Protoplasma (1993) 172: 38–42

PROTOPLASMA



# Statoliths pull on microfilaments

## Experiments under microgravity

**Brigitte Buchen***, **M. Braun**, **Z. Hejnowicz**, and **A. Sievers**

Botanisches Institut, Universität Bonn, Bonn

Received June 29, 1992
Accepted September 2, 1992

Dedicated to Hilton H. Mollenhauer on the occasion of his retirement

**Summary.** Previous videomicroscopy of *Chara* rhizoids during parabolic flights of rockets showed that the weightless statoliths moved basipetally. A hypothesis was offered that the removal of gravity force disturbed the initial balance between this force and the basipetally acting forces generated in a dynamic interaction of statoliths with microfilaments (MFs). The prediction of this hypothesis that the statoliths would not be displaced basipetally during the microgravity phase (MG-phase) after disorganizing the MFs was tested by videomicroscopy of a rhizoid treated with cytochalasin D (CD) immediately before the flight. The prediction was fully supported by the flight experiment. Additionally, by chemical fixation of many rhizoids at the end of the MG-phase it was shown that all rhizoids treated with CD before the flight had statoliths at the same location, i.e., sedimented on the apical cell wall, while all untreated rhizoids had statoliths considerably displaced basipetally from their normal position. Thus, a dynamical interaction involving shearing forces between MFs and statoliths appears highly probable.

**Keywords:** Actin filaments; *Chara* rhizoid; Cytochalasin; Microgravity.

**Abbreviations:** CD cytochalasin D; **g** gravitational acceleration; MF microfilament; MG-phase microgravity phase; TEXUS technological experiments under reduced gravity.

### Introduction

Plants need gravity as a stimulus for oriented growth (Sievers 1991). The unicellular, polar organized rhizoids of the green alga *Chara* respond strictly gravitropically and are favoured cells to study the gravity sensing mechanism (reviews Sievers and Volkmann 1979, Sievers et al. 1991 a). In this rhizoid, gravity is suscepted by heavy particles of $BaSO_4$, the statoliths, which are able to change their position in the rhizoid according to the gravity vector. In normal vertical orientation of the rhizoid, the statoliths are located at a distance of 10–30 μm above the cell tip, their position being dynamically steady (Hejnowicz and Sievers 1981). After removal of the statoliths by basipetal centrifugation, the rhizoids grow but do not bend (Sievers et al. 1991 b). Thus, the correct position of the statoliths complex within the rhizoid and its displacement by gravity are preconditions for final graviresponse.

After treatment of rhizoids by the drugs cytochalasin B or D (CB, CD; see Ohmori et al. 1992 and references therein), the statoliths in normal vertically oriented rhizoids sediment on the apical cell wall, i.e., fall down onto the physically lowest point of the cell (Hejnowicz and Sievers 1981). As shown by labelling with rhodamine-phalloidin, actin filaments form a network in the apical part of the rhizoid (Sievers et al. 1989, 1991 b). It has been proposed that on earth the position of statoliths is balanced by two counteracting forces, the gravitational force and the force mediated by microfilaments (MFs) (Sievers et al. 1991 a, Volkmann et al. 1991). In normal vertical orientation of the rhizoid under 1 **g**, the MFs would be under tension due to the interaction with the statoliths. This hypothesis was strongly supported by in-vivo videomicroscopy of rhizoids during parabolic flights of rockets (Volkmann et al. 1991). Namely, during 6 min of reduced gravity

* Correspondence and reprints: Botanisches Institut, Universität Bonn, Venusbergweg 22, D-W-5300 Bonn 1, Federal Republic of Germany.

