Planta (1997) 203: S69–S78

Planta

Root cytoskeleton: its role in perception of and response to gravity

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Received: 27 June 1996 / Accepted: 26 April 1997

Abstract. We have critically evaluated the possible functions of the plant cytoskeleton in root gravisensing and graviresponse and discussed the evidence that microtubules (MTs) and actin microfilaments (MFs) do not control differential cell growth during bending of roots. On the other hand, MF and MT networks are envisaged to participate in gravisensing because of the mechanical properties of the cytoskeletal structures that interconnect plant cell organelles with the plasma membrane. In restrained gravisensing, forces are suggested to be transmitted to membranes because large-scale gravity-dependent repositioning of organelles is effectively prevented due to the cytoskeleton-mediated anchorage of their envelopes at the plasma membrane. From the cytoskeletal point of view, we can also envisage an unrestrained gravity sensing when cytoskeletal tethers are not strong enough to preserve the tight control over distribution of organelles and the latter, if heavy enough, are allowed to sediment towards the physical bottom of cells. This situation obviously occurs in root cap statocytes because these uniquely organized cells are depleted of prominent actin MF bundles, endoplasmic MT arrays, and ER elements in their internal cytoplasm. Nevertheless, indirect evidence clearly indicates that sedimented root cap statoliths are enmeshed within fine but dynamic MF networks and that their behaviour is obviously under, at least partial, cytoskeletal control. The actomyosin-enriched domain among and around amyloplasts is proposed to increase the perception of gravity due to the grouping effect of sedimenting statoliths. Cytoskeletal links between myosin-rich statoliths, and cell peripheries well equipped with dense cortical MTs, membrane-associated cytoskeleton, as well as with ER elements, would allow efficient restrained gravisensing only at the statocyte cell cortex. As a consequence of cytoskeletal depletion in the internal statocyte cytoplasm and bulk sedimentation of large amyloplasts, restrained gravisensing is spatially restricted to the bottom of the statocyte irrespective of whether roots are vertical or horizontal. This spatial aspect allows for efficient gravisensing via amplification of gravity-induced impacts on the cellular architecture, a phenomenon which is unique to root cap statocytes.

Key words: Actin microfilament – Cytoskeleton – Gravity perception – Gravity response – Microtubule – Root growth

Introduction

Roots typically exhibit positive gravitropism, meaning that sufficient reorientation from the vertical position, above a threshold angle, is compensated by differential flank growth (e.g. Zieschang and Sievers 1991; Ishikawa and Evans 1993; Baluška et al. 1996a). Our current knowledge regarding the mechanisms of perception of and response to gravity in roots is incomplete, despite decades of intense research. Even accepted hypotheses are now being questioned for roots because of their inconsistencies with recent results (e.g. Caspar and Pickard 1989; Sievers and Zieschang 1992; Konings 1995; Masson 1995). The complexity of root gravisensing and graviresponse is complicated by relatively large distances between sites of perception in root cap statocytes (e.g. Sack 1991) and sites of growth responses in the transition (e.g. Baluška et al. 1996a,b) and elongation (e.g. Baluška et al. 1996a) growth zones of the root proper.

The plant cytoskeleton has long been overlooked as an indicator of cellular functions relevant for gravisensing and graviresponse of plant roots. This disregard is surprising if we consider that both microtubules (MTs) as well as actin microfilaments (MFs) are dynamic and ubiquitous, often membrane-associated, structures that are well-known to be essential for a variety of cellular

Abbreviations: ER = endoplasmic reticulum; IP_3 = inositol-1,4,5-trisphosphate; MF = actin microfilament; MT = microtubule

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processes related to perception of and response to gravity. For instance, the plant cytoskeleton is involved in intracellular signalling (Tan and Boss 1992; Xu et al. 1992; Drobak 1993; Lisanti et al. 1994), cellular motilities (Kamiya 1981; Williamson 1993), cell growth (Thimann et al. 1992; Baluška et al. 1997a; Reichelt et al. 1997), and establishment and maintenance of cell and tissue polarities (Hepler and Palevitz 1974; Hensel 1985; 1986b; Baluška et al. 1993a; Shibaoka 1994). Our goal is to critically survey the possible role of the cytoskeleton in gravistimulated root cells. In addition, we will consider all those cytoskeletal functions known from other biological systems that might be relevant for the elucidation of processes controlling gravisensing, signal transduction, and graviresponse of roots.

