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Gravitropism in tip-growing cells

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Abstract. Unicellular tip-growing cells are excellent experimental systems in which to study gravitropism because cell extension, gravity sensing and the gravity response are all confined to the apical dome. Thus various approaches can be used to determine the distinct steps of the short gravitropic signal-transduction chain, which lacks a signal-transmission phase between the gravity-sensing cells and the competent responding target cells. Single-cell systems readily allow in-vivo observation of cellular processes during gravistimulation at 1 *g*, centrifugation, clinostatting and in microgravity, as well as permitting fluorescence labeling. Such diverse studies have revealed fascinating information on the mechanism of gravitropic tip growth, especially on the important role of the cytoskeleton in the positioning of the statoliths and in organizing and adjusting the Spitzenkörper. A hypothesis explaining the negative and positive gravitropism of *Chara* rhizoids and *Chara* protonemata has been put forward, which emphasizes the role of the actin cytoskeleton in the process of gravitropic tip-growth. Differences in the gravitropic responses of single-cell systems, however, reflect a diversity of gravitropic mechanisms, and represent an example of parallel evolution.

Key words: *Chara* (rhizoids, protonemata) – Cytoskeleton – Gravitropism – Moss protonemata – Statoliths – Tip-growth

Introduction

The Earth's gravitational acceleration (9.81 m · s^{-2}) has been present throughout evolution and is used by many plant organs for growth orientation. In higher plants, gravity-sensitive cells called statocytes are continuously stimulated and control the direction of growth by gravity-dependent movements of intracellular masses. A well-known example is the displacement of plastids in the statocytes of root caps (Audus 1962; Sack 1991, 1997, this issue; Sievers et al. 1991b; Sievers and Braun 1996; Perbal et al. 1997, this issue). In order to function as statoliths, the density of these organelles must be greater than that of the surrounding cytoplasm and their gravity-dependent displacement must exceed that which is caused by Brownian motion (Björkman 1988).

Since very small displacements of statoliths from their original equilibrium are sufficient to initiate a gravitropic response, the interaction of statoliths with the cytoskeleton, a highly dynamic network of filamentous proteins, is considered to play a crucial role in the transduction of the physical signal of gravity into a biochemical/physiological signal that leads to a growth response (Sievers et al. 1991b, 1994).

In order to understand how gravity is perceived and how the stimulus is transduced into a physiological chain of events, gravitropically tip-growing cells increasingly receive attention because of the lower complexity of their gravitropic system. The stimulus-response chain is short and limited to the apical zone of a single cell, in contrast to higher-plant organs where the signal is transmitted from stimulus-perceiving cells, the statocytes, to competent responding target cells.

Root hairs, pollen tubes, rhizoids and protonemata of algae, mosses and ferns are typical tip-growing cells; tracheids, epidermal and mesenchyma cells also show tip growth. Gravitropic tip-growth, however, is limited to a small number of tip-growing cell types (Sievers et al. 1996), e.g. protonemata and rhizoids of the characean algae (Fig. 1) and apical protonema cells of the mosses *Ceratodon* (see Fig. 8), *Funaria* and *Physcomitrella*. This paper reviews current knowledge on these well-characterized and intensively studied single-cell systems. Research on gravitropism in other tip-growing cells is sparse. Despite the fact that all such cells are attached to a multicellular organism, the term single-cell system has

Abbreviations: CD = cytochalasin D; *g* = gravitational acceleration (9.81 m · s^{-2}); IML-2 = International Microgravity Spacelab Mission 2; NIZEMI = Niedergeschwindigkeits-Zentrifugenmikroskop (slow-rotating-centrifuge-microscope); TEXUS = technologische Experimente unter Schwerelosigkeit (experiments under reduced gravity)

been used because gravity-perception and gravitropic response occur in the same cell and, therefore, gravitropic tip-growth is only dependent on the cell's own orientation (Sack 1993).

Positive gravitropism in *Chara* rhizoids

The positively gravitropic (downward growing) *Chara* rhizoids are tube-like cells with a highly polarized cytoplasmic zonation (Fig. 1A,C). The large vacuole in the basal zone is surrounded by rotationally streaming cytoplasm. The subapical cytoplasm is relatively stationary and characterized by a great abundance of dictyosomes, predominantly axially oriented endoplasmic reticulum cisternae, mitochondria, plastids and ribosomes. The apical zone contains the statoliths, $BaSO_4$-crystal-filled vacuoles, and the Spitzenkörper (Sievers and Schnepf 1981), which is a tip-growth-organizing complex consisting of an aggregation of endoplasmic reticulum (Bartnik and Sievers 1988), actin filaments (Sievers et al. 1991a) and a dense accumulation of vesicles of different sizes and staining patterns (Bartnik et al. 1990).

The apical crystal-filled vacuoles have been identified as statoliths by basipetal centrifugation of the particles out of the apical zone: the gravitropic response was abolished until the statoliths were retransported to the tip (Buder 1961; Sievers et al. 1991a). In normal vertically growing rhizoids, the statoliths are dynamically positioned 10–30 μm above the apical cell wall (Fig. 1A). After deviation from the plumb line, the positively gravitropic (downward) bending of rhizoids is initiated by sedimentation of the statoliths onto the lower cell flank in the basal part of the apical zone, causing an asymmetric exocytosis of vesicles which leads to differential flank growth (Sievers et al. 1979; see also Fig. 7A). Sedimentation of statoliths or other cell components has not been observed in the subapical zone. During gravitropic bending, the centre of maximal growth remains positioned in the outermost tip of the rhizoids, only the upper flank grows faster than the lower flank. The downward bending stops when the statoliths are symmetrically redistributed.

