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Hypergravity can reduce but not enhance the gravitropic response of *Chara globularis* protonemata

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Dedicated to Prof. Dr. Zygmunt Hejnowicz on the occasion of his 70th birthday

Summary. The relationship between the position of the statoliths and the direction and rate of tip growth in negatively gravitropic protonemata of *Chara globularis* was studied with a centrifuge video microscope. Cells placed perpendicularly to the acceleration vector (stimulation angle 90°) showed a gradual reduction of the gravitropic curvature with increasing accelerations from 1 **g** to 8 **g** despite complete sedimentation of all statoliths on the centrifugal cell flank. It is argued that the increased weight of the statoliths in hypergravity impairs their acropetal transport which is induced when the cell axis deviates from the normal upright orientation. When the statoliths were centrifuged deep into the apical dome at 6 **g** and a stimulation angle of 170° the gravitropic curvature after 1 h was identical to that determined for the same cells at 1 **g** and the same stimulation angle. This indicates that gravitropism in Chara protonemata is either independent of the pressure exerted by the statoliths on an underlying structure or is already saturated at 1 **g**. When the statoliths were moved along the apical cell wall at 8 **g** and the stimulation angle was gradually increased from 170° to 220° the gravitropic curvature reverted sharply when the cluster of statoliths passed over the cell pole. This experiment supports the hypothesis that in Chara protonemata asymmetrically distributed statoliths inside the apical dome displace the Spitzenkörper and thus the centre of growth, resulting in gravitropic bending. In contrast to the positively gravitropic Chara rhizoids, no modifications either in the transport of statoliths during basipetal acceleration (6 **g**, stimulation angle 0°, 5 h) or in the subsequent gravitropic response could be detected in the protonemata. The different effects of centrifugation on the positioning of statoliths in Chara protonemata and rhizoids indicate subtle differences in the function of the cytoskeleton in both types of cells.

Keywords: Centrifuge microscope; *Chara globularis*; Gravitropism; Hypergravity; Protonema; Tip growth.

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Introduction

The green algae *Chara* spp. generate two types of tip-growing cells with opposite gravitropism. Rhizoids are positively gravitropic and grow into the soil to anchor the thallus (Buder 1961, Sievers et al. 1996) whereas protonemata are negatively gravitropic cells which give rise to a new thallus after completing a complex photomorphogenesis (Maeda and Imahori 1968; Hodick 1993, 1994). Both types of cells use small vacuoles with a dense core as statoliths, identical in light and electron microscopy. With X-ray microanalysis the core was identified as comprising crystals of $BaSO_4$ (Schröter et al. 1975; M. Braun, Botanisches Institut, Universität Bonn, pers. commun.). It has been shown that the mineral core is embedded in a matrix of proteins and carbohydrates in rhizoids (Wang-Cahill and Kiss 1995). Statoliths experience acceleration and sediment when the spatial orientation of the cell changes. Sedimentation may be perceived by sensitive structures as a change in pressure or tension exerted by the sedimenting statoliths. In addition, redistribution of the statoliths can generate a gravitropic signal by displacing and rearranging other cellular components (Björkman 1988).

In Chara protonemata and rhizoids the statoliths are not free to sediment to the physically lowest point of the cell, but their position is under control of the cytoskeleton (reviewed by Sievers et al. 1996, Braun 1997). In upward-growing protonemata the statoliths are found up to 100 μm from the tip and are only spo-

radically and briefly transported into the apical dome. Only after tilting a protonema by, e.g., 90° are most or all the statoliths transported into the apical dome where they settle within approximately 15 min. It has been suggested that subsequent reorientation of growth results from the displacement of the Spitzenkörper (tip body), and thus the centre of growth in the apical dome, by the statoliths which temporarily occupy a considerable portion of the lower half of the apical dome (Hodick 1994, Hodick et al. 1998).

This yet hypothetical mechanism differs in key aspects from those proposed for the other tip-growing cells in which gravitropism has been studied in detail. In the positively gravitropic rhizoids of *Chara* spp. the centre of growth is fixed at the cell pole (Sievers et al. 1979). Gravitropic curvature is probably achieved by a subapical blockade by the settled statoliths of exocytosis of Golgi vesicles with cell wall material. Thus the local rate of cell wall extension is reduced at the subapical site compared to the physically upper cell wall (Sievers 1967, Sievers and Schröter 1971). From centrifugation experiments with Chara rhizoids it was concluded that pressure of the sedimented statoliths on the plasma membrane can enhance the gravitropic stimulus (U. Friedrich and Hertel 1973). In the negatively gravitropic protonemata of the moss *Ceratodon purpureus* sedimentation of amyloplasts, i.e., the putative statoliths in these cells, only occurs 20–70 μm from the cell pole (Walker and Sack 1990, 1991; Sack 1993) which makes transmission of the gravitropic signal from the site of sedimentation to the site of growth, i.e., the apical dome, indispensable. The mechanism of gravitropism proposed for the Chara protonema does not consider a blockade of exocytosis beneath sedimented statoliths nor an effect of the pressure of sedimented statoliths on the underlying plasma membrane nor the transmission of a signal from subapically sedimented statoliths as a decisive step in establishing gravitropic curvature.

