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Actomyosin-Mediated Statolith Positioning in Gravisensing Plant Cells Studied in Microgravity

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Abstract

The positioning and gravity-induced sedimentation of statoliths is crucial for gravisensing in most higher and lower plants. In positively gravitropic rhizoids and, for the first time, in negatively gravitropic protonemata of characean green algae, statolith positioning by actomyosin forces was investigated in microgravity ($<10^{-4}$ *g*) during parabolic flights of rockets (TEXUS/MAXUS) and during the Space-Shuttle flight STS 65. In both cell types, the natural position of statoliths is the result of actomyosin forces which compensate the statoliths' weight in this position. When this balance of forces was disturbed in microgravity or on the fast-rotating clinostat (FRC), a basipetal displacement of the statoliths was observed in rhizoids. After several hours in microgravity, the statoliths were loosely arranged over an area whose apical border was in the same range as in 1 *g*, whereas the basal border had increased its distance from the tip. In protonemata, the actomyosin forces act net-acropetally. Thus, statoliths were transported towards the tip when protonemata were exposed to microgravity or rotated on the FRC. In preinverted protonemata, statoliths were transported away from the tip to a dynamically stable resting position. Experiments in microgravity and on the FRC gave similar results and allowed us to distinguish between active and passive forces acting on statoliths. The results indicate that actomyosin forces act differently on statoliths in the different regions of both cell types in order to keep the statoliths in a position where they function as susceptors and initiate gravitropic reorientation, even in cells that had never experienced gravity during their growth and development.

Key words: Actomyosin; *Chara* (rhizoids and protonemata); Fast-rotating clinostat (FRC); Gravisensing; Microgravity; Statolith

Introduction

Gravity, the constant extracellular stimulus, provides organisms with vital information, that is, for oriented growth. Signal-transduction pathways are highly complex and involve the intracellular susception of the stimulus, followed by perception and the transformation of the stimulus into physiological signals and finally the response. In higher plants, gravity-directed sedimentation of starch-filled amyloplasts is the characteristic feature of specialized gravity-perceiving cells, the statocytes (Kiss 2000; Sack 1997; Sievers and others 2001). Cytoskeletal elements restrict sedimentation of amyloplasts in most other cell types; in statocytes, however, the cytoskeleton regulates the dynamic equilibrium

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position of the statoliths and is most likely essentially involved in the early signal-tranduction phase, the transformation of the directional information that derives from sedimenting statoliths into physiological events, two crucial prerequisites for gravisensing (Kiss 2000; Sack 1997; Sievers and others 1991a; Sievers and others 2001). By using the elegant magnetophoresis method to move statoliths in normal vertically oriented roots, coleoptiles, hypocotyls and stems, it was confirmed that statolith displacement is sufficient to induce gravitropic curvature (Kuznetsov and Hasenstein 1996, 1997; Weise and others 2000). However, little is known about whether and how the cytoskeleton interacts with and regulates the positioning of statoliths.

This aspect has been intensively studied in the positively gravitropic (downward growing) rhizoids and the negatively gravitropic (upward growing) protonemata of the characean green algae, where the complete signal-transduction pathway is limited to the only growing part of the cell located at the apical region (Braun and Wasteneys 2000; Sievers and others 1996). In both cell types, gravity-oriented tip growth is based on sedimentation of $BaSO_4$-crystal-filled statoliths. An extensive, complexly arranged actin cytoskeleton (Braun and Wasteneys 1998) interacting with myosin-like proteins (Braun 1996a) not only organizes the tip-growth machinery, but also regulates the position of the statoliths and controls how and where statoliths sediment upon gravistimulation in a cell-type specific manner (Braun 2002; Hodick and others 1998). The well-coordinated regulation of statolith positioning, which is sensitive to actin-disrupting drugs like cytochalasins (Buchen and others 1993; Hejnowicz and Sieves 1981; Sievers and others 1991b), is crucial for both undisturbed tip growth and for the mechanisms of the opposite gravitropic responses in rhizoids and protonemata. The microtubule cytoskeleton plays a crucial role in maintaining the polar cytoplasmic zonation and the arrangement of the actin cytoskeleton, but is not involved in gravisensing and gravitropic tip growth (Braun and Sievers 1994).

Statolith Positioning is Regulated by Gravitational and Actomyosin Forces

In downward growing rhizoids, the statoliths are positioned 10–30 μm above the apical cell wall; actomyosin forces prevent statoliths from settling into the tip. In upward-growing protonemata, the actomyosin system prevents statoliths from sedimenting towards the cell base; it generates forces acting in the opposite direction. In their original position, the weight of the statoliths is dynamically outbalanced by oppositely directed actomyosin forces in both cell types.

