Chapter 31

ACTIN CYTOSKELETON RELATED TO GRAVISENSING IN HIGHER PLANTS

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Abstract: Gravisensing cells (statocytes) from plant root caps are characterized by a polar arrangement of organelles and sedimented amyloplast-based statoliths. Immunofluorescence microscopy fails to visualize prominent actin filaments in statocytes but indicates a highly dynamic cytoskeletal network, composed at least of actin, myosin-like proteins and profilin, surrounding sedimented statoliths. Experiments under microgravity demonstrated that the positioning of statoliths depends on the external gravitational force and on endocellular cytoskeleton-based forces exerted on their surfaces. Accepting the amyloplast-based statolith hypothesis, these results strongly suggest that gravisensing occurs in a close vicinity of statolith surfaces. Experiments with grass nodes revealed transient changes of the signalling molecule IP_3 already few seconds after gravistimulation. The importance of mutants for dissecting the gravity-related signal transduction chains is highlighted.

1.   INTRODUCTION

Gravisensing belongs to the general category of mechanosensing processes which encompass also perception of sound and pressure in animals, and touch and osmosis in plants. The most outstanding feature of mechanosensory perception is the extreme velocity of the process having latencies of less than one millisecond (Kernan & Zuker, 1995). By this characteristic, the mechanosensing process is faster than any other biological process transforming environmental stimuli into cellular responses including photosensing (Hargrave & McDowell, 1993). This special situation is explained by the idea that mechanical forces might be directly transduced to mechanosensitive ion channels of the plasma membrane (e.g. Sachs, 1997;

Spencer et al., 1999) via cytoskeletal elements (e.g. Glogauer et al., 1998), thereby bypassing a need for special receptor molecules (Kernan & Zuker, 1995). Root apices proved to be extremely sensitive towards extremely weak mechanical stimuli (Monshausen & Sievers, 1998).

Gravity-oriented growth of higher plant organs, like roots, is mainly under the control of two signal transduction chains (Volkmann & Tewinkel, 1998). The first one is initiated in specialized gravity sensing cells (known as statocytes) where gravity-induced stimuli transform into a physiological response (for a review see Sack, 1997). The second one ends up in cells of the transition zone (Baluška et al., 1996) which initiates the differential cell elongation (Zieschang & Sievers, 1991). Thus, the transition zone acts as the target tissue which receives root cap signals transmitted from the site of gravisensing. The transition zone cells are uniquely sensitive towards auxin and calcium (Ishikawa & Evans, 1992, 1993) and are induced into their differential elongation at the opposite root flanks, resulting in the gravity-induced re-orientation of the whole root apex (Evans & Ishikawa, 1997). We would like to highlight putative roles of the actomyosin-based cytoskeleton at the gravisensing sites represented by amyloplast surfaces. Currently, a large body of evidence is emerging for the involvement of diverse molecules related to the phosphoinositide metabolism in both signal perception and transduction (for plant cells see Munnik et al., 1998). Finally, we stress the importance of mutants relevant for the disection and exploration of signal transduction chains during root gravisensing.

2.   SITES OF GRAVISENSING

Since the pioneering surgical experiments performed by Juniper et al. (1966) with maize roots, the root cap is well established as site of gravisensing for the underground parts of plants (for genetic evidence see Tsugeki & Fedoroff, 1999). For above-ground plant organs mutants suggest a role of endodermis as site of perception (e.g. Fukaki et al., 1998; Tasaka et al., 1999). In the root cap centre, highly specialized columella cells are characterized by a polar arrangement of cell organelles when the nucleus is mainly localized at the proximal cell pole whereas amyloplasts are sedimented mostly on cortical ER membranes at the distal pole (for review see Volkmann & Sievers, 1979). In spite of recent controversal discussions of 'starchless mutants' in relation to the first step of stimulus transformation, it is accepted that sedimentable particles (statoliths) are involved in an intracellular perception mechanism (for a review see Sack, 1997; for controversal discussion compare Pickard & Ding, 1992; Staves et al.,