In the present study a centrifuge video microscope was used to displace the statoliths in Chara protonemata under simultaneous microscopic observation. By centrifugation the naturally occurring movements of the statoliths could either be supported or counteracted. The pressure of the statoliths on a potentially sensitive structure could be increased and, furthermore, the statoliths could be concentrated in a small volume. Thus video-controlled centrifugation allowed a test of whether increased pressure of sedimented statoliths on the plasma membrane alters the graviresponse and identification of the location where graviperception takes place. In addition, we looked for adaptations of the statolith-positioning system to hypergravity, as observed in Chara rhizoids (Braun and Sievers 1993). In Chara rhizoids statolith positioning was surprisingly stable in hypergravity up to 6 **g** (Braun and Sievers 1993). The different effects of hypergravity on the positioning of statoliths in protonemata are in agreement with the different mechanisms proposed to explain the occurrence of either positive or negative gravitropism in the two closely related cells.

Material and methods

Thalli of *Chara globularis* Thuill. were collected from a fresh-water pond in the Botanical Garden Bonn (Bonn, Federal Republic of Germany). Thallus nodes were isolated by cutting off all adjacent internodes and branchlets. The nodes were embedded in agar gels on microscope slides as described earlier (Hodick 1993) and kept in darkness for two to three weeks. The preparations were then screened in dim green safe light for newly formed protonemata springing from the upper side of the isolated nodes.

For experiments segments were cut from the agar gels which contained a node with one or several outgrowing protonemata. These gel segments (approx. 5 mm by 10 mm, 1 mm thick) were transferred to flat perspex cuvettes for microscopy and centrifugation. The cuvettes were filled with agar to give a new gel of approx. 10 mm by 45 mm by 2 mm, which enclosed the gel fragment with the isolated node and protonemata. After 1 day the protonemata had recovered from the embedding procedure and were grown for approx. 1 week in these cuvettes in darkness.

Centrifugation experiments were carried out on the slow-rotating-centrifuge microscope NIZEMI Labmodel-1 (Dornier GmbH, Friedrichshafen, Federal Republic of Germany). This device makes it possible to expose the cells to accelerations of up to 8 **g** and to observe them simultaneously in video-microscopy. The orientation of the cells relative to the acceleration vector could be changed by rotating the specimen via remote control. Technical details of the centrifuge microscope were presented by Häder et al. (1991) and U. L. D. Friedrich et al. (1996). The cuvettes were mounted on the microscope stage of the centrifuge microscope about 1 h prior to the start of centrifugation. The cells were observed with an objective with long-working distance (Achroplan ×32, N. A. 0.4; Zeiss, Oberkochen, Federal Republic of Germany) and a yellow-green broadband interference filter (Zeiss) inserted into the condenser to exclude wavelengths shorter than 500 nm which can trigger photomorphogenesis in the protonemata. The centrifuge reached the maximal acceleration used (8 **g**) within 1 min. Video-images were recorded with a VHS video recorder and analysed on a flat-screen monitor. All video-micrographs were mounted with the acceleration vector pointing downward.

Stimulation angles were denoted as 0° for cells with their tip oriented against the direction of the acceleration vector, i.e., the natural position, and 180° for the inverted position. Growth rates and bending angles were determined on transparencies by tracing the outlines of the cells from the monitor screen. Growth was measured by the length of a line connecting the cell poles on tracings made in defined, short intervals of time, which gave a good approximation of the elon-

gation rate of the cell axis. Curvature was determined by measuring the angle between the orientation of the cell axis at the beginning of an experiment and the actual axis of the apical dome. This actual axis was constructed as a line passing through the apical cell pole and which had equal distances from cell wall 15 µm behind the cell pole. The statistical significance of differences between the means of samples was probed in a t-test (Parker 1979).

Results

Gravitropism at different accelerations and a stimulation angle of 90°

In order to test whether an increase in mass acceleration had an effect on the gravitropic response of the protonemata the cells were centrifuged for 1 h at accelerations of 2 **g**, 4 **g**, and 8 **g** and the acceleration vector perpendicular to the cell axis (stimulation angle 90°). Then the gravitropic curvature was determined and compared to the curvature of the same cells stimulated under 1 **g** for 1 h. The interval between both runs was always shorter than 20 h. Thus differences in curvature resulting from possible variability in the graviresponses of different samples were ruled out. At 1 **g** all cells selected for the experiments bent by approx. 40° during 1 h of stimulation (Fig. 1). A few cells (approx. 15%) with a response of less than

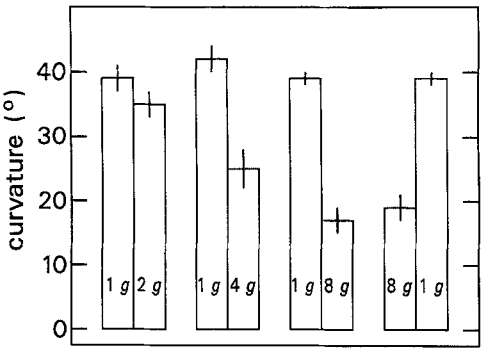

Fig. 1. Effects of hypergravity on the gravitropic responses of Chara protonemata. Each pair of columns presents the mean curvatures of 10 cells (bars represent standard error of the mean), which were gravistimulated at 1 **g** as a control and, after an interval of recovery, in hypergravity at 2 **g**, 4 **g**, and 8 **g**. The stimulation angle was 90° with respect to the cell axis, the curvatures were determined after 1 h of gravistimulation. The 8 **g** experiment was also performed in reverse order, i.e., the 1 **g** control was run after centrifugation, to rule out that refractoriness reduced the curvatures in cells which were gravistimulated twice