This dynamically stable equilibrium position of statoliths is disturbed when one of the two components are altered. Inhibiting the actomyosin-mediated transport in rhizoids and protonemata with cytochalasin D resulted in a settling of statoliths on the apical cell wall and towards the cell base, respectively (Hejnowicz and Sievers 1981; Hodick 1994). After inversion or basipetal centrifugation of rhizoids, an initial displacement of statoliths into the subapical region was followed by an actomyosin-mediated retransport of the statoliths towards their original position near the tip (Braun and Sievers 1993). In rhizoids, when the; gravitational component was abolished in microgravity (Buchen and others 1993; Volkmann and others 1991) and on the fast-rotating clinostat (FRC; Cai and others 1997), the actomyosin component generated a displacement of the statoliths away from the tip. This displacement did not occur in rhizoids that were treated with cytochalasin D to disrupt the actin cytoskeleton (Buchen and others 1993).

Interestingly, the statoliths were not randomly distributed within the cell in the absence of directing gravitational forces. Instead they were kept in a position near the cell tip and they were still able to function as gravity susceptors. Thus, the actomyosin forces not only control the equilibrium position of the statoliths, they also actively rearrange the original position of the statoliths by retransporting statoliths from other regions.

Studies in a stimulus-free environment are obligatory to assess the significance of stimuli in signal-transduction pathways. Gravity, however, cannot simply be switched off, but can be compensated to a certain level, that is, during parabolic flights and in an orbiting space craft (free-fall situations). In this paper, investigations on statolith positioning in characean rhizoids and, for the first time, in protonemata are presented that were performed in microgravity during parabolic flights of sounding rockets (TEXUS/MAXUS), during the Space-Shuttle mission STS (Space Transportation System)-65 and in simulated weightlessness during rotation on the FRC. With these unique methods, we intended to unravel the complexly coordinated regulation of statolith positioning by the actomyosin system which is indispensable for gravitropic orientation in lower and undoubtedly also in higher plants.

Material and Methods

Thalli of the green alga *Chara globularis* Thuill were collected from a pond at the Botanischer Garten (Universität Bonn) and cultured in 10–20 l plastic buckets. We cut segments of two nodes and one internodal cell and embedded them in agar (1.2% (w/v) in distilled water) either for STS-65 in Plexiglas cuvettes or for parabolic rocket flights in vacuum-tight cuvettes that consisted of V_{2A}-steel frame and two Plexiglas windows to initiate development of rhizoids and protonemata. Both cuvette types were handmade by the mechanical workshop, Botanical Institute, University of Bonn. For the experiments performed during the Space-Shuttle mission IML-2 (Second International Microgravity Laboratory) on STS-65, *Chara* nodes without rhizoids were launched into space approximately 30 h after embedding in agar to ensure development in microgravity.

Experiments were performed during different parabolic flights of rockets and during the 16-day Space-Shuttle mission IML-2. TEXUS (Technologische Experimente unter Schwerelosigkeit) and MAXUS (enlarged version of TEXUS) rockets were launched from Esrange, near Kiruna in northern Sweden. They reached altitudes of approximately, 250 km and 800 km and provided microgravity ($<10^{-4}$ *g*) for approximately 6 min and 13 min, respectively. The TEXUS acceleration profile of the launch phase includes a peak thrust acceleration of 7.2 *g*. The mean thrust was 5.5 *g*. The spin of 3.1 Hz was compensated after 60 s by a yo-yo despin, which is accomplished by deploying two weights laterally along tethers to dissipate energy. The MAXUS launch acceleration profile included lower peak and mean accelerations. The TEXUS/MAXUS cuvettes were mounted on a payload module that allowed *in vivo* video-microscopy of cells in one cuvette and chemical fixation of cells in 4 other cuvettes at the beginning and the end of the microgravity phase. The fixation solution contained 5% (v/v) glutaraldehyde and 3% (v/v) formaldehyde in 0.1 M Pipes buffer, pH 7.0. The position of the statoliths in fixed rhizoids and protonemata were also recorded by video-microscopy in the laboratory at Esrange.