Cytoskeletal involvement in root gravisensing

Gravisensing based on cytoskeletally restrained masses – restrained gravisensing. In a typical plant cell, organelles are not freely movable but are cytoskeletally restrained. Due to gravity and the absence of any apparent gravity-dependent repositioning, the mass of restrained intracellular organelles is proposed to exert both pulls and pressures on the cytoskeleton and associated membranes. Therefore, if heavy enough, restrained organelles could function as gravity susceptors whose responses are cytoskeletally transmitted to relevant sensory membranes. We would like to introduce the term restrained gravisensing for those situations when subtle shifts of intracellular masses (organelles) or of whole protoplasts, not associated with obvious visible displacements, generate a signal by stretching or compressing cytoskeletal and membraneous elements (for early hypotheses, see Hejnowicz and Sievers 1981; Björkman 1988; Sievers et al. 1991a).

In restrained gravisensing, the positioning of organelles is under the strict control of the cytoskeleton which interconnects and anchors them at the plasma membrane. Such a system of 'suspended' and cytoskeletally restrained organelles, exerting pressures and pulls on putative sensory membranes, is suitable for gravisensing of the cell's own mass (passive gravistimulation – Barlow 1992). Restrained gravisensing might have evolved in close association with the inception of the cytoskeleton in primitive eukaryotic cells (Barlow 1995). For instance, restrained gravisensing based on the mass of the protoplast and occuring in the absence of intracellular sedimentable statoliths could be proposed for large internodal cells of characean algae. The mass of these protoplasts has been calculated to be sufficient for gravisensing (Wayne et al. 1990; Staves et al. 1992, 1995). The latter experimental system represents a useful model for sensing of gravity forces via the membrane-associated cytoskeleton that is testable by experimentation (but see Ackers et al. 1994). For example, plant integrin homologues seem to interconnect the plasma-membrane-associated cytoskeleton with the plant cell wall (Schindler et al. 1989; Kaminskyj and Heath 1995; Henry et al. 1996; Correa et al. 1996; Katembe et al. 1997). Their competitive inhibition was shown to prevent gravisensing in characean internodal cells (Wayne et al. 1992). These authors proposed that integrin-like proteins, which are expected to be involved in mechanotransduction across the cell periphery (Ingber 1991; Sastry and Horwitz 1993; Wang et al. 1993; Miyamoto et al. 1995), could act as gravireceptors in plant cells (Wayne et al. 1992; see also Katembe et al. 1997).

The concept of restrained gravisensing is substantiated by an inherent suitability of interconnected cytoskeletal networks for rapid transmission of mechanical forces throughout the eukaryotic cell due to the putative tensegrity (Ingber 1993) and percolation (Forgacs 1995) properties of the cytoskeleton. According to Ingber (1993), tensegrity means that the cellular integrity is based on tensional forces which originate from the actomyosin complex and are resisted by mechanically more-robust structures such as MTs and the plasma membrane supported by the extracellular matrix. The percolation concept, as proposed by Forgacs (1995), indicates that the cytoskeletal elements do not necessarily need to be continuous structures but that interconnected shorter fragments are sufficient for the mechanical transmission of signals. Hejnowicz and Sievers (1981) were the first to show that disruption of the F-actin arrays affected statolith positioning in *Chara* rhizoids. Subsequent studies, using rhodamine-phalloidin, revealed the organization of MFs around the statoliths of these cells (Sievers et al. 1989) and confirmed the essential role of F-actin in their positioning (Sievers et al. 1991b, 1996; Braun and Sievers 1993; Buchen et al. 1993; see also Braun 1997, this issue). On the other hand, MTs were reported not to be involved in the gravisensing of *Chara* rhizoids (Braun and Sievers 1994).

In the case of multicellular roots, past attempts to incorporate cytoskeletal elements into existing concepts of gravisensing and gravitropic response were limited by the absence of any knowledge regarding the organization of MTs and F-actin networks in relevant cells. A breakthrough in this respect was achieved recently by the introduction of new sectioning methods suitable for the visualization of MTs and F-actin (Baluška et al. 1992, 1997a; Blancaflor and Hasenstein 1993, 1995a,b; Baskin et al. 1995; Vitha et al. 1997). These immunofluorescence techniques can now provide the critical information concerning cytoskeletal distribution in cells of different root regions which is essential for assessing their involvement in both the perception of and the response to gravity by roots. It is still unknown if intracellular masses that are tethered by the plasma-membrane-anchored cytoskeletal elements function as gravity susceptors (Sack 1991). Nevertheless, specific distributions of both the MF and MT arrays in cells of the root tip, when a centrally located nucleus is densely enmeshed in distinct MT (Baluška et al. 1992) and MF (Baluška et al. 1997a) networks (see also Fig. 1), support this possibility. Until now, the only organisms that seemed to rely on restrained gravisensing for gravitropic movements were basidiomycete fungi as their fruiting