($10^{-4}$ **g**, i.e., microgravity) the weightless statoliths moved approx. 15 µm in the opposite direction to the originally acting gravity vector. According to the hypothesis, the removal of gravity disturbed the initial balance, so the uncompensated basipetal force mediated by MFs brought about the basipetal movement of statoliths. However, it might be argued that the position of statoliths was regulated by a sort of elastic support in which MFs did not participate, and the basipetal displacement of statoliths on weightlessness manifested the elastic behaviour of this support. An additional experiment on a rocket was thus necessary with rhizoids treated with CD immediately before the flight, with videorecording of a rhizoid during the flight and fixing rhizoids by aldehydes at the end of the MG-phase. The prediction of the hypothesis was that the statoliths would not be displaced basipetally during the MG-phase. Lack of displacement would strengthen the hypothesis, occurrence of displacement, however, would invalidate it. This crucial test was performed in the TEXUS-flight 28.

## Materials and methods

Detailed description and data of parabolic flight of rockets (TEXUS), the facilities used for videomicroscopy and the cuvettes used for application of solutions were previously described (Buchen et al. 1991, Volkmann et al. 1991). Microgravity (approx. $10^{-4}$ **g**) was reached 74 s after launch of the rocket and lasted for 347 s during the TEXUS-flight 28 (in Esrange near Kiruna, Sweden; November 23, 1991). The time scale was chosen so that point zero coincided with the beginning of the MG-phase. Rocket lift-off occurred at − 74 s on the scale. Preparation and cultivation of rhizoids of *Chara globularis* Thuill. (Botanical Garden, Bonn, Federal Republic of Germany) was performed as described (Buchen et al. 1991) with little modifications. In short, the rhizoids grew on a 1–2 mm thin layer of 1.2% agar (A 7921, Sigma, Deisenhofen, Federal Republic of Germany) in distilled water on a slide covered with a cover glass. The slides were vertically arranged in a glass staining jar the bottom of which was filled with distilled water to provide high humidity during 5–6 days of culture at room temperature (day 21 °C, night 18 °C) under continuous incandescent light.

For the flight-experiments, the agar layer with the *Chara* internodal segment and rhizoids was transferred into the cuvette for videomicroscopy and into three additional cuvettes which could not be observed during the flight. In order to avoid a shift of the agar during the acceleration phase of the rocket, the borders of the thin agar layer were attached firmly by adding some more agar. The cuvettes were provided with silicon tubings for application of solutions and connected to storage vessels containing the fixation solution. At designated times (after the acceleration phase and at the end of the MG-phase) the valves were opened by electrical signals and the fixation solution injected into the cuvettes. The rhizoids were fixed with a mixture of 6% glutaraldehyde and 3% paraformaldehyde in 0.1 M Pipes buffer, pH 7.0. After recovery of the module (within 1 h), they were observed with a light microscope (Axiophot 405, Zeiss, Oberkochen, Federal Republic of Germany) and photographed.

Into the cuvette chosen for videomicroscopy, 5 ml of 6.5 µg CD/ml in distilled water were injected with a syringe 30 min before launch of the rocket. Into the cuvette for statistical analysis, 5 ml of 5 µg CD/ml were injected approx. 90 min before launch. In both cuvettes, the CD-solution was exchanged by the fixation solution at the end of the MG-phase. The rhizoids in the two other cuvettes serving as controls without CD-application were fixed at the beginning and the end of the MG-phase. The fixation solutions were injected 20 s and 90 s before beginning and end of the MG-phase, respectively, to provide time long enough for diffusion and to guarantee fixation of the cells at microgravity conditions.

## Results

### *Videomicroscopy of the CD-treated rhizoid*

A *Chara* internodal segment with rhizoids showing the characteristic polar cell organization was chosen for the flight experiment. The normal location of the statoliths complex (see Volkmann et al. 1991) and the cytoplasmic streaming in the basal vacuolar part (see Buchen et al. 1991) were taken as criteria for a good status of the rhizoids. After application of the CD-solution, the cuvette was mounted in the flight module and the rhizoids were observed by videomicroscopy. Ten minutes before launch of the rocket, the statoliths in the rhizoids had fallen onto the apical cell wall (Fig. 1, − 674 s). The velocity of the cytoplasmic streaming slowed down, yet did not stop. This situation did not change under 1 **g** (Fig. 1, − 314 s).