The polar cytoplasmic organization and the arrangement of the actin cytoskeleton are dependent on the microtubule cytoskeleton (Fig. 2). Relatively short

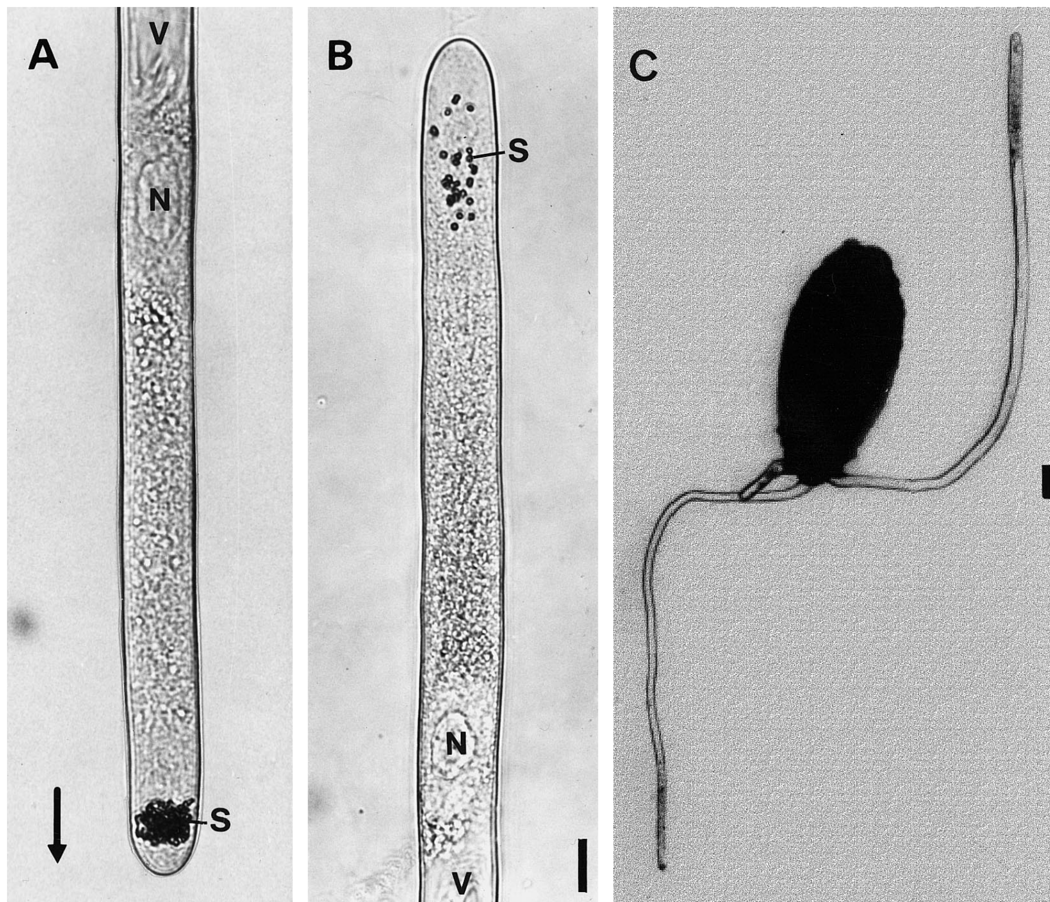

Fig. 1A–C. Micrographs showing a positively gravitropic (downward growing) *Chara* rhizoid (**A**) and a negatively gravitropic (upward growing) *Chara* protonema (**B**, photo: D. Hodick). Both cell types show the same cytoplasmic zonation and contain a large basal vacuole (*V*), a nucleus (*N*) and statoliths (*S*). The gravity vector is indicated by an *arrow*. ×280; bar = 25 μm. **C** An oospore with a rhizoid and a protonema was tilted by 90°; both cell types show their characteristic gravitropic response (photo: D. Hodick, modified) ×50; bar = 100 μm

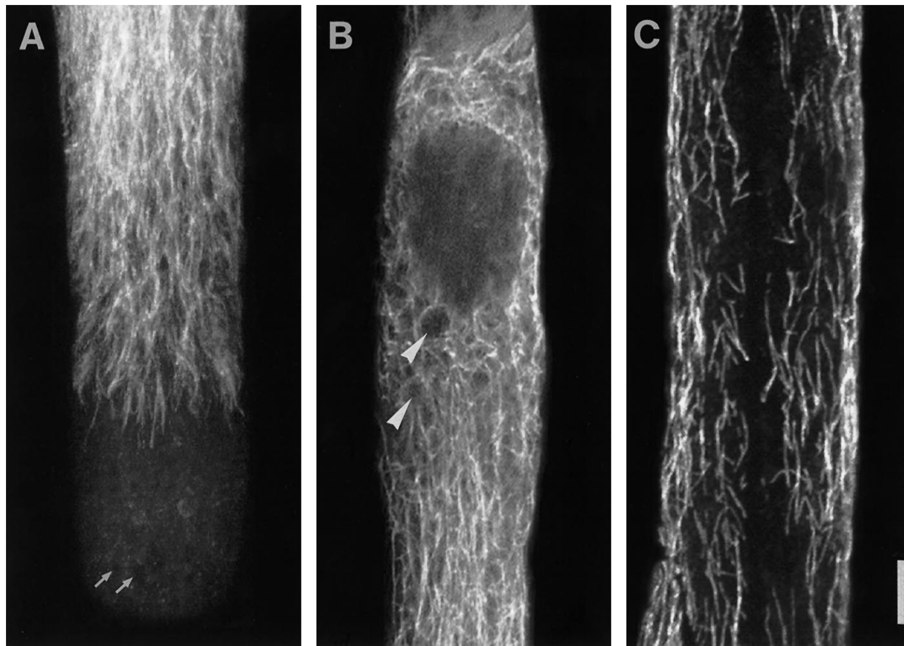

Fig. 2A–C. Immunocytochemical localization of microtubules in the different zones of a *Chara* rhizoid. **A** Anti-tubulin immunofluorescence reveals an extensive three-dimensional meshwork of microtubules in the subapical cytoplasm. Microtubules are not detected by antibodies in the apical zone. The dark areas (*arrows*) represent the position of the statoliths. **B** Spaces in the subapical fluorescence pattern indicate the positions of the nucleus (*N*) and organelles (*arrowheads*). **C** The basal zone is characterized by cortical, predominantly axially oriented microtubules showing interconnections. ×1000; bar = 10 μm

microtubules form a cylinder in the cortical cytoplasm of the basal zone. Microtubules are often less abundant in the indifferent zone, i.e., the zone between those parts of the cytoplasm streaming in opposite directions. In the subapical zone, microtubules fill the whole cytoplasm with an extensive axially oriented network. Microtubules have not been detected in the apical cytoplasm. After application of oryzalin, a highly effective microtubule-depolymerizing drug, the positions of the organelles and the nucleus become unstable and the functional polar organization of the actin cytoskeleton is destroyed (Braun and Sievers 1994). However, the gravitropic tip-growth is not disturbed as long as the statoliths and the components of the Spitzenkörper are not incorporated into the streaming cytoplasm. The lack of stainable microtubules in the apex of *Chara* rhizoids and the undisturbed gravitropic response after application of microtubule-disrupting drugs suggest that microtubules

