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# The Cytoskeleton and Gravitropism in Higher Plants

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## Abstract

The cellular and molecular mechanisms underlying the gravitropic response of plants have continued to elude plant biologists despite more than a century of research. Lately there has been increased attention on the role of the cytoskeleton in plant gravitropism, but several controversies and major gaps in our understanding of cytoskeletal involvement in gravitropism remain. A major question in the study of plant gravitropism is how the cytoskeleton mediates early sensing and signal transduction events in plants. Much has been made of the actin cytoskeleton as the cellular structure that sedimenting amyloplasts impinge upon to trigger the downstream signaling events leading to the bending response. There is also strong molecular and biochemical evidence that the transport of auxin, an important player in gravitropism, is regulated by actin. Organizational changes in microtubules during the growth response phase of gravitropism have also been well documented, but the significance of such reorientations in controlling differential cellular growth is unclear. Studies employing pharmacological approaches to dissect cytoskeletal involvement in gravitropism have led to conflicting results and therefore need to be interpreted with caution. Despite the current controversies, the revolutionary advances in molecular, biochemical, and cell biological techniques have opened up several possibilities for further research into this difficult area. The myriad proteins associated with the plant cytoskeleton that are being rapidly characterized provide a rich assortment of candidate regulators that could be targets of the gravity signal transduction chain. Cytoskeletal and ion imaging in real time combined with mutant analysis promises to provide a fresh start into this controversial area of research.

**Key words:** Actin filaments; Cell elongation; Cytoskeleton; Gravitropism; Microtubules; Signal transduction

## Introduction

Gravity is a fundamental factor that affects the growth and development of all living organisms. The downward bending of roots and the upward bending of shoots is one of the most common examples of how gravity affects plant development. This phenomenon, referred to as gravitropism, has been one of the most intensively studied areas in plant biology. However, despite more than a century of research, several aspects regarding the cellular and molecular mechanisms underlying higher plant gravitropism remain a mystery (Kiss 2000).

The cytoskeleton has been receiving increased attention lately for its possible involvement in plant gravitropism (Baluška and Hasenstein 1997; Volkmann and Baluška 2000). The cytoskeleton is a network of filamentous proteins found in all eukaryotic cells that consists of microtubules, actin filaments (F-actin), intermediate filaments, and their

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associated proteins. In plants, the cytoskeleton has been well documented to play a role in a variety of cellular processes including cellular signaling (Volkmann and Baluška 1999; Nick 1999; Staiger 2000), growth orientation and polarity establishment (Bibikova and others 1999; Mathur and Chua 2000; Wasteneys 2000; Baluška and others 2001c), and sensing of environmental stimuli (Örvar and others 2000; Sangwan and others 2001). Because the phenomenon of plant gravitropism involves the coordinated response of several cells and tissues in a series of steps that include signal transduction, gravity sensing, signal transmission, and differential cellular growth (Kiss 2000), it is likely that the cytoskeleton mediates or is at least partially involved in each of the phases of gravitropism.

In recent years, advances in the fields of molecular biology and genetics have led to an increased understanding of the complexities that underlie gravitropism (Chen and others 1999), of which a major accomplishment has been defining the molecular basis of auxin involvement (Dolan 1998; Palme and Galweiler 1999; Muday 2000). In parallel, new breakthroughs in optical microscopy (Blancaflor and Gilroy 2000) and refinements in tissue fixation, and processing methods for electron microscopy have led to new insights into the structural (Zheng and Staehelin 2001) and signaling components (Scott and Allen 1999; Fasano and others 2001; Johannes and others 2001) involved in gravitropism. Research on the plant cytoskeleton has also advanced rapidly within the past decade. Labeling the plant cytoskeleton in living cells using green fluorescent protein (GFP) technology (Kost and others 2000; Ueda and Matsuyama 2000; Granger and Cyr 2001; Hawes and Satiat-Jeunemaitre 2001) and refinements in preparative methods for light microscopy (Baskin and others 1996; Wasteneys and others 1997; Blancaflor and Hasenstein 2000; Vitha and others 2000; Collings and others 2001) have provided new information on the organization and dynamics of the cytoskeleton in plant cells. Furthermore, a combination of biochemical and molecular approaches have led to the identification and isolation of myriad other proteins in plants that are associated with, and appear to regulate the plant cytoskeleton (Muday 2000; Davies and others 2001; Gibbon 2001).

Although reviews on the cytoskeleton and gravitropism have appeared fairly recently (for example, see Baluška and Hasenstein 1997; Volkmann and Baluška 2000), the tremendous progress made in characterizing the molecular components of the plant cytoskeleton in just the past year alone has opened new and exciting possibilities for future research on plant gravitropism. Therefore another review of this topic is warranted. This article focuses on some of the recent developments in plant cytoskeletal research, and discusses how the cytoskeleton and its associated proteins serve as possible sensors and transducers of the gravity signal.

## The Cytoskeleton in Signal Transduction and Gravity Sensing

The mechanism by which a plant senses gravity continues to be an intensely debated topic with at least two lines of thought proposed to explain this process. The starch-statolith hypothesis is the most widely accepted view of how plants perceive gravity. It suggests that gravity is sensed when dense particles or organelles (statoliths) within the gravity sensing cells (statocytes) settle to the bottom of the cell when the plant is reoriented (Sack 1997). In roots, the putative gravity-sensing cells are found at the center of the root cap (the columella cells) whereas endodermal or bundle sheath cells are the statocytes in shoots (Tasaka and others 1999; Kiss 2000; Johannes and others 2001). In both roots and shoots, the statoliths are starch-filled amyloplasts. The alternative to the starch-statolith hypothesis, which is referred to as the gravitational pressure model, proposes that it is the entire mass of the cytoplasm, rather than sedimenting particles, that enables the plant to sense gravity (Wayne and Staves 1996).

Both models on gravisensing in plants can be supported by the current literature. For example, experiments employing laser or genetic ablation (Blancaflor and others 1998a; Fukaki and others 1998), high gradient magnetic fields (Kuznetsov and Hasenstein 1996; Weise and others 2000), and studies on a variety of gravitropic mutants of *Arabidopsis* (Tasaka and others 1999; MacCleary and Kiss 1999; Kiss 2000) support a plastid-based mechanism for gravity sensing. The gravitational pressure model has also been supported by several lines of experimentation, the bulk of which have been conducted with the single-celled internodes of the green algae *Chara* (Staves 1997), although there has been some experimental support for this model in higher plant roots (Staves and others 1997b). The popularity and general acceptance of the starch-statolith hypothesis over the gravitational pressure model stems from the fact that the former is supported by experimental data from different laboratories whereas experimental data supporting the latter model comes almost exclusively from one

group. Furthermore, the methodology that led to the formulation of the gravitational pressure model has been criticized (Ackers and others 2000). With the continuing debate between the advocates of the starch-statolith and gravitational pressure models, and the current experimental evidence to support each model not totally discounting the other (Wayne and Staves 1996; Kiss 2000), the proposal that multiple mechanisms may exist for plant gravity sensing is an appealing concept (Barlow 1995; Sack 1997).

## Statolith-Based Gravity Signaling and Sensing: The Role of the Actin Cytoskeleton

The starch-statolith hypothesis incorporates more of the cytoskeleton into its proposal, therefore, this will be the model that this review will focus on. In their 1997 article, Baluška and Hasenstein presented two possibilities for cytoskeletal-based gravity sensing in higher plant roots, namely restrained and unrestrained gravity sensing. In restrained gravity sensing, cellular organelles (amyloplasts) are anchored to elements of the cytoskeleton, which in turn are linked to putative plasma membrane receptors and other integral proteins (ion channels and pumps). During gravistimulation, amyloplast sedimentation could induce tensional changes within an interlinked cytoskeletal network, leading to the activation of ion channels that then trigger the downstream signaling cascades and the resulting biochemical changes responsible for the gravitropic growth response (Sievers and others 1991). In unrestrained gravity sensing, the cytoskeletal networks anchoring the amyloplasts to the plasma membrane are not robust enough to hold them in place, therefore amyloplasts readily sediment to the physical bottom of the cell (Baluška and Hasenstein 1997). Amyloplast sedimentation could then impinge on other cellular structures (endoplasmic reticulum, ER) and, like restrained gravity sensing, activate the downstream signaling cascades that lead to gravitropic curvature (Sievers and others 1991; Sack 1997). From detailed measurements of amyloplasts sedimentation dynamics in maize columella cells a new cytoskeletal-based tensegrity model of gravity sensing has also been proposed. This model states that signaling in the columella is triggered by sedimenting amyloplasts that locally disrupt the actin networks, which then activates or inactivates unknown receptors in the plasma membrane (Yoder and others 2001). The recently characterized nodal ER in the columella cells was further postulated to shield local plasma membrane receptors from sedimenting amyloplasts, thereby providing a directional vector to the sensing system (Zheng and Staehelin 2001).

In evaluating the above models, one has to consider other critical physiological data. For example, a study involving intermittent stimulation of cress roots on a clinostat estimated the perception time for gravitropic bending to be less than 1 sec and based on measurements of amyloplast sedimentation velocities, displacement of a statolith by less than 0.5 μm is enough for gravity sensing. The only apparent cellular structure that can sense such small displacements is the cytoskeleton (Hejnowicz and others 1998). Although these data can be used to support the restrained gravity-sensing model and some aspects of the tensegrity model, they are inconsistent with the proposal that the nodal ER networks shield plasma membrane receptors from sedimenting amyloplasts to provide directional cues for gravitropism (Zheng and Staehelin 2001) because amyloplasts do not have to reach the physical bottom of the cell for sensing to occur. However, this does not discount the possibility that these novel ER networks are involved in the later phases of signal transmission.