1997a,b). Recently, Blancaflor et al. (1998) specified the central root cap cells of the *Arabidopsis* roots as the most important gravisensing site.
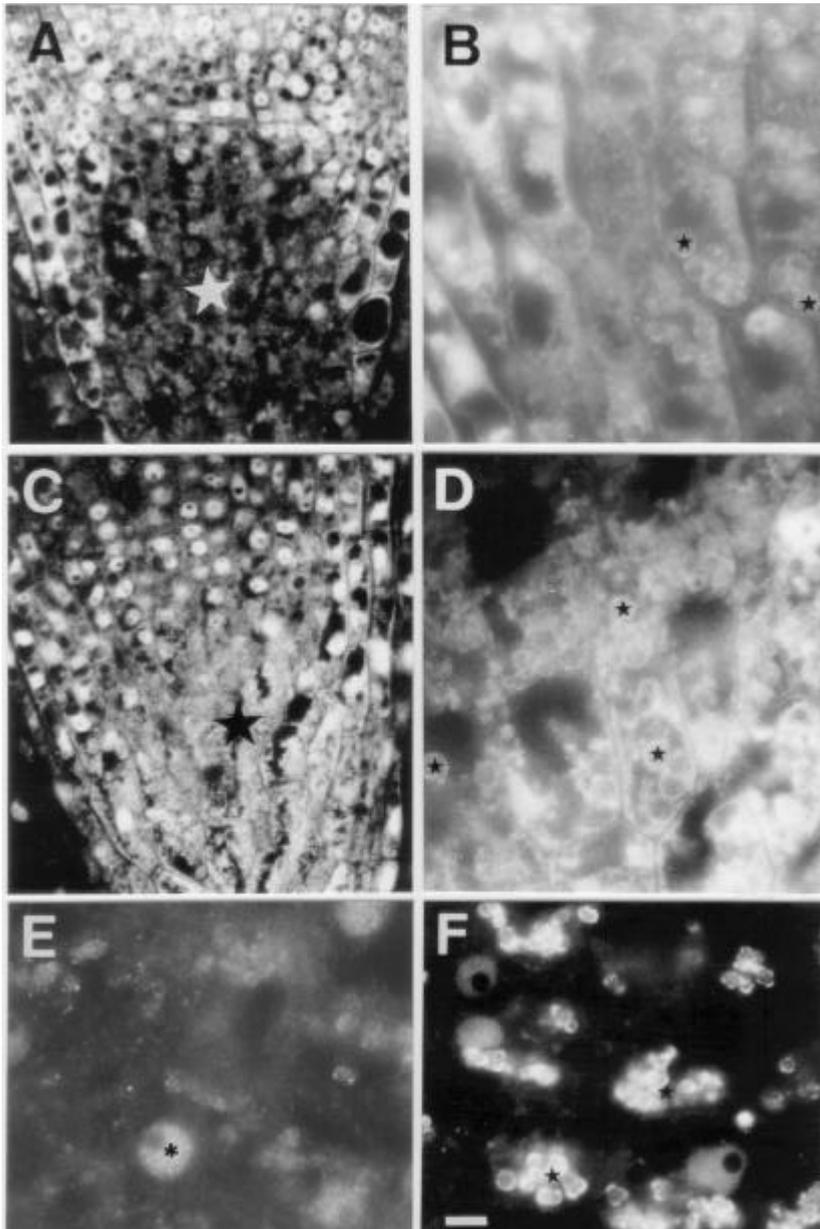
Figure 1. Distributions of actin (A,B), profilin(s) (C,D) reactive to the ZmPRO5 antibody (gift from Chris Staiger), myosin VIII (E), and myosin-like protein (F) reactive to the myosin II antibody (Sigma, M7648) in statocytes (big stars in A, C) of alfalfa (A-D) and maize (E, F) root apices. Note accumulation of actin, profilins, and myosin-like proteins around surfaces of

sedimented amyloplasts (small stars in B, D, F). Myosin VIII does not accumulate around statoliths. Asterisk in E indicates nucleus. Bar = 18 µm for A, C; and 10 µm for B, D, E, F.

Experiments using centrifuges (Wendt et al., 1987) and microgravity (Perbal et al., 1987; for review see Perbal et al., 1997) ruled out the plasma membrane and ER membranes as cell structures directly involved in the process of early stimulus transformation. On the other hand, dynamic cytoskeletal elements, especially those based on the actomyosin complex, have recently come into discussion as sensors and mediators of various environmental signals (for reviews see Volkmann & Baluška, 1999; Staiger, 2000). In general, actomyosin-based networks represent hot candidates for transducing the mechanical force (e.g. Bähler, 1996; Mermall et al., 1998; Volkmann & Baluška, 1999; Staiger, 2000) transformed at the statolith surfaces and passed towards membranes at the cell periphery (Sievers et al., 1989; Sievers et al., 1991). Thus, they might be implicated not only in signal perception but also in propagation of perceived stimuli (for review see Baluška & Hasenstein, 1997).

