Developmental Biology **231,** 113–124 (2001)
doi:10.1006/dbio.2000.0115, available online at http://www.idealibrary.com on IDEAL®

Latrunculin B-Induced Plant Dwarfism: Plant Cell Elongation Is F-Actin-Dependent

F. Baluška,*,†,[1] J. Jasik,‡,§ H. G. Edelmann,*
T. Salajová,¶ and D. Volkmann*

**Zellbiologie der Pflanzen, Botanisches Institut, Rheinische Friedrich-Wilhelms-Universität Bonn, Kirschallee 1, Bonn D-53115, Germany; †Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, Bratislava SK-84223, Slovakia; ‡Department of Plant Physiology, Comenius University, Mlynská dolina, Bratislava SK-84512, Slovakia; §Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg, Cologne D-50829, Germany; and ¶Institute of Plant Genetics, Slovak Academy of Sciences, Akademická 2, Nitra SK-95007, Slovakia*

Marine macrolides latrunculins are highly specific toxins which effectively depolymerize actin filaments (generally F-actin) in all eukaryotic cells. We show that latrunculin B is effective on diverse cell types in higher plants and describe the use of this drug in probing F-actin-dependent growth and in plant development-related processes. In contrast to other eukaryotic organisms, cell divisions occurs in plant cells devoid of all actin filaments. However, the alignment of the division planes is often distorted. In addition to cell division, postembryonic development and morphogenesis also continue in the absence of F-actin. These experimental data suggest that F-actin is of little importance in the morphogenesis of higher plants, and that plants can develop more or less normally without F-actin. In contrast, F-actin turns out to be essential for cell elongation. When latrunculin B was added during germination, morphologically normal *Arabidopsis* and rye seedlings developed but, as a result of the absence of cell elongation, these were stunted, resembling either genetic dwarfs or environmental bonsai plants. In conclusion, F-actin is essential for the plant cell elongation, while this F-actin-dependent cell elongation is not an essential feature of plant-specific developmental programs.

Key Words: **actin; cell elongation; plant dwarfism; plant development.**

INTRODUCTION

Some mature plants can boast with the largest dimensions among all organisms of this planet. For instance, adult sequoia trees can reach up to 90 m in above-ground height. In respect of cell numbers, however, these giant trees do not reach numbers typical for much smaller animals. Further characteristic features of higher plants, making them absolutely unique among all other higher multicellular organisms, are: (1) overall immobility of plants because of their firm anchorage within the soil via highly branched root systems; (2) similar immobility of most of their cells, which are firmly linked together via their cell walls; (3) plant-specific vacuome-based cell elongation, which at least partially overcomes negative impacts of plant immobility. This unique cell growth mode distinguishes higher plants from all other eukaryotic organisms and allows dramatic size increases of postmitotic plant cells, when their cellular volumes can expand in extreme situations up to 20,000 times (Cosgrove, 1987).

The sessile nature of higher plants precludes escaping from unfavorable environments and this makes adaptability and developmental plasticity for their most critical features. For instance, environment-induced differential cell elongations along plant organs result in their rapid repositionings, thus allowing harmonization of plant development according to the actual environmental situations. This environmental adaptability is effectively supported by highly sensitive sensoring systems, which continuously monitor a broad range of environmental properties and transduce this information into relevant physiological processes. Among these latter processes, cell elongation proves to be an extremely interesting one, in that endogenous

[1] To whom correspondence should be addressed at Rheinische Friedrich-Wilhelms-Universität Bonn, Botanisches Institut, Zellbiologie der Pflanzen, Kirschallee 1, Bonn D-53115, Germany. Fax: 49-228-732-677. E-mail: baluska@uni-bonn.de.

0012-1606/01 $35.00

control over this process is tightly linked to sensing of such environmental factors like light (Devlin *et al.*, 1996; Waller and Nick, 1997; Whitelam *et al.*, 1998), gravity (Barlow and Rathfelder, 1985; Ishikawa and Evans, 1993; Baluška *et al.*, 1996a), circadian rhythms (Dawson-Day and Millar, 1999), moisture (Sharp *et al.*, 1988; Takahashi, 1997), temperature (Baluška *et al.*, 1993a; Gray *et al.*, 1998), and mechanical stimulation (Okada and Shimura, 1990; Ishikawa and Evans, 1992).

In the early 1990s preliminary evidence accumulated, indicating that cell elongation might be F-actin-dependent. Thimann *et al.* (1992) reported that elongation of oat coleoptile cells dropped by about 50% after cytochalasin D-induced disintegration of F-actin. Additional studies indicate that F-actin-mediated links between cell elongation and stimulus-responsive morphogenesis of higher plants are closely related to actions of phytohormones. For instance, auxin response and graviresponse of coleoptiles are altered in the rice mutant *Yin-Yang*, which can be phenocopied by mild cytochalasin D treatment (Wang and Nick, 1998; Waller *et al.*, 2000). All these data suggest that the actin cytoskeleton is inherently linked, perhaps via direct interactions, with stimulus-responsive modulations of plant cell elongation, induced via diverse environmental signals (Nick, 1999; Waller *et al.*, 2000). Unfortunately, there are still many concerns with this attractive concept.

To test this highly attractive concept, we have taken advantage of maize root apices (Baluška *et al.*, 1992, 1993a,b, 1994, 1996a,b, 1997, 2000a,b) and of the specific and efficient F-actin drug latrunculin B (Spector *et al.*, 1983, 1989; Schatten *et al.*, 1986). At the biochemical level, latrunculins have a well-understood mode of action, which ends up in a complete shift from F-actin to G-actin (Coué *et al.*, 1987; Ayscough, 1998a; Ayscough *et al.*, 1997; Morton *et al.*, 2000; for plant cells see Gibbon *et al.*, 1999; Kandasamy and Meagher, 1999). We extended our studies on several other plant systems to confirm the generality of data obtained with cells of maize root apices. Our present developmental analysis of F-actin-devoid plant cells reveals that the plant cell elongation is F-actin-dependent.