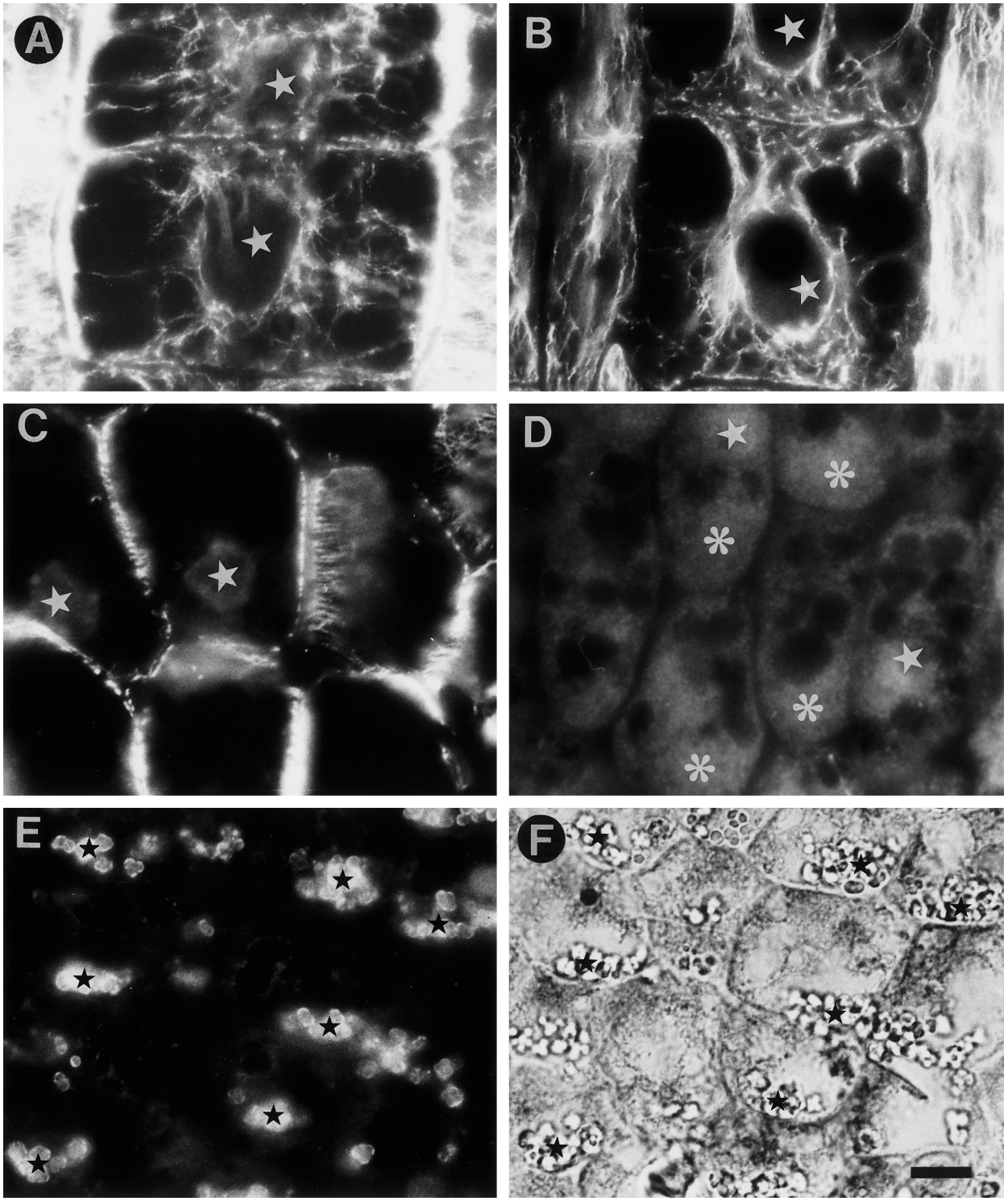

Fig. 1. A–F Typical distributions of MTs (**A, C**) and actin-based cytoskeleton (**B, D**) in early postmitotic maize root cells (metaxylem elements) of the root proper (**A, B**) and in statocytes of the root cap (**C–F**). Stars in **A–D** indicate positions of nuclei. *Snowflakes* in **D** show actin-rich domains localized preferentially at distal statocyte poles in association with sedimented amyloplast-based statoliths; dark roundish structures correspond to nuclei and provacuoles. **E** Myosin-related proteins are associated with the statolith surface in cytoskeleton-depleted maize root statocytes. **F**. Differential interference contrast version of the same image as shown in **E**. For details on the immunofluorescence see Baluška et al. (1992, 1996c and Vitha et al. 1997) and on the myosin antibody (Sigma, M7648) see Braun (1996) and references cited therein. Bar = 10 μm; × 1000

bodies lack any sedimentable structures whereas an intact actin cytoskeleton is essential for graviperception in these organisms (Monzer 1995; Moore et al. 1996).