The first videorecords under microgravity showed no change in the overall structural organization of the rhizoid compared to that one under 1 **g**. The statoliths complex remained on the physically lowest site of the rhizoid (Fig. 1, 42 s, 166 s, 286 s after onset of the MG-phase) and did not move basipetally during the whole MG-phase. The shape of the statoliths complex did also not change or spread over a larger cell-area (Fig. 1). Considering the location of the nucleus, its distance from the apical cell wall (apical and basal border of the nucleus at a distance of 214 and 264 µm, respectively) remained the same under 1 **g** and the MG-phase, i.e., the nucleus was not displaced. As an internal marker for the viability of the rhizoid cytoplasmic streaming was taken. The streaming velocity had decreased drastically until the end of the experiment, but did not completely stop. In the cell area where the acropetal streaming turned into the basipetal streaming, cytoplasm accumulated.

### *CD-treated rhizoids fixed at the end of the MG-phase*

After application of 5 µg CD/ml, the rhizoids in another cuvette were first observed under 1 **g** conditions in order

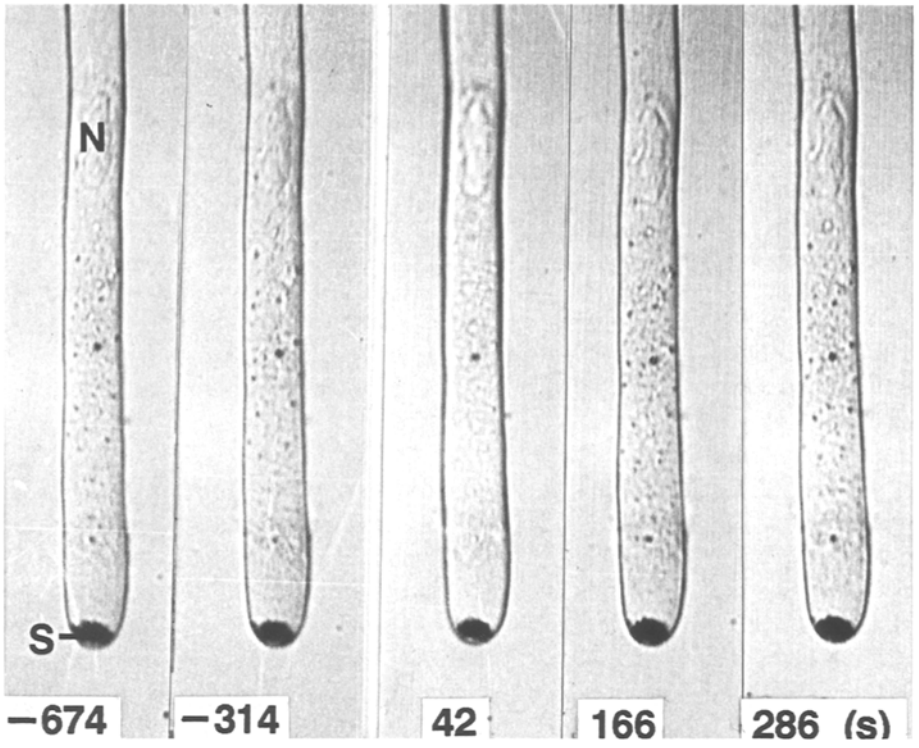
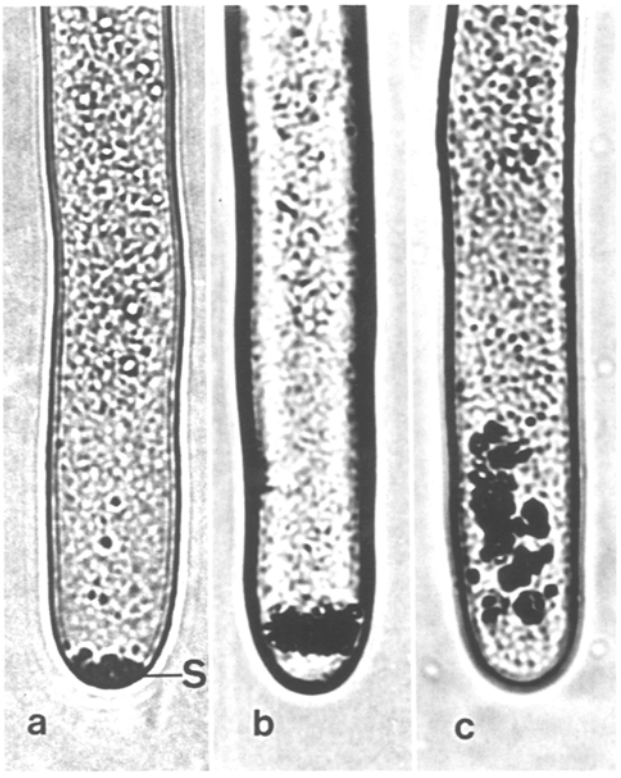



