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Cortical Actin Filaments Potentially Interact with Cortical Microtubules in Regulating Polarity of Cell Expansion in Primary Roots of Maize (*Zea mays* L.)

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Abstract

Evidence is accumulating implicating cortical microtubules in the directional control of cell expansion. However, the role of actin filaments in this process is still uncertain. To determine the involvement of actin in cell elongation, the organization of actin filaments in primary roots of maize (*Zea mays* L.) was examined by use of an improved fluorochrome-conjugated phalloidin-labeling method. With this method, a previously undetected state of actin organization was revealed in the elongation and maturation zone of maize roots. Fine transversely oriented cortical actin was observed in all cells of the elongation zone, including the epidermis, cortex, and vascular tissues. The orientation of cortical actin shifted from a predominantly transverse orientation to oblique, longitudinal, and/or random arrangements as the cells matured. The reorientation of cortical actin in maturing root cells mimics the behavior of cortical microtubules reported in other studies. Furthermore, roots treated with the microtubule-stabilizing drug taxol improved the quality of actin preservation as evidenced by the thicker bundles of cortical actin. This suggested that taxol was also capable of stabilizing the cortical actin networks. The elongation of roots exposed to 1 μM Latrunculin B, an actin-disrupting drug, was inhibited, and after 24 h the roots exhibited moderate swelling particularly along the elongation zone. Latrunculin B also caused microtubules to reorient from transverse to oblique arrays. The results from this study provide evidence that cortical microtubules and actin filaments respond in a coordinated way to environmental signals and may well depend on both elements of the cytoskeleton.

Key words: Actin; Cell elongation; Cytoskeleton; Latrunculin B; Microtubules; Phalloidin; Roots; Taxol; *Zea mays*

Introduction

Plant organs attain a particular shape by controlling the growth of individual cells. Although a plant cell grows primarily by turgor pressure–driven expansion, it is the rigid wall surrounding the cytoplasm that ultimately determines the direction in which the cell grows. Among the components of the cell wall, the high tensile strength of cellulose microfibrils resists turgor forces and therefore limits

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growth to one direction. The characteristic cylindrical shape of many plant cells results from the preferential deposition of cellulose microfibrils normal to the major axis of elongation (Green 1980). Cortical microtubules are believed to play a key role in cell elongation by providing the template for the directional deposition of cellulose microfibrils (Fisher and Cyr 1998; Lloyd and others 2000). However, relatively little is known about the function of actin in the elongation of plant cells.

Roots are ideal systems for studies on the mechanisms of cell elongation because of the clear distinctions that can be made between developmental zones along the axis (for example, meristem, elongation and maturation zones) and cylindrically arranged tissue. The ability to observe these developmental stages in a single root has allowed correlative studies between a specific type of cytoskeletal structure and the stage of cellular development (for review see Baluška and Barlow 2000; Baluška and others 1998). For example, by focusing on cells of the elongation and maturation zones, it has been shown that the orientation of cortical microtubules correlates with the growth status of the cell. The transverse microtubules typically observed in the rapidly elongating cells of the root elongation zone shift to an oblique or longitudinal orientation as the cells mature (Blancaflor and Hasenstein 1995b; Liang and others 1996). An equivalent scenario for actin filaments, however, has never been demonstrated in root cells.

Imaging the organization of actin in roots has relied almost exclusively on fluorescence microscopy and the application of specific antibodies and/or fluorescently labeled phalloidin probes to chemically fixed roots (Blancaflor and Hasenstein 2000). Using these methods, the highly vacuolated root cells of the elongation and maturation zones have been shown to contain an actin network that consisted primarily of longitudinally oriented bundles (Baluška and others 1997b; Blancaflor and Hasenstein 1997). Although such studies have provided significant insights into the organization and possible function of actin in roots, it is becoming increasingly clear that not all microfilament structures are optimally preserved by chemical fixation (Blancaflor and Hasenstein 2000; Collings and others 2001). The fine transverse arrays of actin at the plasma membrane are particularly prone to disruption by methods that use aldehyde fixation. These fine cortical actin arrays could only be imaged with improved labeling techniques that use protein cross-linkers (Sonobe and Shibaoka 1989; Traas and others 1987), microinjection with rhodamine-phalloidin (Cleary 1995), and more recently through the use of an actin-binding protein fused to the green fluorescent protein (GFP; Kost and others 1998). Although good preservation of cortical actin with aldehyde-based fixatives was reported (Collings and others 1998), the large number of studies on the deleterious effects of aldehydes on preserving the more delicate actin strands cannot be ignored.