During the IML-2 Space-Shuttle mission, cuvettes were transferred from a Biorack Type I storage container to the NIZEMI (Niedergeschwindigkeits-Zentrifugenmikroskop) flight module, a video-microscope centrifuge constructed by Dornier GmbH, Friedrichshafen, Germany, on behalf of the Deutsches Zentrum für Luft- und Raumfahrt (DLR). Experimental data were recorded on video tapes. Ground controls were performed in the Hangar L at the Kennedy Space Center, Florida, USA, and by using the fast-rotating videomicroscope clinostat (FRC) at the microgravity user support center (MUSC) of the Deutsches Zentrum für Luft- und Raumfahrt (DLR) in Cologne, Germany. Rhizoids and protonemata were rotated with 90 rpm centered in the axis of the clinostat and observed by videomicroscopy. Movements of 18–45 statoliths per rhizoid were tracked by capturing video frames at intervals of 15 s to several min. The positions of the statoliths were determined on digital images (TIF-files) and mean values of the movement of the individual statoliths were calculated. The speed of the acropetal and basipetal movement of all statoliths analyzed is given as a range of mean values in Results and Discussion. Statistical analysis and image processing were done by using Excel (version 97, Microsoft), Photoshop (version 5, Adobe, Mountain View, USA) and Corel Draw (version 9, Corel Corporation, Dublin, Ireland).

Results and Discussion

Actomyosin Forces Preserve Graviresponsiveness in Microgravity by Active Statolith Positioning in Rhizoids.

In the normally tip-downward growing *Chara* rhizoids at 1 *g*, the statoliths are kept in a dynamic equilibrium position in the form of a flat disc-shaped complex 10–30 μm above the apical cell wall (Figure 1A). Individual statoliths frequently escape from this complex, perform saltatory and trembling movements preferentially in both axial directions and return to the complex. During the 6-min microgravity phase of the parabolic flight of a TEXUS rocket, the statoliths almost doubled their original distance from the tip and the shape of the complex became axially extended (Figure 1B) as was described in Volkmann and others (1991). After the 13-min microgravity phase of a parabolic MAXUS flight, statoliths had further increased their distance from the tip (Figures 1C, 2).

After 30 h in microgravity in the orbiting Space Shuttle during STS-65, some statoliths had returned acropetally and they became loosely arranged over an area of 11–44 μm basal to the tip. They still showed saltatory and trembling movements, but no net transport (Figures 1D, 2). Interestingly,

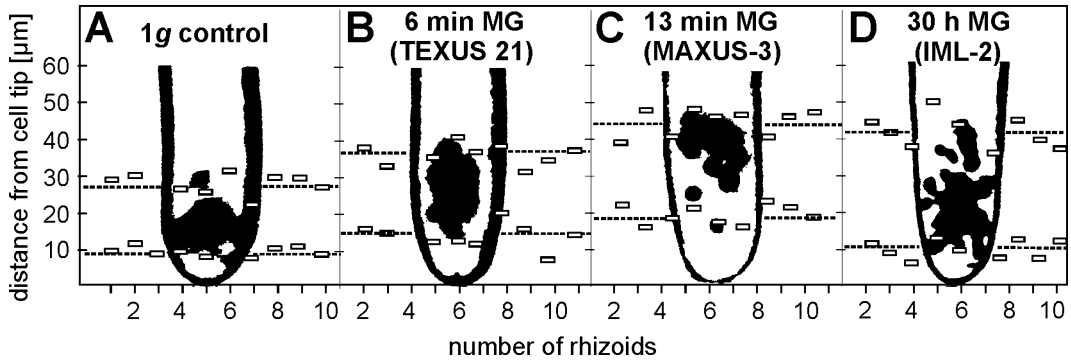

Figure 1. The positions of the statoliths in *Chara* rhizoids are shown under normal 1 *g*-conditions (**A**), at the end of the 6-min microgravity (MG) phase of a TEXUS-rocket flight (**B**), at the end of the 13-min microgravity phase of a MAXUS-rocket flight (**C**), and after 30 h on the orbiting Space Shuttle Columbia during IML-2 mission STS-65 (**D**). The apical and basal borders of the statolith complexes of individual rhizoids are indicated by rectangles; the dotted lines represent mean values.

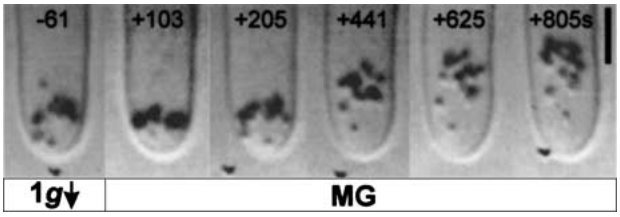

Figure 2. Series of micrographs showing the position of statoliths in a *Chara* rhizoid before launch of the MAXUS-3 rocket and the basipetal displacement of statoliths during the microgravity (MG) phase of the parabolic flight. For easier comparison of the statolith positions, the rhizoid tips were arranged on a horizontal line. Seconds on the images denote time before and after launch (t = 0 s); microgravity conditions (10^{-4} *g*) from t = 71 s to t = 841 s. Bar: 20 µm.

after 30 h in microgravity, the apical border of the statolith complex was in the same range again as in 1 *g*. This position of the statoliths was dynamically stable, and without any gravistimulation, the cells grew straight or slightly undulating.