From studies with animal cells, we know that MFs bind to integral plasma-membrane proteins, at specific peripheral domains forming adhesion sites, which are

connected to components of the extracellular matrix (Luna and Hitt 1992; Wang et al. 1993). Centrifugal forces rupture these adhesion sites (Thoumine et al. 1996) indicating that they might function as mechanical sensors of the plasma membrane. The possible significance of focal adhesion complexes for the perception of gravity is also indicated by their active role in signal transduction across the cell periphery (Sastry and Horwitz 1993; Pavalko and Otey 1994) and in controlling the spatial organization of MFs (Miyamoto et al. 1995). Importantly, the integrity of F-actin networks was shown to affect formation of these peripheral focal adhesion complexes (Miyamoto et al. 1995). Also, the caveolae signalling system of the plasma membrane, based on membrane subdomains enriched with signalling molecules such as G-proteins, is linked with the actin cytoskeleton (Lisanti et al. 1994; Fujimoto et al. 1995). In addition, the actin-binding protein profilin was reported to be closely associated with the phosphoinositide signal-transduction system, directly controlling the availability of polyphosphoinositides for second-messenger production (Drobak 1993). Inositol-1,4,5-trisphosphate (IP_3) is known to be involved in the mobilization of calcium from intracellular storage sites (Berridge and Irvine 1989) while IP_3 receptors have been identified as calcium channels at the plasma membrane. F-actin was reported to connect these IP_3 receptors with ryanodine-binding calcium channels located at membranes of internal calcium storage sites (Kraus-Friedmann 1994). The latter author proposed that structural changes to the actin-based cytoskeleton are responsible for the activation of calcium channels, causing a quick increase in cytoplasmic calcium levels. A rapid but transient release of calcium into the cytoplasm was also elicited by mechanical stimulation of plant membranes (Thonat et al. 1993). Moreover, recent publications suggest that F-actin-dependent plasma-membrane adhesion sites also exist in brown algae (Henry et al. 1996), while F-actin is closely asociated with transmembrane integrins (Tamkun et al. 1986; Kaminskyj and Heath 1995) which co-distribute with stretch-activated calcium channels in tip-growing lower-plant cells (Garrill et al. 1992, 1993; Levina et al. 1994). Importantly, a large portion of actin is bound to cellular membranes and this actin can consist of unique isoforms (i.e. Janßen et al. 1996).

Microtubules may also be suitable for restrained gravisensing. For example, in animal systems, MTs were reported to bind specifically to proteins associated with the signal transduction across the plasma membrane (Offringa and Bierer 1993; Roychowdhury et al. 1993). Moreover, putative links between the MT cytoskeleton and signalling pathways based on phosphoinositide were suggested for both animal and plant cells (Bartolo and Carter 1992; Surridge and Burns 1992). Since phosphoinositide signalling pathways induce modulation of intracellular calcium, which is suspected to affect the graviperception of roots (Sievers et al. 1984; Evans et al. 1986; Sievers and Busch 1992), MTs could be involved in restrained gravisensing. In addition, dynamic MTs may act as sensors of weak physical fields (Tabony and Job 1992a,b) which have been hypothesized to be relevant for gravisensing and cellular morphogenesis (Tabony and Job 1992a,b).

Gravisensing based on statolith sedimentation – unrestrained gravisensing. Restrained gravisensing may not be substantial enough to initiate the cascade of events that leads to the induction of differential elongation along the upper and lower root flanks in higher plants. In order to allow more efficient gravisensing, complex multicellular roots have evolved a rather advanced gravity sensing mechanism in their root cap statocytes. These specialized cells located in the root cap center appear to be depleted of endoplasmic MTs and prominent MF bundles deeper in their cytoplasm (Baluška et al. 1997b; Blancaflor and Hasenstein 1997). The latter cytoskeletal elements are responsible for the positioning as well as for the mobilities of larger organelles in plant cells (Williamson 1993). This does not mean that there are absolutely no cytoskeletal elements in the internal statocyte cytoplasm, activities of which are indicated via numerous indirect data (see Perbal et al. 1997, this issue). Nevertheless, they are obviously not robust enough to sustain an efficient control over the positioning and mobilities of internal organelles. Weakening of the cytoskeletal constraints on the statoliths allows them, if they are heavy enough, to reposition along the gravity vector and to sediment to the physical bottom. This new feature introduces a gravity-dependent spatial aspect into the intracellular architecture of some specialized plant cells, such as root cap statocytes, which enables them to accomplish a more-efficient gravity perception. In a more-general way, we would like to propose that unrestrained gravisensing occurs whenever the gravity forces affect, in an apparent way, the positioning of organelles.