A method that uses a protein cross-linker and glycerol permeabilization (Olyslaegers and Verbelen 1998) of Alexa-Fluor phalloidin was recently applied to Vibratome-sectioned root caps of several plant species to label actin arrays in the gravity-sensing columella cells of roots (Collings and others 2001). The distinct actin networks that were demonstrated in the columella cells were previously undetected using conventional methods of immunolabeling (Baluška and others 1997a; Blancaflor and Hasenstein 1997). By applying this method to cells in the elongation and maturation zones of maize roots, fine arrays of cortical actin that were not previously detected in aldehyde-fixed roots (Baluška and others 1997b; Blancaflor and Hasenstein 1997) were imaged. The transversely oriented arrays of cortical actin in the elongation zone shifted to oblique, longitudinal, and/or random orientations in the maturation zone. Application of Latrunculin B severely inhibited root elongation, induced radial swelling, and disrupted the actin arrays in the elongation zone, whereas taxol improved the stability of the cortical actin networks. The data indicate that root elongation requires an intact actin cytoskeleton and that the fine cortical actin network in the elongation zone could interact with cortical microtubules in regulating anisotropic growth.

Materials and Methods

Plant Material and Treatments

Seeds of maize (*Zea mays* L. cv. Merit) were germinated and grown vertically in opaque plastic trays at 22°C as previously described (Blancaflor and Hasenstein 1993). Stock solutions of Latrunculin B (5 mM; CalbioChem, La Jolla, CA) and taxol (10 mM; Molecular Probes, Eugene, OR) were prepared in 100% dimethyl sulfoxide (DMSO, Sigma). A working solution of 1-μM Latrunculin B and 20-μM taxol was prepared by adding the appropriate volume of stock solution to deionized water. Three-day-old seedlings with straight roots were selected and transferred to 1.5-mL microfuge tubes containing working solutions of the drugs, with the terminal 15 mm of the roots immersed in the solutions. After 2 h, seedlings with straight roots were selected and processed for microscopy as described later. Separate sets of seedlings were used for growth analysis. Roots immersed

in deionized water with the corresponding amount of DMSO were used as controls.

Growth Measurements

Seedlings treated with Latrunculin B were transferred to 9-cm Petri dishes lined with wet filter paper. Growth of vertically oriented roots was monitored by capturing images of the roots every 10 min for 3 h with a Hamamatsu C240075i camera (Hamamatsu, Tokyo, Japan) running the Optimas 6.0 image acquisition software (Optimas Corporation, Bothel, WA). The length of the roots was measured from digitized images using MetaMorph 4.01 image processing software (Universal Imaging Corp, West Chester, PA)

Alexa Fluor-Phalloidin Labeling of Actin

Labeling of actin filaments using Alexa-Fluor phalloidin was essentially as described in Collings and others (2001). The terminal 8 mm of control, Latrunculin B, and taxol-treated roots were cut and immediately attached to a Vibratome 1000 (Technical Products International, St. Louis, MO). Median longitudinal sections (60-µm thick) were immediately immersed in PME (50 mM Pipes, 4 mM $MgSO_4$, 10 mM EGTA) buffer, pH 6.9, containing 300 µM 3-maleimidobenzoyl-*N*-hydroxy-succinimide ester (MBS) for 30 min. This was followed by incubating the sections in PME buffer (without MBS) containing 0.1-µM Alexa Fluor-phalloidin (Molecular Probes, Eugene, OR), 0.3-M mannitol and 2% glycerol (v/v) for 5 min. After incubation, root sections were washed once with PME buffer, mounted on glass slides, and immediately observed under a confocal microscope. For comparison, a separate set of roots was processed using the conventional methods of aldehyde fixation described in Blancaflor and Hasenstein (1997).