The kinetics of the displacement of the statoliths (Figure 3A) shows an initial, slightly acropetal shift resulting from the launch accelerations and subsequently a strong basipetal displacement of statoliths with an average speed being in the range of 1.8–2.4 µm min^{-1} during the 6-min microgravity phase of TEXUS flights and the first 6 min of the MAXUS flight. In the second half of the 13-min MAXUS flight, the basipetal movement drastically slowed down and the average speed was reduced to 0.6–0.8 µm min^{-1}. This indicates that further away from the tip in the subapical region, actomyosin forces are active that do not support a further basipetal displacement, but redirect statoliths towards the tip (Figures 2, 3A). Basipetal displacement of statoliths was also observed in rhizoids, which were rotated on the FRC, although this transport was slower and steadier (Figure 3A). The average speed was in the lower range of 0.8–1.0 µm min^{-1}. The final position of FRC-rotated statoliths, however, resembled that of statoliths in microgravity-exposed rhizoids. This well-coordinated regulation of the position of statoliths by actomyosin forces guarantees that even in the absence of gravitational forces, statoliths are still located in a region close to the tip where prospective accelerations induce a displacement that finally results in gravitropic reorientation.

Rhizoids, which had developed in microgravity during STS-65 aboard the Space Shuttle Columbia, exhibited a dispersed arrangement of statoliths, as shown in Figure 1D. When microgravity-developed rhizoids were exposed to 1 *g* conditions by centrifugating the cells acropetally with the aid of the video-microscope centrifuge (NIZEMI; Friedrich and others 1996), their statoliths were displaced to a position similar to that of normally tip downward-growing rhizoids at 1 *g* (Figure 3B); they formed a similar flat, disc-like complex. The average speed was in the range of 1.4–1.8 µm min^{-1}. This position of the statoliths remained dynamically stable over the following 20 min. When centrifugation was stopped, the statoliths were retransported back to their original microgravity position (Figure 3B) with an average speed ranging from 1.5 to 1.7 µm min^{-1}. This indicates that the actomyosin system of microgravity-developed rhizoids has principally the same transport properties, and under the same gravity conditions accomplishes similar statolith positioning as ground controls.

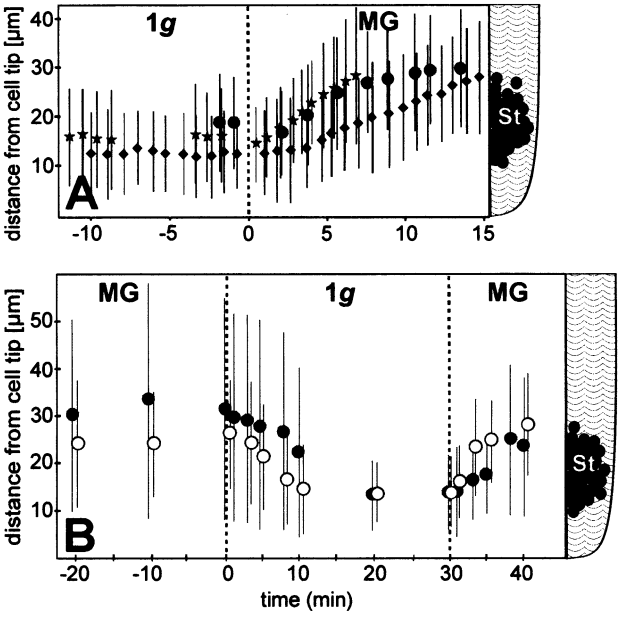

Figure 3A,B. (**A**) Kinetics of the displacement of statoliths (mean values ± SD, n = 18–26) in *Chara* rhizoids during the 6-min microgravity phase (MG) of the TEXUS-37 flight (★), during the 13-min microgravity phase of the MAXUS-3 flight (●), and during 15 min rotation on the FRC (◆). At time point t = 0 min the rocket was launched and the FRC was switched on. (**B**) Kinetics of the statoliths displacement (mean values ± SD, n = 22) in two microgravity grown rhizoids caused by an acceleration of 1 *g* for 30 min (t = 0–t = 30 min) applied acropetally with the video-microscope centrifuge NIZEMI in microgravity aboard the orbiting Space Shuttle Columbia (STS-65). St = statoliths.