30° were not considered further. Increasing the acceleration to 2 **g** reduced the curvature slightly to 91% of the 1 **g** control, which is not statistically significant ($P > 0.1$). At 4 **g** and 8 **g** the gravitropic curvature was reduced to 60% and 42%, respectively, of the 1 **g** con-

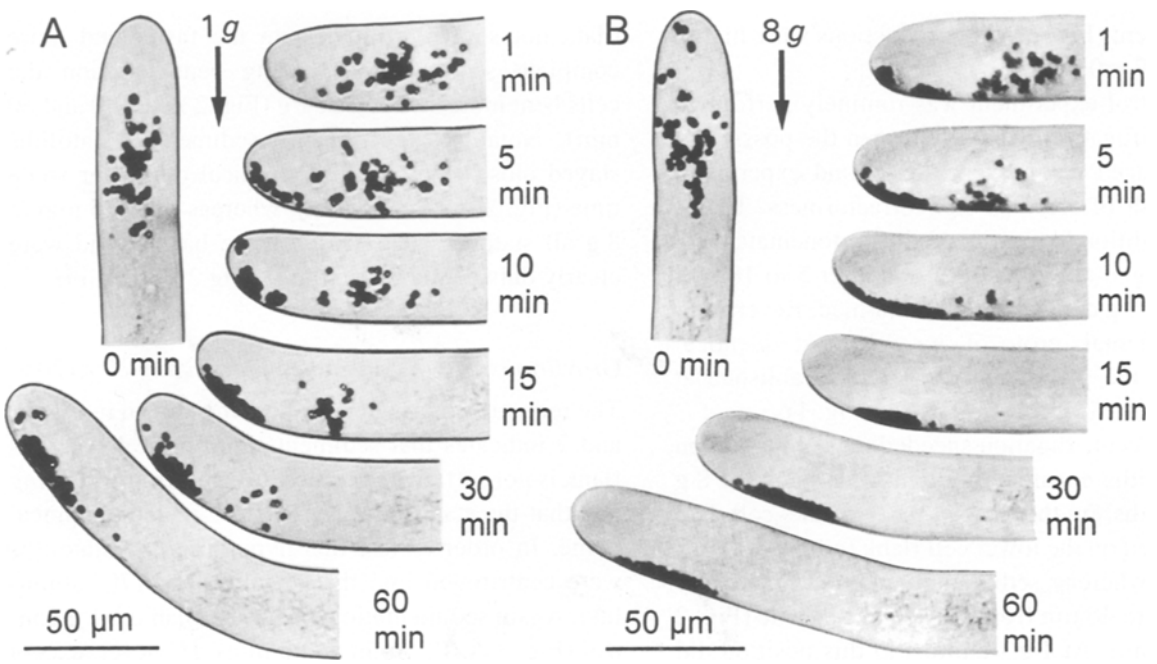

Fig. 2 A, B. Video-micrographs of the gravitropic response of the same protonema at 1 **g** (**A**) and at 8 **g** (**B**) with a stimulation angle of 90°. In both sequences the time point 0 min presents the cell in the normal upright position at 1 **g** before the onset of stimulation. Lateral stimulation at both 1 **g** and 8 **g** induced massive transport of statoliths into the apical dome. However, at 8 **g** the statoliths were soon removed from the apical dome (**B**, 15 min onwards) and the ensuing cell curvature was much reduced compared to the 1 **g** control (**A**)