MATERIALS AND METHODS

Plant Material

Maize root apices. Maize grains (*Zea mays* L. cv. Alarik), obtained from Force Limagrain (Darmstadt, Germany), were soaked for 6 h and left for germination in well-moistened rolls of filter paper for 4 days in darkness at 20°C. Seedlings with straight primary roots, 50–70 mm long, were selected and growing root apices were excised for fixation. For experimental treatments, seedlings were transferred to containers with latrunculin B (10 μM, 1 h) obtained from Calbiochem (Bad Soden, Germany). Root apices were fixed either immediately after this treatment or after further 12 h of their growth in latrunculin-free environment of filter paper rolls.

Arabidopsis thaliana seedlings. After sterilization in 70% ethanol for 30 s and by 5% calcium hypochlorite for 20 min, seeds of *Arabidopsis thaliana*, ecotype Columbia, germinated on medium with MS (Murashige and Skoog, 1962) salts together with 5 g/L sucrose in 9-cm broad, vertically placed petri dishes. The medium was solidified by 0.7% agar and the pH of the medium was adjusted to 5.8 before sterilization by autoclaving. Latrunculin B was dissolved in ethanol and added to sterile medium. The final concentration of ethanol used for application of latrunculin B was 0.05% (v/v) of the medium. Cultures were kept in the dark or under 12-h daylength (40 mE/m^{-2}/s^{-1}) at 22°C. Lengths of roots and hypocotyls were estimated after 2 weeks of cultivation. For studying effects of latrunculin B on actin, seeds were germinated on nylon mesh placed on the above-mentioned medium and under the 12-h daylength. After 4 days nylon mesh with seedlings was replaced on the medium containing 1 μM latrunculin B for another 24 h.

Rye coleoptiles and seedlings. Rye seedlings (*Secale cereale* L. cv. Marder II; Lochow-Petkus, Bergen, Germany) were grown as described in Edelmann and Köhler (1995). In brief, caryopses were planted in moist vermiculite in covered plastic boxes, kept for 3 days in darkness at 25 ± 0.5°C, and harvested under laboratory light conditions. Segments (10 mm long) were cut 3–4 mm below tips of 3- to 3.6-cm-long coleoptiles and allowed to extend under aeration in a dark room (with temporary green safety light) at 25 ± 0.5°C. For longitudinal growth measurements, harvested coleoptile segments were abraded according to Lüthen *et al.* (1990). The segments were incubated in distilled water or in 10^{-5} M auxin (IAA) by addition of an appropriate stock solution (10^{-3} M). Elongation kinetics were determined with a ruler by measuring lengths of six segments lined up on straight stainless steel needles. The effects of latrunculin B on the morphogenesis of dark-grown seedlings of rye was tested by growing caryopses on filter paper soaked with either distilled water or 10 μm latrunculin B solution in covered glass beakers for 3 days.

Embryogenic cultures Abies alba x Abies cephalonica hybrid. Embryogenic cultures of *Abies alba* x *Abies cephalonica* hybrid were obtained and maintained as described by Salajová *et al.* (1996). Shortly, cultures were established in July 1991 from *Abies alba* Mill. female megagametophytes with developing embryos after pollination with *Abies cephalonica*. Embryogenic cultures were regularly transferred every 2 to 3 weeks into 9-cm petri dishes containing medium supplemented with 20 g/L sucrose, 1000 mg/L casein hydrolysate, 500 mg/L glutamine, 1000 mg/L myo-inositol, and 1 mg/L BAP (for further details see Salajová *et al.*, 1996). The medium was solidified with 0.7% agar and kept in the dark at 25°C. In experiments with latrunculin B, cultures were cultivated in 5-cm petri dishes with 15 ml medium of the composition as mentioned above. Medium pH was adjusted to 5.8 before sterilization by autoclaving. Latrunculin B was dissolved in ethanol and added to the sterile medium. The final concentration of ethanol used for application of latrunculin B was 0.05% (v/v) of the medium. The lengths of suspensor cells in early embryos, as characterized by Salajová *et al.* (1996) and Jasik *et al.* (1995), were measured after 2 weeks of cultivation.

Indirect Immunofluorescence

Maize root apices and whole *Arabidopsis* seedlings were processed, using Steedman's wax, for indirect immunofluorescence as described in detail previously (Baluška *et al.*, 1992, 1997; Vitha *et al.*, 2000a,b). Longitudinal sections (7 μm thick) were incubated with the anti-actin antibody (clone C4 from ICN, Costa Mesa, CA) and FITC-conjugated anti-mouse IgGs (Sigma Chemical, St. Louis,