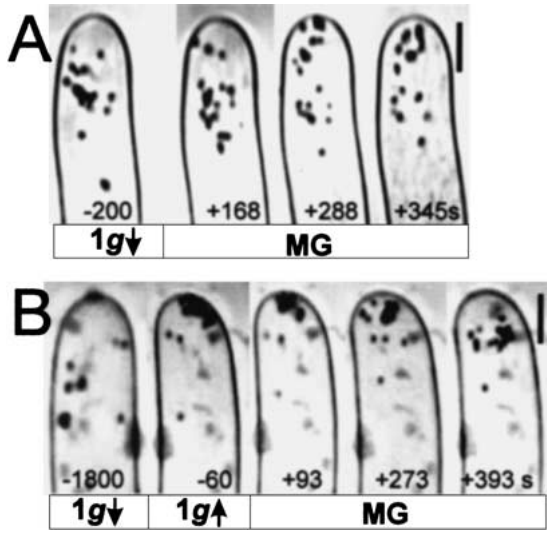

Figure 4A,B. (**A**) Series of micrographs showing the position of statoliths in a *Chara* protonema before launch of the TEXUS-rocket and the acropetal displacement of statoliths during the microgravity (MG) phase of the parabolic flight. (**B**) This protonema was inverted 30 min prior to launch and most statoliths sedimented on the apical cell wall. During the 6-min microgravity phase, the statoliths were lifted from the apical cell wall and gathered a short distance from the tip. For easier comparison of the statolith positions, the rhizoid tips were arranged on a horizontal line. Seconds on the images denote time before and after launch (t = 0s); microgravity conditions (< 10^{-4} *g*) from t = 75 s to t = 425 s. Bars: 20 µm.

Actomyosin Forces Preserve Graviresponsiveness in Microgravity by Active Statolith Positioning in *Chara* Protonemata

During the 6-min microgravity phase of a TEXUS flight, a displacement of statoliths was observed in *Chara* protonemata, which was not basally directed like in rhizoids but apically, towards the tip (Figures 4A, 5A). The average speed of the acropetal displacement was 2.3 µm min^{-1}. Some statoliths transiently approached the apical cell wall avoiding only a small area at the very tip. During simulation of weightlessness by rotation on the FRC, the statoliths also moved acropetally (Figure 5A), but their average speed was considerably slower, ranging from 0.8 to 1.2 µm min^{-1}. After about 20–30 min on the FRC, the distribution of the statoliths resembled that in rhizoids after 6 min in microgravity; in each of the cells, some statoliths settled on the apical cell wall close to the tip at this time. Interestingly, after 40 min, the distance of the statoliths from the tip increased considerably again (Figure 5A).

Inverting protonemata 30 min prior to launch of the TEXUS rocket or prior to switching on the FRC resulted in a transport of statoliths into the apical dome (Figures 4B, 5B). After being transiently arranged in a disc-shaped complex resembling that of normally tip-downward growing rhizoids, they settled asymmetrically on an apical flank close to the center of growth at the tip, which goes along with the beginning of the gravitropic redirection of the cell tip (Figure 4B; see also Hodick 1994; Hodick and others 1998). At this time, the protonemata were launched and the FRC was started. During the 6-min microgravity phase, the statoliths were lifted from the apical cell wall and displaced basipetally with an average speed of 0.8–1.0 µm min^{-1}. In contrast, statoliths in FRC-rotated protonemata started to move basipetally only after a delay of several min, but reached a new dynamically stable position after about 25 min, which was in the same range (15–25 µm basal to the tip) as the statoliths after the 6-min microgravity phase. Therefore, a