**Fig. 1.** Series of photographs of the apical part of a *Chara* rhizoid. The rhizoid was in-vivo videorecorded. CD was applicated 30 min before launch of the rocket (launch = −74 s). Under 1 **g**, the statoliths complex (*S*) sedimented on the apical cell wall (−674 s, −314 s = pre-launch). During the MG-phase (42 s to 286 s), the shape and the location of the statoliths did not change. The MG-phase began at 0 s. *N* Nucleus. Diameter of the rhizoid = 25 μm




to ensure that they were affected by the drug. In all rhizoids the statoliths sedimented on the apical cell wall as was the case with the rhizoid recorded by videomicroscopy (Fig. 1). The streaming velocity also decreased. At the end of the MG-phase, the CD-solution was exchanged by the aldehyde-fixative. This was also done in the cuvette taken for videomicroscopy. Former tests had shown that the polar organization and the position of the statoliths complex in the rhizoids was completely stabilized by this fixation and no shifting of the statoliths occurred even if the cuvette was jolted. Measurements of the distance of the center, the apical and the basal border of the statoliths complex from the apical cell wall in different rhizoids clearly showed

**Fig. 2.** A *Chara* rhizoid treated with CD and fixed at the end of the MG-phase (**a**) and untreated rhizoids for controls fixed at the beginning (**b**) and at the end of the MG-phase (**c**). The tips of the rhizoids were positioned on a horizontal line for easier comparison of the location of the statoliths (*S*). These spread over a large cell area during the MG-phase (**c**) compared to rhizoids fixed at the beginning of the MG-phase (**b**) or compared to CD-treated rhizoids (**a**). Diameter of the rhizoids = 25 μm