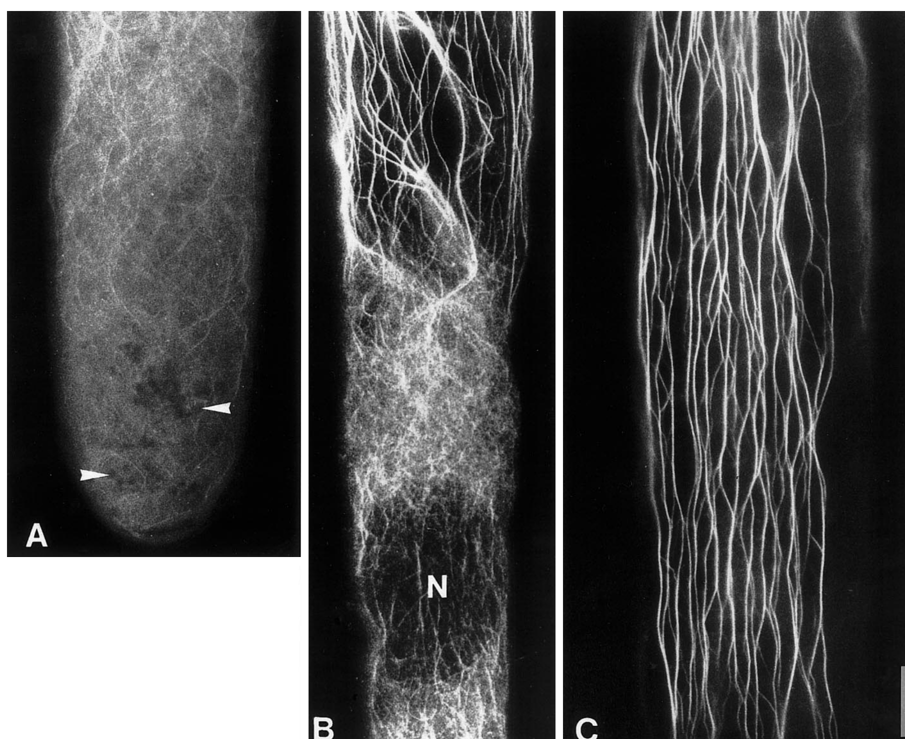

Fig. 3A–C. Fluorescence micrographs of *Chara* rhizoids after staining with rhodamine phalloidin. **A** The apical and subapical zones contain a dense network of very fine actin filaments encircling the statoliths (*arrowheads*). **B** The network of fine actin filaments surrounds the nucleus (*N*) and converges into the thick bundles of the basal zone. **C** Thick undulating bundles of actin filaments arranged in two files are characteristic of the basal zone where actin is responsible for generating rotational cytoplasmic streaming. ×1350; bar = 10 μm

are not directly involved in the primary events of gravitropism (Hejnowicz and Sievers 1981; Braun and Sievers 1994). Instead, cytoplasmic streaming and the positioning and transport of statoliths are mediated by the actin cytoskeleton (Sievers et al. 1991a; Braun and Sievers 1993).

In the basal zone, actin filaments forming two files of thick undulating bundles are involved in generating the rotational cytoplasmic streaming, one file of actin-filament bundles for each streaming direction. Actin filaments in the subapical and apical zones form an extensive network of very fine bundles encircling the nucleus and the statoliths (Fig. 3). Using a confocal microscope, actin filament bundles can be detected that converge into a brightly fluorescing spot in the apical dome of the rhizoid. Another population of actin filament bundles radiates from this spot towards the apical membrane. The position of the bright spot corresponds exactly to the position of the dense endoplasmic reticulum aggregation which may represent the centre of the Spitzenkörper. Myosin-related proteins have been detected by indirect immunofluorescence on the surface of statoliths in the apical zone and in the form of diffusely fluorescing strands in the endoplasm of the basal zone, making it likely that the actomyosin system is responsible for organelle motility in rhizoids (Braun 1996a).

The intimate interaction of statoliths with the actin cytoskeleton has been proven by (i) cytochalasin treatment, (ii) translocation of statoliths using optical tweezers, (iii) centrifugation experiments, (iv) experiments in microgravity: TEXUS (sounding rockets) flights and (v) the Spacelab IML-2 mission, and (vi) clinostatting.

(i) After application of the actin-destabilizing drugs cytochalasin B or D, the integrity of the Spitzenkörper was destroyed and tip-growth was inhibited; the statoliths sedimented onto the apical cell wall (Hejnowicz and Sievers 1981; Bartnik and Sievers 1988). Cytochalasins also inhibited the retransport of statoliths towards the tip after basipetal centrifugation or inversion (Sievers et al. 1991a). The statoliths were retransported to their original position and tip-growth recovered after removal of the drug.

(ii) Leitz et al. (1995) initiated bending of rhizoids by using optical tweezers to translocate statoliths laterally to one flank of the rhizoids. The laser output power necessary for moving statoliths in the basal direction was higher than that for moving statoliths in the apical direction. Low laser output power was needed for a lateral displacement of statoliths. In cytochalasin-treated rhizoids, the lowest power was sufficient for moving statoliths in all directions. Thus, the laser-tweezer experiments prove that actin filaments interact with statoliths mainly in the two axial directions. Very weak interactions in lateral directions allow the unimpeded sedimentation of statoliths onto the lower cell flank during gravistimulation.

(iii) Acropetal and basipetal accelerations up to 6.5 *g* were applied by means of the slow-rotating-centrifuge-microscope NIZEMI (Braun and Sievers 1993). A conventional centrifuge was utilized to apply higher accelerations. During acropetal centrifugation (1.4–6.0 *g*), the statoliths were displaced into a new stable position nearer to the cell vertex (12.0–6.5 μm from the apical cell wall, respectively). The statoliths did not sediment onto the apical cell wall and were not retransported to their original position (Fig. 4A). However, within 30 s after stopping centrifugation, the statoliths were lifted back to their original position. Thus, during acropetal centrifugation (< 6 *g*), the actin filament system controlling the position of statoliths behaves like an elastic system. Sedimentation of statoliths on the apical cell wall and reduction of growth rates of the rhizoids were only observed during acropetal accelerations higher than 50 *g*.