Immunofluorescence Staining of Microtubules

Labeling of microtubules was as described in Blancaflor and Hasenstein (1993). The terminal 8 mm of control and drug-treated roots were placed in separate vials containing 4% formaldehyde in PME buffer, pH 6.9, and 5% (v/v) DMSO for 2 h. After extensive washing in PME buffer, 60-µm median longitudinal sections were cut with a Vibratome and sections secured onto glass coverslips using a thin film of agar as described in Brown and Lemmon (1995). The sections were digested in a cocktail of wall-degrading enzymes in PME buffer (1% cellulase; 0.01% pectolyase, and 0.1% BSA, all w/v) for 10 min followed by a 20-min incubation in 1% (v/v) Triton X-100. The sections were then incubated overnight in a monoclonal rat antiyeast alpha tubulin diluted 1:100 (clone YOL1/34, Accurate Chemicals, Westbury, NY) followed by a 2-h incubation in a secondary antibody diluted 1:200 (goat anti-rat IgG conjugated to FITC, Sigma Chemicals). Root sections were then mounted in phosphate-buffered saline (PBS), pH 8.5, containing 20% (v/v) Mowiol 4-88 (Calbiochem, La Jolla, CA) and 0.1% (w/v) phenylenediamine and left overnight at room temperature before observing with a confocal microscope.

Confocal Microscopy

Microtubules and actin in the root sections were imaged with a Bio-Rad 1024ES confocal laser scanning microscope (Bio-Rad Hercules, CA) equipped with a 63x, 1.2 numerical aperture (N.A.) water-immersion objective. Optical sections were acquired at 0.3-µm intervals, with each section being Kalman averaged three times. Alexa-Fluor and FITC were excited at 488 nm with emission from 500–550 nm. All images were processed using Adobe Photoshop 5.0LE (Adobe Systems Inc., Mountain View, CA) and printed on an Epson 900 ink jet printer (Epson America Inc., Torrance, CA).

Results

Fine Cortical Actin Arrays in Cells of the Elongation and Maturation Zones

Cells in the outer cortex of the elongation zone of roots fixed in formaldehyde and labeled with Alexa-Fluor phalloidin revealed actin bundles that were predominantly oriented in the longitudinal direction (Figure 1A; see also Blancaflor and Hasenstein 1997). The corresponding cells in roots cross-linked with MBS and permeabilized with glycerol revealed a more extensive actin network. In addition to the longitudinally oriented actin bundles, randomly oriented subcortical bundles and fine transversely oriented cortical filaments were detected (Figure 1B). The fine actin filaments in the cell cortex could be observed in all cell types of the elongation zone, including the vascular parenchyma (Figure 1C) and epidermis (data not shown). The fluorescence signal from these transverse cortical actin arrays was generally weaker than the thicker subcortical and transvacuolar actin cables (Figure 1C). However, the transversely oriented cortical actin network in cells closer to the root apex such as those in the distal elongation zone (Figure 1D) and meristem (data not shown; see Collings and others 2001) were brighter and thus easier to observe.