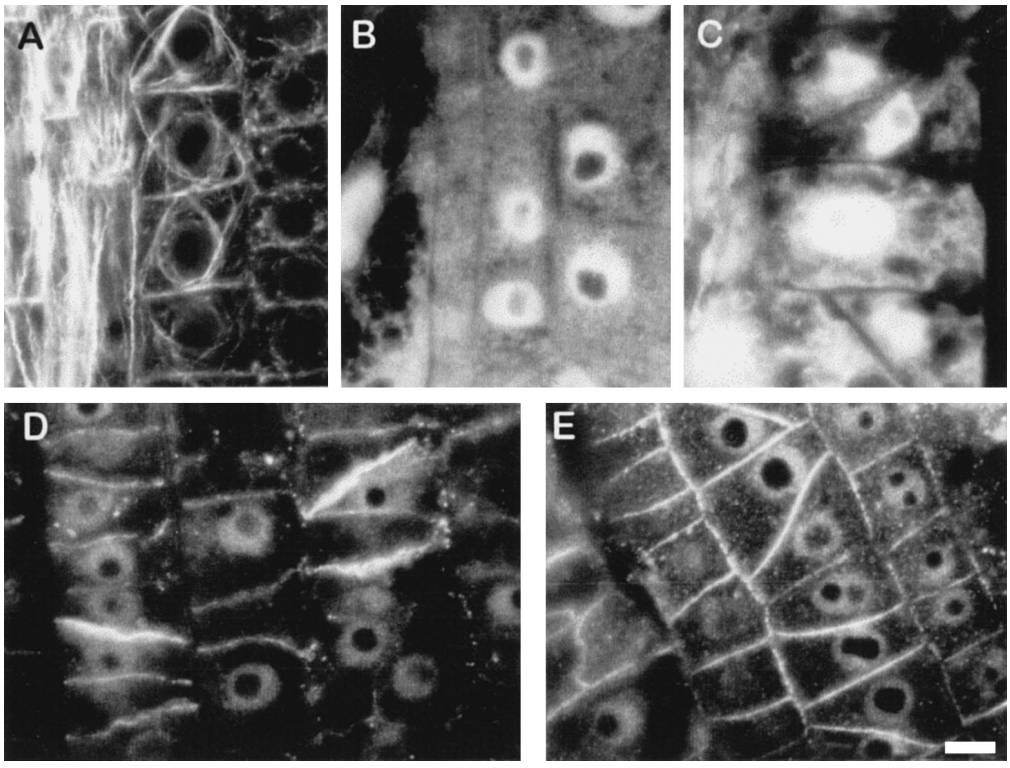


FIG. 1. Actin in maize root cells. (A–C) Actin in cells of the transition zone in control (A) and latrunculin B-treated (10 μM for 1 h (B); 10 μM for 1 h followed by 12 h in latrunculin-free environment of filter paper rolls (C) roots. F-actin networks are absent in the cytoplasm and diffuse fluorescence (G-actin-latrunculin B complexes) accumulate in nuclei of latrunculin-treated roots (B, C). (D, E) Plasma membrane-associated myosin VIII (Reichelt *et al.*, 1999) accumulates abundantly at misplaced cytokinetic cell plates (D) and postmitotic cells walls (E) in F-actin-devoid root cells (10 μM of latrunculin B for 12 h). Bar = 12 μm.

MO) diluted 1:200 and 1:100, respectively, for 1 h at room temperature. For myosin labeling, polyclonal antibody raised against unconventional myosin VIII (Reichelt *et al.*, 1999), diluted 1:100, was followed by FITC-conjugated anti-rabbit IgGs also diluted 1:100. Fluorescence was examined with an Axiovert 405M microscope (Zeiss, Oberkochen, Germany) equipped with epifluorescence and standard FITC exciter and barrier filters (BP 450–490, LP 520). Photographs were taken on Kodak T-Max films rated at 400 ASA.

RESULTS

Latrunculin B Rapidly Depolymerizes F-Actin

Figure 1A shows the F-actin arrays as observed in cells of maize root apices before treatment (see also Baluška *et al.*, 1997). After 1 h of exposure to 10 μM of latrunculin B, all cells of the maize root apex lost their F-actin, which transformed into diffuse fluorescence of the cytoplasm accumulating within nuclei (compare Figs. 1A and 1B). This latter feature was true for all nuclei found in all sections. Transfer of latrunculin-treated (2 h) maize root apices into latrunculin B-free environment for 12 h did not allow repolymerization of actin filaments (AFs) (Fig. 1C). This finding indicates that, during longer treatments, most cellular G-actin becomes complexed with latrunculin B in the form of stable complexes. Latrunculin B at 1 μM removed most F-actin throughout *Arabidopsis* seedlings (compare Figs. 2A, 2C, and 2E with Figs. 2B, 2D, and 2F), when only large bundles remained in parenchymatic cells of root stele (Figs. 2B and 2D) and some actin-positive rods in stem apex and cotyledones cells (Fig. 2F). As compared with maize, that requires 10 μM for maximal effects (see below), this higher sensitivity of *Arabidopsis* toward latrunculin B probably relates to the much smaller statures of *Arabidopsis* organs. For instance, the root diameter in *Arabidopsis* is about 0.1 mm, while in maize it is about 1 mm.

Long-Term Experiments: Latrunculin B-Induced Dwarfism

Easy handling of *Arabidopsis* seedlings grown on agar allowed us to assess latrunculin B-mediated effects at the level of a whole plant in long-term experiments. To show the effects of latrunculin B on the overall seedling habitus and on morphogenesis, we germinated seeds and performed

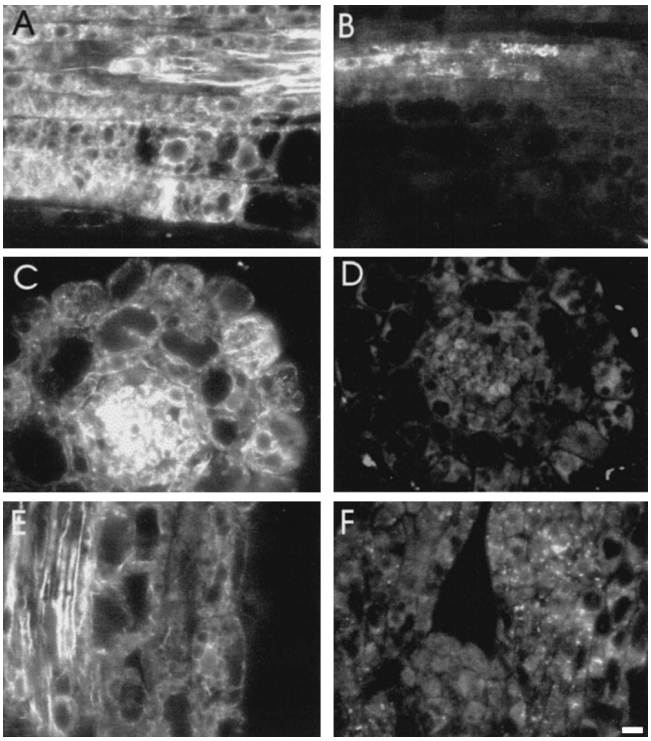


FIG. 2. Actin in *Arabidopsis*. (A–D) Distributions of the actin cytoskeleton in root cells. (A, B) Longitudinal sections through the transition zone and distal part of the elongation region. (C, D) Transversal sections of roots on the border of transition zone and elongation region. (E, F) Distributions of the actin cytoskeleton in cells of the hypocotyl (E) and of the shoot apex with cotyledons (D) in control seedlings (A, C, E) and seedlings treated with 1 μM latrunculin B for 24 h (B, D, F), both of the same age. Note the generally fainter signal and absence of filamentous structures (F-actin) in cells of latrunculin B-treated seedlings. Bar = 7 μm.