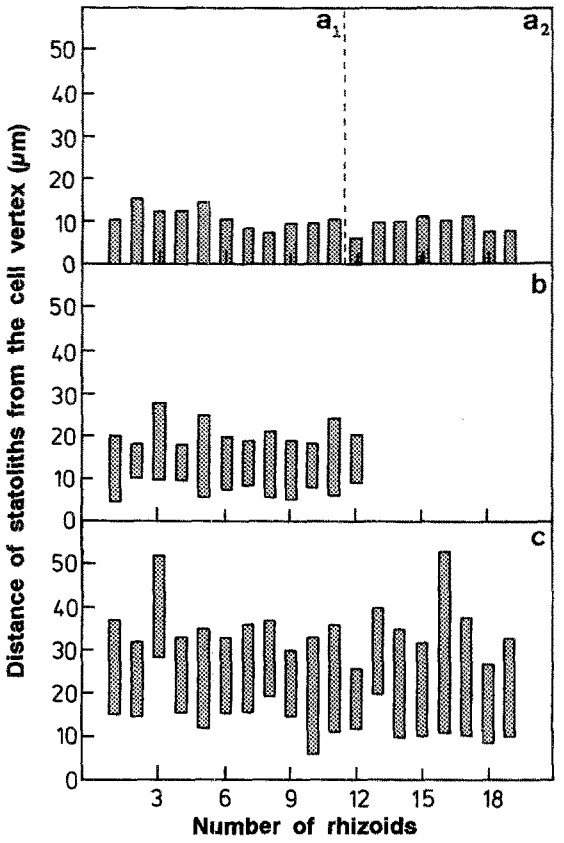



**Fig. 3.** Quantitative analysis of the location of statoliths of CD-treated rhizoids fixed at the end of the MG-phase ($a_1$ and $a_2$) and of untreated rhizoids fixed at the beginning (**b**) and at the end of the MG-phase (**c**). The apical and basal distance of the statoliths complexes from the apical cell wall (vertex) are indicated for individual rhizoids. CD was applied at 1 g in concentrations of 6.5 µg/ml 30 min ($a_1$) and 5 µg/ml 90 min ($a_2$) before the beginning of the MG-phase, respectively. No basipetal displacement occurred in CD-treated rhizoids in contrast to the untreated controls

that the statoliths did not move basipetally during the MG-phase (Fig. 2 a, compare controls Fig. 2 b and c). The same result was obtained for all CD-treated rhizoids (Fig. 3 $a_1$ and $a_2$). The extension of the densely packed statoliths in axial direction reached in most rhizoids 10 µm, maximal 15 µm (Fig. 3 $a_1$ and $a_2$). Thus, the result obtained by in-vivo videomicroscopy of one CD-treated rhizoid was confirmed by those obtained for many CD-treated rhizoids which had been fixed at the end of the MG-phase.

*Control rhizoids not treated with CD, but fixed at the beginning and at the end of the MG-phase, respectively*

The rhizoids fixed just after the acceleration phase of the rocket, i.e., at the very beginning of the MG-phase, showed the statoliths complex positioned nearly normally with the center of the statoliths complex located approx. 15 µm above the apical cell wall (Fig. 2 b). The apical border of the statoliths complex had a distance of 5–10 µm and the basal border of 18–20 µm from the cell vertex (three rhizoids 24–27 µm; Fig. 3 b); the difference between both borders was approx. 12 µm. In most rhizoids fixed at the end of the MG-phase, the statoliths spread over an area of approx. 20 µm in the axial direction (Fig. 3 c). An extreme case of statoliths distribution (over an area of approx. 40 µm) is shown in Fig. 2 c. Only one rhizoid (out of 19) had few statoliths located as close as 6 µm distant from the cell vertex while the basal border of the statoliths complex reached 33 µm above the cell vertex. However, one rhizoid had the apical zone free of statoliths as long as 28 µm (Fig. 3 c). In another rhizoid (no. 16, cf. Fig. 3 c), the apical and basal borders of the statoliths complex were 11 µm and 52 µm, respectively. Thus, the experiment with the fixation of rhizoids at the end of the MG-phase fully confirmed the results obtained for two rhizoids by in-vivo videomicroscopy in former flight experiments (Volkmann et al. 1991) and documented as well the differences between untreated and CD-treated rhizoids.

## Discussion

The present study proves the prediction made on the basis of the hypothesis that in plant cells statoliths are in dynamic interaction with MFs (Sievers et al. 1991 a, Volkmann et al. 1991). The statoliths do not move basipetally on sudden and drastic reduction of gravity after CD-caused disorganization of MFs. This indicates that the MFs are an important part of the cytoplasmic structure which keeps the statoliths in a proper position within the cell. The way in which this is achieved is unknown. One possibility is an interaction involving shearing forces between MFs and organelles. Such a hypothesis fits the observations that (*i*) statoliths move in rhizoids, often in a saltatory way (Hejnowicz and Sievers 1981, Sievers et al. 1991 b), and (*ii*) that they move basipetally in rhizoids as well as in higher plant statocytes during a relatively long time when the gravity force is reduced in rocket experiments (Volkmann et al. 1991). Another possible way of the involvement of MFs in keeping the statoliths in position in a rhizoid is a static elastic support for them. In this case, the gravity force in the normal vertical orientation of the rhizoid would be counteracted statically by the reaction force of the underlying cytoskeleton being elastically deformed.