Despite the attractiveness of the above models, the precise relationships among amyloplast sedimentation, the cytoskeleton, plasma membrane receptors, ion fluxes, and the downstream components (for example, auxin redistribution) of the gravity signal transduction pathway have been difficult to characterize. The current experimental evidence, which has relied heavily on imaging the organization of the cytoskeleton in gravity-sensing cells or the use of substances that perturb the cytoskeleton, have so far been inconclusive and sometimes contradictory. Because actin has been the most intensively studied cytoskeletal component in terms of gravity sensing, it is not surprising that most of the controversies lie with the actin cytoskeleton. For example, reports describing the root columella cells as lacking prominent F-actin bundles (Baluška and others 1997; Driss-Ecole and others 2000a; Figure 1A) certainly support the unrestrained gravity-sensing model, because the depletion of internal F-actin bundles in the columella cytoplasm could provide a suitable intracellular environment for amyloplast sedimentation (Baluška and Hasenstein 1997). However, depending on the method used for labeling, there are reports demonstrating distinct F-actin bundles in the root columella (Hensel 1986; White and Sack 1990; Collings and others 2001; Figure 1B), and statocytes

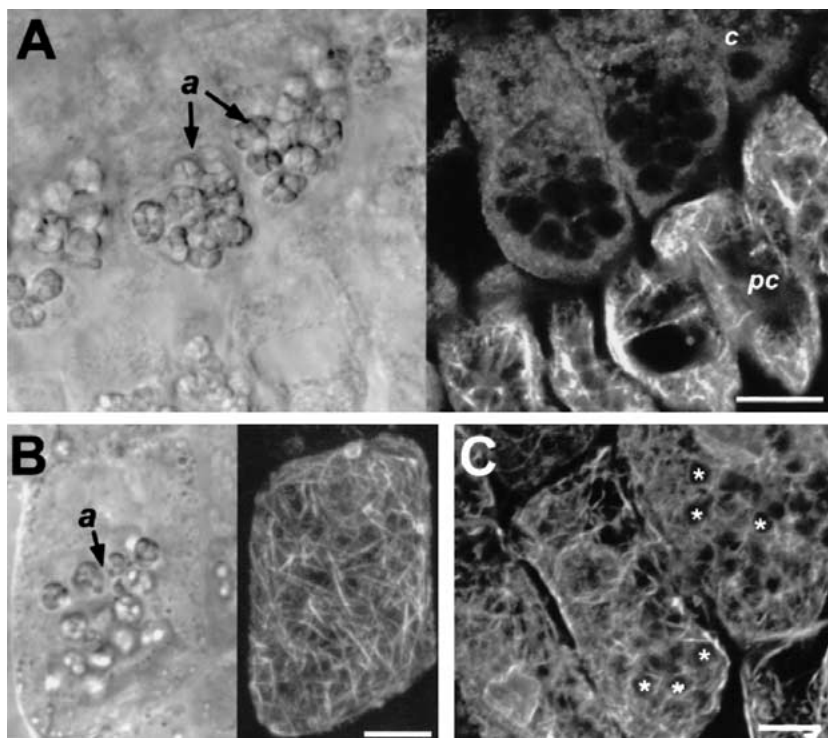


**Figure 1.** Fluorescent labeling of actin in the columella cells of flax roots with anti-actin antibodies and Alexa-fluor-phalloidin. **(A)** Columella cells fixed in formaldehyde display strong fluorescence and distinct actin filaments in the peripheral cap region (*pc*) while only diffuse fluorescence around the amyloplasts (*a*) is observed in the columella (*c*). **(B)** Direct labeling of columella cells with Alexa fluor-phalloidin after cross-linking with MBS (Collings and others 2001) reveals distinct actin filaments. The fluorescence image is a projection of 15 optical sections taken with a confocal microscope. **(C)** A single optical section shows short but distinct bundles of actin surrounding the amyloplasts (*). Bars = 10 μm.

of shoots (White and Sack 1990; Volkmann and others 1993; Collings and others 1998a; Yamamoto and Kiss 2002). These observations suggest that restrained gravity sensing may also occur in higher plants, as is the case in the unicellular *Chara* rhizoids and protonemata (Sievers and others 1996). Like the unrestrained gravity-sensing model, the tensegrity model gives importance to results showing diffuse actin labeling in the columella, which is further substantiated in cryofixed electron microscopy studies of columella cells (Yoder and others 2001). Although the lack of prominent F-actin bundles in the columella region could explain gravity-sensing in roots, it does not account for the gravity sensing apparatus in shoots that have statocytes containing distinct F-actin bundles (White and Sack 1990; Volkmann and others 1993; Collings and others 1998a; Yamamoto and Kiss 2002). Moreover, the results demonstrating distinct actin filaments in the columella add to the uncertainty as to which type of gravity-sensing occurs in the root (Collings and others 2001 and references therein).

The contrasting results obtained in visualizing the actin cytoskeleton in the root columella (Baluska and others 1997; Collings and others 2001) (Figure 1) may be due to the more dynamic nature of actin in these cells, which could be under tight regulation by a plethora of actin binding proteins (ABPs), whose activity in turn may be tightly regulated by other effector molecules (Gibbon 2001; see discussion below). The ability to test the above models will depend significantly on reliable imaging of organizational changes in the actin cytoskeleton during gravistimulation, a task that could prove difficult to accomplish because rapid changes in actin organization during gravistimulation may not be detected by the fixation methods typically used. The application of GFP-based cytoskeletal reporters (Kost and others 2000) combined with rapid freeze fixation techniques (Yoder and others 2001) could prove to be a solution to this problem.

Actin-disrupting drugs have been used extensively to study the involvement of the actin cytoskeleton in gravitropism. Cytochalasin has been shown to block the changes in membrane potential

and proton efflux patterns associated with root gravitropism (Sievers and others 1995; Monshausen and others 1996). Furthermore, the polarized distribution of plastids (Hensel 1985), nuclei (Lorenzi and Perbal 1990), and the morphology of the recently identified nodal ER networks (Zheng and Staehelin 2001) in the columella are affected by cytochalasin, with the maintenance of this polarity being a prerequisite for gravity sensing (Sievers and Heyder-Caspers 1983; Busch and Sievers 1990). However, the effect of actin-disrupting drugs on gravitropism has been variable. Cytochalasin treatment had no observable effect on curvature in rice, maize, and cress roots (Staves and others 1997a), but was shown to marginally increase or induce premature bending in roots and shoots (Blancaflor and Hasenstein 1997; Nick and others 1997), or delay the onset of curvature in *Lepidium* and cress roots (Wendt and others 1987; Monshausen and others 1996). The unpredictable results obtained with cytochalasin therefore cannot be used to conclusively support the different cytoskeletal-based gravity-sensing models, although it has been argued that such results are consistent with the tensegrity model (Yoder and others 2001). It is anticipated that some of the more potent actin-disrupting drugs such as latrunculin B (Bibikova and others 1999; Baluška and others 2001b) or jasplakinolide, used in combination with the measurement of other parameters to test gravisensitivity (for example, presentation time), will provide more consistent results as to how actin disruption affects gravity sensing.

Interestingly, it was shown recently that latrunculin B led to the promotion of gravitropism in inflorescence stems and hypocotyls of *Arabidopsis* (Yamamoto and Kiss 2002), which is similar to the effect of cytochalasin B on root and shoot curvature (Blancaflor and Hasenstein 1997; Nick and others 1997). The promotion of curvature may be due to increased plastid movement resulting from the disruption of the F-actin network (Yamamoto and Kiss 2002) and is consistent with reports showing that cytochalasin or latrunculin increased the rate of amyloplast sedimentation in statocytes of roots (Sievers and others 1989; Yoder and others 2001) and shoots (Nick and others 1997). Although this provides indirect evidence that statoliths are anchored or suspended by a network of actin, a recent report showing that cytochalasin D increased the viscosity of the cytoplasm and slowed down the velocity of amyloplast displacement in lentil columella cells further complicates the interpretation of studies that involve the use of these actin-disrupting drugs (Driss-Ecole and others 2000b). Nevertheless, it would be interesting to see how substances that induce F-actin polymerization affect gravitropism.

Myosin-like proteins and the actin monomer binding protein profilin have been localized to amyloplasts using immunofluorescence techniques (Baluška and Hasenstein 1997; Volkmann and Baluška 2000). The localization of myosin-related proteins at the surface of sedimenting amyloplasts coupled with the observation that the active transport of amyloplasts under microgravity is disrupted by cytochalasin is indicative of the involvement of the actomyosin system in gravity sensing (Volkmann and others 1999; Volkmann and Baluska 2000). This is also the case in tip-growing cells of *Chara* rhizoids and protonemata (Sievers and others 1996). Therefore the interaction between statoliths and the actomyosin system may be a universal mechanism in gravity sensing.

Molecular genetic approaches are also pointing to the cytoskeleton as a mediator of the early gravity-signaling events. The *arg1* (altered response to gravity) gene encodes a DnaJ-like protein whose C-terminal domain contains motifs with sequence similarity to cytoskeletal-binding proteins, and a mutation in this gene alters gravitropism (Sedbrook and others 1999). Moreover, the molecular components of the auxin transport pathway in plants, which is well established to be an important player in gravitropism (Dolan 1998; Rashotte and others 2000), is being rapidly characterized (Palme and Galweiler 1999; Muday 2000). The auxin efflux carrier encoded by *AtPIN1* (PIN-FORMED) in *Arabidopsis* localizes to the basal plasma membrane of stem vascular tissue (Gälweiler and others 1998), whereas a related transporter encoded by *AtPIN2* shows reversed polarity, localizing to the basal ends of cortical cells in *Arabidopsis* roots (Müller and others 1998). A naphthylphthalamic acid (NPA)-binding protein that appears to modulate the activity of the auxin efflux carrier binds to F-actin (Hu and others 2000) and this binding is inhibited by cytochalasin treatment (Butler and others 1998). A recent report that could have additional implications for studies on the cytoskeleton and gravitropism is that the *PIN1* efflux carrier cycles rapidly between the plasma membrane and an unknown cellular compartment, with this cycling dependent on an intact actin cytoskeleton (Geldner and others 2001). Furthermore, the auxin uptake carrier (*AUX1*), in addition to its location in the plasma membrane of root protophloem cells, was also detected in specific columella cells (Swarup and others 2001), particularly in columella layers that are important for gravity sensing (Blancaflor and others 1998a). The localization of *AUX1* in the columella

appeared to be dynamically regulated because the *AUX1* signal was detected either in the cytoplasm or the cell periphery (Swarup and others 2001). This is an important result because it provides a link between the sensing apparatus in the root cap and the responding cells in the elongation zone. It is possible that the polarized distribution, targeting, and turnover of both the auxin uptake and efflux carriers in different cells and tissues are defined and maintained by interactions with the cytoskeleton. The apparent dynamic nature of these carriers makes them excellent candidates for modifying auxin transport patterns during the gravity response.