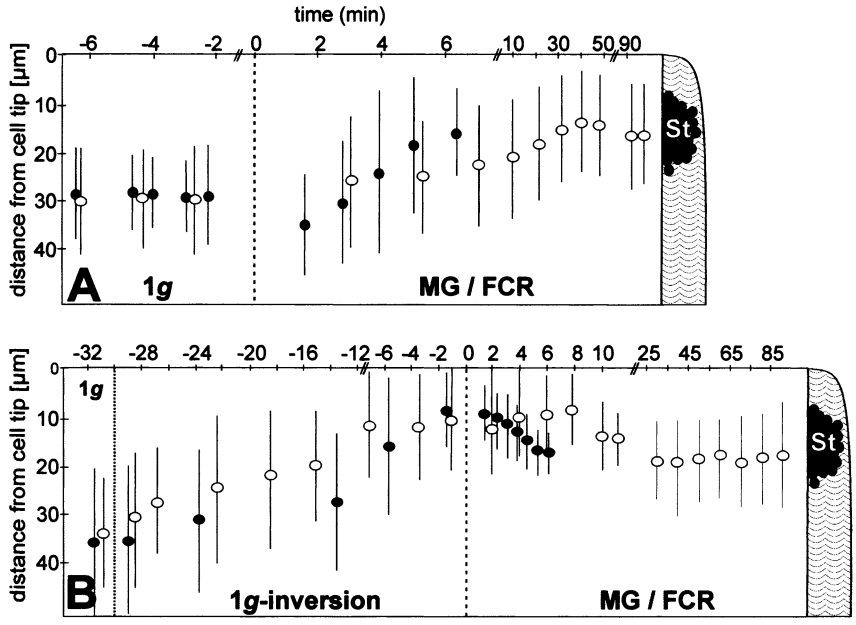

Figure 5A,B. (**A**) Kinetics of the displacement of statoliths (mean values ± SD, n = 19) in *Chara* protonemata during the 13-min microgravity (MG) phase of the TEXUS-37 rocket (closed circles) and during rotation on the FRC (open circles). (**B**) Kinetics of the statoliths displacement (mean values ± SD, n = 18) in *Chara* protonemata that were inverted for 30 min and subsequently exposed to the 6-min microgravity phase of TEXUS-37 (closed circles) and rotated on the FRC (open circles). St = statoliths. At time point 0 min the rocket was launched (microgravity conditions: t = 1.26 to t = 7.07 min) and the FRC was switched on.

force must exist in a small apical-most tip region that protects the center of growth by generating a basipetal transport of statoliths.

It is notable that the gravity-supported acropetal displacement during inversion (Figure 5B) was in the same range or even slower than the acropetal displacement of statoliths when the gravity force was abolished in normally vertically oriented protonemata (Figure 5A). This indicates that the vectorial actomyosin-mediated statolith transport can be induced by gravity, but that the transport rate itself is not considerably affected by the gravitational component.

All these actomyosin-mediated statolith displacements comprise acropetal and basipetal transport components. The mean values of the acropetal and basipetal components of the statolith movements in protonemata recorded at 15-s intervals are shown in Figure 6. At 1 *g*, both transport components are dynamically balanced on a similar level (Figure 6; 1 *g*) and do not generate a net-transport of statoliths. After inversion, the acropetal component strongly predominated the basipetal component in protonemata, which resulted in the acropetal transport of the statoliths into the tip (Figure 6B). During the 6-min microgravity phase of TEXUS-37, a predominating acropetal transport component caused a displacement of the statoliths towards the tip (Figure 6A; MG); whereas in pre-inverted protonema, sedimented statoliths were removed from the tip by a predominating basipetal transport component (Figure 6B; MG).

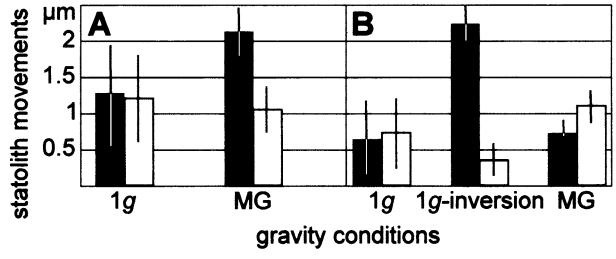

Figure 6. Mean values (± SD) of the acropetal (black) and basipetal (white) transport components of the statolith movements in protonemata during 6 min at 1 *g* and during the 6-min microgravity (MG) phase of TEXUS 37 (**A**) and during 10 min at 1 *g*, during the 30 min of inversion at 1 *g*, and during the 6-min microgravity phase of TEXUS-37 (**B**). The statolith movements were recorded by videomicroscopy. Video frames were captured in intervals of 15 s and acropetal and basipetal track elements were analyzed while the small lateral components of the track elements were ignored.