the whole postembryonic development in the presence of latrunculin B. Intriguingly, morphologically normal but extremely small seedlings resulted from the long-term (2 weeks) latrunculin B exposure (Fig. 3C and insets in Figs. 3A and 3B). The only minor alterations are slightly swollen hypocotyls and opening of cotyledones and young leaves in the dark. This latrunculin B-mediated dwarfism of *Arabidopsis* provides evidence that, in sharp contrast to plant cell elongation, F-actin is not essential for the polarity of growth and for the performance of overall plant morphogenesis. The same situation is true for growth and morphogenesis of rye seedlings devoid of F-actin in their cells (Fig. 4).

Latrunculin B Impairs Growth of Arabidopsis as a Result of Inhibition of Cell Elongation

Latrunculin B proved to be extremely effective in the inhibition of growth of *Arabidopsis* roots and hypocotyls; already, 1 μM of latrunculin B induced maximal inhibition (Fig. 5A). The most prominent effect was detected in dark-grown hypocotyls, where 1 μM of latrunculin B inhibited the organ extension by about 90% (Fig. 5A).

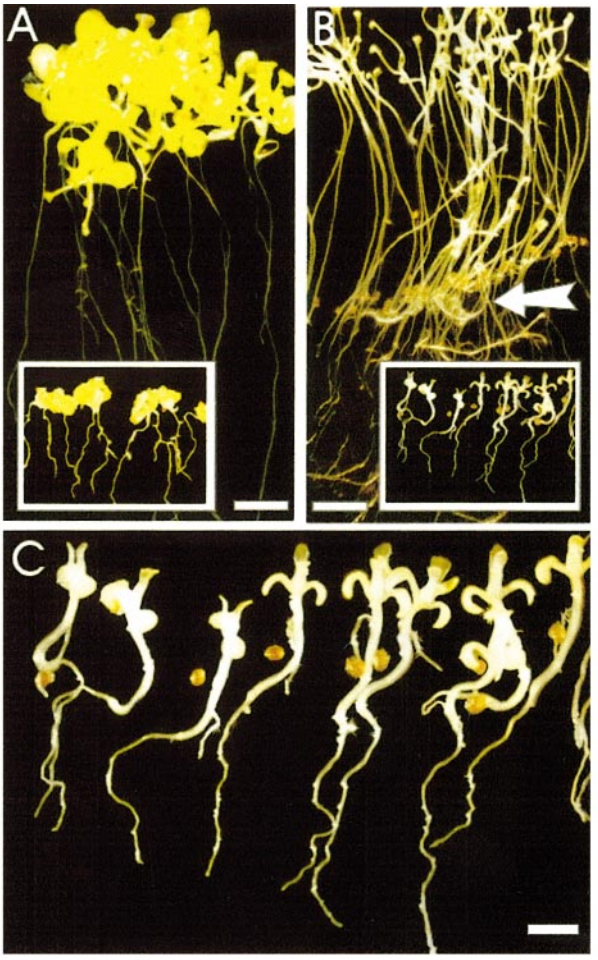
We also analyzed root and hypocotyl epidermis cells for their easy accessibility and our data confirmed that latrunculin B specifically inhibits cell elongation (Figs. 6A and 6B). In roots, the light regime has only slight effects on growth rates and latrunculin B elicits approximately 40% inhibition of cell elongation both in the dark and in the light (Fig. 6A). More conspicuous effects were detected in hypocotyls. In the dark, prominent cell elongation is effectively inhibited, resulting in a decrease of cell lengths by about 80% (Fig. 6B). On the other hand, light-grown hypocotyl cells grow only slightly and, here, latrunculin B caused only a 20% decrease of cell lengths (Fig. 6B).

F-Actin-Devoid Maize Root Cells Divide Further but Fail to Accomplish Rapid Cell Elongation

Latrunculin B effectively inhibits maize root growth in a concentration-dependent manner when the lowest concentration showing maximal effects was 10 μM. At this concentration, root extension was inhibited by about 70% and a further increase in latrunculin B concentration could not induce additional inhibition of maize root extension (Fig. 5B). Importantly, roots reached this low growth rate within 2 h of treatment and then maintained this rate for at least a further 12 h (data not shown). Because of the absence of rapid cell elongation, growth of F-actin-devoid maize roots is accomplished exclusively via the slow cytoplasmic cell growth. Both mitosis and cytokinesis continued further, as demonstrated by numerous short cells filling up the former elongation region encompassing the first 7 mm. Nevertheless, cell division planes are often skewed (Figs. 1D and 1E). Morphometric analysis of cell lengths, widths, and shapes

FIG. 3. Latrunculin B-induced dwarf seedlings of *Arabidopsis*. Long-term absence of F-actin, imposed by continuous treatment (throughout germination and subsequent growth) with 1 μM latrunculin B, results in dwarfed habitus of *Arabidopsis* seedlings. This situation is clearly obvious for both light-grown (12/12-h photoperiod) (A) and dark-grown, etiolated seedlings (B). Inserts depict latrunculin B-induced dwarfs, which are morphologically normal, as it is obvious from image (C) showing dark-grown dwarfs in larger magnification. Arrow in (B) points to the root-hypocotyl border. Bar = 5 mm (A, B) and 1.1 mm (C).