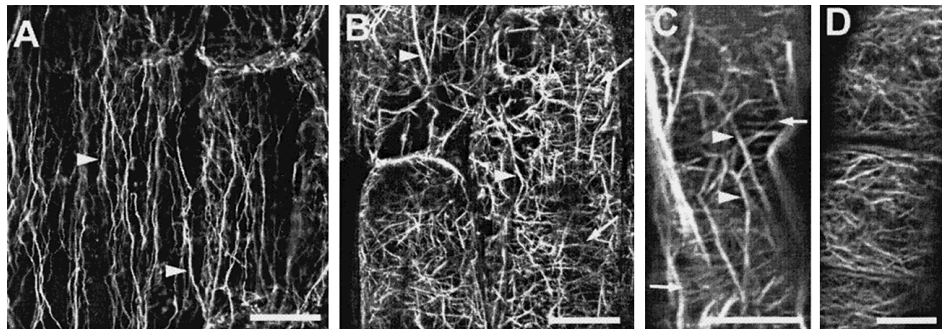

Figure 1. Comparison of two labeling methods to image actin organization in the elongation zone of maize roots. (**A**) and (**B**) are projections of 20 optical sections taken at 0.3-μm intervals. (**C**) is a projection of 10 optical sections taken at 0.3-μm intervals, whereas (**D**) is a single optical section. The root tip is toward the bottom of the page for all images. (**A**) Longitudinal section of a maize root fixed in 2% formaldehyde and labeled with Alexa-Fluor Phalloidin. Only the thick longitudinally oriented subcortical actin bundles could be imaged (arrowheads). (**B**) Longitudinal section of a maize root after MBS cross-linking and glycerol permeabilization of Alexa-Fluor phalloidin. In addition to the longitudinally oriented subcortical bundles (arrowheads), fine transverse actin filaments in the cortex can be imaged (arrows). (**C**) A vascular cell in the elongation zone. The transversely oriented cortical actin filaments (arrows) exhibit a weak fluorescent signal in contrast to the thicker longitudinal actin cables (arrowheads). (**D**) Cells closer to the root tip (approximately 2 mm from the tip) generally show a brighter fluorescence of their transverse cortical actin. Scale bar = 25 μm (**A** and **B**); 10 μm (**C** and **D**).

The orientation of cortical actin in the elongation zone observed in this study was similar to the orientation of cortical microtubules reported by other investigators (Baluška and others 1992; Blancaflor and Hasenstein 1993, 1995a,b; Liang and others 1996). The alignment of microtubules has been shown to shift from transverse to oblique and/or longitudinal orientations when cells reached the maturation zone. To determine whether a similar phenomenon occurs for actin, the orientation of the cortical actin network in the maturation zone was compared with that of the elongation zone. Although transverse cortical actin was consistently observed in cells of the elongation zone (approximately 3–5 mm from the root tip; Figure 2A), its orientation shifted to oblique or longitudinal directions in cells located 6–8 mm from the root tip (Figure 2B and C). This region of the root corresponded approximately to the region where cell growth is declining and where cortical microtubules undergo a similar type of reorientation (Baluška and others 1992; Blancaflor and Hasenstein 1993, 1995a,b; Liang and others 1996).

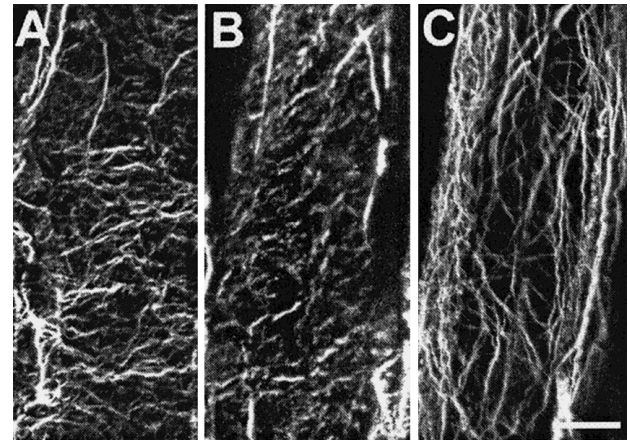

Figure 2. Reorientation of cortical actin filaments in cells of vertically growing maize roots revealed by MBS cross-linking and glycerol-facilitated permeabilization of Alexa-Fluor phalloidin. The root tip is toward the bottom of the page for all images. All images are projections of five optical sections taken at 0.1-μm intervals. (**A**) A cell in the elongation zone (approximately 4 mm from the root tip) displays fine cortical actin filaments oriented perpendicular (transverse) to the major growth axis of the cell. (**B** and **C**) In the maturation zone (6–8 mm from the root tip) cortical actin filaments shift to oblique (**B**) and longitudinal orientations (**C**). Scale bar = 10 μm.

Latrunculin B Inhibits Elongation and Induces Radial Expansion in Roots

The actin-disrupting drug, Latrunculin B caused a reduction in the elongation of maize roots. Although untreated roots elongated at a rate of about 1.25 mm/h, roots exposed to Latrunculin B elongated at a rate of 0.5 mm/h (Figure 3). Twenty-four hours after Latrunculin B treatment, moderate swelling in the elongation zone was observed in the roots (Figure 4).