Sievers et al. (1991a) and Volkmann et al. (1991) proposed that the sedimented root cap statoliths remain tethered, although less efficiently, to the plasma membrane by MFs. The attractive hypothesis that this tethering contributes to root gravisensing was based on numerous indirect observations suggesting that the root cap statoliths are enmeshed in actin-containing cytoskeletal elements (see Perbal et al. 1997, this issue). In particular, sedimented statoliths sometimes do not contact the underlying ER membranes (Barlow et al. 1984) and accomplish saltatory movements (Sack et al. 1986), indicating that they might be dynamically suspended via F-actin-containing structures. Statoliths moved closer to the distal statocyte cell wall after treatment of cress roots with the anti-MF drug cytochalasin B (Hensel 1985, 1987). Sophisticated rocket-flight experiments indicated that the cytoskeletal forces can partially counteract the gravity force (Volkmann et al. 1991; Buchen et al. 1993). Moreover, cytochalasin D treatment impaired the gravity-induced lowering of intracellular membrane potentials of the ionic current in statocytes of cress root caps (Sievers et al. 1995). Since these potential changes are the earliest indicators of root reorientation, such effects suggest a direct role of the actin cytoskeleton in perception of gravity by plant roots. In accordance with this model, disruption of actin

MFs with cytochalasin D inhibited the gravity-induced differential proton secretion in *Phleum* roots (Monshausen et al. 1996).

In spite of this correlative evidence, we still lack convincing experimental proof for a direct role of the actin-based cytoskeleton in the perception of gravity by roots. In addition, pretreatment of roots with cytochalasin B does not prevent the graviresponse (Wendt et al. 1987; but see Monshausen et al. 1996). This indicates that intact actin MF bundles, which are fragmented in root-proper cells treated with cytochalasin D (Baluška et al. 1997a), are not essential for root graviperception and graviresponse. But all experiments with cytochalasins must be interpreted with caution as it is not known to what extent the actin-based cytoskeleton is disintegrated. In contrast to MT-drugs, cytochalasins do not fully depolymerize MFs and cause only their fragmentation (Schliwa 1982; Cooper 1987), which is followed by tissue-specific recoveries of MF networks and bundles in maize root cells still under cytochalasin D treatment (Baluška unpublished results). Therefore, short dynamic membrane-associated F-actin fragments, that are presumed to interconnect statoliths with each other and with the relevant sensory membranes, may be affected only slightly by these drugs. Tensegrity-based signal transduction (Ingber 1993; Wang et al. 1993) would still be possible even if the actin-based cytoskeleton consisted only of short but interconnected MF fragments (Forgacs 1995).

Electron-microscopic and immunofluorescence studies did not reveal prominent F-actin bundles or dense networks in root cap statocytes (reviewed by Sack 1991), although such cytoskeletal structures were visualized in all postmitotic cells of the root proper (Baluška et al. 1997a). Reports indicating distinct F-actin networks throughout the statocyte cytoplasm are scarce and inconclusive. Firstly, fluorochrome-conjugated phalloidin has been applied in vivo to root cap slices in order to visualize the statocyte F-actin (Hensel 1989). Because phalloidin stabilizes actin MFs (Cooper 1987) this may have artificially increased the amount of F-actin. Secondly, root caps were kept under prolonged enzymatic treatments to facilitate cell separation prior to F-actin visualization and this may have changed the physiological state of the root cap statocytes (White and Sack 1990). Recently, dense F-actin networks of maize root cells have been consistently visualized, either with monoclonal antibodies applied to sections taken of Steedman wax-embedded root segments (Baluška et al. 1997a) or with rhodamine-phalloidine staining of non-embedded, but fixed, root preparations (Blancaflor and Hasenstein 1997). In root cap statocytes, both these techniques showed only diffuse cytoplasmic labeling instead of extensive F-actin networks and bundles as found in all other postmitotic cells of the maize root apex. This also corresponds well with previous attempts to visualize the actin cytoskeleton in statocytes of intact root caps (Hensel 1986a; Koropp and Volkmann 1994).