FIG. 4. Latrunculin B-induced dwarf seedlings of rye. Long-term absence of F-actin, imposed by continuous latrunculin B exposure throughout germination and seedling growth, also induces seedling dwarfism in rye. Here we present examples of dark-grown seedlings in the presence (three seedlings on the left side) and the absence (three seedlings on the right side) of latrunculin B for 3 days. Bar = 7 mm.



3

4

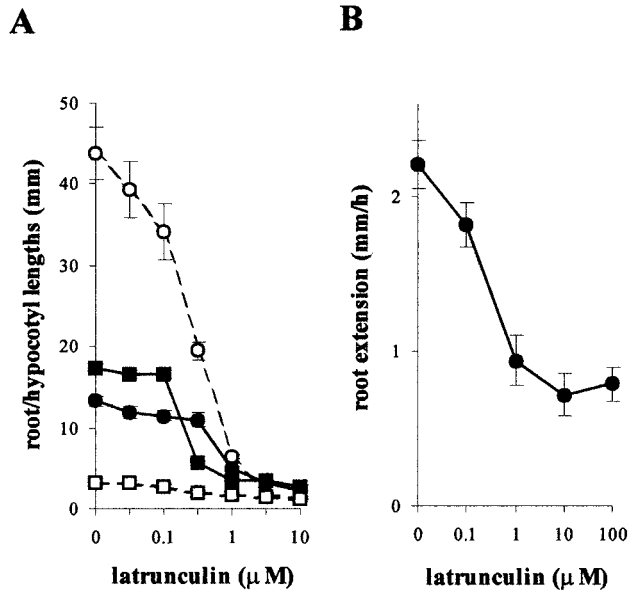


FIG. 5. Effects of latrunculin B on root and hypocotyl growth. (A) Concentration-dependent effects of latrunculin B on growth of *Arabidopsis* hypocotyls (empty squares and circles) and roots (full squares and circles). Root and hypocotyl lengths were evaluated after 2 weeks of seedling cultivation in dark (circles) and under 12-h dark/12-h light photoperiod (squares). (B) Concentration-dependent effects of latrunculin B on maize root extension. For cultivation details and latrunculin B treatment, see Materials and Methods.

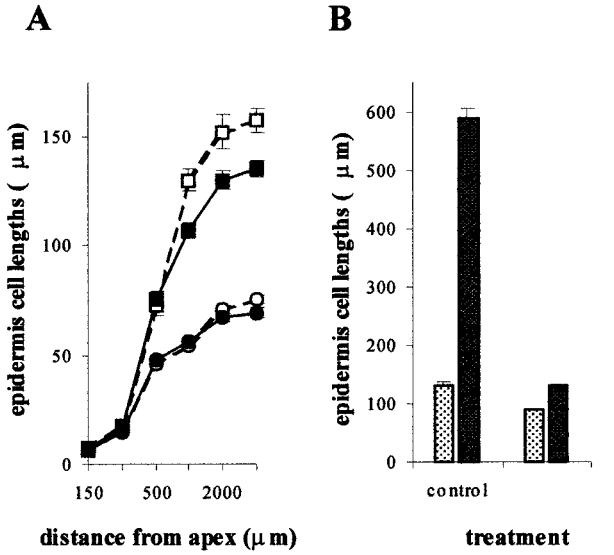


FIG. 6. Effects of latrunculin B on epidermis cell lengths. (A) Root epidermis cells. Roots of *Arabidopsis* seedlings grown 2 weeks on medium with 1 μM latrunculin B (circles) or without latrunculin B (squares). Seedlings were germinated and kept either in dark (full circles and squares) or under 12-h dark/12-h light photoperiod (empty circles and squares). (B) Epidermis cell lengths of hypocotyls in *Arabidopsis* seedlings grown on medium with 1 μM latrunculin B and of control seedlings. Seedlings were germinated and cultivated for 2 weeks either in dark (hatched columns) or under 12-h dark/12-h light photoperiod (tinted columns).

reveals that cell lengths of mature cortical cells attained only about 50% of control values in roots devoid of F-actin for 12 h (Table 1). On the other hand, cell widths were not affected significantly. This means that the final cell volumes of latrunculin-treated cells are decreased as a result of the inhibited cell elongation (Table 1).

Effects of Latrunculin B on Auxin-Induced Elongation of Coleoptile Segments

To further probe the generality of latrunculin B-mediated effects on cell elongation, we have taken the advantage of *Secale* coleptile segments because their cells can be easily induced into rapid cell elongation via exposure to exogenous auxin. In good accordance with data on maize roots, extension of rye coleoptiles is also sensitive toward latrunculin B, although higher concentrations (approximately one magnitude higher) are required for coleoptiles to elicit effects comparable to those recorded in roots (Fig. 7A). This is, again, apparently related to the size and unique morphology of coleoptiles. The impermeable cuticle of coleoptiles, although partially abraded, hinders effective penetration of latrunculin B into their cells. Nevertheless, during 6 h of incubation in 100 μM of latrunculin B, the coleoptile extension dropped to a value of about 28% of that of the control level. Importantly, this level of latrunculin B completely abolished the auxin-induced increase in the rate of the cell elongation in segments of rye coleoptiles (Fig. 7B).

Inhibition of Cell Elongation in Embryogenic Cultures of Abies

Owing to their simple cultivation *in vitro* and pronounced cell elongation (Jasik *et al.*, 1995; Salajová *et al.*, 1996), somatic embryos of *Abies alba* x *Abies cephalonica*

TABLE 1

Effects of Latrunculin B on Final Lengths and Volumes of Cortex Cells in Maize Roots

	Lengths	Volumes
Control	169 ± 3	344,340 ± 14,454
2 h	143 ± 5	290,925 ± 12,392
6 h	118 ± 3	221,321 ± 11,987
2 + 12 h	61 ± 2	163,030 ± 10,211

Note. Under exposures to 10 μM latrunculin B, both final cell lengths (μm) and volumes (μm^3) decrease (results are means ± SE; for details of the morphometry analysis see Baluška *et al.*, 1993a,b, 1996a) prominently with the increasing exposure times.

