Planta (1989) 179:275–278

Planta

Short communication

Statoliths and microfilaments in plant cells

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Abstract. Microfilaments have been demonstrated in rhizoids of *Chara fragilis* Desvaux by labelling of actin with rhodamine-conjugated phalloidin. Each rhizoid contains thick microfilament-bundles arranged longitudinally in the basal region. In the subapical and apical regions, much thinner bundles exist which contact the statoliths and encircle them in the form of a dense envelope. In root statocytes from *Lepidium sativum* L. the presence of an actin network is indicated by the fact that application of cytochalasin B (25 $\mu g \cdot ml^{-1}$ for 4 h) results in an approximately threefold increase in the rate of statolith (amyloplast) sedimentation relative to controls. It is concluded that in gravity-perceiving plant cells statoliths may trigger the transduction mechanism via actin filaments.

Key words: *Chara* rhizoid – Graviperception – *Lepidium* root – Microfilament – Statolith

Recently, Caspar and Pickard (1989) demonstrated that the presence of starch in plastids is unnecessary for graviperception in a mutant of *Arabidopsis thaliana* L. However, amyloplasts are necessary for full gravitropic sensitivity, and starchless, non-sedimented plastids may also function as statoliths (Kiss et al. 1989). Therefore the possibility arises that the position and the translocation of statoliths in gravity-perceiving cells may be dependent on and influenced by elements of the cytoskeleton. To investigate this possibility we have, as a first step, studied actin filaments in two cell types which transduce the gravity stimulus.

The ultrastructure of the *Chara* rhizoid, as well as its gravitropic reaction chain, has been examined in detail (for review see Sievers and Schnepf 1981). A group of small vacuoles filled with barium-sulfate crystals, positioned approx. 10–30 μm above the cell apex, functions as statoliths. In a rhizoid growing downwards the statoliths are in a dynamical balance with respect to the forces acting upon them: gravity and the interaction with the cytoskeleton (Hejnowicz and Sievers 1981). When the position of the rhizoid is changed to horizontal, the balance is disturbed and the statoliths sediment onto the lower part of the plasma membrane. The statoliths play a major role in establishing differential growth, resulting in a positive gravitropic curvature of the rhizoid (Sievers et al. 1979). When the rhizoid is oriented downwards the position of the statoliths is relatively stable as a 5-min apically directed centrifugation at $50 \cdot g$ is necessary to shift the statoliths to the cell apex (Friedrich and Hertel 1973). The first evidence for an involvement of microfilaments (MF) in stabilizing the position of statoliths came from experiments with cytochalasin B (CB; Hejnowicz and Sievers 1981), which causes sedimentation of the statoliths in an apical direction. Recently, Bartnik and Sievers (1988) described a spherical aggregate of endoplasmic reticulum (ER) located beneath the statoliths. The ER aggregate disappears after treatment with CB or phalloidin, and this is followed by sedimentation of the statoliths, demonstrating that the integrity of the polar organization of the cell is actin-dependent.

The presence of MFs in *Lepidium* root statocytes has been claimed on the basis of the results

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Abbreviations: CB = cytochalasin B; ER = endoplasmic reticulum; MF = microfilament

of experiments with cytochalasins (Hensel 1985; Wendt and Sievers 1986; Wendt et al. 1987) and proved by rhodaminyl-phalloidin labelling (Hensel 1989). Microfilaments play a role in stabilizing the positions of the nucleus and of the distal ER complex, and are involved in ER-retransport after centrifugation. Their significance for graviperception is discussed but not yet clear.

Plant material. Chara fragilis Desvaux (Botanical Garden, University of Bonn, FRG) was induced to form rhizoids as described by Bartnik and Sievers (1988). Seeds of *Lepidium sativum* L. (Chrysant, Bonn, FRG) were soaked in tap water for 30 min and then germinated for 24 h (21 ± 1° C, darkness) with their roots growing downwards.

F-actin staining. Chara rhizoids of approx. 1 cm length were dipped into a solution of 30 nM rhodamine-phalloidin (Molecular Probes, Eugene, Ore., USA) dissolved in 0.2 M Sörensen phosphate buffer, pH 7.0 (Tewinkel et al. 1989). After 2 min of staining the rhizoids were layered onto slides for observation (epifluorescence microscope, Zeiss, Oberkochen, FRG). Micrographs were taken on Agfapan 400 films (Agfa, Leverkusen, FRG).