However, it is not easy to explain on this basis the nearly constant mean velocity of statoliths displacement during approx. 6 min after reducing gravity. This is too long a period for a "normal" elastic expansion. It should, however, be mentioned that there exist materials, like "Saran-Wrap" foil, which after deformation return very slowly to the initial form (Feynman et al. 1963). There are probably electric dipol moments "frozen" in a viscous matrix of such a material and the interaction between the dipols and the matrix might explain the slow elastic expansion. Maybe the deformation of the cytoskeleton is of such a type. However, until now there is no experimental support for such a property of the cytoskeleton. If, instead of static elastic forces, shearing forces are involved in the interaction between statoliths and MFs, myosin is the likely mediator. Thus, future studies should take into account the myosin-mediated interaction.

Whatever the interaction between statoliths and MFs is, it brings about a tension of the MFs. When on earth, the gravity vector changes with respect to the plant axis, the balance between the gravity force and the interacting force is disturbed. In consequence, the change in gravity must bring about a change in the tension of the MFs (symmetry, magnitude). As MFs are associated with other elements of the cytoskeleton, e.g., microtubules, and are anchored at cellular membranes (Niggli and Burger 1987, Lichtscheidl et al. 1990, Ding et al. 1991), such a change of MF-tension could explain how a differential signal might be achieved during graviperception (Sievers et al. 1991 a). The present study, in its part concerning the rhizoids fixed during the MG-phase, confirms the observation made on individual rhizoids in former rocket flights (Volkmann et al. 1991) that the statoliths move in direction opposite to the originally acting gravity vector upon gravity reduction. There is no doubt that this phenomenon generally occurs in rhizoids under microgravity conditions.

## Acknowledgements

Financial support by the AGRAVIS-project (DARA, Bonn, and Ministerium für Wissenschaft und Forschung, Düsseldorf) is gratefully acknowledged. We thank the excellent technical support by the TEXUS team (MBB/ERNO, Bremen, Federal Republic of Germany).

## References

Buchen B, Hejnowicz Z, Braun M, Sievers A (1991) Cytoplasmic streaming in *Chara* rhizoids. Studies in a reduced gravitational field during parabolic flights of rockets. Protoplasma 165: 121–126

Ding B, Turgeon R, Parthasarathy MV (1991) Microfilament organization and distribution in freeze-substituted tobacco plant tissues. Protoplasma 165: 96–105

Feynman RP, Leighton RB, Sands ML (1963) The Feynman lectures on physics, vol 2. Addison-Wesley, Reading, MA

Hejnowicz Z, Sievers A (1981) Regulation of the position of statoliths in *Chara* rhizoids. Protoplasma 108: 117–137

Lichtscheidl IK, Lancelle SA, Hepler PK (1990) Actin-endoplasmic reticulum complexes in *Drosera*. Their structural relationship with the plasmalemma, nucleus, and organelles in cells prepared by high pressure freezing. Protoplasma 155: 116–126

Niggli V, Burger MM (1987) Interaction of the cytoskeleton with the plasma membrane. J Membrane Biol 100: 97–121

Ohmori H, Toyama S, Toyama S (1992) Direct proof that the primary site of action of cytochalasin on cell motility processes is actin. J Cell Biol 116: 933–941

Sievers A (1991) Gravity sensing mechanisms in plant cells. In: Halstead TW, Todd P, Powers JV (eds) Gravity and the cell. American Society for Gravitational and Space Biology, Washington, DC, pp 43–50 (ASGSB Bulletin, vol 4)

– Volkmann D (1979) Gravitropism in single cells. In: Haupt W, Feinleib ME (eds), Physiology of movements. Springer, Berlin Heidelberg New York, pp 567–572 [Pirson A, Zimmermann MH (eds) Encyclopedia of plant physiology, NS, vol 7]

– Kruse S, Kuo-Huang L-L, Wendt M (1989) Statoliths and microfilaments in plant cells. Planta 179: 275–278

– Buchen B, Volkmann D, Hejnowicz Z (1991 a) Role of the cytoskeleton in gravity perception. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic Press, London, pp 169–182

– Kramer-Fischer M, Braun M, Buchen B (1991 b) The polar organization of the growing *Chara* rhizoid and the transport of statoliths are actin-dependent. Bot Acta 104: 103–109

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