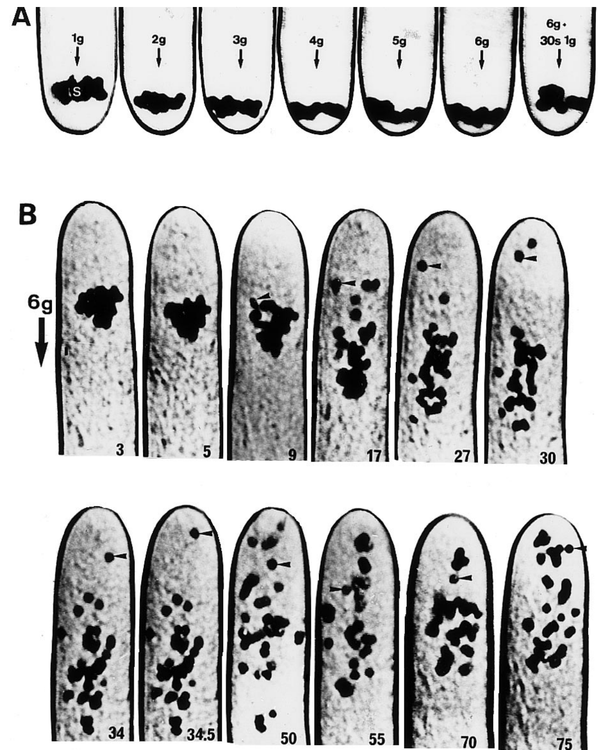

Fig. 4A,B. Series of micrographs of two representative *Chara* rhizoids showing the displacement of statoliths during acropetal (**A**) and basipetal centrifugation (**B**). **A** During acropetal centrifugal accelerations (1–6 *g*), the distance between the statoliths (*S*) and the cell vertex decreased with increasing accelerations, and the statolith complex became flatter. The micrograph on the *far right* represents the position of statoliths after each acceleration >1 *g* plus 30 s at 1 *g*. **B** Permanent basipetal centrifugation at 6 *g* (indicated by the *arrow*) for 75 min. The statoliths were displaced into the subapical zone. At 17 min, some statoliths had been retransported acropetally, whereas the main group of statoliths continued moving basipetally. The maximal distance from the cell vertex was achieved at 34 min. Later, all statoliths were retransported acropetally, different statoliths moving in different directions (*arrowheads* point to the same statolith). ×1050; bar = 20 μm. (After Braun and Sievers 1993)

When not only the amount but also the direction of the acceleration was changed in comparison to the natural condition, i.e., during inversion and basipetal centrifugation (1.0–6.5 *g*), the statoliths were displaced into the subapical zone (up to 90 μm from the apical cell wall); after 15–20 min, the retransport of statoliths to

the apex against the direction of acceleration started (Fig. 4B). Finally, the original position in the tip was re-established against the direction of continuous centrifugal force. Retransport of statoliths was observed against accelerations $< 70\ g$. Basipetal centrifugation $> 70\ g$ resulted in a displacement of all statoliths into the basal zone of the rhizoid where they were cotransported in the streaming cytoplasm. It is concluded that the actin filament system is able to adapt to higher mass accelerations in order to restore gravitropic responsiveness and that the actin cytoskeleton of the growing rhizoids is polarly organized.

(iv) Experiments in microgravity provided direct proof that actin filaments exert forces on the statoliths. The position of statoliths in rhizoids that were launched in normal vertical orientation was observed during the parabolic phase of TEXUS flights (Volkmann et al. 1991). When gravity was reduced to $10^{-4}\ g$ (microgravity), the "weightless" statoliths moved in the basal direction (Fig. 5). The original distance to the tip was nearly doubled within 6 min of microgravity. Therefore, it must be concluded that on Earth the position of statoliths in rhizoids depends on the balance of two forces, i.e., the gravitational force and the counteracting force mediated by the actin cytoskeleton. According to Newton's third principle, the reduction of one of these forces must necessarily cause a displacement of the statoliths into a new dynamically stable position. This conclusion is supported by the observation that the statoliths in rhizoids which had been treated with cytochalasin D (CD) prior to launch (Fig. 5) were not displaced during the microgravity phase of the TEXUS flight (Buchen et al. 1993).

(v) During the IML-2 mission aboard the Space-shuttle Columbia, *Chara* rhizoids were observed by videomicroscopy under long-term microgravity conditions and under different centrifugal accelerations provided by NIZEMI in order to study the behaviour of statoliths and to determine threshold values of sensitivity for accelerations.

Due to the absence of a directional acceleration stimulus, in microgravity, rhizoids grew out from the node in random orientation, showing their characteristic structural polarity (Fig. 6). Thus, it has been demonstrated that tip-growing single cells follow their genetic program in development and morphogenesis. Automorphogenesis is a developmental principle independent of gravity, as is the case in higher plants (Volkmann et al. 1986; Laurinavičius et al. 1996).

In microgravity-grown rhizoids, the distribution of statoliths was similar to that found in rhizoids after 6-min of microgravity during TEXUS flights. The statoliths became spread over an area of about 50 μm at a greater distance from the vertex than in 1 *g* controls. Thus, after only a short period of microgravity, a new dynamically stable balance of forces, exerted on statoliths by gravity and by actin filaments, had been established.

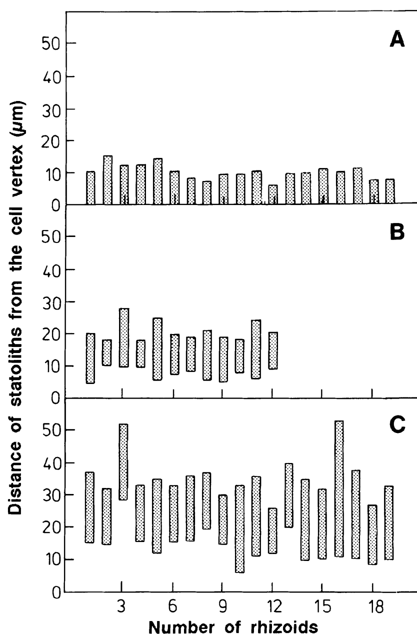

Fig. 5A–C. Localization of statoliths in CD-treated rhizoids fixed at the end of the microgravity phase (**A**) and of untreated rhizoids fixed at the beginning (**B**) and the end of the microgravity phase (**C**) during the parabolic flight of a TEXUS rocket. The apical and basal distances of the statoliths complex from the apical cell wall (vertex) are shown for individual rhizoids. Cytochalasin D was applied at 1 *g* in concentrations of 5–6.5 $\mu g \cdot ml^{-1}$ 30 min prior to launch. In contrast to untreated rhizoids, no basipetal displacement of statoliths occurred in CD-treated rhizoids. (After Buchen et al. 1993)