Sedimentation kinetics. Roots of *L. sativum*, 24 h old, were treated with 25 $\mu g \cdot ml^{-1}$ CB (dissolved in 1% dimethylsulfoxide) for 4 h and then inverted for 0.5, 1, 2, 5 and 10 min. Subsequently, they were fixed in situ (2% aqueous $KMnO_4$) in the inverted position. Micrographs from serial sections out of the median plane of the root cap were used to determine the position of amyloplasts relative to the cell walls. Sedimentation rates were calculated by dividing mean distances by the given sedimentation time.

Chara rhizoids contain MF bundles in different patterns related to their location in the cell. In the basal region, thick bundles run preferentially parallel to the long axis of the cell (Fig. 1; *b*). Their function is correlated to cytoplasmic streaming (Hejnowicz et al. 1985). The subapical and the apical region are characterized by much thinner MF bundles (approx. one-fifth of the diameter of thick bundles) which are predominantly oriented in the longitudinal direction. However, they form a three-dimensional network which is especially dense in the zone where the statoliths are located (Fig. 1; *s*). The MFs encircle each statolith, giving the image of a bright fluorescent envelope. The statoliths are not labelled themselves. It seems evident that MFs act in establishing the balance of forces acting on statoliths (Hejnowicz and Sievers 1981; Bartnik and Sievers 1988).

Evidence for the existence of an MF network in another gravity-sensing system, in statocytes from *Lepidium* roots, comes from the examination of the sedimentation kinetics of statoliths (amyloplasts) after CB treatment. Statoliths move on average 2.9-times faster in statocytes from CB-treated roots relative to statocytes from control roots; however, values within individual statocytes vary with their position in the statenchyma (Table 1). Maximum velocities for CB-treated roots are 3.3 $\mu m \cdot min^{-1}$ and for controls 1.3 $\mu m \cdot min^{-1}$, respectively. Close proximity of MFs to amyloplasts has also been described in statocytes from barley and maize coleoptiles (White and Sack 1986) in which, however, in contrast to the *Lepidium* root statocytes, protoplasmic streaming occurs.

Although the *Chara* rhizoid, as a single cell, and the *Lepidium* root, as a multicellular organ, represent gravitropic systems which are different in principle (for reviews see Volkmann and Sievers 1979; Sievers and Schnepf 1981), there are some similarities. In both cases the statoliths are embedded in a network of actin filaments. In the case of the rhizoid a rather dense envelope of filaments encircles each statolith, indicating a considerable degree of support which together with an ER aggregate (Bartnik and Sievers 1988) acts against its sedimentation in the apical direction of the cell. The corresponding support in root statocytes is represented by an ER complex and MFs at the distal cell pole (Volkmann and Sievers 1979; Hensel 1987). There is no doubt that CB destroys the filament network and thereby reduces the force exerted on statoliths by microfilaments, and also reduces the viscosity of the cytosol. In consequence, in the downward-oriented *Chara* rhizoid the statoliths sediment in the apical direction (Hejnowicz and Sievers 1981). In *Lepidium* statocytes, where there is not much space for sedimentation in the distal direction, the sedimentation rate in the proximal direction is increased upon inversion of the root. This may be important for graviperception.

It is still not known if the statoliths destroy the network of filaments during sedimentation when the organism is tilted out of the vertical direction, or if they sediment together with the surrounding, and possibly attached, MFs. The movement of statoliths may trigger graviperception by causing both, mechanical disruption followed by an active reorganization and-or stretching and subsequent relaxation of filament bundles. If the filament network is not destroyed by moving statoliths, actin filaments would be able to focus and transmit stress to their anchorage sites located at the plasma membrane and-or at ER membranes. These sites may function as Ca^{2+} pumps which exist in ER membranes (Sievers et al. 1984). Also, stress-activated ion-channels have been held responsible for graviperception (Edwards and Pickard 1987). Indeed, Wendt and Sievers (1989) recently demonstrated that the gravisensitivity of roots is dependent on the calcium concentration