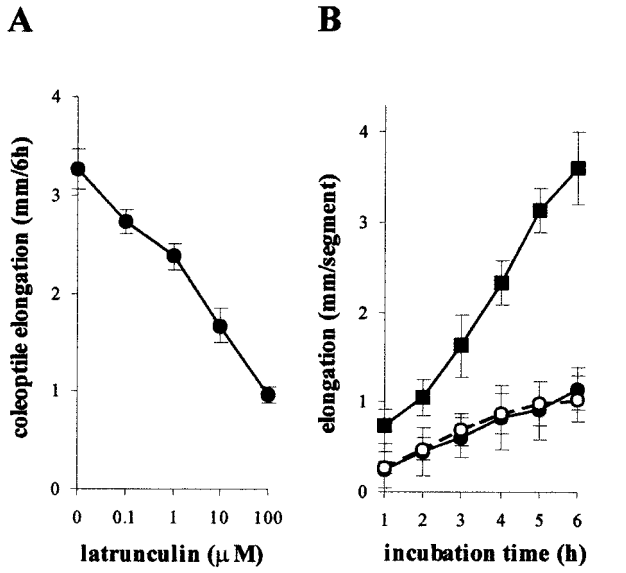


FIG. 7. Effects of latrunculin B on elongation of rye coleoptile cells. (A) Concentration-dependent effects of latrunculin B on elongation of coleoptiles during 6-h incubation periods. (B) Extension of abraded 1-cm-long coleoptile segments incubated in distilled water (open circles), auxin (full squares), and auxin plus latrunculin B (full circles).

hybrid represent an excellent model object for studies on cell elongation. Both early and late embryos are suitable for studies of different aspects of plant cell elongation processes. After application of ABA, embryonal masses on both latrunculin B-containing and latrunculin B-free media produced late embryos (Figs. 8A and 8B). Those late embryos which developed on latrunculin B-containing media exhibited standard anatomy (data not shown) and morphology, with only slightly swollen shoot apical meristems and cotyledones (Fig. 8B). Nevertheless, cells of both hypocotyls and cotyledones failed to elongate properly, which resulted in shorter barrel-like appearances of late embryos as formed in the presence of latrunculin B (Fig. 8B).

For cell length analysis, suspensor cells proved to be suitable. Suspensor cells in early embryos arise from small isodiametric cells localized proximally in embryonal masses and elongate strongly during formation of early embryos (Jasik *et al.*, 1995). This suspensor cell elongation is effectively inhibited with latrunculin B (Figs. 8C and 8D) and cell lengths were affected in a concentration-dependent manner (Fig. 9).

DISCUSSION

In the present study, latrunculin B is confirmed to be a powerful drug for destroying F-actin in higher plant cells organized within intact tissues. This finding is in good agreement with recent biochemical data showing high affinity of latrunculin B to maize pollen G-actin, which results in depolymerization of F-actin because of a very high turnover of this polymer (Gibbon *et al.*, 1999). Indirect immunofluorescence analysis, using specific actin antibody, showed that all cell types of maize root apices and *Arabidopsis* seedlings depolymerize all their F-actin within 60 min (although most cells already lose F-actin after 20 min; data not shown) of exposures to low concentrations of latrunculin B. Simultaneously, extension of maize root apices treated with latrunculin B decreases prominently in a concentration-dependent manner. Morphometric analysis of maize root apices reveals that root cell elongation is particularly latrunculin B-sensitive: F-actin-depleted root cortex cells attain only approximately 50% of their final cell lengths. In contrast, the slow cytoplasmic cell growth, which accompanies cell divisions and continues in the transition zone (Baluška *et al.*, 1996b), proceeds effectively further in root cells devoid of actin filaments (AFs). Moreover, in contrast to yeast and animal cells, both mitosis and cytokinesis of maize root cells are completed in the absence of F-actin, although cell division planes are positioned irregularly. All this ends up in a large number of short and aberrantly shaped cells filling up the whole root growth

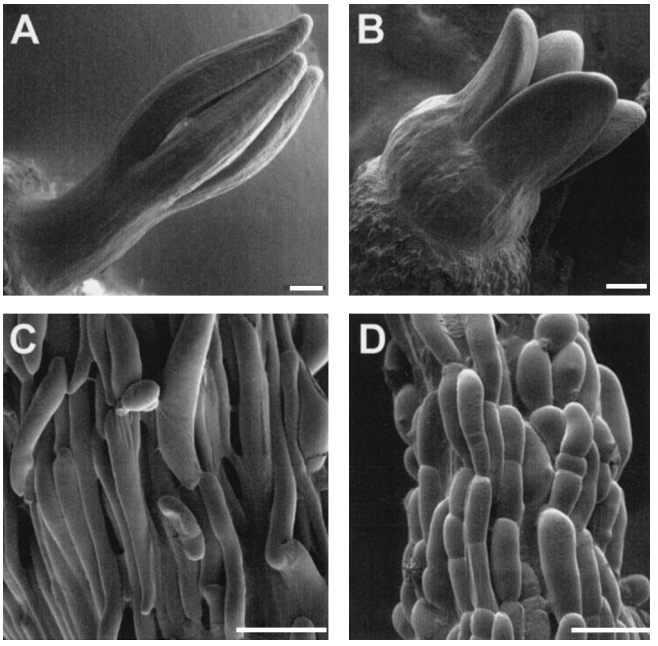


FIG. 8. Latrunculin B effects on *in vitro* embryogenesis in *Abies alba* × *Abies cephalonica* hybrid. (A, B) Long-term presence of latrunculin B alters dimensions of late embryos, which compared with control embryos (A), have shorter hypocotyls and cotyledones (B). (C, D) Latrunculin B inhibits elongation of suspensor cells of early embryos. (C) Elongating suspensor cells of the early embryo in control culture. (D) Short suspensor cells of early embryos in culture supplemented with 1 μM latrunculin (see also Fig. 3C). Bar = 250 μm (A, B), 120 μm (C), 150 μm (D).