The actin arrays in roots treated with Latrunculin B were investigated using the actin labeling method described previously. After 2 h of exposure to La-

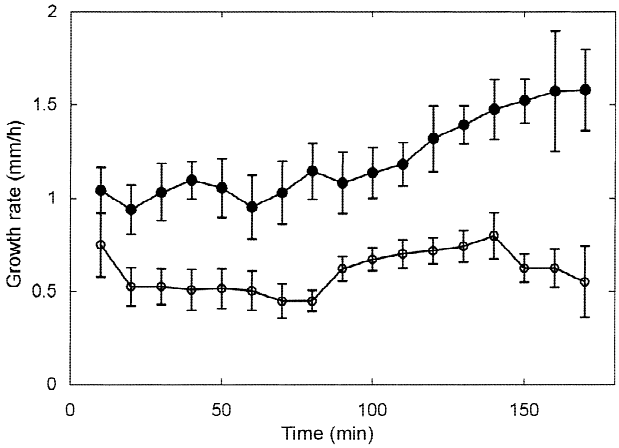

Figure 3. Growth rate of maize primary roots exposed to 1 μM Latrunculin B (○). Controls (●). Values are means ± SE for seven roots.

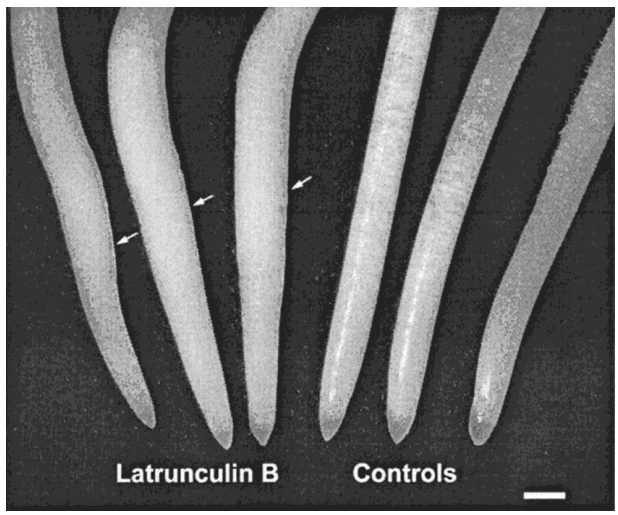

Figure 4. Morphology of maize primary roots exposed for 2 h in 1 μM Latrunculin B and grown in humidified Petri dishes for 24 h. Root swelling is apparent at 3–6 mm from the root tip (arrows) after Latrunculin B treatment. Scale bar = 1 mm

trunculin B, disruptions in the filamentous actin structures were observed in all regions of the root. However, the severity of Latrunculin B's effect varied from cell to cell. Outer cortical cells in the elongation zone were particularly sensitive to the effect of Latrunculin B as evidenced by the accumulation of fluorescent aggregates and the loss of most filamentous staining with the exception of a few subcortical actin bundles (Figure 5A). On the other hand, cells in the vascular region retained a larger number of filaments, but these were mostly fragmented (Figure 5B).

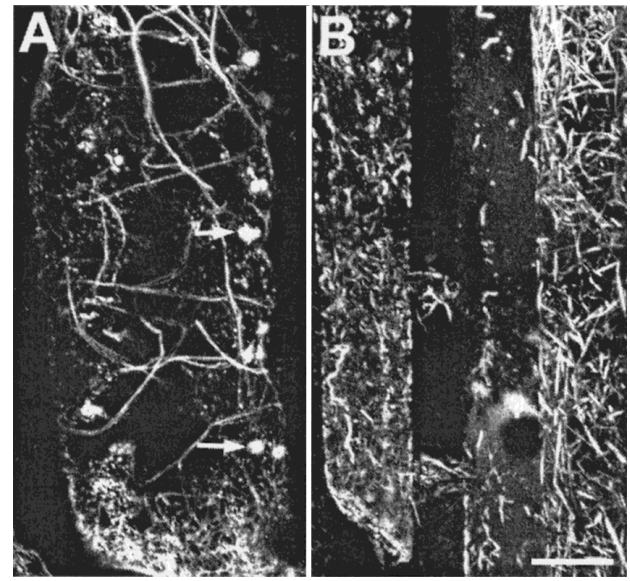

Figure 5. Latrunculin B disrupts actin distribution in maize roots. Roots were incubated in 1 μM Latrunculin B for 2 h and processed for microscopy with the MBS cross-linking and glycerol permeabilization method. Images are projections of 10 optical sections taken at 0.3-μm intervals, and the root tip is toward the bottom of the page for both images. (**A**) An outer cortical cell in the elongation zone shows a reduction in the amount of filamentous staining and the proliferation of fluorescent aggregates (arrows). (**B**) Cells in the vascular cylinder retain more of the filamentous staining patterns, but most of the filaments are fragmented. Scale bar = 20 μm.