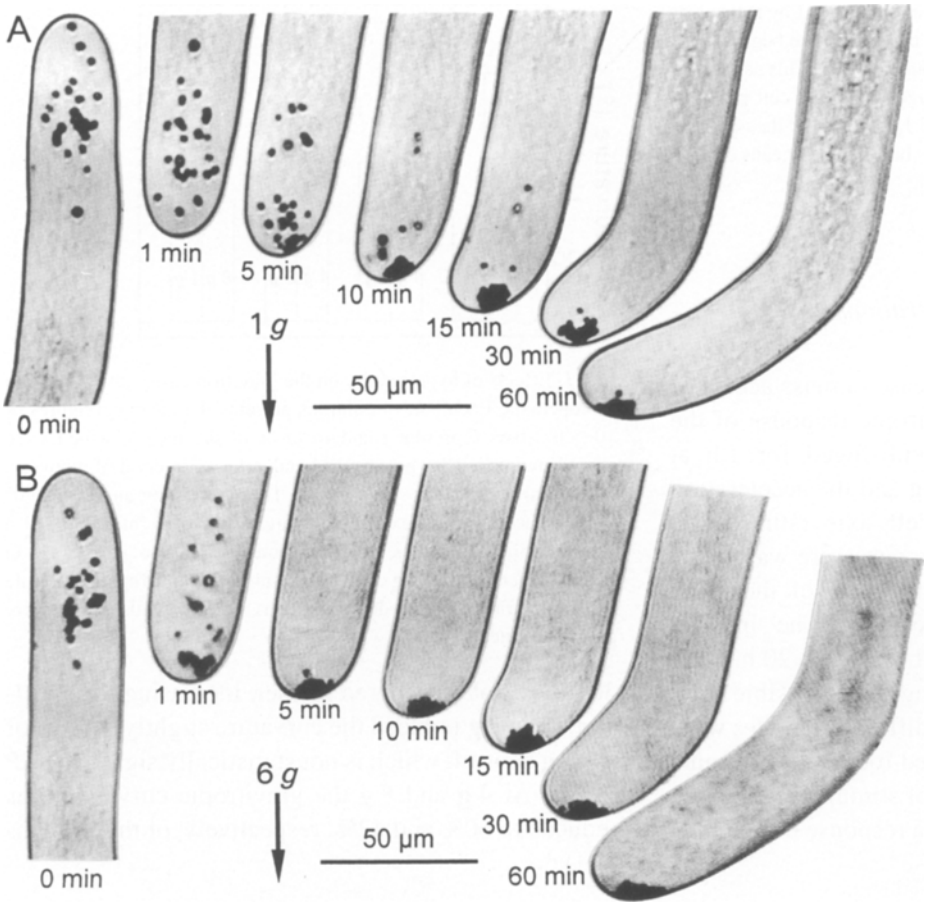

Fig. 3 A, B. Video-micrographs of the gravitropic responses of the same protonema at 1 **g** (**A**) and at 6 **g** (**B**) with a stimulation angle of 170°. In both sequences the time point 0 min presents the cell in the normal upright position at 1 **g** before the onset of stimulation. In both experiments most or all statoliths settled on the apical cell wall slightly lateral to the cell axis. At 1 **g** sedimentation and the appearance of a first asymmetry in cell shape lagged approx. 5 min behind the stimulation at 6 **g** (**B**). But from 15 min onwards both statolith distribution and curvature were very similar

trol experiment (Fig. 1), these reductions were highly significant ($P < 0.01$).

The 1 **g** control experiment was routinely performed before centrifugation, which left open the possibility that the reduced response in the second experiment was the result of some sort of refractoriness. Therefore in an additional experiment the protonemata were first centrifuged at 8 **g** and 90° and after 5 to 18 h of recovery the 1 **g** controls were performed. Reversal of the experimental protocol gave almost identical results, i.e., the gravitropic curvature established at 8 **g** was only 49% of the 1 **g** control (Fig. 1).

As expected centrifugation speeded up sedimentation of the statoliths considerably and after 1 min at 8 **g** most statoliths in the apical part of the cell had already settled on the lower cell flank (compare Fig. 2 A with B) whereas sedimentation proceeded more slowly at 30 to 40 µm from the apical cell pole (Fig. 2 B, 1 to 10 min). At 1 **g** statoliths in this position did not settle even after 30 min of gravistimulation (Fig. 2 A, 30 min). These statoliths were probably trapped between massive stacks of endoplasmic reticulum, which run through the cytoplasm except at the apex (data not shown). But despite the faster and more complete sedimentation during centrifugation the cells bent more strongly at 1 **g** (Fig. 2 A, B; 30 and 60 min). Notably, at 1 **g** many sedimented statoliths stayed close to or inside the apical dome for some time (Fig. 2 A, 5 to 15 min), whereas after 15 min at 8 **g** all statoliths had settled more basally and were clearly outside the apical dome (Fig 2 B, 15 min).

*Gravitropism at 6 **g** and a stimulation angle of 170°*

The reduction of gravitropic bending shown in Figs. 1 and 2 indicates that sedimentation on the lower cell flank is not sufficient to cause full gravitropic bending and that the statoliths have to settle inside the apical dome. In order to test this hypothesis the statoliths were centrifuged into the apical dome (170° stimulus). Again sedimentation was faster than the 1 **g** control (Fig. 3 A, B; 1 min and 5 min). However, except for a time delay of approx. 5 min the distribution of statoliths after sedimentation was almost identical at 1 **g** and 6 **g**. Also the gravitropic curvatures were almost the same after 1 h of stimulation with 58 ± 13°

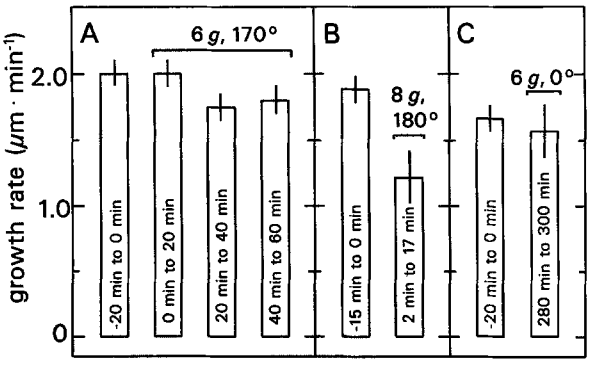

Fig. 4 A–C. Effects of centrifugation on the growth rates of protonemata. The growth rates (bars represent standard error of the mean; n = 10) were calculated from the cell elongation determined for the different intervals of time given in each column, with negative time values denoting measurements before the onset of stimulation, i.e., at 1 **g** and the normal, upright position of the cell. **A** Cells were centrifuged at 6 **g** and a stimulation angle of 170° for 1 h. **B** Cells were exposed to 8 **g** for 17 min and measurements started 2 min after the onset of centrifugation, i.e., after most or all statoliths had settled in the apical dome (cf. Fig. 3). **C** Cells were centrifuged at 6 **g** acting in a basal direction (stimulation angle 0°) for 5 h and measurements were made over the final 20 min