3.   CYTOSKELETAL ELEMENTS AS A BASIS FOR STATOLITH SHORT-RANGE MOTIONS

Sedimentation of statoliths is obviously facilitated by the specific cytoskeletal status of root cap statocytes which differ from all other root cells. Until now, endoplasmic microtubules (MTs) and bundles of actin filaments (AFs) have not been observed in this unique type of root cap cells (Baluška et al., 1997). Statolith sedimentation, however, is not just a simple one-way process executed progressively during development. Rather, as can be observed using high resolution video microscopy, it represents a complex form of the endocellular motility accomplished via continuous short-range motions (Sack et al., 1986; Volkmann et al., 1999). Dynamic statoliths occur often in clusters (Smith et al., 1997), typically showing saltatory movements (Sack et al., 1986). This situation has also been reported for barium sulfate-filled statoliths in unicellular graviresponding rhizoids of the green alga *Chara* (Hejnowicz & Sievers 1981). Statoliths motions are not randomized, as it is typical for non-sedimentable amyloplasts, but are preferentially correlated according to the direction of the gravity vector. Thus, in developing statocytes, short-range motions of amyloplasts are progressively channelled into directed motions which ultimately culminates in their sedimentation. In other words, oriented gravity-dependent statolith motions overcome the noise of their constitutive random motions (Volkmann et al., 1999).

Actomyosin-driven transport of cell structures and organelles, especially of chloroplasts, is well documented for algae such as *Nitella* or *Acetabularia* (Williamson, 1993; Menzel, 1994; Grolig & Pierson, this volume; Takagi, this volume) as well as for ferns and higher plants (Sato et al., 1999; Kandasamy & Meagher, 1999). Using monospecific antibodies, it can be demonstrated that root cap cells, especially statocytes, possess a highly specific status in comparison to cells from other root tissues concerning the expression of actin and its related proteins, myosin and profilin (Fig. 1). In spite of intensive studies using different immunocytological techniques, visualization of prominent AFs in root statocytes is still lacking (for recent literature compare Baluška & Hasenstein, 1997). In contrast, other cell types of the root proper have been shown to be rich in filamentous actin, distinctly organized in different cell types (Baluška et al., 1997; Vitha et al. 1997; Vitha et al., 2000; Baluška et al., this volume; Vitha et al., this volume). Recently, Blancaflor and Hasenstein (this volume) were able to visualize F-actin networks within isolated statocytes of *Arabidopsis* roots using a special technique combining phalloidin fluorochromes with glycerol incubation. On the other hand, prominent F-actin cables were observed in statocytes of coleptiles (White & Sack, 1990) and hypocotyls (Volkmann et al., 1993) after phalloidin labelling. However, these statocytes of above-ground plant organs are distinguished by large central vacuoles and by cytoplasmic streaming which is driven by the actomyosin system (Grolig & Pierson, this volume). The latter situation is completely different to cytoplasmic-rich root cap statocytes lacking any cytoplasmic streaming. Diffuse actin signal in root statocytes surrounds sedimented statoliths. This suggests dense, but short, dynamic F-actin elements surrounded by abundant G-actin in these cells (compare Fig. 1). Interestingly, if statocytes of maize and barley root caps were labelled for F-actin after their enzymatic release from cap tissues, then distinct AF networks were found (White & Sack, 1990). This latter finding indicates that the actin cytoskeleton in root cap statocytes is capable of rapidly assembling a more robust system if these cells are treated with cell wall digesting enzymes which, thereby, change the cellular tensegrity system (Ingber, 1997; Chicurel et al., 1998). A powerful tool for visualization of the plant actin cytoskeleton has been developed using a Green Fluorescence Protein (GFP)-talin construct which visualizes even actin oligomers (Kost et al., 1998; Kost et al., this volume). Using this in vivo approach, dense meshworks of presumably oligomeric AFs are implicated around sedimented statoliths (Mathur Jaideep, personal communication).

Concerning the motor molecule myosin, essential information is relatively rare for higher plants. Using heterologous antibodies against myosin II from chicken muscle, prominent labelling was localized in close vicinity to statoliths of cress root statocytes (Wunsch & Volkmann, 1993;

compare also Fig. 1). Similar observations have been reported for statoliths of maize root caps (Baluška & Hasenstein, 1997) and *Chara* rhizoids (Braun, 1996). Profilin, the actin-binding protein which is essential for the organization of F-actin (Staiger et al., 1997; Gibbon & Staiger, this volume), is detectable around statoliths (Fig. 1). All this suggests that dynamic actin-based cytoskeleton surrounds the statolith envelopes being probably the structural basis for continuous saltatory motions of statoliths even in their sedimented state (Sack et al., 1986; for review compare Volkmann et al., 1999).