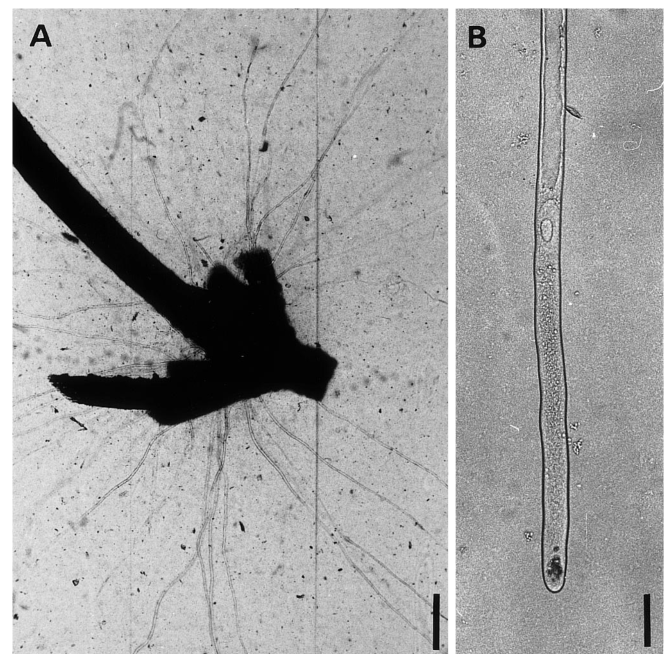

Fig. 6. A Rhizoids growing out from a *Chara* node during 16 d under microgravity conditions aboard the Space-shuttle Columbia (IML-2 mission). Due to the absence of a directional acceleration force, rhizoids emerge from the node in random orientation. ×10; bar = 1 mm. **B** Except for the position of the statoliths, microgravity-grown rhizoids show the same cytoplasmic zonation as 1 *g* controls (see Fig. 1A). The greater distance of the nucleus from the tip in the microgravity-grown rhizoid is within the range of normal variability, indicating a fast growth rate. ×200; bar = 50 μm

The first experiments relating to the gravisensitivity of *Chara* rhizoids were performed during the Spacelab mission IML-2. The application of 1 *g* for 4 min and 0.5 *g* for 30 min perpendicular to the axis of the rhizoids growing in microgravity resulted in an asymmetrical distribution of statoliths and a sedimentation of some statoliths onto the centrifugal cell flank (data not shown). This displacement of statoliths was followed by a weak bending of the rhizoids. Acceleration doses of 1 *g* for 1 min and 0.1 *g* for 30 min proved to be below the threshold value; they did not cause asymmetrical distribution of statoliths and, consequently, did not result in a bending reaction. Thus, the threshold value for gravisensitivity can be expected to be >0.1 *g* and <0.5 *g*. There was no obvious difference in the ultrastructural features of rhizoids which had grown under microgravity conditions for at least 3 d and 1 *g* controls (Braun et al. 1996). It is concluded that a shift to microgravity condition does not disturb or destroy the cellular organization and does not abolish the gravitropic responsiveness.

(vi) During rotation on the horizontal axis of slow- and fast-rotating clinostats simulating the effect of weightlessness, basipetal displacement of statoliths occurred which was similar to that during TEXUS flights (Cai et al. 1997). The centre of the statolith complex remained in a new relatively stable position for approx. 30 min. The following acropetal displacement of statoliths resembled the movement of statoliths during basipetal centrifugation, indicating that rotation on the clinostats caused a reorganization of the actin filament system.

In conclusion, cytoskeletal elements are involved in the maintenance of the structural polarity of the gravisensitive tip-growing *Chara* rhizoids, as well as in the positioning and movements of statoliths and in the final growth response. In both axial directions, the movements of statoliths are dynamically controlled by the actin cytoskeleton which results in a relatively stable position of statoliths in normal vertically oriented rhizoids. In the lateral direction, however, the movements of statoliths are only weakly controlled by actin filaments, allowing the statoliths to sediment onto the lower flank after the rhizoid is positioned horizontally. Statolith sedimentation onto the lower cell flank is not accompanied by a rearrangement of the actin filaments in the apical zone; statoliths appear to fall through the actin filaments without dragging them along.

Negative gravitropism in *Chara* protonemata

In darkness, *Chara* nodes and oospores regenerate negatively gravitropic protonemata instead of positively gravitropic rhizoids (Fig. 1, 7). When exposed to light, the single-celled protonemata stop tip-growth, divide and regenerate the green thallus with nodes and internodes. *Chara* rhizoids and dark-grown protonemata show a close morphological similarity (Hodick 1993). Both cell types have the same shape, contain statoliths and exhibit the same cytoplasmic zonation. The opposite direction of gravitropic growth is the remarkable difference.

Recently, a gravitropic reaction chain has been presented for the negative gravitropism of protonemata, in which not only the subapical differential flank growth (bending by bowing) is considered, but also the displacement of the growth centre (bending by bulging) caused by an asymmetrical intrusion of sedimenting statoliths into the apical dome very close to the cell vertex (Hodick 1994; Sievers et al. 1996). Such an actin-mediated intrusion of statoliths was observed during gravistimulation of protonemata at 1 *g* (Fig. 7B), but not during gravistimulation of rhizoids at 1 *g* (Fig. 7A). However, when centrifugal accelerations of 50–250 *g* were applied to rhizoids at stimulation angles of 5–90° between the acceleration vector and the rhizoid axis (Fig. 7C), the statoliths were pressed asymmetrically onto the centrifugal flank of the apical cell wall (Braun 1996b). In contrast to the positively gravitropic bending at 1 *g*, the rhizoids grew away from the acceleration vector by forming a sharp bend, which is similar to the negatively gravitropic response of *Chara* protonemata; then rhizoids stopped bending and, in the second phase, grew straight in directions clearly deviating from the direction of acceleration.

These results indicate that gravitropic bending of rhizoids during enhanced centrifugal accelerations is influenced not only by subapical differential flank

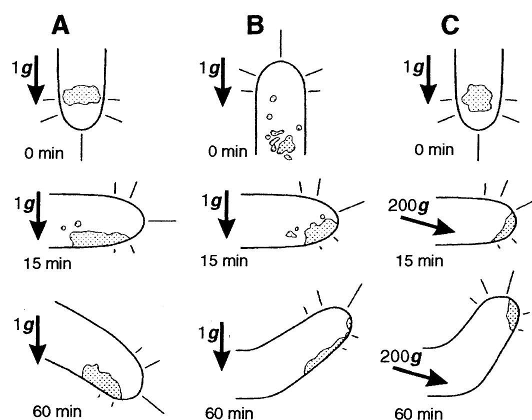

Fig. 7A–C. Gravitropic response of two *Chara* rhizoids (**A, C**) at different accelerations and stimulation angles and a *Chara* protonema (**B**) at 1 *g*. Experimentally evaluated (**A**) and hypothetical extension rates (**B, C**) are indicated by the different lengths of the five lines positioned normal to the surface of the apical dome. **A** After turning the rhizoid into the horizontal position at 1 *g*, the statoliths (*dotted area*) are sedimented onto the lower cell flank at a considerable distance from the vertex (t = 15 min). Downward bending of the rhizoid is the result of differential flank growth (t = 60 min). **B** After turning the protonema into the horizontal position, the statoliths are located very close to the vertex due to sedimentation and an additional acropetal transport (t = 15 min). Upward bending is caused by a displacement of the growth centre from the vertex of the protonema towards the upper flank (t = 60 min). **C** In the rhizoid which is centrifuged at 200 *g* with the acceleration vector at an oblique angle to the cell axis, the statoliths are pressed asymmetrically onto the apical cell wall (t = 15 min) and the rhizoid grows away from the acceleration vector (t = 60 min) which is very similar to the negatively gravitropic response of protonemata (**B**, t = 60 min)