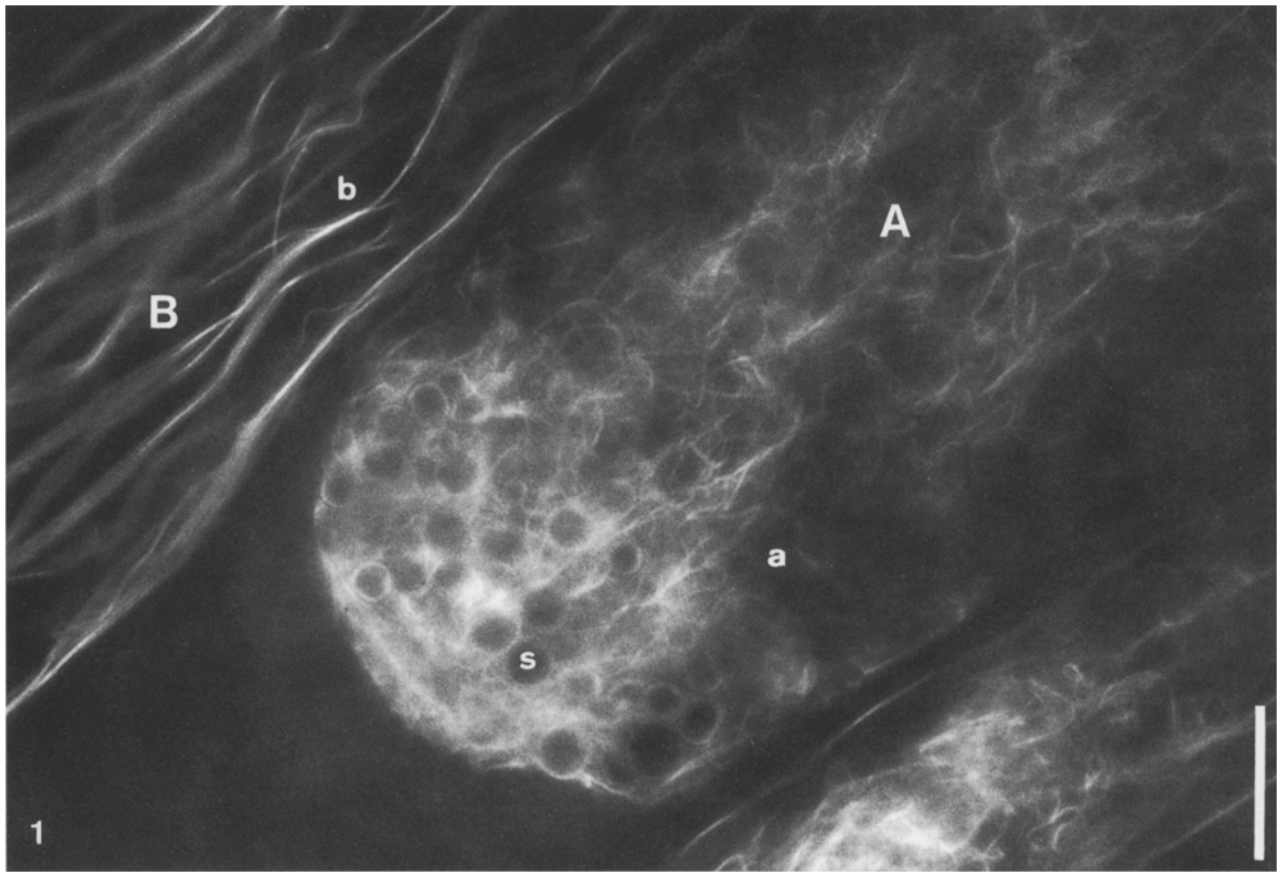

Fig. 1. Actin filaments in the basal (*b*) and in the subapical and apical (*a*) regions of vertically grown *Chara* rhizoids stained by rhodamine-phalloidin. In the basal cell zone, containing the central vacuole, bundles of filaments are arranged longitudinally (shown in a part of the rhizoid *B*). The subapical and apical zones of another rhizoid (*A*) contain thinner filaments forming a three-dimensional network, leaving spherical holes (*s*) in the apical part as revealed by focussing. The latter are produced by a dense envelope of actin filaments surrounding the statoliths, which do not contain actin themselves. ×2000; *bar* = 10 μm

Table 1. Sedimentation rates ($\mu m \cdot min^{-1}$) of the physically lower amyloplasts in central statocytes from CB-treated *Lepidium* roots and controls (during the first minute after inversion of the roots; $n = 18$)

Storey of statocytes[a]	CB-treated roots	Control roots
II	3.3	0.2
III	3.1	1.1
IV	1.5	1.3
Mean values	2.6	0.9

[a] See Volkmann and Sievers 1979

in statocytes. It should be mentioned that in both gravity-sensing cell types, the *Lepidium* root statocyte and the *Chara* rhizoid, a special ER complex or an ER aggregate, respectively, is located beneath the statoliths.

The authors thank Drs. B. Buchen, Z. Hejnowicz and W. Hensel for stimulating discussions. This work was supported by the Bundesminister für Forschung und Technologie, Bonn.

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Received 5 April; accepted 5 May 1989