4. STATOLITHS BEHAVIOUR UNDER MICROGRAVITY CONDITIONS: ACTOMYOSIN COUNTERACTS GRAVITY

Epxeriments under microgravity showed that the actual position of statoliths in statocytes depends on two forces, the external gravitational force and the endocellular cytoskeletal forces, both of which impinge on the statoliths (Lorenzi & Perbal, 1990; Volkmann et al., 1991). In these experiments, the possibility to switch from higher **g** levels to microgravity has proven to be very important. Experiments investigating cress and lentil roots (Perbal et al., 1986; Volkmann et al., 1986; Perbal & Driss-Ecole, 1989; Lorenzi & Perbal, 1990; Laurinavièius et al., 1996) indicated that statoliths under microgravity did not show random distributions as might be expected, but showed remarkable shifts in direction towards the proximal statocyte pole implicating active motion of these organelles. Direct evidence for actively driven movements of statoliths came from experiments on rockets when, after launch accelerations of some **g**, gravitational conditions changed immediately to microgravity. Under these experimental conditions, statoliths moved in the opposite direction to the originally acting gravity vector within a few minutes (Volkmann et al., 1991). These results were confirmed by observations made during long term experiments (Perbal & Driss-Ecole, 1994; Laurinavièius et al., 1996; Smith et al., 1997; for review see Volkmann et al., 1999). Corresponding behaviour of statoliths from the *Chara* rhizoid was directly observed in *orbit* by tele-communication (Volkmann et al., 1991). In the presence of cytochalasin D, this motion did not occur and statoliths remained in their launch position (Buchen et al., 1993; for review see Sievers et al., 1996). Interestingly, one actin isoform strongly decreased under short-term microgravity conditions (Janßen et al., 1996).

5. THRESHOLD VALUES AS INDICATIONS FOR STIMULUS TRANSFORMATION IN CLOSE VICINITY OF STATOLITH ENVELOPES

Microgravity experiments offered possibilities to study important paramaters like threshold values under controlled conditions of sensor physiology. For plant material cultivated entirely under microgravity conditions, the minimum dose under continuous stimulation (presentation time tp; for details compare Perbal et al., 1997) has been estimated to be 20–30 **g**×sec for cress roots (Volkmann & Tewinkel, 1996), and by extrapolation from microgravity data to be 27 **g**×sec for lentil roots (Perbal & Driss-Ecole, 1994). Correlations of this threshold value with the position of statoliths show that these moved very slightly in the direction of the stimulating gravity vector, generally less than 1 μm. Thus, one must conclude that stimulus transformation occurs in close vicinity to the statolith envelopes. On the basis of these results, it can be hypothesized that gravity perceiving cells are transforming the gravity stimulus into endocellular signalling pathways by cytoskeleton-mediated measuring of the actual positions of continuously repositioning statoliths.

6. SECOND MESSENGER MOLECULES INVOLVED IN GRAVITROPIC SIGNAL TRANSDUCTION CHAINS

Important molecules related to signalling are second messengers like phosphoinositides and calcium (e.g. Yang, 1996). Components of the phosphoinositol signalling pathway have recently been investigated in maize nodes after their gravistimulation (Perera et al., 1999). Already within 10 seconds after gravistimulation, inositol 1,4,5-trisphosphate (IP_3) increases transiently in the faster-growing lower half. Additionally, the activity of phosphatidylinositol 4,5 bisphosphate kinase (PIP_2 kinase) increased transiently within 10 minutes. These results indicate up-regulation of PIP_2 biosynthesis during the gravisensing-graviresponding process. At the moment, however, it is unclear to which signal transduction chain these events are pertinent. Intriguingly, plastids are unique with respect of some lipidic molecular species (e.g. Miège & Maréchal, 1999) which might be relevant for signalling associated with gravisensing.

Regulation of calcium channels via cytoskeletal elements and second messengers is becoming increasingly evident in plant signal transduction chains (Thuleau et al., 1998). Changes of cytosolic free calcium have been documented for *Arabidopsis* roots after stimulation by touch (Legué et al.,

1997) and gravity (Davies et al., 1999). The same is true for calmodulin (see Braam et al., 1997). Cyclopiazonic acid, specific inhibitor of ER-Ca^{2+}-ATPases inhibited the graviresponse of cress roots but not their growth (Sievers & Busch, 1992). For tip-growing pollen tubes there is a general agreement that the two second messenger molecules, calcium and IP_3, are essential for signalling-based tip growth (Franklin-Tong et al., 1996; Staiger, 2000; Vidali & Hepler, this volume).