There are three possibilities, not mutually exclusive, for the inability to visualize MF bundles in root cap statocytes. First, current methods may not preserve intact F-actin networks in statocytes. Second, statocytes may contain a dynamic actin-based cytoskeleton, consisting of numerous short but interconnected F-actin elements and high G-actin levels, precluding the visualization of distinct F-actin networks or cables. Third, blockage of the actin-antibody binding sites by MF-associated proteins is also feasible, although this was reported to be more relevant for the rhodamine-phalloidin technique (Jackson and Heath 1993). However, both monoclonal antibodies (Baluška et al. 1997a) and the rhodamine-phalloidin technique (Blancaflor and Hasenstein, 1997) revealed the same images in all cells of maize root apices. With respect to the absence of F-actin cables, a situation similar to that in statocytes occurs in germinating zygotes of brown algae, which show predominantly diffuse actin (Kropf et al. 1989), and in budding yeast cells, which display only actin patches beneath the growing portions of plasma membrane (Mulholland et al. 1994). In this respect, the finding that the actin cytoskeleton is composed of unique actin isoforms in the root cap cells (MacLean et al. 1990; An et al. 1996) is both interesting and perhaps of some relevance.

After approaching the bottom of the statocyte sedimented root cap statoliths associated with dynamic MF networks obviously interact with the dense arrays of cortical MTs. Indirect experimental evidence for this is that depolymerization of all MTs enhances the gravity-dependent mobility of statoliths in inverted cress root cap statocytes (Baluška et al. 1997b). Extensive interactions among statolith-associated cytoskeletal elements, cortical MTs, ER elements, and membrane-associated cytoskeleton would suggest that, at the statocyte periphery, the unrestrained gravisensing of root cap statocytes also involves 'remnants' of the restrained gravisensing system. In other words, despite the cytoskeletally unrestrained sedimentability of statoliths in the statocyte interior, the cytoskeleton-based restraining of statolith mobilities and positioning becomes effective at the statocyte periphery where fine actin microfilaments get efficient support from cortical MT arrays and numerous ER elements. Enmeshment of statoliths within fine and extremely dynamic networks of actin MFs, interacting with putative myosins at their surfaces, could be relevant for effective transmissions of perceived stimuli towards the cortical cytoskeleton and plasma membrane. Numerous indirect data support the existence of an actomyosin-based cytoskeleton in the internal cytoplasm of statocytes (see also Perbal et al. 1997, this issue). The discovery of starchless root mutants lacking distinct statolith sedimentability but preserving, although at reduced levels, their abilities to sense and respond to gravity has challenged the classical statolith-based theory of root graviperception (Caspar and Pickard 1989; Kiss and Sack 1989; Kiss et al. 1989). By preserving some features of the unrestrained gravisensing, like a partial free mobility of their starchless plastids, the restrained gravisensing could be expected to be much more efficient in statocytes of these mutants. This would then allow gravity to be sens to a certain extent in these roots, leading to a graviresponse even in