The illustration in Figure 7 summarizes the actomyosin and gravity forces acting on statoliths in normal vertically oriented, inverted, and horizontally positioned rhizoids and protonemata. In the

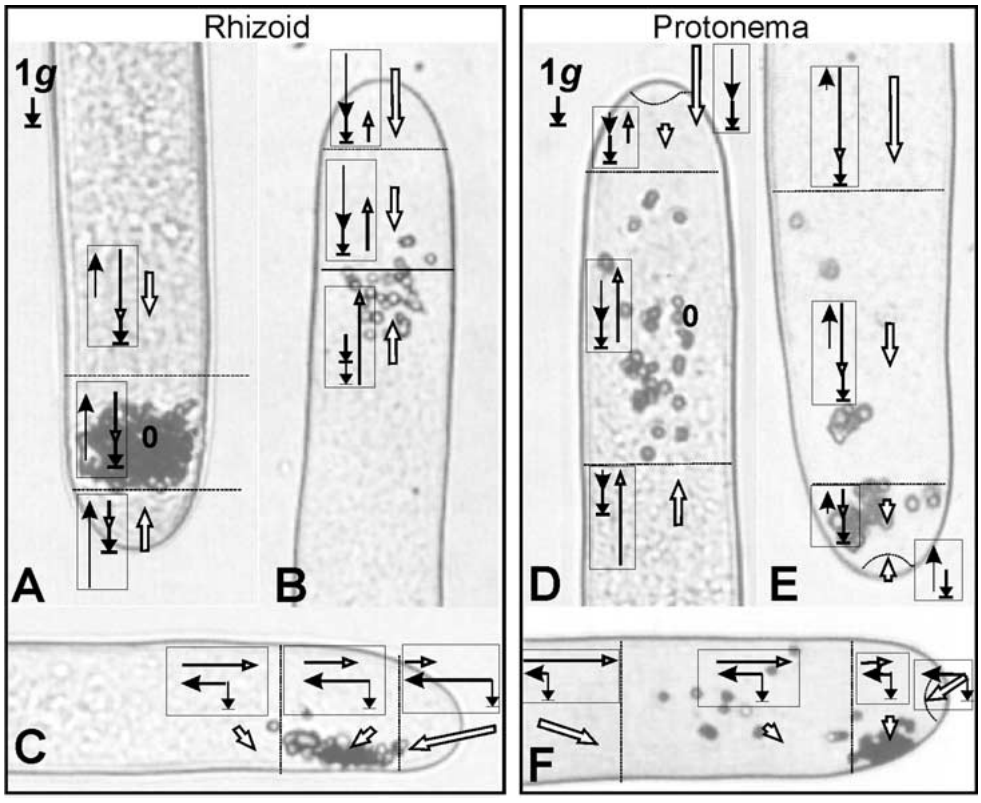

Figure 7. The illustration summarizes the actomyosin and gravitational forces acting on statoliths in the different regions of vertically oriented, inverted, and horizontally positioned *Chara* rhizoids (**A**–**C**) and protonemata (**D**–**F**)**.** The gravity force is indicated by the truncated arrows, basipetal and acropetal actomyosin forces by arrows with black and white arrowheads, respectively. The resulting force acting on the statoliths is indicated by the white arrows with black outlines. In the original position of the statoliths in normal vertically oriented cells, the forces compensate each other; the resulting force is zero and the statoliths remain in their dynamically stable equilibrium position.

statolith region of downward-growing rhizoids and upward-growing protonemata, net-basipetally and net-acropetally-acting actomyosin forces compensate for the weight of the statoliths, respectively. There is still vigorous movement of the individual statoliths in this region, but no net transport of statoliths. In the other regions of both cell types, the components of the actomyosin forces act in such a way that statoliths are redirected towards the statolith region. The outermost tip regions in both cell types are especially well-protected against statolith intrusion. When the cells are tilted from vertical, the concerted action of the actomyosin components and gravity directs statoliths to specific areas at the plasma membrane where they initiate the gravitropic responses (Braun 2002; Sievers and others 1996).

Actomyosin Forces Direct Sedimenting Statoliths to Distinct Graviperception Sites

The actomyosin forces acting differently on statoliths in rhizoids and protonemata have important implications for how and where statoliths sediment. Upon gravistimulation, the sedimenting statoliths are directed to specific, statolith-sensitive areas of the plasma membrane, the graviperception sites, where statolith sedimentation initiates the mechanisms of the opposite gravitropic responses. Forcing statoliths to settle outside these locally restricted, statolith-sensitive areas did not result in the initiation of the gravitropic responses in either cell type (Braun 1996b; Braun 2002).

After horizontal positioning of rhizoids, the sedimenting statoliths mainly followed the gravity vector and settled onto the lower cell flank of the statolith region (10–30 μm basal to the tip). When cells were rotated in angles other than 90°, however, statoliths did not simply follow the gravity vector. Instead, they were actively redirected to the same plasma membrane area of the statolith region (10–30 μm basal to the tip). In this area, sedimented statoliths caused differential flank growth by locally reducing the rate of exocytosis at this site (Braun 1997; Sievers and others 1996).