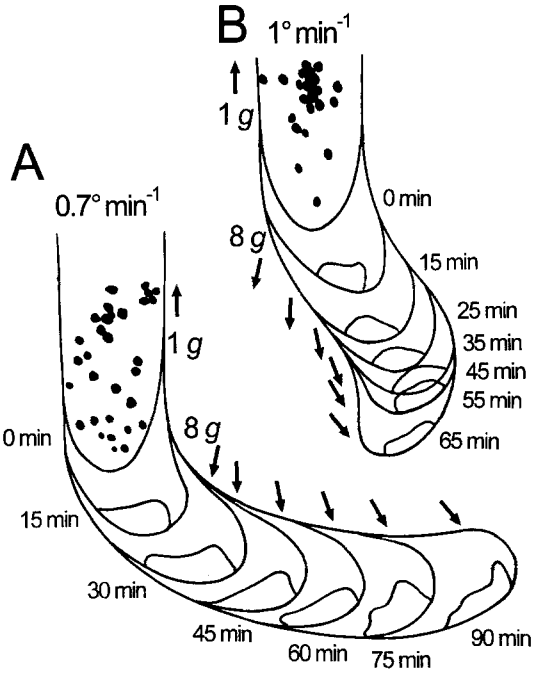

Fig. 5 A, B. Gravitropic responses with steadily increasing stimulation angles. The cells were centrifuged at 8 **g** and stimulation angle of 170° for 15 min to induce a distinct initial curvature. The time from the start of the centrifugation is presented with the corresponding outline of the cell and of the cluster of statoliths. Arrows indicate the direction of acceleration. After 15 min the stimulation angle was increased by rotating the stage of the centrifuge microscope. **A** Stimulation angle was increased by 10° every 15 min (i.e., 0.7°/min) and the cell kept bending in the same direction for 90 min. **B** Stimulation angle was increased by 10° every 10 min (i.e., 1°/min). From 35 min to 55 min growth rate declined, simultaneously the cluster of statoliths moved from a position left of the cell pole to a position right of the cell pole. This movement coincided with a reversal of the direction of curvature

at 1 **g** and 57 ± 13° (mean with standard deviation; n = 10) at 6 **g**. Due to the large stimulation angle of 170° the sedimented statoliths covered a considerable portion of the plasma membrane of the apical dome throughout the centrifugation experiment (Fig. 3 B). Nevertheless tip-growth measured as the elongation rate of the cell axis during bending was not inhibited (Fig. 4 A): the small difference in rate after 20 min of centrifugation was not statistically significant ($P > 0.1$).

*Gravitropism at 8 **g** and steadily increasing stimulation angles*

In order to decide whether statoliths induced gravitropic bending while they were moving towards the tip or after they had settled in the apical dome, the gravitropic responses of cells were studied with the statoliths sedimented in different positions within the apical dome. Protonemata were first centrifuged at 8 **g** and a stimulation angle of 170° for 15 min to accumulate all statoliths on one flank of the apical dome. The stimulation angle was then increased in steps of 10° in different intervals of time. In Fig. 5 A the specimen was rotated by 10° every 15 min (i.e., 0.7°/min) and bent by approx. 90° within 1 h. Throughout centrifugation the position of the cluster of statoliths with respect to the cell pole remained constant. Another cell was tilted by 10° every 10 min (i.e., 1°/min; Fig. 5 B). After increasing the stimulation angle from 170° to 200° (t = 45 min) the direction of curvature reverted abruptly and the cell bent sharply in the opposite direction. This reversal of gravitropic curvature occurred reproducibly when rotating the cells with 1°/min or more (Fig. 6). During rotations with 1°/min the direction of growth changed abruptly by 61 ± 10° (mean with standard deviation; n = 6) within 20 min. Before this reorientation the cluster of statoliths, which was sedimented on the flank of the apical dome (Fig. 5 B, 25 to 45 min) was slightly displaced. The cluster slid from a position slightly lateral of the cell axis across the cell pole immediately before the reversal of curvature occurred.

In about half the cells observed the growth rate fell transiently before the reversal of curvature while the other cells continued to grow uninhibited. This may

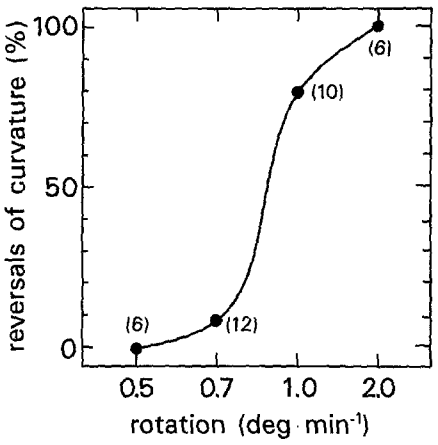

Fig. 6. Dependence of the occurrence of reversals in gravitropic curvature on the rate of increase in stimulation angle. Cells were centrifuged at 8 **g** and the stimulation angle was increased from 170° to 220° at different speeds. The ordinate presents the percentage of centrifuged cells (total numbers in parentheses) which reversed the direction of their gravitropic bending, as shown in Fig. 5 B