Pharmacologic Agents Modify Actin and Microtubules

A conventional approach to study the interaction between microtubules and actin makes use of pharmacologic agents that specifically disrupt either actin filaments or microtubules (Collings and Allen 2000). To determine whether the similar alignment between cortical actin and microtubules in maize roots is due to interactions between these two cytoskeletal components, actin distribution in roots treated with taxol and microtubule organization in roots treated with Latrunculin B were examined.

Although the general pattern of actin distribution in root cells treated with taxol did not change, there was a significant improvement in the state of cortical actin preservation. Whereas the cortical actin filaments in control roots showed a generally weak fluorescent signal (Figure 1), the cortical actin networks in taxol-treated roots were brightly labeled (Figure 6). In addition, taxol-treated roots displayed thicker actin arrays in a variety of cell types in the elongation (Figure 6A and B) and maturation zones

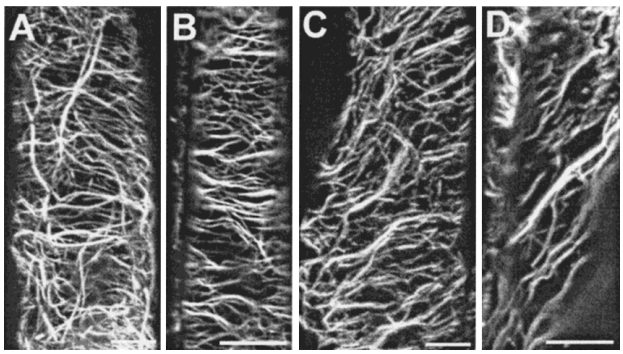

Figure 6. Taxol treatment improves the stability of cortical actin in the elongation and maturation zone. Images are projections of 3–10 optical sections taken at 0.1-μm intervals. The root tip is toward the bottom of the page. An outer cortical cell (**A**) and vascular cell (**B**) in the elongation zone show well-defined bundles of transversely -oriented actin. (**C** and **D**) Cells in the maturation zone also display brighter and thicker cortical actin bundles that are obliquely oriented. Scale bars = 10 μm.

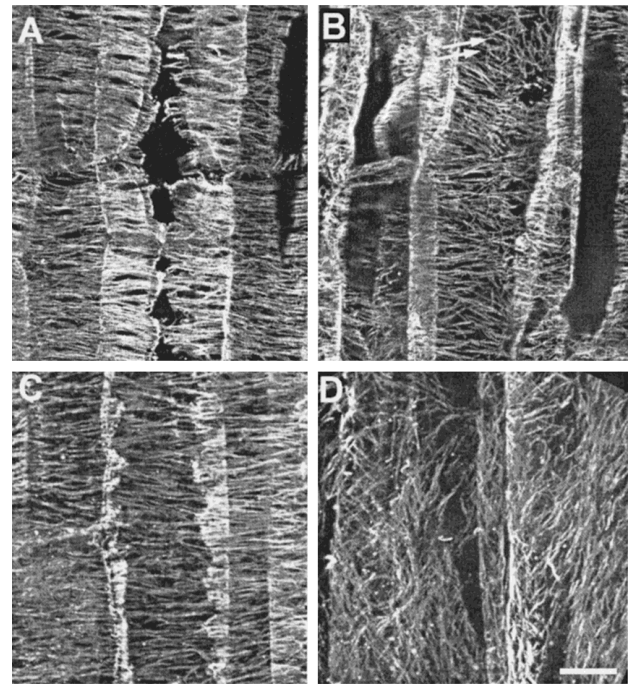

Figure 7. Effect of Latrunculin B on microtubule orientation. All images are projections of 11 optical sections taken at 0.3-μm intervals. The root tip is toward the bottom of the page. (**A** and **B**) Cortical cells in the distal elongation zone (2–3 mm from the root tip). Microtubules in control (**A**) and Latrunculin B–treated roots (**B**) show predominantly transverse orientation. Cells of Latrunculin B–treated roots, however, also contain some randomly oriented strands (arrows in **B**). (**C** and **D**) Cortical cells in the proximal elongation zone (4–6 mm from the root tip). Although cells in control roots still display transverse microtubules (**C**), cells in Latrunculin B–treated roots contain obliquely oriented microtubules (**D**). Scale bar = 20 μm.