growth, as it is the case at 1 *g*, but mainly by the centripetal displacement of the growth centre, as is proposed for the negative gravitropism of *Chara* protonemata. The rhizoid stopped bending when a dynamic equilibrium between bowing (displacement of the growth centre) and bending (differential flank growth) was achieved (Braun 1996b). This hypothesis is supported by electron-microscopic images of centrifuged rhizoids showing a centripetal displacement of the endoplasmic reticulum aggregate (Braun 1996b). It seems likely that the differences in the sedimentation behaviour of statoliths in protonemata and rhizoids, and the different growth responses, are mainly based on different properties of the actin cytoskeleton which, in these cell types, appears to be involved in the anchorage of the growth-organizing-complex, i.e., the Spitzenkörper, in the tip (Hodick 1994).

Negative gravitropism in moss protonemata

In apical protonema cells of the mosses *Ceratodon*, *Funaria* and *Physcomitrella*, the negatively gravitropic (upward) bending (Fig. 8) in darkness is less well understood than that of *Chara* (Sack 1993; Sack et al. 1997). In contrast to the unicellular *Chara* protonema which can reach a length of several centimeters, moss protonemata

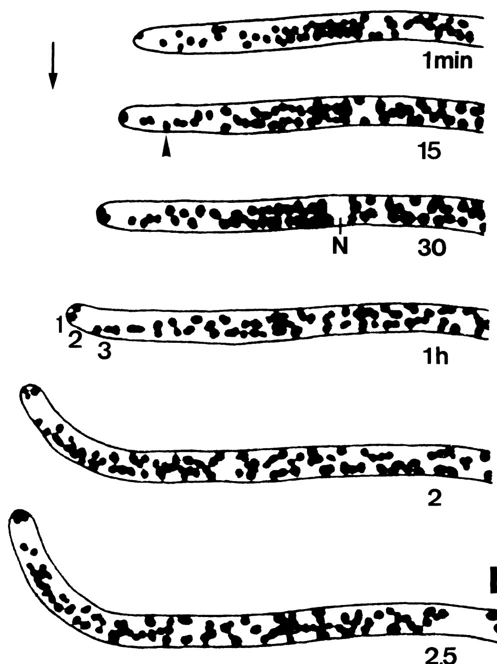

Fig. 8. Negative gravitropism of a horizontally positioned apical protonema cell of the moss *Ceratodon* growing in darkness. The *black spots* represent the starch-containing plastids. Three plastid zones found in the apical part of the cell are numbered (at 1 h). Plastid sedimentation (*arrowhead* at 15 min) in zone 3 preceded the start of upward bending. The accumulated plastids close to the cell tip (zone 1) and the plastids in the basal part of the cell do not show gravity-dependent movements. Zone 2 is a relatively plastid-free zone. The gravity vector is indicated by the *arrow*. *N*, nucleus. ×200; bar = 20 μm. (Modified after Sack, 1993, with permission)

cells divide frequently and only the apical cell responds gravitropically. The cytoplasmic organization and the differential distribution of organelles in apical protonema cells are as complex as in *Chara* rhizoids, *Chara* protonemata and other tip-growing cells (Sievers and Schnepf 1981; Walker and Sack 1995b). Common features are the Spitzenkörper (the dense accumulation of secretory vesicles close to the tip and actin filaments) and a great abundance of Golgi stacks basal to the apex. However, the conspicuous aggregation of endoplasmic reticulum that is found in *Chara* rhizoids (Bartnik and Sievers 1988) and *Chara* protonemata has not been observed in the apical dome of moss protonemata.

Throughout the apical cell of moss protonemata both microtubules and actin filaments form three-dimensional networks with a predominantly axial orientation but converging towards the tip. In *Ceratodon*, microtubules rather than actin filaments seem to be involved in maintaining the cell shape and gravitropic response which are both sensitive to anti-microtubule drugs, whereas actin filaments are necessary for tip extension (Doonan et al. 1988). A reorientation of actin filaments was reported during the phototropic response in *Ceratodon* caulonemal tip cells (Meske and Hartmann 1995). In contrast, reorientation of actin filaments was not detected after gravistimulation of this moss (Walker and Sack 1995a). However, during the graviresponse there was a conspicuous enrichment of microtubules in the lower cell flank in the plastid-free zone behind the tip (Fig. 9); these microtubules may play a role in the intracellular signal transmission to the growing tip (Schwuchow et al. 1990; Walker and Sack 1990; Young and Sack 1992; Sack 1993).

Sedimentation of starch-filled plastids takes place at a considerable distance from the apical dome and even during inversion (tip down orientation) the plastids that sediment are excluded from the apex (Schwuchow and Sack 1993). Therefore, a direct interaction of plastids with a growth-organizing structure in the apical dome is unlikely; and since the gravitropic response in moss protonemata is inhibited by visible light (Young and Sack 1992), it is obvious that there must be intermediate steps in intracellular signal transmission. In addition, gravitropism in moss protonemata is complicated by a slight downward curvature (wrong-way curvature) before the protonemata start to grow upward (Young and Sack 1992). A role for an apical accumulation of plastids around the "centre of the new direction of growth", as shown by statoliths in negative gravitropism, could not be demonstrated (Walker and Sack 1991; Young and Sack 1992).