indicate that statoliths sedimenting upon the cell pole inhibited tip-growth. This was tested further in an experiment in which the protonemata growth rates were determined during centrifugation at 8 **g** and a stimulation angle of 180°. The cells were inverted immediately before the start of centrifugation and then rotated to follow the changes in the direction of acceleration which occur until a rotor reaches its nominal speed. Thus it was ensured that the gravity vector was always perfectly acropetal throughout the experiment. The statoliths could be pressed upon the plasma membrane at the cell pole which resulted in an average 36% reduction of the growth rates during the initial 15 min of centrifugation (Fig. 4 B). In some cells the tips showed concomitant transverse growth (not shown). Thereafter measurements could not be continued because the tips of most of the cells started to bend randomly with respect to the optical plane of the microscope and the statoliths no longer covered the cell pole. A smaller, 21% reduction in growth rate could be provoked by acropetal centrifugation at 6 **g** (not shown).

Gravitropism after basipetal centrifugation

Protonemata were exposed to an acceleration of 6 **g** and a stimulation angle of 0° for 5 h. Under these conditions the cells kept growing against the vector of acceleration and their growth rates were only slightly affected (Fig. 4 C) except for 2 cells which stopped

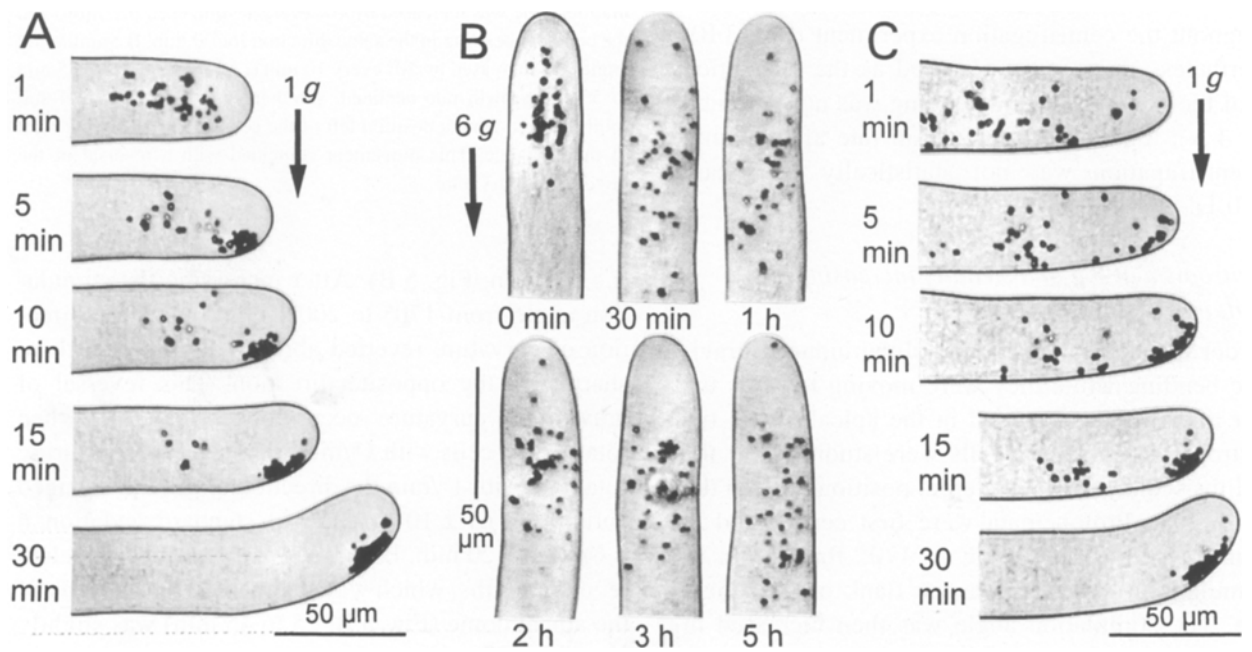

Fig. 7 A–C. Effects of basipetal centrifugation on the distribution of statoliths and on gravitropism. **A** A protonema was first gravistimulated at 1 **g** (stimulation angle 90°) and showed the normal acropetal transport of statoliths and curvature. **B** Then the same cell was centrifuged at 6 **g** in a basal direction (stimulation angle 0°) for 5 h. **C** Most statoliths were dislocated in the basal direction with some fluctuation in their distance from the tip, but even after 5 h single statoliths were found inside the apical dome. Immediately after the end of centrifugation the cell was gravistimulated a second time at 1 **g**, stimulation angle 90°. The statoliths were rapidly transported into the apical dome and after 10 min curvature started with no visible difference to the sequence recorded before centrifugation (**A**)

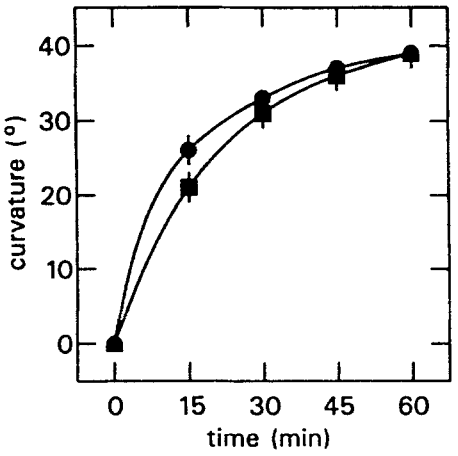

Fig. 8. Effects of basipetal centrifugation (6 **g**, 0°, 5 h) on the time courses of the gravitropic bending of protonemata. The gravitropic response of 10 cells (bars represent the standard error of the mean) to a stimulation at 1 **g** (stimulation angle 90°) was determined before (●) and after centrifugation (■)

growing and were not included in the statistics. The distance of the statoliths from the tip increased (Fig. 7 B) varying markedly between individual cells, but even after 5 h of continuous centrifugation some statoliths were transported against the centrifugal acceleration into the apical dome. However, enhancement of the acropetal transport of statoliths as it occurred in basipetally centrifuged rhizoids (Braun and Sievers 1993) was not observed in protonemata.