### Microtubules as Gravity Sensors

The involvement of microtubules in gravity sensing and signaling also remains obscure. Like actin-disrupting drugs, microtubule inhibitors modify the rate of amyloplast sedimentation and inhibit auxin transport in rice coleoptiles. This suggests that microtubules could transmit the pressure of amyloplast sedimentation to auxin transport proteins in the plasma membrane (Godbole and others 2000). Furthermore, the observations that microtubule disruption inhibits gravitropic bending in maize and rice coleoptiles are consistent with the above findings (Nick and others 1991; Godbole and others 2000). Although microtubule disruption also affects amyloplast sedimentation in roots (Baluška and others 1997), these drugs do not affect root bending (Baluška and others 1996a; Hasenstein and others 1999) or auxin transport (Hasenstein and others 1999), indicating that a different mechanism for microtubule involvement in root gravity signaling may exist. This situation could be compared to observations made in rhizoids and protonemata of the green algae *Chara*, wherein the opposite responses of these two cell types are based on different properties of the cytoskeleton (Braun and Wasteneys 1998).

The possibility that microtubules act as gravity sensors and transducers can also be inferred from observations of microtubule rearrangements in fixed plant cells. In intact plant organs, however, it is often difficult to separate the early sensing and signaling events from the accompanying response, since individual cells within the tissue could be influenced by signals from its neighbors. A way around this problem has been to use single-celled protoplasts so that the direct effect of an applied directional force could be analyzed. Using tobacco protoplasts subjected to centrifugation, it was demonstrated that microtubules align parallel to the direction of the applied centrifugal force vector and elongated at an axis of about 90° to the force vector. Furthermore, protoplasts with depolymerized microtubules elongated in random directions, indicating that microtubules are necessary for transducing the directional forces resulting from centrifugation (Wymer and others 1996). Similar experiments with *Brassica* hypocotyl protoplasts showed that exposure to hypergravity (7 or 10 *g*) resulted in the rapid reorganization of microtubules into arrays parallel to the direction of the centrifugal force. On the other hand, simulating weightlessness with a two-dimensional clinostat increased the frequency of randomly organized microtubules (Skagen and Iversen 1999). These data, taken together with *in vitro* studies showing that the self-assembly of tubulin subunits into microtubules is dependent on gravity, raise the possibility that a simple biological molecule such as a microtubule could function as a gravity receptor (Papaseit and others 2000), and that transient biophysical forces such as those imposed by gravity may be sufficient to affect microtubule alignment in intact plant organs (Wymer and others 1996).

## The Gravitropic Response: is Differential Growth Mediated by the Cytoskeleton?

### Microtubules

The possibility that microtubules are involved in regulating the gravity-induced differential growth of plant organs is based on the hypothesis that microtubules provide the template that controls cellulose microfibril deposition in the developing cell wall by orienting the cellulose synthesizing complexes located at the plasma membrane (referred to as the ''alignment hypothesis''; Baskin 2001). The high tensile strength of cellulose microfibrils could then restrict growth to a direction that is perpendicular to the predominant orientation of microfibrils and microtubules (Green 1980).

Developing roots provide a good example of the close correlation between microtubule orientation and the directionality of growth (Barlow and Baluška 2000). Along the root elongation zone, cortical microtubules are arranged in strictly transverse orientations and as the cells mature, microtubules shift to longitudinal orientations, which coincide with a reduction in cellular growth rates (Figure 2A). Microtubules have been implicated in the graviresponse of plants because of studies showing that they reorient from transverse to longitudinal arrays along the slower growing, lower side of

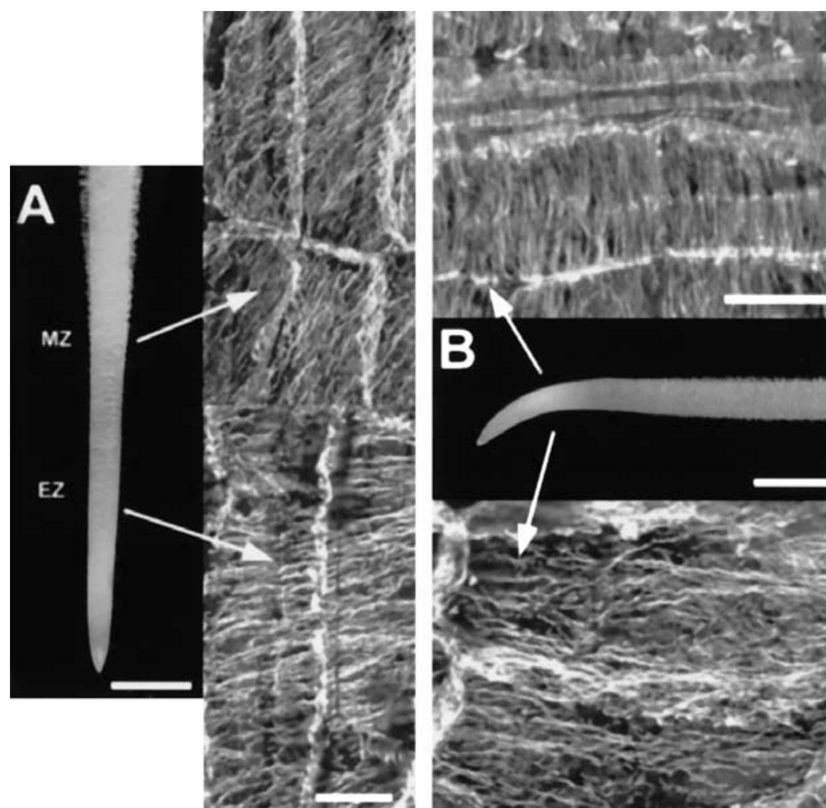


**Figure 2.** Microtubule orientation in vertical and graviresponding *Medicago truncatula* primary roots. **(A)** Microtubules in the elongation zone (EZ) are transverse with respect to the long axis of the root. Microtubules shift to oblique arrays in cells of the maturation zone (MZ). **(B)** The cells on the faster growing upper side of graviresponding roots continue to display transverse microtubules while cells on the slower growing lower side display longitudinal microtubules. The shift in microtubule orientation corresponds with the declining growth of cells. Bars = 1 mm (for root images); 10 μm (for fluorescent images).

downward bending roots (Blancaflor and Hasenstein 1993, 1995; Figure 2B), and from longitudinal to transverse in the faster growing, lower side of upward bending shoots (Nick and others 1990; Himmelspach and others 1999). By virtue of the alignment hypothesis, it was suggested that microtubules may be actively regulating wall properties (that is, the orientation of microfibril deposition) that lead to a modification in cell growth, and ultimately to gravitropic curvature (Blancaflor and Hasenstein 1993). However, there are several studies in roots that argue against this explanation. First, a time course analysis of microtubule reorientation during root gravitropic bending revealed that microtubules reorient only after root curvature has commenced (Blancaflor and Hasenstein 1995). Second, maize roots whose microtubules were depolymerized or stabilized (that is, prevented from reorienting) with microtubule disrupting drugs were still capable of gravitropic curvature (Baluška and others 1996a; Hasenstein and others 1999). Third, roots of the *BOTERO1*(*Bot 1*) mutants of *Arabidopsis*, which are characterized by disorganized cortical microtubule networks in the elongation zone, still exhibit a gravity response (Bichet and others 2001). Although a recent study reported that reducing the level of α-tubulin expression by antisense approaches resulted in reduced ability of *Arabidopsis* roots to respond to gravity, differences in the angle of curvature were only detected after 12 h of reorientation, indicating that it is the later stages of gravitropism that are affected by microtubule disruption (Bao and others 2001). There is no doubt that microtubules are parallel to newly deposited cellulose microfibrils. However, numerous exceptions have been reported (Baskin 2001), therefore microtubules alone may not be responsible for regulating root growth (Granger and Cyr 2001) or for the changes in cell wall properties that lead to differential growth during the graviresponse. The modification in cell wall properties could involve other wall components such as expansins, which have recently been shown to be differentially expressed in opposite

sides of graviresponding roots (Zhang and Hasenstein 2000).

In contrast to roots, maize and rice coleoptiles exposed to microtubule disrupting drugs exhibit a reduction in the upward bending response (Nick and others 1991), and a disruption in auxin transport patterns (Nick and others 1997; Godbole and others 2000), suggesting that microtubules may be important for the graviresponse at least in aboveground plant organs. However, a detailed study of the growth kinetics and microtubule organization in maize pulvinus revealed no differences in microtubule orientation between opposite flanks of the stem during gravistimulation, leading to the conclusion that microtubules do not directly control gravitropic bending, at least in the maize pulvinus system (Collings and others 1998a). Alternatively, microtubule reorientation during gravitropic bending could be a response to the mechanical strain of bending rather than an active modulator of the growth response. For example, experiments that prevented the upward curvature of maize coleoptiles, or forced the coleoptiles to bend down instead of up, either stopped microtubules from reorienting, or caused microtubule orientations that were opposite to the orientations observed in normal graviresponding coleoptiles (Fischer and Schopfer 1998). Similarly, microtubules in epidermal cells of sunflower hypocotyls reoriented in the direction of maximal wall stress, that is, longitudinal stress, caused microtubules to reorient parallel to the direction of stress (Hejnowicz and others 2000). However, a recent study by Himmelspach and Nick (2001) demonstrated that microtubule reorientation in maize coleoptiles induced by gravistimulation can precede gravitropic curvature. Restricting the bending and growth of coleoptiles by attaching them to glass slides showed that growth inhibition in the absence of gravitropic stimulation does not cause microtubules to reorient. Based on these findings, it was concluded that microtubules could respond to gravity directly, and that microtubule reorientation during gravitropism is not merely a consequence of mechanical bending stresses as previously described (Fischer and Schopfer 1998), but an active modulator of gravitropism. This conclusion is further substantiated by *in vivo* observations of microtubule rearrangements in living corn coleoptiles (Himmelspach and others 1999).