In contrast to an earlier report (Jenkins et al. 1986), a weak plastid sedimentation was also detected in negatively gravitropic protonemata of *Physcomitrella* (Schwuchow et al. 1995), which suggests that plastid sedimentation is commonly related to gravitropism in moss protonemata. Gravitropic reorientation of *Ceratodon* protonemata was completely abolished by centrifugating the plastids out of the tip, which proves the role of the plastids as statoliths (Walker and Sack 1991). Plastid sedimentation in the basal zone was not correlated with

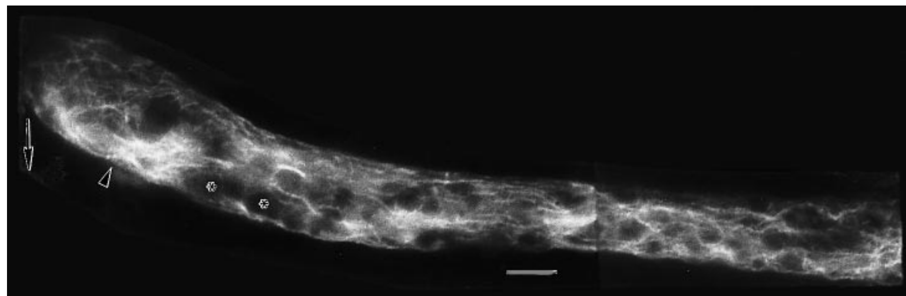

Fig. 9. Immunofluorescence microtubule labeling of a *Ceratodon* apical protonema cell. The tip of the protonema is towards the left. The protonema was horizontal for several hours, as is evidenced by the upward curvature and by the sedimented plastids distinguishable by the absence of tubulin immunofluorescence (*). There is a rich network of microtubules, including in the apical dome. Note the brighter fluorescence from the enrichment in microtubules located in the lower flank of the plastid-free zone (*arrowhead*). The gravity vector is indicated by the *arrow*. ×1250; bar = 10 μm. (Reproduced, with permission, from Sack 1993)

gravitropic response; it must occur in a specific region close to the tip, as is the case in *Chara* rhizoids.

Recently, mutants of *Physcomitrella* and *Ceratodon* have been isolated, whose protonemata show a reversed polarity of gravitropism (Jenkins et al. 1986; Knight and Cove 1989; Sack et al. 1997). Positively gravitropic mutants of *Ceratodon* show initial and mitotic reversals during which they temporarily grow up (Wagner et al. 1996). *Physcomitrella* wrong-way mutants have been genetically analysed (Knight et al. 1991) and provide opportunities for a molecular approach to study the mechanism of gravitropic tip-growth.

Conclusion and future outlook

Studies on gravitropically tip-growing cells reveal the involvement of cytoskeletal elements in the maintenance of polarized cytoplasmic organization and in the processes of gravitropism.

Further experiments in microgravity should improve our knowledge on the cytoskeleton-mediated regulation of gravitropic tip-growth. In order to answer the question of whether the new dynamically stable position of statoliths in *Chara* rhizoids is already achieved after 6 min of microgravity (TEXUS flights), observation of the translocation of statoliths during a longer-lasting microgravity period is necessary. Only from such experiments can conclusions on the mechanical and functional interaction of the actin filaments with the (weightless) statoliths be drawn. And only such microgravity experiments will allow us to decide whether and after what time the system is able to adapt to new gravity conditions. Centrifugation experiments in microgravity represent an excellent approach to measure threshold values for the gravisensitivity of tip-growing cells and to allow evaluations of the strength of the actin filaments as well as the in-vivo biophysics of the actomyosin system.

The opposite directions of gravitropic bending of *Chara* protonemata and *Chara* rhizoids may be based on different properties of the actin cytoskeleton in two very similar cell types. Differences in the positioning and transport of statoliths, as well as in the anchorage of the Spitzenkörper in the apical dome, may reflect differences in function and strength of the actin and other cytoskeletal elements. In addition, it is well documented that the exocytosis of cell wall material focused on a very small area in tip-growing cells requires a steep gradient of calcium concentrations (Herth et al. 1990; see also Sinclair and Trewavas 1997, this issue). Actin-mediated processes (reviewed by Kuroda 1990) and cellular polarity are also influenced by calcium, which makes it likely that calcium is essential for the different mechanisms of polarized growth of rhizoids and protonemata. Therefore, in future experiments, the roles of calcium, the cytoskeleton and associated proteins in the regulation of positive and negative gravitropism have to be studied. *Chara* rhizoids and *Chara* protonemata provide a chance to define the parameters that play a role in the determination of polar growth. At least from the point of view of the cytoskeletal elements, gravitropic tip-growing cells provide excellent opportunities for the analysis of the general mechanism of graviperception and might serve as model systems for gravitropism in higher plants.

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References

Audus LJ (1962) The mechanism of the perception of gravity by plants. Symp Soc Exp Biol 16: 197–226

Bartnik E, Sievers A (1988) In-vivo observation of a spherical aggregate of endoplasmic reticulum and of Golgi vesicles in the tip of fast-growing *Chara* rhizoids. Planta 176: 1–9

Bartnik E, Hejnowicz Z, Sievers A (1990) Shuttle-like movements of Golgi vesicles in the tip of growing *Chara* rhizoids. Protoplasma 159: 1–8

Björkman T (1988) Perception of gravity by plants. Adv Bot Res 15: 1–41

Braun M (1996a) Immunolocalization of myosin in rhizoids of *Chara globularis* Thuill. Protoplasma 191: 1–8