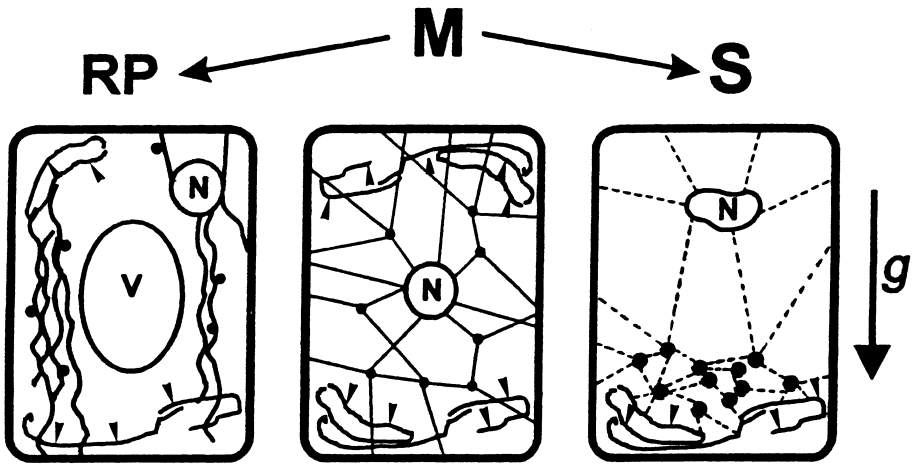

Fig. 2. Schematic depiction of the actin cytoskeleton in different cell types of the maize root apex. The typical meristematic cell (*M*) exhibits a complex F-actin network (*straight lines*) that interconnects plastids (*black spheres*), the nucleus (*N*), and membranes. The elongating cells in the root proper (*RP*) typically contain longitudinal F-actin bundles (*curled lines*) which appear to have, due to their curled appearance, reduced tension. Plastids (*black spheres*) central vacuole (*V*) and nucleus (*N*) are depicted. Postmitotic development into statocytes (*S*) is characterized by the absence of internal F-actin bundles; however, numerous indirect data indicate the possible presence of fine networks of dynamic actin microfilaments (*dashed lines*). Interconnected fragments of F-actin are enriched around statoliths (*black spheres*) and nucleus (*N*). *Black arrowheads*, position of ER elements; *g*, gravity vector

the absence of unrestrained gravisensing associated with the statolith sedimentation. It would be highly desirable to characterize the statocyte cytoskeleton in these mutants in order to obtain further insight in this respect.

In a line with the above reasoning, immunofluorescence of myosin-like proteins showed distinct domains of grouped statoliths within the cytoskeleton-depleted cytoplasm of maize root statocytes (Fig. 1E). Plastid membranes from cress root statocytes have a similar enrichment of myosin-related proteins (Wunsch and Volkmann 1993). Interestingly, using the same antibody, distinct accumulations of myosin-related proteins were also found around the statoliths of *Chara* rhizoids (Braun 1996). Since *Chara* rhizoid statoliths are not amyloplast-based but represent barium sulphate-containing vesicles, the abundant presence of myosin-related proteins could be viewed as a general feature of statoliths in plant cells. The actomyosin-based aggregation of statoliths would create an ostensibly unified group with a larger functional mass. Recent findings indicate that unconventional myosins are involved in signal transduction (Bähler 1996), providing the theoretical background for a model which incorporates actomyosin-based interactions into the concept of restrained gravisensing. All these features could explain the rapid perception time of a few seconds reported for root cap statocytes (Behrens et al. 1985).

Developmentally generated polarity of root cap statocytes is known to be essential for gravisensing. In cress and lentil roots, this structural polarity is both established as well as maintained by the cytoskeleton. The MFs are involved in the distal accumulation of ER elements (Hensel 1985, 1987) and in the proximal positioning of the nucleus (Hensel 1985; Lorenzi and Perbal 1990). Intact MT arrays appear to be essential for statocyte polarity (Hensel 1984, 1986b). In contrast, the statoliths are not part of the structural polarity of statocytes, they always sediment to the physical base of the cell. However, they abandon this position in microgravity (Volkmann et al. 1991; Buchen et al. 1993; Perbal et al. 1997, this issue).

Finally, we would like to stress the unique character of both the tubulin- and actin-based cytoskeletons in root cap statocytes (Figs. 1, 2). The depletion of endoplasmic MT arrays (Baluška et al. 1997b) apparently allows an unobstructed sedimentation of statoliths (see Schwuchow and Sack 1994). Similarly, when statoliths of the *Chara* rhizoid were centrifuged away from the apical zone to the subapical MT-enriched zone, they lost their sedimentation properties (Braun and Sievers 1993, 1994; also Braun 1997, this issue). The original behavior was re-established as they again reached the MT-depleted apical zone. We would like to propose that in plant cells depletion of internal F-actin bundles and stable networks (Baluška et al. 1997b) is directly responsible for the lack of ER networks deeper in the statocyte cytoplasm (Barlow et al. 1984) as their spatial organization relies on support from the actomyosin complex (Liebe and Quader 1994; Lichtscheidl 1995; Liebe and Menzel 1995). Partial actin MF depletion in statocytes might be expected to contribute to the formation of their large amyloplasts since plastid division seems to be F-actin dependent both in lower and higher plants (Hashimoto 1992). Last but not least, depletion of MF bundles, MT networks, and of ER membranes, reduces viscosity (Pollard 1976) of the internal statocyte cytoplasm, which provides an intracellular environment inherently suitable for the sedimentation of statoliths.

