrease in the concentration of cytosolic Ca^{2+} at the lower subapical cell flank (S. Gilroy, Penn State University, U.S.A., pers. commun.) which most likely results from the local inhibition of calcium channels in the area of statolith sedimentation. The subsequent reduction of the rate of exocytosis of secretory vesicles causes differential growth of the opposite cell flanks, resulting in the positively gravitropic curvature (Fig. 4) (Sievers et al. 1979).

Although calcium is likely to play a role also in gravity sensing of higher plants, several studies failed to show gravity-induced changes in cytosolic Ca^{2+} in higher-plant statocytes (Legué et al. 1997). This result may be due to the limited accessibility of the cells and/or the techniques that are unable to show very small or highly localized changes (Boonsirichai et al. 2002).

Conclusions and outlook

The progress that has been made in the unravelling of gravitropic signalling pathways underlines the significance of single-cell model systems for our understanding of how plants use gravity as a guide for orientation. Recent experiments including those that have been performed on centrifuges, on clinostats, and in particular those that have been performed in the almost stimulus-free environment of microgravity greatly improved our knowledge on the role of the actin cytoskeleton and its associated proteins in the early processes of gravity susception and gravity perception in plant gravity-sensing cells.

The rapidly increasing number of actin-binding proteins that are identified and localized in characean rhizoids and protonemata illuminates the complexity of how the actin cytoskeleton is organized and dynamically remodelled in order to execute the diversity of functions in the processes of polarized growth, statolith positioning, gravity sensing, and the gravitropic response in a specialized single cell.

Taking into consideration the results obtained from the unicellular model systems and the indications for actin–statolith interactions in higher-plant statocytes from microgravity experiments, it is tempting to speculate that actin might play similar roles in the early processes of gravity sensing in higher plants. Since disrupting the actin cytoskeleton in statocytes does not prevent gravity sensing, actin may not be essential for the sensoric process per se, but actin may have a fine-tuning function by acting as a guiding system for sedimenting statoliths. Thereby, actin could ensure an adequate, most beneficial graviresponse by avoiding unfavorable and inappropriate responses to transient changes in the orientation of the organ with respect to the gravity vector. Work is in progress to elucidate actin–statolith interactions and the mode of gravireceptor activation also in higher-plant statocytes which would greatly enhance our knowledge of the early decisive phases of gravity sensing in higher plants.

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