Other factors that induce microtubules to reorient in plants include ethylene (Baluška and others 1993) and applied electrical fields (Blackman and Overall 1995). Electric currents are associated with gravitropism and are likely due to calcium and proton fluxes (Weisenseel and Meyer 1997). Calcium currents were detected specifically in cells of the elongation zone of *Arabidopsis* roots (Kiegle and others 2000) while a drop in apoplastic pH of the elongation zone accompanied gravitropic bending (Fasano and others 2001). Microtubules appear to modulate calcium channel activity (Thion and others 1998), and recent studies implicating ethylene in gravitropism (Madlung and others 1999) add to the variety of stimuli that can alter microtubule reorganization during plant graviresponse. Although it is far from clear how all these stimuli are integrated into the realignment of microtubules during gravitropic organ bending, the graviresponse system of plants promises to be an attractive system for studying the mechanisms of microtubule redistribution and cell-cell communication in intact plant organs (Overall and others 2001).

## Actin Filaments

*Longitudinal actin bundles and cell elongation.* In contrast to microtubules, the role of actin in gravity-induced differential growth has received less attention despite recent evidence showing that actin filaments are involved in the regulation of cell elongation (Baluška and others 2000; Waller and others 2000). In elongating plant cells, the most commonly visualized actin networks are thick subcortical bundles that are typically oriented in longitudinal directions. In maize coleoptiles, these actin bundles condense during phases of growth inhibition, whereas a network of finer longitudinal bundles assembles during phases of rapid cell elongation (Waller and Nick 1997). The inhibition of growth in oat coleoptiles resulting from a variety of treatments was also demonstrated to be correlated with an increase in actin bundling (Thimann and others 1992), while the tension and stability of F-actin increased when the growth of soybean suspension cells and maize roots cells was inhibited by aluminum (Grabski and Schindler 1995; Blancaflor and others 1998b). Based on these results, it was proposed that actin regulates cell elongation by mechanically constraining cell elongation, by influencing microtubule alignment, or by controlling vesicle transport to areas of active cell wall growth (Waller and others 2000). Additional work is needed to determine if any of these proposals are valid and it is likely that a variety of actin-binding proteins (APBs) are involved in this process (see discussion below).

A similar mechanism was proposed for the role of longitudinal actin bundles in roots, and appears to be most significant in a region of the root referred to as the transition zone (Baluška and others 2001c).

The transition zone (formerly called the postmitotic isodiametric growth zone) is a region between the meristematic and elongation zone where cells exhibit similar rates of extension in both width and length (Baluška and others 1996b). This assumption was challenged recently by detailed kinetic studies analyzing longitudinal and radial root growth rates (Baskin and Beemster 1997). Nevertheless, the physiological significance of this root region cannot be totally discounted in view of the distinctive growth properties it displays in response to a variety of environmental stimuli, including auxin and gravity (Ishikawa and Evans 1993).

Like studies on shoots, most of the assumptions on the significance of longitudinal actin bundles in roots have come from immunofluorescence techniques. Using these techniques, the transition zone cells of maize roots showed the existence of straight actin bundles attached laterally to the nucleus. During the onset of rapid cell elongation, these straight actin bundles took on a wrinkled appearance. Such changes in the state of actin were proposed to drive cellular elongation by affecting the mechanical stretching of the plasma membrane and the activity of ion channels that drive cell elongation (Baluška and others 2000). The significance of these actin bundles in the transition zone is further supported by a study on a dwarf mutant of maize (*lilliputian*) that exhibits, among other things, a thickening of the root apex and the absence of cell elongation in the root cortex. Immunofluorescence labeling revealed that the straight actin bundles in the transition zone of this mutant were severely disrupted, and the thickening of the root apex induced by long-term incubation in Latrunculin B mimicked the phenotype of the *lilliputian* mutant (Baluška and others 2001a,b).

Despite the correlation between the state of actin bundling and elongation growth, graviresponding maize roots revealed no differences in the organization of the longitudinally oriented actin bundles between the faster growing upper and slower growing lower flanks of maize roots (Blancaflor and Hasenstein 1997). Similarly, no changes in the distribution of actin bundles in gravistimulated maize pulvinus were observed (Collings and others 1998a). However, unlike the more highly ordered microtubules, organizational changes in F-actin have been difficult to assess, and any changes (or lack of changes) in the organization of the longitudinal actin bundles in plant tissues, especially after harsh methods of tissue preparation, can be subject to overinterpretation (Blancaflor and Hasenstein 2000). Alternatively, changes in the organization of longitudinal actin bundles during differential growth could be very subtle and not easily detected by the current fluorescence microscopy methods. One possibility is that modifications in the tension of the actin bundles occur during differential cell elongation. Such a scenario has been demonstrated in cells that have been exposed to a variety of growth-modifying compounds using optical trapping techniques (Grabski and others 1998 and references therein). The application of these techniques to the problem of plant gravitropism might well reveal modifications in the state of the actin cytoskeleton during differential cell growth.

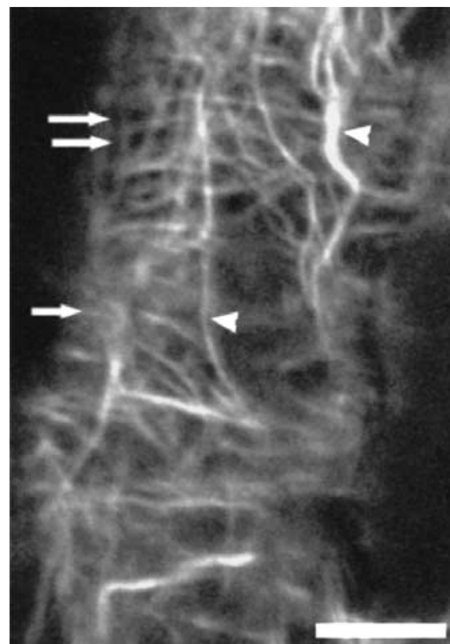

**Figure 3.** Fluorescence image of an elongating root cell of maize labeled with Alexa-fluor phalloidin after MBS cross-linking (Blancaflor 2000). Two types of actin filaments are present in elongating root cells: thick subcortical longitudinal bundles (arrowheads) and fine cortical transverse arrays (double arrows). Both types of F-actin arrays could play a role in regulating cell elongation. Bar = 25 μm **(A)**; 10 μm **(B)**.

## Cortical Actin and the Regulation of Differential Growth

In addition to the longitudinal actin bundles, a fine network of transversely oriented actin located on the outer cortex (Figure 3) has been imaged in elongating cells by using a variety of techniques, including immunofluorescence (Collings and others 1998a,b), phalloidin labeling (Blancaflor 2000; Blancaflor and Hasenstein 2000; Collings and others

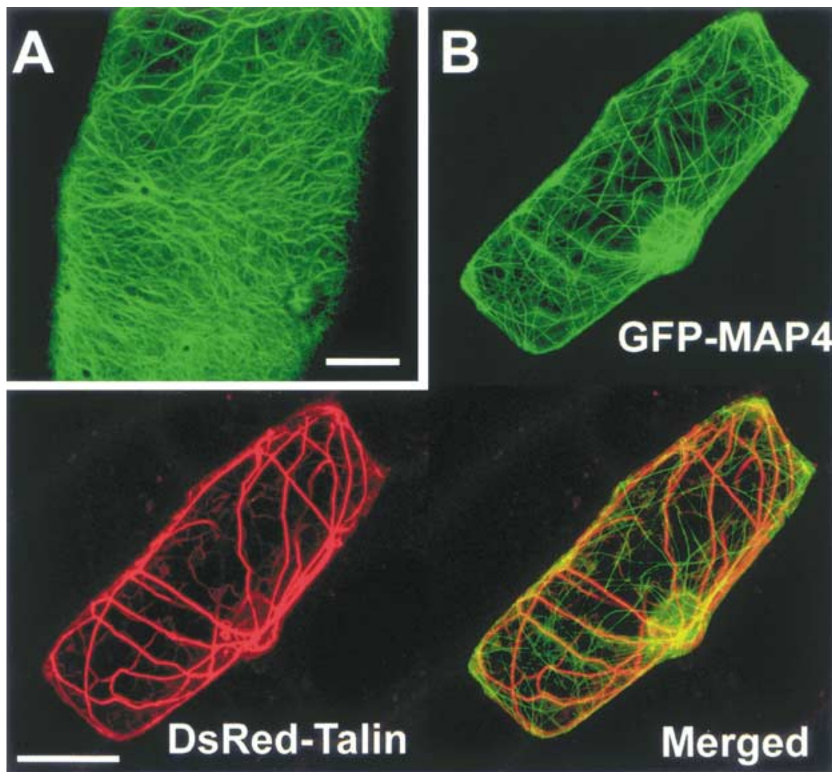


**Figure 4.** Fluorescent protein constructs that bind to the plant cytoskeleton promise to yield substantial information on cytoskeletal dynamics and function during higher plant gravitropism. **(A)** eGFP-mouse talin construct transiently expressed in a living onion epidermal cell reveals fine cortical actin networks. **(B)** Co-bombardment of a GFP-MAP4 construct (Granger and Cyr 2001) and red fluorescent protein (DsRed)-mouse talin construct allows double labeling of microtubules and F-actin in living onion cells. The availability of spectral variants of fluorescent proteins can therefore be used to study the interaction between the different components of the plant cytoskeleton. Bars = 10 μm **(A)**; 50 μm **(B)**.