(Figure 6C and D) with their respective transverse and oblique alignment still intact.

Cells in the distal and central elongation zone (approximately 2–4 mm from the root tip) of control roots (Figure 7A) and roots treated with Latrunculin B (Figure 7B) displayed transversely oriented cortical microtubules. Although microtubules in cells of Latrunculin B–treated roots were predominantly transverse, randomly oriented bundles were often detected within the population of transversely oriented microtubules (Figure 7B). The effect of Latrunculin B on microtubule arrangement was more pronounced in the proximal elongation zone (4–6 mm from the root tip). Although control roots still displayed transverse microtubules in this region (Figure 7C), Latrunculin B–treated roots contained obliquely oriented microtubules (Figure 7D).

Discussion

Previous reports have proposed the involvement of actin in cell elongation, but the primary focus of these studies has been on the role of the longitudinally oriented subcortical and transvacuolar actin cables. For example, Baluška and others (1997b) described conical clusters of actin bundles in maize root cells preparing for elongation followed by the appearance of wrinkled cables of longitudinally oriented actin as the cells made their transition from postmitotic growth to rapid elongation. Similarly, Waller and Nick (1997) showed that longitudinal actin cables in maize coleoptile cells condense during phases of growth decline and loosen during the onset of cell elongation. The inhibition of growth in oat coleoptiles was also shown to be associated with an increase in actin bundling (Thimann and others 1992), which was suggested to impart tension on the plasma membrane, leading to the inhibition of growth (Grabski and others 1998; Waller and Nick 1997). Alternatively, it was proposed that the polymerization of the longitudinal actin bundles provided the mechanical forces needed to stretch the polysaccharide matrix of the cell wall, allowing the protoplast to extend (Thimann and others 1992).

The predominance of studies implicating the longitudinal actin bundles in cell elongation has been due to the ease by which these thick actin bundles could be preserved by standard methods of aldehyde fixation. But modifications in tissue preparation techniques have revealed the existence of a finer

network of transversely oriented cortical actin in a variety of cell types (Baluška and others 1997b; Blancaflor and Hasenstein 1997; Blancaflor and Hasenstein 2000; Collings and others 2001; Traas and others 1987). In chemically fixed roots, however, demonstration of transverse cortical actin has been limited to cells in the meristematic region (Baluška and others 1997b; Blancaflor and Hasenstein 1997; McCurdy and Gunning 1990). Transverse cortical actin in elongating root cells was only revealed when aldehyde fixation was avoided (Hush and Overall 1992). The cortical actin network in actively elongating cells is more likely to contribute to growth control because of its proximity to the plasma membrane and microtubules (Collings and Allen 2000; see below). By applying an improved MBS cross-linking and glycerol permeabilization protocol that avoided the use of aldehyde-based fixatives (Collings and others 2001), this study shows that vacuolated cells of the elongation and maturation zones of maize roots also contain fine arrays of cortical actin. This, therefore, facilitated the design of experiments that looked into the function of these cortical actin arrays in the elongation growth of roots.

A major drawback of actin imaging without fixation is that the applied Alexa Fluor phalloidin could shift the balance from G-actin (globular actin) to filamentous actin (F-actin). Although this could partially explain the appearance of a more extensive F-actin network in the elongation zone, the observations of transversely oriented cortical actin in elongating cells of chemically fixed tobacco suspension cells (Collings and others 1998; Sonobe and Shibaoka 1989) and cells transiently expressing a (GFP)-talin fusion protein (Kost and others 1998) strongly indicate that the fine transverse cortical actin arrays are genuine and a normal component of elongating root cells. Furthermore, this method of actin labeling contributes little to artificial actin polymerization (Collings and others 2001).