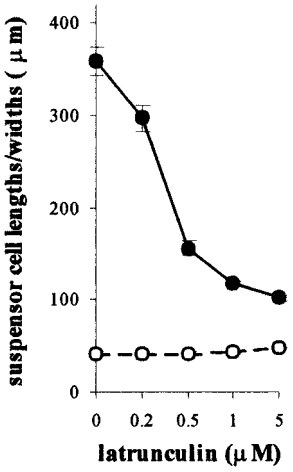


FIG. 9. Latrunculin B-mediated inhibition of suspensor cell elongation in *Abies in vitro* embryogenesis. Concentration-dependent inhibition of suspensor cell elongation (full circles) during early *in vitro* embryogenesis of *Abies alba* × *Abies cephalonica*. In contrast to cell lengths, cell widths are unaffected by latrunculin B (open circles).

region when maize root apices grow in the absence of F-actin for 12 h. F-actin dependency of plant cell elongation has been confirmed during germination of rye seedlings and young *Arabidopsis* seedlings, as well as for auxin-induced cell elongation-based extension of rye coleoptile segments. Intriguingly, the long-term depletion of F-actin throughout germination results in miniaturized *Arabidopsis* and rye seedlings (Figs. 6 and 7). These latrunculin B-induced dwarfs are phenotypically similar to several dwarf mutants, indicating that latrunculin B can phenocopy these mutants.

Latrunculin B as a Plant F-Actin Drug of Choice

The most often used F-actin drugs, cytochalasins, have a complex mode of action, which is not yet thoroughly understood (e.g., Cooper, 1987). Nevertheless, it is clear that they disturb complex dynamic processes at barbed ends of AFs in cooperation with numerous actin-binding proteins (e.g., Ayscough, 1998b). Responses of plant cells to cytochalasins range from the expected depolymerization of AFs to unexpected stimulation of actin polymerization, which either causes overstabilization of AFs (Williamson, 1978; Foissner and Wasteneys, 1997) or results in aberrant AF arrays (e.g., Williamson and Hurley, 1986; Palevitz, 1988; Lancelle and Hepler, 1988; Collings *et al.*, 1995).

This critical drawback of experimental drug studies on the plant actin cytoskeleton can be avoided by the application of a new generation of F-actin drugs, represented by macrolide toxin latrunculins isolated from the red sea sponge *Latrunculia magnifica* (Spector *et al.*, 1983; Schatten *et al.*, 1986; Coué *et al.*, 1987). Latrunculins have a straightforward mode of action, which is relatively well understood: they bind to G-actin, forming G-actin–latrunculin complexes, which are no longer available for polymerization (Coué *et al.*, 1987; Ayscough *et al.*, 1997; Ayscough, 1998a; Gibbon *et al.*, 1999; Morton *et al.*, 2000). In the case of very dynamic actin cytoskeleton, as found in most eukaryotic cells, intracellular latrunculins rapidly disintegrate all F-actin. The higher reliability of latrunculins versus cytochalasins (Spector *et al.*, 1989) was also noted in *Drosophila* cells, in which latrunculin A proved to be much more effective and allowed reinterpretation of F-actin-dependent events during asymmetric cell divisions of neural precursor cells (Knoblich *et al.*, 1997). These authors applied rather high (200 μM) concentrations of latrunculin A, but even this is without side effects (Ayscough *et al.*, 1997). To date all performed studies show that latrunculin B is extremely effective in all plant cell types tested so far (for lower plants see, e.g., Gupta and Heath, 1997; Alessa and Kropf, 1999; for higher plants see Gibbon *et al.*, 1999; Kandasamy and Meagher, 1999; Zonia *et al.*, 1999; and this study). We confirmed this for maize roots and *Arabidopsis* seedlings. Intriguingly, G-actin accumulated within nuclei of latrunculin-treated maize root cells, indicating that changed conformation of G-actin after its latrunculin binding (Morton *et al.*, 2000) interferes with the nuclear export of G-actin (Wada *et al.*, 1998). In fact, G-actin is a nucleocytoplasmic shuttle protein whose predominant cytoplasmic localization is based on two functional nuclear export sequences found in all known types of G-actin (Wada *et al.*, 1998).

F-Actin-Devoid Plant Cells Perform Mitosis, Cytokinesis, and Slow Cytoplasmic Growth but They Fail in Vacuome-Based Rapid Cell Elongation

Plant morphogenesis is determined by aligning of cell division planes and by the polarity and rate of subsequent postmitotic cell expansion (e.g., Barlow, 1994). Cortical microtubules (MTs) and nascent cellulosic microfibrils are well known to play the decisive role in orienting plant cell expansion (for roots see, e.g., Baluška *et al.*, 1992, 1993b). On the other hand, the importance of AFs in controlling plant growth polarities remains obscured, although numerous studies indicate that AFs are not absolutely essential for the accomplishment of mitosis and cytokinesis (e.g., Palevitz, 1980; Cho and Wick, 1990; Valster and Hepler, 1997). Published data obtained with cytochalasins suggest that AFs might be involved in the execution of vacuome-based plant cell elongation (Thimann and Biradivolu, 1994; Thimann *et al.*, 1992; Waller and Nick, 1997; Waller *et al.*, 2000). Nevertheless, this attractive possibility requires further testing with more specific F-actin drugs and a wider range of plant cell types.

Using latrunculin B as an F-actin disrupter, here we have taken advantage of several plant systems developed and/or used in our laboratories as suitable model systems for testing the possible role of F-actin in plant cell elongation. For instance, latrunculin B-treated maize root cells devoid

of F-actin accumulate within the postmitotic transition zone (this phenomenon is already evident after short exposures: see Baluška *et al.,* 1997; Volkmann and Baluška, 1999), and perform only partial cell elongation (this study) when compared with appropriate cells of control roots. In good accordance with these root data, extension of coleoptiles is also impaired and auxin-induced cell elongation of rye coleoptile cells is, in fact, completely inhibited by latrunculin B exposures. This clearly supports our conclusion that latrunculin B effectively inhibits plant cell elongation.