2001), microinjection (Cleary 1995), and GFP-talin fusions (Kost and others 2000; Figure 4A). Since the orientation of cortical actin filaments parallels the transverse alignment of cortical microtubules, their involvement in elongation could be mediated through their interaction with microtubules (Collings and others 1998a,b). There have been a substantial number of reports employing biochemical, pharmacological and electron microscopy approaches to demonstrate actin-microtubule interactions in plants, leading to the hypothesis that an actin-microtubule feedback system is involved in regulating plant morphogenesis (Collings and Alien 2000 and references therein). Interestingly, by microinjecting the actin monomer binding protein ADF (see discussion below), the organization of actin in plant cells can be modified from predominantly longitudinal bundles to fine transverse arrays that parallel cortical microtubules (Hussey and others 1998).

The possibility that cortical actin filaments play a role similar to that of cortical microtubules in controlling cell morphogenesis was demonstrated in apical cells of the brown alga *Sphacelaria*. These cells lack cortical microtubules, but contain cortical actin filaments that are mutually aligned with cellulose microfibrils, suggesting that at least in these cells, actin may play a role in the oriented deposition of cellulose microfibrils (Karyophyllis and others 2000). The effect of actin-disrupting drugs on plant roots (Baskin and Bivens 1995; Blancaflor 2000; Baluška and others 2001b) and the parallel alignment and demonstrated interaction of cortical actin and microtubules in some plant cell types (Collings and others 1998b), indicates that cortical actin may, in coordination with microtubules, influence cellulose deposition in higher plants.

Although gravity-induced reorientation of cortical microtubules in both roots and shoots has been reported (Figure 1), rearrangements in cortical actin during gravitropism has not been investigated. Since cortical actin undergoes reorientations similar to microtubules in developing roots (Blancaflor 2000), it is possible that the cortical actin networks

are also modified during the graviresponse. With the availability of fluorescent probes to label actin in living cells (Kovar and others 2001; Kost and others 2000) combined with less harsh methods of tissue fixation (Blancaflor and Hasenstein 2000) and cryofixation (Zheng and Staehelin 2001), this question will hopefully be addressed in the future. Simultaneous imaging of cortical actin and microtubules in living plant cells using different spectral variants of fluorescent protein reporters should also reveal how these two components of the cytoskeleton interact during gravitropic bending (Figure 4B).

## Cytoskeletal Binding Proteins: Cellular Targets of the Gravity Signal Transduction Chain

The reorganization of the cytoskeleton in response to a variety of internal and external stimuli is likely to be mediated by the activity of other proteins that bind to actin and/or microtubules. However, the involvement of cytoskeletal-binding proteins in mediating plant gravitropism has been relatively unexplored. It is anticipated that this will change within the next few years due to considerable progress being made in defining the molecular, biochemical, and cytological characteristics of several plant cytoskeletal-binding proteins, some of which appear to be regulated by signaling intermediates and second messengers suspected to be involved in transmitting the gravity signal.

### Actin Binding Proteins

Cytoskeletal binding proteins can be classified into either actin binding proteins (ABPs) or microtubule-associated proteins (MAPs). The best characterized ABPs in plants are the actin monomer-binding proteins, profilin, and actin depolymerizing factor (ADF/cofilin). Both proteins are encoded by multigene families, and have been demonstrated to have dramatic effects on the organization of the plant actin cytoskeleton (Gibbon 2001). Recent *in vivo* studies involving profilins and ADF are consistent with actin's role in modulating cell expansion, and therefore strengthen its position as a player in regulating differential cell growth. For example, *Arabidopsis* plants overexpressing ADF are characterized by a reduction in growth, and accompanied by the disappearance of thick longitudinal actin cables in the affected cells. On the other hand, underexpression of ADF stimulated cell expansion and organ growth while inducing the formation of thick longitudinally oriented actin bundles (Dong and others 2001). These results are consistent with the idea that the longitudinal actin cables are part of the machinery that drives directional cell expansion (Waller and others 2000; Baluška and others 2000), and ADF could be responsible for regulating the state of these longitudinal actin bundles. Similarly, it was shown that over or underexpressing profilin (PFN-1) in *Arabidopsis* resulted in abnormal cell elongation and cell shape, but caused no defects in the organization of the actin cytoskeleton (Ramachandran and others 2000). This stands in contrast to a previous study showing that microinjection of profilin in plant cells caused the breakdown of actin filaments (Staiger and others 1994). Nonetheless, the observation that overexpression of profilin disrupts normal patterns of plant growth is consistent with profilin and thus actin's role in cell elongation. The absence of changes in the actin cytoskeleton upon overexpression of profilin could indicate that it may be regulating a class of actin filaments other than the thick longitudinal cables (Ramachandran and others 2000). The dynamic cortical actin networks that are often more difficult to image may be a likely target of this protein (Collings and Allen 2000; Blancaflor 2000).

The application of novel biochemical and cell biological approaches as well as the rapid growth of plant genome databases are also leading to the isolation and characterization of other classes of ABPs in plants, including fimbrins (Kovar and others 2001), villins (Tominaga and others 2000), and myosins (Yokota and Shimmen 2000; Liu and others 2001). In contrast to profilins and ADFs, which are actin monomer binding proteins, villins and fimbrins are actin filament cross-linking proteins. Though villins appear to function by crosslinking plant actin filaments into thick bundles (Tominaga and others 2000), microinjecting fluorescently labeled fimbrins decorate a more dynamic cortical actin network, which interestingly is disrupted by coinjections with profilin (Kovar and others 2001).

The possibility that these cytoskeletal-binding proteins may be modulating the reorganization of the cytoskeleton during gravistimulation is attractive in view of reports demonstrating that molecules induced during gravistimulation modulate the activity of these proteins. For example, changes in cytoplasmic pH have been documented to occur in the columella cells of *Arabidopsis* (Scott and Allen 1999; Fasano and others 2001) and pulvinal cells of maize (Johannes and others 2001) during early stages of gravitropism. Furthermore, the differential pH changes observed in graviresponding *Phleum*

roots is abolished by cytochalasin D treatment (Monshausen and others 1996). Because ADF has a greater tendency to depolymerize F-actin at elevated pH (Gibbon 2001), the rapid cytoplasmic alkalinization of the gravity-sensing cells observed in both roots and stems (Fasano and others 2001; Johannes and others 2001) could alter the dynamics of the actin cytoskeleton in the statocytes.

Profilin and ADF are also known to interact with elements of the phosphoinotiside signaling system (Gibbon 2001), while gravistimulation causes an increase in inositol 1,4-5 triphosphate levels, suggesting that cytoplasmic calcium fluxes are involved in gravitropism (Perera and others 1999, 2001). Villins and villin-like homologues in plants exhibit calcium dependence (Yokota and Shimmen 2000), and the activity of actin as well as other ABPs such as ADF are modulated by phosphorylation and dephosphorylation events (Grabski and others 1998; Yokota and others 2000; Gibbon 2001). Although direct measurements of cytoplasmic calcium changes during root gravitropism could not be detected by quantitative fluorescence imaging methods (Legue and others 1997), there is evidence suggesting a role for calcium-dependent protein kinases (Lu and Feldmann 1997), phosphatases (Rashotte and others 2001), and calmodulin (Sinclair and others 1996) in plant gravitropism. A challenge for gravitropism researchers will be to determine how the different signaling molecules interact with each other during plant gravitropism. The diverse molecular components that affect the plant cytoskeleton make it a good candidate for integrating the different regulatory elements that appear to be part of the gravitropic signaling cascades.

## Microtubule associated proteins

Like actin, microtubule reorganization during differential cellular growth could involve regulatory and structural proteins that interact with and control the polymerization and/or depolymerization of microtubules. There has been tremendous progress recently in characterizing a number of MAPs in plants, some of which have been shown to be essential for organizing cortical microtubules. Much of the progress has been on the analysis of *Arabidopsis* mutants that exhibit abnormal cell elongation. For example, *Arabidopsis* mutants that were defective in the mechanical strength of interfascicular fibers (*fra2*) and showed reduced elongation in the hypocotyls (*Botero1*) were recently identified. Both mutants contained disrupted cortical microtubules, and molecular cloning revealed that the gene products *fra2* and *Bot1* are similar to the microtubule-severing protein katanin (Burk and others 2001; Bichet and others 2001). Furthermore, an extremely laborious screen involving immunofluorescence allowed the isolation of mutant *Arabidopsis* seedlings that had aberrant microtubule patterns under restrictive temperatures. This led to the identification of MOR1, a MAP that has significant similarities to a family of MAPs in animal cells represented by *Xenopus* MAP215. MOR1 was shown to contain 10 putative HEAT repeats that could modulate protein-protein or protein-plasma membrane interactions (Whittington and others 2001). A gene encoding a protein that localizes to regions of overlapping microtubules was also recently identified, suggesting that it may regulate microtubule bundling (Smertenko and others 2000). The protein translational regulator EF1-α bundles plant microtubules in a calcium-calmodulin-dependent manner (Durso and Cyr 1994), and associates with the actin cytoskeleton (Collings and Allen 2000 and references therein). Cytoskeletal binding proteins such as EF-1 α could potentially mediate actin-microtubule interactions in the cortex of plant cells, but the exact mechanisms for such interactions (Collings and Allen 2000), or whether actin-microtubule interactions are significant for gravitropism remains to be determined.