In gravistimulated protonemata, a strong acropetal transport resulted in sedimentation of the statoliths to the apical plasma membrane close to the center of growth at the very tip (Hodick and Sievers 1998; Hodick and others 1998). Statoliths induce the negative graviresponse only when they are settled on this specific plasma membrane area (Braun 2002). The negatively gravitropic mechanism is based on a statolith-induced repositioning of the growth center towards the upper flank, where a bulge forms and the new outgrowth occurs (Braun 1997; Sievers and others 1996). The negatively gravitropic pathway of protonemata seems to be physiologically more complex than the positive gravitropism in rhizoids and involves ion-channel regulation, calcium redistribution, and, most likely, the activation of regulatory, actin-associated proteins (Braun and Richter 1999; Braun 2001).

It should be noted that the actomyosin-mediated statolith positioning might even be more complexly regulated than discussed above. The illustration in Figure 7 does not consider a recently discussed gravity-dependent activation of a mechanism that modifies acropetal and basipetal statolith-transport components. During basipetal centrifugation and after inverting rhizoids, an increase of the acropetal transport component might be necessary to return statoliths to and then keep them in their original position in order to reestablish graviresponsiveness (Braun and Sievers 1993; Sievers and others 1991b). A similar increase of the acropetal actomyosin-transport component has been proposed to occur in protonemata after gravistimulation that generates a fast displacement of statoliths towards the tip (Hodick and Sievers 1998; Hodick and others 1998). Additional support for such adaptational modifications of the actomyosin forces comes from data presented in this paper (Figure 5A). When inverted protonemata with sedimented statoliths were brought to microgravity, the statoliths were transported away from the tip to a resting position where they did not interfere with tip growth. However, when non-inverted protonemata were brought to microgravity or were rotated on the FRC, statoliths were not transported from their original subapical position to this resting position, but were transported further into the apex. They might end up in the same resting position after a prolonged period of microgravity, but it remains unclear how they are able to pass the resting position and proceed to the apical cell wall before they are transported back again. Future research is needed to find unambiguous evidence for such adaptational modifications of acropetally or basipetally acting actomyosin forces and how these changes might be accomplished physiologically.

Conclusion

Recent results have shown that the equilibrium position of statoliths is under the control of actomyosin forces which counteract gravity in characean rhizoids. This study presents investigations on the complex regulation of the actomyosin-mediated statolith positioning in the oppositely gavirespondingcharacean rhizoids and protonemata. By acting differently in the different regions of characean rhizoids and protonemata, the actomyosin forces direct statoliths to and keep them in a position where they fulfill their specific role as susceptors in the gravitropic pathway. In this dynamically stable equilibrium position, the weight of the statoliths is just compensated by actomyosin forces. Disturbing the balance of forces by tilting the cells from the vertical, results in an actomyosin- and gravity-directed sedimentation of statoliths to specific graviperception sites at the plasma membrane, where the mechanisms of the opposite gravitropic responses are initiated. Research in microgravity represents a unique method to distinguish between active and passive components of forces acting on statoliths and to reveal the significance of the forces for the function of the biological gravisensors.

Investigating the molecular mechanisms of the actin actions (that is, the regulation of the statolith transport and positioning, the vesicle delivery and the organizing functions) raises questions on how these multiple actions are coordinated at a confined area in a single cell. The diverse actions could be based on different actin populations or on a complex interaction and regulation of different motor proteins. Considering the present investigations, it is tempting to suggest that actomyosin interactions with the amyloplast-statoliths play a much greater role in the early phases of the signaling pathway of gravitropism in higher plants than is widely assumed. Sedimentation of amyloplast-statocytes is also not simply a gravity-directed falling to the bottom; instead, the statolith dynamics indicate extensive interactions with the actin cytoskeleton. Sedimentation can be complicated by actomyosin-based cytoplasmic streaming, such as in statocytes of coleoptiles and pulvini (Johannes and others 2001; Sack and Leopold 1985; White and Sack 1990), and even sedimenting statoliths in root cap statocytes seem to follow certain pathways with varying velocities (Sack and others 1985; Sievers and others 1991a; Yoder and others 2001). The initial interac-

tions with the actin cytoskeleton may be crucial for eliciting the early events of the gravitropic pathway that finally lead to the gravitropic response (Sievers and others 2001). Thus, analyzing the molecular mechanisms of how actin interacts with statoliths may yield a new understanding of the gravitropic signaling pathway in lower and higher plants.

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