Braun M (1996b) Anomalous gravitropic response of *Chara* rhizoids during enhanced accelerations. Planta 199: 443–455
Braun M, Sievers A (1993) Centrifugation causes adaptation of microfilaments; studies on the transport of statoliths in gravity sensing *Chara* rhizoids. Protoplasma 174: 50–61
Braun M, Sievers A (1994) Role of the microtubule cytoskeleton in gravisensing *Chara* rhizoids. Eur J Cell Biol 63: 289–298
Braun M, Buchen B, Sievers A (1996) Fixation procedure for transmission electron microscopy of *Chara* rhizoids under microgravity in a Spacelab (IML-2). J Biotechnol 47: 245–251
Buchen B, Braun M, Hejnowicz Z, Sievers A (1993) Statoliths pull on microfilaments. Experiments under microgravity. Protoplasma 172: 38–42
Buder J (1961) Der Geotropismus der Characeenrhizoide. Ber Dtsch Bot Ges 74: (14)–(23)
Cai WM, Braun M, Sievers A (1997) Displacement of statoliths in *Chara* rhizoids during horizontal rotation on clinostats. Acta Biol Exp Sinica, 30: 147–155
Doonan JH, Cove DJ, Lloyd CW (1988) Microtubules and microfilaments in tip growth: evidence that microtubules impose polarity on protonemal growth in *Physcomitrella patens*. J Cell Sci 89: 533–540
Hejnowicz Z, Sievers A (1981) Regulation of the position of statoliths in *Chara* rhizoids. Protoplasma 108: 117–137
Herth W, Reiss H-D, Hartmann E (1990) Role of calcium ions in tip growth of pollen tubes and moss protonema cells. In: Heath IB (ed) Tip growth in plant and fungal cells. Academic Press, San Diego, pp 91–118
Hodick D (1993) The protonema of *Chara* fragilis Desv.: regenerative formation, photomorphogenesis, and gravitropism. Bot Acta 106: 388–393
Hodick D (1994) Negative gravitropism in *Chara* protonemata and rhizoids: a model integrating the opposite gravitropic responses of protonemata and rhizoids. Planta 195: 43–49
Jenkins GI, Courtice GRM, Cove DJ (1986) Gravitropic responses of wild type and mutant strains of the moss *Physcomitrella patens*. Plant Cell Environ 9: 637–644
Knight CD, Cove DJ (1989) The genetic analysis of tropic responses. Environ Exp Bot 29: 57–70
Knight CD, Futers TS, Cove DJ (1991) Genetic analysis of a mutant class of *Physcomitrella patens* in which the polarity of gravitropism is reversed. Mol Gen Genetics 230: 12–16
Kuroda K (1990) Cytoplasmic streaming in plant cells. Int Rev Cytol 121: 267–307
Laurinavičius R, Stockus A, Buchen B, Sievers A (1996) Structure of cress root statocytes in microgravity (Bion 10 mission). Adv Space Res 17: 91–94
Leitz G, Schnepf E, Greulich KO (1995) Micromanipulation of statoliths in gravity-sensing *Chara* rhizoids by optical tweezers. Planta 197: 278–288
Meske V, Hartmann E (1995) Reorganization of microfilaments in protonemal tip cells of the moss *Ceratodon purpureus* during the phototropic response. Protoplasma 188: 59–69
Perbal G, Driss-Ecole D, Tewinkel M, Volkmann D (1997) Statocyte polarity and gravisensitivity in seedling roots grown in microgravity. Planta 203: S57–S62
Sack FD (1991) Plant gravity sensing. Int Rev Cyt 127: 193–252
Sack FD (1993) Gravitropism in protonemata of the moss *Ceratodon*. Mem Torrey Bot Club 25: 36–44
Sack FD (1997) Plastids and gravitropic sensing. Planta 203: S63–S68
Sack FD, Wagner TA, Kern VD (1997) Gravitropism in moss protonemata. J Bryol, in press
Schwuchow J, Sack FD (1993) Effects of inversion on plastid position and gravitropism in *Ceratodon* protonemata. Can J Bot 71: 1243–1248
Schwuchow J, Sack FD, Hartmann E (1990) Microtubule distribution in gravitropic protonemata of the moss *Ceratodon*. Protoplasma 159: 60–69
Schwuchow JM, Kim D, Sack FD (1995) Caulonemal gravitropism and amyloplast sedimentation in the moss *Funaria*. Can J Bot 73: 1029–1035
Sievers A, Schnepf E (1981) Morphogenesis and polarity of tubular cells with tip growth. In: Kiermayer O (ed) Cytomorphogenesis in plants (Cell biology monographs, vol 8). Springer, Wien New York, pp 265–299
Sievers A, Braun M (1996) The root cap: structure and function. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots – the hidden half. 2nd edn, Marcel Dekker, New York, pp 31–49
Sievers A, Heinemann B, Rodriguez-Garcia MI (1979) Nachweis des subapikalen differentiellen Flankenwachstums im *Chara*-Rhizoid während der Graviresponse. Z Pflanzenphysiol 91: 435–442
Sievers A, Braun M, Hejnowicz Z (1994) Gravity and the cytoskeleton. In: Proc Fifth Eur Symp on Life Sci Res in Space, Arcachon. ESA Publications Division, ESA SP-366, Noordwijk, pp 15–17
Sievers A, Buchen B, Hodick D (1996) Gravity sensing in tip-growing cells. Trends Plant Sci 1: 273–279
Sievers A, Kramer-Fischer M, Braun M, Buchen B (1991a) The polar organization of the growing *Chara* rhizoid and the transport of statoliths are actin-dependent. Bot Acta 104: 103–109
Sievers A, Buchen B, Volkmann D, Hejnowicz Z (1991b) Role of the cytoskeleton in gravity perception. In: Lloyd CW (ed) The cytoskeletal basis for plant growth and form. Academic Press, London, pp 169–182
Sinclair W, Trewavas AJ (1997) Calcium in gravitropism. A re-examination. Planta 203: S85–S90
Volkmann D, Behrens HM, Sievers A (1986) Development and gravity sensing of cress roots under microgravity. Naturwissenschaften 73: 438–441
Volkmann D, Buchen B, Hejnowicz Z, Tewinkel M, Sievers A (1991) Oriented movement of statoliths studied in a reduced gravitational field during parabolic flights of rockets. Planta 185: 153–161
Wagner TA, Cove DJ, Sack FD (1996) A positively gravitropic mutant mirrors the wild-type protonematal response in the moss *Ceratodon*. In: Suge H (ed) Plants in space biology, Institute of genetic ecology, Tohoku University, Japan, pp 53–60
Walker LM, Sack FD (1990) Plastids as possible statoliths in gravitropic protonemata of the moss *Ceratodon purpureus*. Planta 181: 71–77
Walker LM, Sack FD (1991) Recovery of gravitropism after basipetal centrifugation in protonemata of the moss *Ceratodon purpureus*. Can J Bot 69: 1737–1744
Walker LM, Sack FD (1995a) Microfilament distribution in protonemata of the moss *Ceratodon*. Protoplasma 189: 229–237
Walker LM, Sack FD (1995b) Ultrastructural analysis of cell component distribution in the apical cell of *Ceratodon* protonemata. Protoplasma 189: 238–248
Young JC, Sack FD (1992) Time-laps analysis of gravitropism in *Ceratodon* protonemata. Am J Bot 79: 1348–1358

Additional references (this issue)

Baluska F, Hasenstein KH (1997) Root cytoskeleton: its role in perception of and response to gravity. Planta 203: S69–S78
Häder D-P, Hemmersbach R (1997) Graviperception and graviorientation in flagellates. Planta 203: S7–S10
Hoson T, Kamisaka S, Masuda Y, Yamashita M, Buchen B (1997) Evaluation of the three-dimensional clinostat as a simulator of weightlessness. Planta 203: S187–S197
Kern VD, Mendgen K, Hock B (1997) *Flammulina* as a model system for fungal graviresponses. Planta 203: S23–S32
Ruyters G, Scott TK (1997) Future research in plant biology in space: summary of critical issues and recommendations of the workshop. Planta 203: S211–S213
Scherer GFE (1997) General discussion on graviperception. Planta 203: S107–S111