Another finding that could have important implications on studies of gravitropism and plant signaling in general is the discovery that a 90-kD protein with microtubule binding activity encodes a phospholipase D (PLD) (Gardiner and others 2001). Phospholipases are an important class of membrane-based signaling molecules shown to regulate a wide variety of plant developmental processes including cell elongation and plant responses to auxin (Wang 2001). The plant hormone auxin causes cortical microtubule rearrangements in elongating plant cells (Nick and others 1990; Blancaflor and Hasenstein 1995), and microtubule reorientation during differential growth has been suggested to result from the development of auxin asymmetry (Nick 1999). Auxin rapidly up-regulates a K+ channel in maize coleoptiles, with the expression pattern of this channel closely following the asymmetric changes in auxin concentration during gravitropism. The asymmetry in the expression of K+ channels could facilitate differential cell turgor between opposite flanks of the coleoptile, which could then drive gravitropic bending (Philippar and others 1999). The maintenance of auxin asymmetry via auxin transport proteins is facilitated by the actin cytoskeleton (Muday 2000) and auxin transport itself is inhibited by microtubule disrupting drugs (Godbole and others 2000). Interestingly,

abscisic acid (ABA) signaling in guard cells, which involves the activation of K+ efflux into the cell to regulate turgor, involves PLD (Jacob and others 1999). Although the role of ABA in gravitropism is still controversial (Konings 1995), the observation that gravitropic stimulation activates intermediates of the phospholipid signaling system (Perrera and others 2001), and the close association between components of phospholipid signaling and the cytoskeleton (Gibbon 2001; Gardiner and others 2001), opens up exciting areas for investigating hormonal and cytoskeletal regulation of plant gravitropism. A challenge for the future will be to demonstrate that modifications in the expression, distribution, and/or activity of cytoskeletal binding proteins occur in response to gravistimulation.

## Future Prospects

It is clear that we are still a long way from unraveling the role of the cytoskeleton in higher plant gravitropism. However, this review of recent developments on the plant cytoskeleton and gravitropism shows that the gravitropic phenomenon is an elegant system for probing into the mechanisms of cytoskeletal alignment, cytoskeletal based signal transduction, plant hormone action, and plant growth regulation. The traditional methods of studying gravitropism and the cytoskeleton are being supplemented with emerging cell biological techniques such as GFP fusions, caged probes, and a battery of other advanced microscopy techniques (Blancaflor and Gilroy 2000; Fasano and others 2001; Hawes and Satiat-Jeunemaitre 2001). Standard immunofluorescence protocols to image the plant cytoskeleton have provided snap shot images of cytoskeletal rearrangements during gravitropic bending. Although providing valuable information, these techniques are limited in their temporal resolution. Additional insight into the function of the cytoskeleton during gravitropism could be gained from a detailed examination of cytoskeletal dynamics in real time. Although this has been accomplished with microinjection techniques (Himmelspach and others 1999), it is limited in that only single cells at the plant surface can be imaged. The use of fluorescent protein-reporters that decorate the plant cytoskeleton in living cells provides a powerful tool for accomplishing this goal (Figure 4). Fluorescent protein sensors to monitor cytoplasmic calcium and pH have been engineered and applied to study signaling in guard cells (Allen and others 1999), root development (Moseyko and Feldmann 2001), and root gravitropism (Fasano and others 2001). With caged probe technology we should be able to introduce cytoskeletal inhibitors or regulators into plants expressing these physiological sensors, and have control over their temporal and spatial release. Since expressing fluorescent protein constructs in plants is now a routine procedure, simultaneous imaging of ion changes and cytoskeletal redistribution *in vivo* during the graviresponse will also be possible. These advanced cell biological approaches aided by mutant analysis (Firn and others 2000) and large scale genomics initiatives should lead to a better understanding of how the cytoskeleton regulates the century-old problem of gravitropism in higher plants.

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## REFERENCES

Ackers D, Buchen B, Hejnowicz Z, Sievers A. 2000. The pattern of acropetal and basipetal cytoplasmic streaming velocities in *Chara* rhizoids and protonemata, and gravity effect on the pattern as measured by laser-Doppler-velocimetry. Planta 211:133–143.

Allen GJ, Kwak JM, Chu S, Llopis J, Tsien RY, Harper JF, Schroeder JI. 1999. Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. Plant J 19:735–747.

Baluška F, Brailsford RW, Hauskrecht M, Jackson MB, Barlow PW. 1993. Cellular dimorphism in the maize root cortex: involvement of microtubules, ethylene and gibberellin in the differentiation of cellular behaviour in postmitotic growth zones. Bot Acta 106:394–403.

Baluška F, Hauskrecht M, Barlow PW, Sievers A. 1996a. Gravitropism of the primary root of maize: a complex pattern of differential cellular growth in the cortex independent of the microtubular cytoskeleton. Planta 198:310–318.

Baluška F, Volkmann D, Barlow PW. 1996b. Specialized zones of development in roots: view from the cellular level. Plant Physiol 112:3–4.

Baluška F, Hasenstein KH. 1997. Root cytoskeleton: its role in perception of and response to gravity. Planta 203:S69–S78.

Baluška F, Kreibaum A, Vitha S, Parker JS, Barlow PW, Sievers A. 1997. Central root cap cells are depleted of endoplasmic microtubules and actin microfilament bundles: implications for

their role as gravity-sensing statocytes. Protoplasma 196:212–223.

Baluška F, Barlow PW, Volkmann D. 2000. Actin and myosin VIII in developing root apex cells. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, 457–476.

Baluška F, Busti E, Dolfini S, Gavazzi G, Volkmann D. 2001a. *Lilliputian* mutant of maize lacks (cell elongation and shows defects in organization of actin cytoskeleton. Dev Biol 236:478–491

Baluška F, Jasik J, Edelman HG, Salajova T, Volkmann D. 2001b. Latrunculin B-induced plant dwarfism: plant cell elongation is F-actin dependent. Dev Biol 231:113–124.

Baluška F, Volkmann D, Barlow PW. 2001c. A polarity crossroads in the transition growth zone of maize root apices: cytoskeletal and developmental implications. J Plant Growth Regul 20:170–181.

Bao Y, Kost B, Chua N-H. 2001. Reduced expression of α-tubulin genes in *Arabidopsis thaliana* specifically affects root growth and morphology, root hair development and root gravitropism. Plant J 28:145–157.

Barlow PW. 1995. Gravity perception in plants: a multiplicity of systems derived by evolution. Plant Cell Environ 18: 951–962.

Barlow PW, Baluska F. 2000. Cytoskeletal perspectives on root growth and morphogenesis. Annu Rev Plant Physiol Plant Mol Biol 51:289–322.

Baskin TI, Bivens NJ. 1995. Stimulation of radial expansion in *Arabidopsis* roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism. Planta 197:514–521.

Baskin TI, Miller DD, Vos JW, Wilson JE, Hepler PK. 1996. Cryofixing single cells and multicellular specimens enhances structure and immunocytochemistry for light microscopy. J Micros 182:149–161.

Baskin TI, Beemster GTS. 1997. On the post-mitotic isodiametric growth zone in roots. In: Lynch JP, Flores HE, Eissenstat D, editors. Radical biology: advances and perspectives on the function of plant roots. American Society of Plant Physiologist. Rockville, Maryland, p 343–345.

Baskin TI. 2001. On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. Protoplasma 215:150–171.

Bibikova TN, Blancaflor EB, Gilroy S. 1999. Microtubules regulate tip growth and orientation in root hairs of *Arabidopsis thaliana.* Plant J 17:657–665.

Bichet A, Desnos T, Turner S, Grandjean O, Hofte H. 2001. *BOTERO1* is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis.* Plant J 25:137–148.

Blackman LM, Overall RL. 1995. Electric fields affect the orientation of cortical microtubules and cells expansion in pea callus. Protoplasma 189:256–266.

Blancaflor EB, Hasenstein KH. 1993. Organization of cortical microtubules in graviresponding maize roots. Planta 191:231–237.

Blancaflor EB, Hasenstein KH. 1995. Time course and auxin sensitivity of microtubule reorientation in maize roots. Protoplasma 185:72–82.

Blancaflor EB, Hasenstein KH. 1997. The organization of the actin cytoskeleton in vertical and graviresponding primary roots of maize. Plant Physiol 113:1447–1455.

Blancaflor EB, Fasano JM, Gilroy S. 1998a. Mapping the functional roles of cap cells in the response *of Arabidopsis* primary roots to gravity. Plant Physiol 116:213–222.

Blancaflor EB, Jones DL, Gilroy S. 1998b. Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. Plant Physiol 118:159–172.

Blancaflor EB. 2000. Cortical actin filaments potentially interact with cortical microtubules in regulating polarity of cell expansion in primary roots of maize (*Zea mays* L.). J Plant Growth Reg 19:406–414.

Blancaflor EB, Gilroy S. 2000. Plant cell biology in the new millennium: new tools and new insights. Am J Bot 87:1547–1560.

Blancaflor EB, Hasenstein KH. 2000. Methods for detection and identification of F-actin organization in plant tissues. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 601–618.

Braun M, Wasteneys GO. 1998. Distribution and dynamics of the cytoskeleton in graviresponding protonema and rhizoids of Characean algae: exclusion of microtubules and a convergence of actin filaments in the apex suggest an actin-mediated gravitropism. Planta 205:39–50.

Burk DH, Liu B, Zhong R, Morrison WH, Ye Z-H. 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. Plant Cell 13:807–827.

Busch MB, Sievers A. 1990. Hormone treatment of roots causes not only a reversible loss of starch but also of structural polarity in statocytes. Planta 181:358–364.

Butler JH, Hu S, Brady SR, Dixon MW, Muday GK. 1998. In vitro and in vivo evidence for actin association of the naphthylphthalamic acid-binding protein from zucchini hypocotyls. Plant J 13:291–301.

Chen R, Rosen E, Masson PH. 1999. Gravitropism in higher plants. Plant Physiol 120:343–350.

Cleary. AL. 1995. F-actin redistribution at the division site in living *Trandescantia* stomatal complexes as revealed by microinjection of rhodamine-phalloidin. Protoplasma 185:152–165.

Collings DA, Winter H, Wyatt SE, Allen NS. 1998a. Growth dynamics and cytoskeletal organization during stem maturation and gravity-induced stem bending in *Zea mays* L. Planta 207:246–258.