Convincing data are accumulating that mitogen activated protein (MAP) kinases, another typical component of signal transduction chains well known from yeast to animals, play crucial roles in sensing of diverse environmental factors in plants (Yang, 1996; Bögre et al., 1996) For instance, in alfalfa leaves, MMK4 MAP kinase becomes activated within 1 minute already after 2 s of mechanical stimulation, (Bögre et al., 1996). MAP kinases have been identified as universal multipurpose signalling tools performing important functions, among others, as mediators of mechanosensitive responses (e.g. Heberle-Bors & Hirt, 1994; Bögre et al., 1996; Hirt, 1997; Jonak et al., 1999). However, the crucial question remains again open; to which of the signal transduction chains these molecules are related and how they interact with the cytoskeleton.

7.   ROLE OF MUTANTS IN DISSECTING AND EXPLORATING GRAVITY-RELATED SIGNAL TRANSDUCTION CHAINS

Recent genetical evidence for participation of cytoskeletal elements in gravisensing came from an *Arabidopsis* mutant showing altered response to gravity (ARG, Sedbrook et al., 1999; Rosen et al., 1999). The authors found that the *ARG1* locus encodes for a 45 kDa protein (DnaJ-like protein) containing a coiled-coil region homologous to coiled-coils found in cytoskeleton-interacting proteins. Interestingly, this gene is expressed in all plant organs investigated so far and the encoded protein is related to a conserved molecule involved in signal transduction in *Caenorhabditis elegans.* Therefore, *ARG1* gene might code for a phylogenetically primitive component of the signal transduction chains associated with the process of gravity sensing in plants (for evolutionary considerations compare Barlow, 1995). The authors (Rosen et al., 1999) do, however, not exclude a more general role of the DnaJ-like protein. For instance, it might be a component of macromolecular chaperone complexes having direct impacts on protein folding, trafficking, and degradation; but only indirect effects on gravisensing signal transduction processes. A number of agravitropic mutants are related to auxin physiology suggesting a global role of these

genes in graviresponses (Chen et al., 1998; Luschnig et al., 1998; Godbolé et al., 1999; Marchant et al., 1999) but their involvement in the gravisensing is unclear. In this respect mutants of the PIN family dissecting the auxin transport mechanisms and pathways (e.g. Müller et al., 1998; Palme & Gälweiler, 1999) are becoming highly important.

The recent view of plasmodesmata as gateable channels (Ding, 1998; Overall et al., this volume), including the plasmodesmal localization of molecules like actin (White et al., 1994; Baluška et al., this volume), myosin VIII (Reichelt et al., 1999; Reichelt & Kendrick-Jones, this volume), epitopes reactive to heterologous myosin II antibody (Radford & White, 1998), and ER-based calreticulin (Baluška et al., 1999), sheds some light on the intercellular signal transmission. Interestingly, root cap statocytes lack myosin VIII and calreticulin at their plasmodesmata but these molecules can by recruited there during plasmolysis (Baluška & Volkmann, in preparation). The possible relevance of this phenomenon for the root cap gravisensing remains to be explored.

8.   CONCLUSIONS AND OUTLOOK

If we accept the starch statolith hypothesis, then it must be concluded that the transformation of gravity stimulus, i.e. gravisensing, has to occur in a close vicinity to the statolith envelopes which are surrounded by a highly dynamic cytoskeletal network based putatively on oligomeric F-actin. First evidences emerge that metabolites of the phosphatidylinositol pathway are involved in the gravity-related signal transduction chains. In addition, mechanosensation of gravity might be tightly linked to those MAP kinases which are activated via mechanical stimuli. All this calls for more intensive investigations of interactions among components of the actin cytoskeleton, diverse phosphoinositide signalling molecules, calcium, and MAP kinases. These complex investigations might include the following approaches:
— identification of functional actin isovariants of the highly diverse plant actin gene family (Meagher et al., 1999; this volume);
— identification of additional relevant cytoskeletal elements of plants, like actin-related proteins (Arps), which are critical organizers of the actin dynamics (Machesky & Gould, 1999). The Arp2 gene has already been identified in *Arabidopsis* genome (Klahre & Chua, 1999);
— testing the amyloplast-based statoliths as putative excitable organelles capable of generating and conveying calcium signals; and their envelopes as suitable assembly sites of protein complexes performing gravity perception: putative gravisensing signalosome (for conserved COP9 signalosome see

e.g. Freilich et al., 1999; Mundt et al., 1999; for signalplex in relation to the phototransduction cascade see Montell 1999);
— exploration of possible participation of dynamic plastid stroma-filled tubules (stromules) which are abundant around chlorophyll-free plastids of roots (Köhler et al., 1997; Köhler & Hanson, 2000);
— selection of mutants which are directly dissecting gravity-related signal transduction chains.

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