The data show two lines of evidence indicating that the observed cortical actin filaments are involved in root elongation. First, the cortical actin network modified their orientation in response to the changing growth status of the cell. From a predominantly transverse alignment in the elongation zone, these actin arrays shifted to an oblique and longitudinal pattern in the maturation zone where cell growth began to decline. This is apparently the first report demonstrating this phenomenon in roots. Second, experiments with the actin-disrupting drug Latrunculin B inhibited root elongation and induced radial expansion. Although Latrunculin B has been shown to inhibit growth in tip-growing cells (Bibikova and others 1999; Gibbon and others 1999), there has been only one report describing its effect on whole plant organs (Volkmann and Baluška 1999). Like Latrunculin B, cytochalasin, another actin-disrupting agent, was capable of inhibiting the elongation of roots and shoots (Baluška and others 1997b; Blancaflor and Hasenstein 1997; Thimann and others 1992; Volkmann and Baluška 1999). However, only a few reports show that actin disruption leads to root swelling (Baskin and Bivens 1995; Thomas and others 1973). The observation that Latrunculin B disrupts actin, inhibits elongation, and stimulates moderate radial swelling in maize roots extends previous observations suggesting that actin contributes to the control of anisotropic growth in roots (Baskin and Bivens 1995).

The close association between cortical actin and microtubules has been demonstrated in several cell types (Collings and Allen 2000 and references therein). The possibility that interactions between these two cytoskeletal components occur in roots comes from the observation that the reorientation of cortical actin mimics the realignment of cortical microtubules (Baluška and others 1992; Blancaflor and Hasenstein 1993; 1995b; Liang and others 1996). This indicates that cortical actin and microtubules are tightly interlinked such that the reorientation of one leads to the reorientation of the other. However, because the method to label microtubules was not optimal for preserving the delicate cortical actin network, imaging both components of the cytoskeleton in the same root section was not possible. Therefore, it could not be determined conclusively whether cortical actin reorientation occurs in precisely the same region as the reorientation of cortical microtubules. Refinement of labeling techniques that optimize the imaging of both cytoskeletal components and determination of whether factors that induce microtubule reorientation (Blancaflor and Hasenstein 1993, 1995a,b) also reorient the cortical actin network should help clarify this issue. Furthermore, the application of actin and microtubule-binding GFP fusions (Kost and others 1998; Marc and others 1998) will likely be beneficial for understanding how microtubules and actin interact in developing roots.

Another observation in this study suggesting that microtubules and actin interact comes from experiments that use cytoskeletal inhibitors. Taxol treatment improved the labeling of the cortical actin arrays by inducing extensive bundling. This observation indicates that the stabilization of microtubules by taxol resulted in a corresponding stabilization of the cortical actin network. This is consistent with

previous reports showing that taxol increased the stability of actin filaments in rye root tips in response to cold (Chu and others 1993). In addition, Collings and others (1998) demonstrated that when microtubules in elongating BY-2 cells are stabilized with taxol, actin is stabilized as well.

Because root swelling can also be induced by microtubule disrupting agents (Baskin and others 1994; Hasenstein and others 1999), it is possible that the effect of Latrunculin B on radial expansion might be mediated through a mechanism whereby the disruption of actin results in the depolymerization or remodeling of the microtubule cytoskeleton. The data presented here show that Latrunculin B affects the organization of microtubules in roots by causing their premature reorientation from transverse to oblique arrays, which is consistent with other studies using different cell types. For example, in developing cotton fibers (Seagull 1990) and azuki bean coleoptiles (Takesue and Shibaoka 1998), cytochalasin D treatment favored the development of oblique and longitudinal microtubules. More recently, microtubules in living epidermal cells of transgenic *Arabidopsis* hypocotyls detected by use of a GFP-tubulin fusion protein formed random arrays after treatment with cytochalasin B (Ueda and Matsuyama 2000). Therefore, like the effect of microtubules on actin, these data show that the arrangements of actin arrays influence the organization of microtubules.

In summary, the results presented in this study indicate that the anisotropic growth of roots depends on both the cortical actin and microtubule cytoskeleton. Pharmacologic data and the observation that both cortical actin and microtubules exhibit a growth-dependent reorientation support the proposal by Collings and Allen (2000) that these two components of the cytoskeleton interact in the control of plant morphogenesis.

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