Collings DA, Asada T, Allen NS, Shibaoka H. 1998b. Plasma membrane-associated actin in Bright Yellow 2 tobacco cells. Plant Physiol 118:917–928.

Collings DA, Allen NS. 2000. Cortical actin interacts with the plasma membrane and microtubules. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 145–164.

Collings DA, Zsuppan G, Alien NS, Blancaflor EB. 2001. Demonstration of prominent actin filaments in the root columella. Planta 212:392–403.

Davies E, Stankovic B, Azama K, Shibata K, Abe S. 2001. Novel components of the plant cytoskeleton: a beginning to plant 'cytomics'. Plant Science 160:185–196.

Dolan L. 1998. Pointing roots in the right direction: the role of auxin transport in response to gravity. Genes Dev 12:2091–2095.

Dong C-H, Xia G-X, Hong Y, Ramachandran S, Kost B, Chua N-H. 2001. ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in *Arabidopsis*. Plant Cell 13:1333–1346.

Driss-Ecole D, Vassy J, Rembur J, Giuvarc'h A, Ptoteau M, Dewitte W, Perbal G. 2000a. Immunolocalization of actin in root statocytes of *Lens culinaris* L. J Expt Bot 51:512–528.

Driss-Ecole D, Jeune B, Prouteau M, Julianus P, Perbal G. 2000b. Lentil root statoliths reach a stable state in microgravity. Planta 211:396–405.

Durso NA, Cyr RJ. 1994. A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1α. Plant Cell 6:893–905.

Fasano JM, Swanson SJ, Blancaflor EB, Dowd PE, Kao T-H, Gilroy S. 2001. Changes in root cap pH are required for the gravity response of the *Arabidopsis* root. Plant Cell 13:907–921.

Firn RD, Wagstaff C, Digby J. 2000. The use of mutants to probe models of gravitropism. J Exp Bot 51:1323–1340.

Fischer K, Schopfer P. 1998. Physical strain-mediated microtubule reorientation in the epidermis of gravitropically or phototropically stimulated maize coleoptiles. Plant J 15:119–123.

Fukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey PN, Tasaka M. 1998. Genetic evidence that the endodermis is essential for shoot gravitropism in Arabidopsis thaliana. Plant J 14:425–430.

Gälweiler L, Guan C, Mueller A, Wisman E, Mendgen K, Yephremov A, Palme K. 1998. Regulation of polar auxin transport by *AtPIN1* in *Arabidopsis* vascular tissue. Science 282:2226–2230.

Gardiner JC, Harper JDI, Weerakoon ND, Collings DA, Ritchie S, Gilroy S, Cyr RJ, Marc J. 2001. A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13:2143–2158.

Geldner N, Frimi J, Stierhof Y-D, Jurgens G, Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413:425–428.

Gibbon BC. 2001. Actin monomer-binding proteins and the regulation of actin dynamics in plants. J Plant Growth Regul 20:103–112.

Godbole R, Michalke W, Nick P, Hertel R. 2000. Cytoskeletal drugs and gravity-induced lateral auxin transport in rice coleoptiles. Plant Biol 2:176–181.

Grabski S, Schindler M. 1995. Aluminum induces rigor within the actin network of soybean suspension cells. Plant Physiol 108:897–901.

Grabski S, Arnoys E, Busch B, Schindler M. 1998. Regulation of actin tension in plant cells by kinases and phosphatases. Plant Physiol 116:279–290.

Granger CL, Cyr RJ. 2001. Spatiotemporal relationships between growth and microtubule orientation as revealed in living root cells of *Arabidopsis thaliana* transformed with the green-fluorescent protein gene construct *GFP-MBD*. Protoplasma 216: (in press).

Green PB. 1980. Organogenesis—a biophysical view. Ann Rev Plant Physiol 31:51–82.

Hasenstein KH, Blancaflor EB, Lee JS. 1999. The microtubule cytoskeleton does not integrate auxin transport and gravitropism in maize roots. Physiol Plant 105:729–738.

Hawes CR, Satiat-Jeunemaitre B. 2001. Trekking along the cytoskeleton. Plant Physiol 125:1119–122.

Hejnowicz Z, Sondag C, Alt W, Sievers A. 1998. Temporal course of graviperception in intermittently stimulated cress roots. Plant Cell Environ 21:1293–1300.

Hejnowicz Z, Rusin A, Rusin T. 2000. Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyls. J Plant Growth Regul 19-31-44.

Hensel W. 1985. Cytochalasin B affects the structural polarity of statocytes from cress roots *Lepidium sativum* L. Protoplasma 129:178–187.

Hensel W. 1986. Demonstration of microfilaments in statocytes of cress roots. Naturwissenschaften 73:510–511.

Himmelspach R, Wymer CL, Lloyd CW, Nick P. 1999. Gravity-induced reorientation of cortical microtubules observed in vivo. Plant J 18:449–453.

Himmelspach R, Nick P. 2001. Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212:184–189.

Hussey PJ, Yuan M, Calder G, Khan S, Lloyd CW. 1998. Microinjection of pollen-specific actin-depolymerizing factor, ZmADF1, reorientates F-actin strands in *Tradescantia* stamen hairs. Plant 114:353–357.

Hu S, Brady SR, Kovar DR, Staiger CS, Clark GB, Roux SJ, Muday GK. 2000. Identification of plant actin binding proteins by F-actin affinity chromatography. Plant J 24:127–137.

Ishikawa H, Evans ML. 1993. The role of the distal elongation zone in the response of maize roots to auxin and gravity. Plant Physiol 102:1203–1210.

Jacob T, Ritchie S, Assman SM, Gilroy S. 1999. Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. Proc Natl Acad Sci USA 96:12192–12197.

Johannes E, Collings DA, Rink JC, Alien NS. 2001. Cytoplasmic pH dynamics in maize pulvinal cells induced by gravity vector changes. Plant Physiol 127:119–130.

Karyophyllis D, Katsaros C, Galatis B. 2000. F-actin involvement in apical cell morphogenesis of Sphacelaria rigidula (Phaeophycea): mutual alignment between cortical actin filaments and cellulose microfibrils. Eur J Phycol 35:195–203.

Kiegle E, Gilliham M, Haseloff J, Tester M. 2000. Hyperpolarisation-activated calcium currents found only in cells from the elongation zone of *Arabidopsis thaliana* roots. Plant J 21:225–229.

Kiss JZ. 2000. Mechanisms of the early phases of plant gravitropism. Crit Rev Plant Sci 19:551–573.

Konings H. 1995. Gravitropism of roots: an evaluation of progress during the last three decades. Acta Bot Neerl 44:195–233.

Kost B, Spielhofer P, Mathur J, Dong C-H, Chua N-H. 2000. Non-invasive F-actin visualization in living plant cells using a GFP-mouse talin fusion protein. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 637–659.

Kovar DR, Gibbon BC, McCurdy DW, Staiger CJ. 2001. Fluorescently-labeled fimbrin decorates a dynamic actin filament network in live plant cells. Planta 213:390–395.

Kuznetsov OA, Hasenstein KH. 1996. Intracellular magnetophoresis of amyloplasts and induction of root curvature. Planta 198:87–94.

Legue V, Blancaflor E, Wymer C, Perbal G, Fantin D, Gilroy S. 1997. Cytosolic free calcium in *Arabidospis* roots changes in response to touch but not gravity. Plant Physiol 114:789–800.

Lorenzi G, Perbal G. 1990. Actin filaments responsible for the location of the nucleus in the lentil statocyte are sensitive to gravity. Biol Cell 68:259–263.

Liu L, Zhou J, Pesacreta TC. 2001. Maize myosins: diversity, localization and function. Cell Motil Cytoskel 48:130–148.

Lu Y-T, Feldman LJ. 1997. Light-regulated gravitropism: a role for, and characterization of, a calcium/calmodulin-dependent protein kinase homolog. Planta 203:S91–S97.

MacCleery SA, Kiss JZ. 1999. Plastid sedimentation kinetics in roots of wild-type and starch-deficient mutants of *Arabidopsis*. Plant Physiol 120:183–192.

Madlung A, Behringer FJ, Lomax TL. 1999. Ethylene plays multiple non-primary roles in modulating the gravitropic response in tomato. Plant Physiol 120:897–906.

Mathur J, Chua N-h. 2000. Microtubule stabilization leads to growth reorientation in *Arabidopsis* trichomes. Plant Cell 12:465–477.

Monshausen GB, Zieschang HE, Sievers A. 1996. Differential proton secretion in the apical elongation zone caused by gravistimulation is induced by a signal from the root cap. Plant Cell Environ 19:1408–1414.

Moseyko N, Feldman LJ. 2001. Expression of pH-sensitive green fluorescent protein in *Arabidopsis thaliana.* Plant Cell Environ 24:557–563.

Muday GK. 2000. Maintenance of asymmetric cellular localization of an auxin transport protein through interaction with the actin cytoskeleton. J Plant Growth Regul 19:385–396.

Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K. 1998. *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO J 17:6903–6911.

Nick P, Bergfeld R, Schäfer E, Schopfer P. 1990. Unilateral reorientation of microtubules at the outer epidermal wall during photo-and gravitropic curvature of maize coleopiles and sunflower hypocotyls. Planta 181:162–168.

Nick P, Schäfer E, Hertel R, Furuya M. 1991. On the putative role of microtubules in gravitropism of maize coleoptiles. Plant Cell Physiol 32:873–880.

Nick P, Godbole R, Wang QY. 1997. Probing rice gravitropism with cytoskeletal drugs and cytoskeletal mutants. Biol Bull 192:141–143.

Nick P. 1999. Signals, motors, morphogenesis – the cytoskeleton in plant development. Plant Biol 1:169–179.

Örvar BL, Sangwan V, Omann F, Dhindsa RS. 2000. Early steps in cold sensing by plant cells: role of actin cytoskeleton and membrane fluidity. Plant J 23:785–794.

Overall RL, Dibbayawan TP, Blackman LM. 2001. Intercellular alignments of the plant cytoskeleton. J Plant Growth Regul 20:162–169.