In order to study the effect of basipetal centrifugation on the positioning of statoliths the gravitropic response of protonemata was recorded at 1 **g** and a stimulation angle of 90° before and immediately after centrifugation. Within 10 min many statoliths had already settled in the apical dome in both runs and a gravitropic curvature was visible (Fig. 7 A, C). During the initial 10 min the distribution of statoliths differed slightly, in that after centrifugation the statoliths entered the apical dome at a higher position and moved closer to the apical cell wall. But after only 15 min of gravistimulation the distributions of statoliths before and after centrifugation were indistinguishable (Fig. 7 A, C). The time-courses of gravitropic bending were almost identical before and after centrifugation (Fig. 8) with the small difference of 5° at $t = 15$ min not being statistically significant ($P > 0.1$).

Discussion

The gravitropism of Chara protonemata was studied in hypergravity in order to test the hypothesis that the gravitropic curvature of these cells is induced and controlled by the presence of statoliths at a distinct position inside the apical dome. Under several conditions the cells responded in accordance with the hypothesis.

Lateral centrifugation (stimulation angle 90°) reduced the gravitropic bending of the protonemata consistently and in a dose-dependent manner. This effect seemed paradoxical at first since, according to the dose–response rule, a gravitropic stimulus should be stronger in hypergravity than at 1 **g**, as shown for multicellular organisms (Volkmann and Sievers 1979). The reduced bending in Chara protonemata during lateral centrifugation could be explained as a composite response comprising positive and negative gravitropism similar to the one which could be enforced upon Chara rhizoids by centrifuging the statoliths into the apical dome (Braun 1996). Analogously, subapically sedimented statoliths in the protonema could inhibit the growth of the underlying cell wall due to a blockade of exocytosis. This would cause downward bending by differential subapical growth, similar to the positively gravitropic response of the rhizoid. As a result, the normal, negatively gravitropic response of the protonema would be partially offset by inhibition of growth on the lower cell wall, a positively gravitropic response. However, this explanation conflicts with the observations that (1) in protonemata the sedimentation of statoliths during lateral centrifugation (Fig. 2 B from 15 min onwards) occurs outside the apical dome and is thus more basal than in gravistimulated rhizoids, and (2) a mixed positively and negatively gravitropic response should be more pronounced with the distribution of statoliths observed during gravistimulation in 1 **g** (Fig. 2 A; 5–15 min). In this situation the statoliths actually cover the growing lower flank of the apical dome. Nevertheless the upward curvature is much stronger than during centrifugation (Fig. 2 B).

Alternatively, the reduced gravitropic response in Chara protonemata during lateral centrifugation could result from the increase in weight of the statoliths. This increase makes transport of statoliths into the apical dome more difficult as soon as the protonema starts growing upwards against the vector of acceleration. This interpretation is in accordance with the observed distribution of statoliths shown in Fig. 2. The strong reduction of the gravitropic curvature, despite the complete sedimentation of statoliths on the centrifugal cell flank, indicates that lateral displacement of statoliths without simultaneous trans-

port into the apical dome is not a strong enough signal in the stimulus-response chain of Chara protonemata. In this respect gravitropism in Chara protonemata differs considerably from that in the protonemata of the moss *Ceratodon purpureus*. In the latter subapical sedimentation of amyloplasts generates a signal which is transmitted to the growth machinery in the apical dome and triggers gravitropic bending. A recent model coupling enrichment in microtubules proximal to the sedimented statoliths (Schwuchow et al. 1990) to the redistribution of dictyosomes could not be confirmed (Walker and Sack 1997).

Hypergravity increases the weight of statoliths and thus the pressure they exert on the structures upon which they sediment. However, acropetal centrifugation (170° stimulus) did not increase the gravitropic response. This indicates that gravitropism in Chara protonemata either does not depend upon the pressure exerted by sedimented statoliths or that a potentially pressure-sensitive system is already saturated at a mass acceleration of 1 **g**. Since the gravitropic response was observed during permanent gravistimulation it was not possible to decide whether the response, i.e., the reorientation of the cell axis, or a preceding step in graviperception are limiting the extent of the curvature. Gravistimulation in hypergravity followed by curvature without a gravitropic stimulus, e.g., after removing the statoliths from the very tip by basipetal centrifugation, was not feasible with protonemata due to an autotropic response (data not shown), which indicates that the Chara protonema has an immediate "memory loss" (Brown 1992) with respect to any preceding gravistimulation.