Possible involvement of the cytoskeleton in root gravitropic response

The appealing concept that the MT cytoskeleton might be involved in differential elongation of graviresponding

roots is based on observations of a parallel alignment between cortical MTs and newly deposited cellulose microfibrils (reviewed in Giddings and Staehelin 1991). The transverse orientation of cortical MTs results in microfibril deposition that facilitates cell elongation perpendicular to the orientation of cellulose microfibrils (Green 1980). The MT cytoskeleton shows inherent dynamic instability (Mitchison and Kirschner 1984) and rapid turnover rates (Hush et al. 1994), enabling plant cells to respond quickly to endogenous and exogenous signals by eliciting developmental responses (Baluška et al. 1993a,b; Shibaoka 1994; Blancaflor and Hasenstein 1995a,b). The intimate involvement of MTs in control of cell growth polarity and in sensing of environmental perturbations makes them ideally suited to integrate multiple events leading to gravitropic response. Reports that MT arrays reorient in cortical cells along the concave side of graviresponding roots (Blancaflor and Hasenstein 1993) as well as in above-ground organs (Nick et al. 1990) and that auxin, the presumptive hormonal signal for the gravitropic response, also causes MT depolymerization (Blancaflor and Hasenstein 1995a; Baluška et al. 1996c), conform with this concept. Also, plant roots do respond to various MT-perturbing drugs. For instance, treatments that depolymerize MTs, including colchicine, IAA and oryzalin, induce swelling of the root tip but not of the root cap (Baluška et al. 1995; 1996c). The swelling originates in the postmitotic transition zone of the root apex (Baluška et al. 1994; 1996b) where the differential cell growth of graviresponding roots is located (Zieschang and Sievers 1991; Ishikawa and Evans 1993; Baluška et al. 1996a). However, later investigations showed that reorientation of cortical MT arrays in cells of gravistimulated roots occurred only after the onset of root gravitropic response (Blancaflor and Hasenstein 1995a) and that cortical MTs may reorient only secondarily, due to mechanical (Zandomeni and Schopfer 1994) and osmotic (Blancaflor and Hasenstein 1995b) stresses. These observations were confirmed in a study on the role of MTs in the graviresponse of maize root apices (Baluška et al. 1996a). It was found that roots devoid of MTs developed root curvatures after their reorientation in the gravity field. In accordance with this, the depolymerization of cortical MT arrays by both oryzalin and colchicine does not disturb growth anisotropy of elongating maize root cells for at least two hours after complete disintegration of their MT arrays (Baluška et al. 1996a).

The possible function of the actin cytoskeleton in the root graviresponse is less well studied. Thimann et al. (1992) proposed that plant cell elongation is causally linked to the polymerization of actin. Moreover, specific plasma-membrane localization of unconventional plant myosin of class VIII (Reichelt et al. 1997), intracellular organization of F-actin, and effects of actomyosin inhibitors on the cell growth in postmitotic growth regions (Baluška et al. 1997a), all indicate a role of the actomyosin-based activities in the elongation of root cells, especially in its initial phases (Baluška et al, 1996b; 1997a; Reichelt et al. 1997). An involvement of the actin-based cytoskeleton in the control of root growth would be consistent with results showing that plasma-membrane-binding activity of the auxin-transport inhibitor, naphthylphthalamic acid, is associated with the actin cytoskeleton (Cox and Muday 1994). Despite the appealing idea that F-actin or actomyosin complexes may be directly involved in the control of root extension, available experimental evidence suggests that, like MT arrays, intact F-actin networks and bundles are not directly responsible for the root gravitropic response. Firstly, a careful examination of F-actin networks in cells of maize root apices during their bending did not reveal any cytologically detectable differences along the concave and convex root sides (Blancaflor and Hasenstein, 1997). Secondly, despite the fragmentation of the F-actin and of reduced root growth rates, curvatures of gravistimulated cytochalasin-treated roots can even exceed those of the control roots (Wendt et al. 1987 for cress roots; Blancaflor and Hasenstein (1997) for maize roots; but see Monshausen et al. 1996 for *Phleum* roots). In contrast, the auxin transport inhibitor naphthylphthalamic acid had no detectable effects on F-actin organization, reduced the root growth rate similar to cytochalasins, but efficiently blocked the differential cell growth (Blancaflor and Hasenstein, 1997). Therefore, we can conclude that both MT arrays and F-actin bundles are not involved in the control of differential cell growth in graviresponding roots.

Supported by a fellowship from the Alexander von Humboldt-Stiftung (Bonn, Germany), by the Grant Agency of Slovak Academy of Sciences (Bratislava, Slovakia) and by AGRAVIS (F.B.). Support to AGRAVIS by Deutsche Agentur für Raumfahrtangelegenheiten (DARA, Bonn) and Ministerium für Wissenschaft und Forschung (Düsseldorf) is gratefully acknowledged. F.B. is also grateful to Dieter Volkmann and Andreas Sievers (University of Bonn) and to Peter W. Barlow (University of Bristol) for stimulating discussions. Supported by NASA grants NAGW-3565 and NAGIO-0190 (K.H.H.).

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