Palme K, Galweiler L. 1999. PIN-pointing the molecular basis of auxin transport. Curr Opin Plant Biol 2:375–381.

Papaseit C, Pochon N, Tabony J. 2000. Microtubule self-organization is gravity dependent. Proc Natl Acad Sci 97:8364–8368.

Perera I, Heilmann I, Boss WF. 1999. Transient and sustained increases in inositol 1,4,5-triphosphate precede the differential growth response in gravistimulated maize pulvini. Proc Natl Acad Sci USA 96:5838–5843.

Perera I, Heilman I, Chang SC, Boss WF, Kaufman PB. 2001. A role for inositol 1,4,5-triphosphate in gravitropic signaling and retention of cold-perceived gravistimulation of oat shoot pulvini. Plant Physiol 125:1499–1507.

Philippar K, Fuchs I, Luthen H, Hoth S, Bauer CS, Haga K, Thiel G, Ljung K, Sandberg G, Bottger M. 1999. Auxin-induced K+ channel expression represents an essential step in coleoptile growth and gravitropism. Proc Natl Acad Sci USA 96:12186–12191.

Ramachandran S, Christensen HEM, Ishimaru Y, Dong C-H, Chao-Ming W, Cleary AL, Chua H-H. 2000. Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis.* Plant Physiol 124:1637–1647.

Rashotte AM, Brady S, Reed R, Ante S, Muday GK. 2000. Basipetal auxin transport is required for gravitropism in roots of *Arabidopsis*. Plant Physiol 122:481–490.

Rashotte AM, DeLong A, Muday GK. 2001. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. Plant Cell 13:1683–1697.

Sack FD. 1997. Plastids and gravitropic sensing. Planta 203: S63–S68.

Sangwan V, Foulds I, Singh J, Dhindsa RS. 2001. Cold-activation of *Brassica napus* BN115 promoter is mediated by structural changes in membranes and cytoskeleton, and requires $Ca^{2+}$ influx. Plant J 27:1–12.

Scott AC, Allen NS. 1999. Changes in cytosolic pH within *Arabidopsis* root columella cells play a role in the early signaling pathway for root gravitropism. Plant Physiol 121:1291–1298.

Sedbrook JC, Chen R, Masson PH. 1999. ARG1 (altered response to gravity) encodes a DNAJ-like protein that potentially interacts with the cytoskeleton. Proc Natl Acad Sci 96:1140–1145.

Sievers A, Heyder-Caspers L. 1983. The effect of centrifugal acceleration on the polarity of statocytes and on graviperception of cress roots. Planta 157:64–70.

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

Sievers A, Buchen B, Volkmann D, Hejnowicz Z. 1991. Role of cytoskeleton in gravity perception. In: Lloyd CW, editor. The cytoskeletal basis of plant growth and form. Academic Press, London, p 169–182.

Sievers A, Sondag C, Trebacz K, Hejnowicz Z. 1995. Gravity induced changes in intracellular potentials in statocytes of cress roots. Planta 197:392–398.

Sievers A, Buchen B, Hodick D. 1996. Gravity sensing in tip-growing cells. Trends Plant Sci 1:273–279.

Sinclair W, Oliver I, Maher P, Trewavas A. 1996. The role of calmodulin in the gravitropic response of *Arabidopsis thaliana* agr-3 mutant. Planta 199:343–351.

Skagen EB, Iversen T-H. 1999. Simulated weightlessness and hyper-g results in opposite effects on the regeneration of the cortical microtubule array in protoplasts from *Brassica napus* hypocotyls. Physiol Plant 106:318–325.

Smertenko A, Saleh N, Igarashi H, Mori H, Hauser-Hahn I, Jinag CJ, Sonobe S, Lloyd CW, Hussey PJ. 2000. A new class of microtubule associated proteins in plants. Nat Cell Biol 2:750–753.

Staiger CJ, Yuan M, Valenta R, Shaw PJ, Warn RM, Lloyd CW. 1994. Microinjected profilin affects cytoplasmic streaming in plant cells by rapidly depolymerizing actin microfilaments. Curr Biol 4:215–219.

Staiger CJ. 2000. Signaling to the actin cytoskeleton in plants. Ann Rev Plant Physiol Plant Mol Biol 51:257–288.

Staves MP. 1997. Cytoplasmic streaming and gravity sensing in Chara internodal cells. Planta 203:S79–S84.

Staves MP, Wayne R, Leopold AC. 1997a. Cytochalasin D does not inhibit gravitropism in roots. Am J Bot 84:1530–1535.

Staves MP, Wayne R, Leopold AC. 1997b. The effect of external medium on gravitropic curvature of rice (*Oryza saliva*, Poaceae) roots. Am J Bot 84:1522–1529.

Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennet M. 2001. Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the *Arabidopsis* root apex. Genes Dev 15:2648–2653.

Tasaka M, Kato T, Fukaki H. 1999. The endodermis and shoot gravitropism. Trends Plant Sci 4:103–107.

Thimann KV, Reese K, Nachmias VT. 1992. Actin and the elongation of plant cells. Protoplasma 171:153–166.

Thion L, Mazars C, Nacry P, Bouchez D, Moreau M, Ranjeva R, Thuleau P. 1998. Plasma-membrane depolarization-activated calcium channels, stimulated by microtubule-depolymerizing drugs in wild-type *Arabidopsis thaliana* protoplasts, display constitutively large activities and a longer half-life in ton2 mutant cells affected in the organization of cortical microtubules. Plant J 13:603–610.

Tominaga M, Yokota E, Vidali L, Sonobe S, Hepler PK, Shimmen T. 2000. The role of plant villin in the organization of the actin cytoskeleton, cytoplasmic streaming and the architecture of the transvacuolar strand in root hair cells of *Hydrocharis*. Planta 210:836–843.

Ueda K, Matsuyama T. 2000. Rearrangement of cortical microtubules from transverse to oblique or longitudinal in living cells of transgenic *Arabidopsis thaliana*. Protoplasma 213:28–38.

Vitha S, Baluška F, Jasik J, Volkmann D, Barlow PW. 2000. Steedman's wax for F-actin visualization. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 619–636.

Volkmann D, Winn Borner U, Waberzeck K. 1993. Graviresponsiveness of cress seedlings and structural status of presumptive statocytes from the hypocotyls. J Plant Physiol 142:710–716.

Volkmann D, Baluška F. 1999. Actin cytoskeleton in plants: from transport networks to signaling networks. Micros Res Tech 47:135–154.

Volkmann D, Baluška F, Lichtscheidl I, Driss-Ecole D, Perbal G. 1999. Statoliths motions in gravity-perceiving plant cells: Does actomyosin counteract gravity? FASEB J 13:S143–S147.

Volkmann D, Baluška F. 2000. Actin cytoskeleton related to gravisensing in higher plants. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 557–571.

Waller F, Nick P. 1997. Response of actin microfilaments during phytochrome-controlled growth of maize seedlings. Protoplasma 200:154–162.

Waller F, Wang Q-Y, Nick P. 2000. Actin and signal-controlled cell elongation in coleoptiles. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: a dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 477–496.

Wang X. 2001. Plant phospholipases. Annu Rev Plant Physiol Plant Mol Biol 52:211–231.

Wasteneys GO, Willingale-Theune J, Menzel D. 1997. Freeze shattering: a simple and effective method for permeabilizing higher plant cells walls. J Micros 188:51–61.

Wasteneys GO. 2000. The cytoskeleton and growth polarity. Trends Plant Sci 3:503–511.

Wayne R, Staves MP. 1996. A down to earth model of gravisensing or Newton's Law of gravitation from the apple's perspective. Physiol Plant 98:917–921.

Weisenseel MH, Meyer AJ. 1997. Bioelectricity, gravity and plants. Planta 203:S98–S106.

Weise SE, Kuznetsov OA, Hasenstein KH, Kiss JZ. 2000. Curvature in *Arabidopsis* inflorescence stems is limited to the regions of amyloplast displacement. Plant Cell Physiol 41:702–709.

Wendt M, Kuo-Huang L, Sievers A. 1987. Gravitropic bending of cress roots without contact between amyloplasts and complexes of endoplasmic reticulum. Planta 172:321–329.

White RG, Sack FD. 1990. Actin microfilaments in presumptive statocytes of roots caps and coleoptiles. Amer J Bot 77:17–26.

Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO. 2001. MOR1 is essential for organizing cortical microtubules in plants. Nature 411:610–613.

Wymer CL, Wymer SA, Cosgrove DJ, Cyr RJ. 1996. Plant cell growth responds to external forces and the response requires intact microtubules. Plant Physiol 110:425–430.

Yamamoto K, Kiss JZ. 2002. Disruption of the actin cytoskeleton results in the promotion of gravitropism in inflorescence stems and hypocotyls of *Arabidopsis*. Plant Physiol 128: (in press).

Yoder TL, Zheng H-Q, Todd P, Staehelin LA. 2001. Amyloplast sedimentation dynamics in maize columella cells support a new model for the gravity-sensing apparatus of roots. Plant Physiol 125:1045–1060.

Yokota E, Imamichi N, Tominaga, Shimmen T. 2000. Actin cytoskeleton is responsible for the change of cytoplasmic organization in root hairs induced by a protein phosphatase inhibitor, calyculin A. Protoplasma 213:184–193.

Yokota E, Shimmen T. 2000. Characterization of native actin binding proteins from pollen. Myosin and the actin bundling proteins, 135-ABP and 115-ABP. In: Staiger C, Baluška F, Volkmann D, Barlow PW, editors. Actin: A dynamic framework for multiple plant cell functions. Kluwer Academic Publishers, Dordrecht, The Netherlands, p 103–118.

Zhang N, Hasenstein KH. 2000. The distribution of expansions in graviresponding maize roots. Plant Cell Physiol 141:1305–1322.

Zheng H-Q, Staehelin LA. 2001. Nodal ER, a novel form of ER found exclusively in gravity-sensing columella cells. Plant Physiol 125:252–265.