In fact, an upper limit for the curvature of Chara protonemata became evident in the experiments with constantly increasing stimulation angles (Fig. 5 A). During this type of stimulation the position of the statoliths within the apical dome remained almost constant over a long time. Nevertheless bending was clearly discontinuous with the highest rate observed during the initial 15 min of stimulation (43°), and a rapid decline to 17°, 10°, and 8° during the following 15 min intervals. This might indicate that the initial acropetal transport of the statoliths from their resting position into the apical dome generates a stronger signal for tip reorientation than the more static position of the statoliths during the following slow rotation of the cell. However, this contradicts the observations made during the reversal of curvature (Fig. 5 B) when statolith displacement is very small and occurs only inside the apical dome. Nevertheless this small displacement evokes the strongest bending response observed so far in Chara protonemata (61 ± 10° within 20 min, mean with standard deviation; n = 6).

The strong decline of the bending rate despite almost unchanged distribution of the statoliths inside the apical dome (Fig. 5 A) may result from an upper limit for the curvature a protonema can generate. Accordingly, only deviations in the direction of growth up to this limiting angle can be performed in a sharp bend. If gravistimulation continues after bending, i.e., if the tip of the cell has not yet reached upright orientation, the old cell axis may exert an increasing resistance against further bending. A structural basis is provided by the cytoskeleton of the nonvacuolated portion of the cell. The dense cytoplasm contains a framework of microtubules (Braun and Wasteneys 1998) which has to be bent to follow the reorientation of the tip. Perhaps the mechanical deformation of the cytoplasm and especially of the microtubules limits the curvature, e.g., by limiting the range over which the Spitzenkörper can be displaced laterally.

During oblique acropetal centrifugation (6 **g**, 170°) growth rate was not reduced despite the sedimentation of the statoliths on the plasma membrane inside the apical dome. Only when statoliths settled symmetrically upon the cell pole (stimulation angle 180°, 6–8 **g**) was tip growth inhibited at least transiently, while sometimes the tip diameter increased. We assume that during acropetal centrifugation (180°) the statoliths occupy the area of the plasma membrane where cell wall expansion and the rate of exocytosis are maximal (cf. Hejnowicz and Sievers 1971, Sievers et al. 1979) and from which statoliths are always excluded at 1 **g**, even during inversion of the cell (Hodick 1994). The nature of this area is not clear, but it seems unlikely that it reflects the only site of exocytosis of cell wall material. But covering the plasma membrane with statoliths some micrometres lateral to this area did not cause changes in the growth rate. Probably the simultaneous displacement of the Spitzenkörper and thereby the centre of growth makes a new area of plasma membrane available for unrestrained exocytosis, thus making the effects of reduced exocytosis below the settled statoliths on the growth rate negligible.

In basipetal centrifugation the distance between statoliths and the apical cell pole increased with large cell-to-cell variability (not shown). This variability probably reflects the differences in statolith size, with a doubling of the diameter of a statolith increasing its volume and thus the force acting on it eightfold.

Immediately after centrifugation the gravitropic curvature developed with only a very small delay in timing, with no pronounced inhibition of sedimentation, as was observed in Chara rhizoids after basipetal centrifugation (Braun and Sievers 1993). But as in rhizoids, the relocated statoliths entered the apical dome before complete sedimentation took place. This is typical for statoliths being transported towards the tip from a position far from the tip (see Hodick 1994: fig. 6 B). Effects of long-term centrifugation on growth and development were not tested, but the growth rates of the tip-growing protonemata of the fern *Adiantum capillus-veneris* remained stable during 8 days of basipetal centrifugation at 10 **g** (Kasahara et al. 1995).

When comparing the effects of moderate hypergravity on Chara protonemata and rhizoids it becomes evident that the statoliths in protonemata are less restricted in their movements. Moderate acropetal centrifugation can press the statoliths upon the plasma membrane at the cell pole, basipetal centrifugation can move the statoliths far away from the apex. In contrast, the statolith position in rhizoids is under rigid cytoskeletal control. The position is almost stable against moderate acropetal centrifugation, and relatively high accelerations (50–200 **g**) are necessary to press the statoliths into the apical dome and to evoke transverse growth of the tip (Braun and Sievers 1993). During basipetal centrifugation adaptational changes of the cytoskeleton occur to ensure the relocation of the statoliths to their normal position (Braun and Sievers 1993). This relocation is crucial in rhizoids because otherwise the cells would no longer be able to correct their direction of growth after inversion (tips pointing upwards). Since the position of the statoliths is controlled by the actin cytoskeleton (Hejnowicz and Sievers 1981, Hodick 1994), the differences in statolith positioning between protonemata and rhizoids indicate subtle differences in the function and perhaps in the organization of their cytoskeleton, which cannot yet be visualised microscopically (Braun and Wasteneys 1998).

One aspect remains to be mentioned which results from the reduction of the gravitropic response of Chara protonemata in hypergravity. By extrapolation it must be assumed that in reduced gravity the gravitropic response to a 90° stimulus will be higher than at 1 **g**. This assumption is in accordance with the finding that the protonemata can produce stronger curvatures (e.g., 58°/h at 1 **g** and a stimulation angle of 170°) when gravity does not pull the statoliths out of the apical dome as it does as soon as a cell starts bending upwards during a 90° stimulation at 1 **g**, bending only about 40°/h. Perhaps experiments in micro- and hypogravity could help to elucidate the interactions between statoliths and the growth machinery in the Chara protonema.

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