Previously, we showed that maize root cells require a specific preparatory phase to switch their postmitotic growth from slow cell expansion into rapid cell elongation (Baluška *et al.,* 1994, 1996b). There is also genetic evidence for the existence of two types of postmitotic plant growth modes, that is, a slower cytoplasmic cell expansion which is replaced by a rapid vacuome-based elongation. The *STP1* gene of *Arabidopsis* was shown to be required for the rapid plant cell elongation but not for the slow postmitotic growth (Baskin *et al.,* 1995). Interestingly, *STP1* mutant seedlings showed severe dwarfism, closely resembling latrunculin B-induced dwarfism of *Arabidopsis* seedlings (see below). A relevant observation in this respect is that F-actin arrays reorganize conspicuously in cells of the root apex transition zone (Baluška *et al.,* 1997, 2000a) and this reorganization appears to be related to the actomyosin-dependent onset of the rapid cell elongation (Baluška *et al.,* 1997, 2000a; Volkmann and Baluška, 1999). The switch from the slow cell expansion into the rapid cell elongation is accomplished in a unique root growth region termed the transition zone (Baluška *et al.,* 1994; 1996b) and it happens as a sudden event (Ivanov and Maximov, 1999). An attractive possibility, that does not exclude other possible scenarios, is that cells of the transition zone activate transcription of actin isoforms involved in the rapid cell elongation (Meagher *et al.,* 1999a,b, 2000; see below for further discussion).

The critical question is how F-actin supports the vacuome-based plant cell elongation. Most important in this respect, as evidenced by prominent accumulation of short F-actin-devoid cells throughout the former elongation region (2–8 mm from the root cap junction), the slow cytoplasmic growth apparently continues during long-term absence of F-actin in root cells. This important finding suggests that processes like Golgi vesicle production, transport, and exocytosis are not absolutely dependent on the intact F-actin cytoskeleton. In fact, recent data reveal that vesicles can be transported via both the actin- and tubulin-based cytoskeletons in eukaryotic cells (e.g., Brown, 1999). Obviously some other F-actin-dependent processes are critical for plant cell elongation. It is known that the actin cytoskeleton controls ion channel activities not only in animal cells but also in plant cells (e.g., Hwang *et al.,* 1997, 2000). It could be speculated that, in the absence of an intact F-actin cytoskeleton, the plasma membrane and tonoplast of plant cells are possibly unable to support sufficiently high uptake and transport of ions and water to allow vacuome-based cell elongation. In accordance with this tentative notion, the *det3* dwarf mutant of *Arabidopsis* shows defects in cell elongation and the *DET3* gene was shown to encode subunit C of the vacuolar H^+-ATPase (Schumacher *et al.,* 1999). Interestingly in this respect, vacuolar H^+-ATPases of animal cells bind AFs with high affinity via their B subunits (Lee *et al.,* 1999; Holliday *et al.,* 2000).

In support of the ion channel hypothesis, F-actin drugs cytochalasin D (Miller *et al.,* 1999) and latrunculin B (Baluška *et al.,* 2000b) inhibit root hair formation after the bulge outgrowth stage. This particular phenotype resembles the root hair mutant of *Arabidopsis*, lacking root hairs but forming bulges, which was recently cloned as a potassium channel (Liam Dolan, personal communication). F-actin drugs also cause almost immediate inhibition of the tip growth (for pollen tubes, see Gibbon *et al.,* 1999), which is associated with altered distributions of vacuoles and cytoplasm in root hair apices (Miller *et al.,* 1999; Ovecka *et al.,* 2000).

Latrunculin B-Induced Dwarfism of Rye and Arabidopsis Seedlings

To support the above-noted results, we performed detailed analyses of the impact of long-term (2 weeks) latrunculin B exposures on intact seedlings of *Arabidopsis* during their whole postembryonic development. These latrunculin B-grown *Arabidopsis* seedlings show severe dwarfism, resembling with their habitus numerous dwarf mutants (e.g., Németh *et al.,* 1998; Salchert *et al.,* 1998; Cheng *et al.,* 2000). Latrunculin B, however, allowed formation of morphologically more or less normal seedlings, suggesting that cell divisions and growth polarities maintain their patterns established during embryogenesis (Jürgens, 1996; Jürgens *et al.,* 1997). Support for the critical importance of an intact F-actin system for building of normally grown large plants comes from experiments with transgenic *Arabidopsis* seedlings, which often obtain dwarfed habitus when they have disturbed their actin cytoskeleton (Xia *et al.,* unpublished data in Carlier *et al.,* 1997). Slight disturbances like swelling of hypocotyls or opening of cotyledons and young leaves in the dark are difficult to interprete because of possibilities of some secondary effects of long-term absence of F-actin. Interestingly, defects in the *ELD1* gene result not only in generally impaired cell elongation but also in continuation of shoot development in the dark-grown *Arabidopsis* seedlings (Cheng *et al.,* 2000).

Stimulus-responsive plant cell elongation is tightly linked to signaling pathways based on phytohormones. For instance, *Arabidopsis* seedlings deficient in steroid hormones (Salchert *et al.,* 1998) and auxin transport (Mattsson *et al.,* 1999) exhibit dwarfed phenotypes (see Fig. 1 in Mattsson *et al.,* 1999). On the other hand, overexpression of the auxin-binding protein 1 resulted in increased cell sizes (Jones *et al.,* 1998). Interestingly in this respect, the *ACT7*

actin gene of *Arabidopsis* is strongly expressed in elongating cells, contains putative phytohormone response elements, and responds sensitively to auxin (McDowell *et al.*, 1996). Typically, the overall body-plan of miniature plants remains more or less normal, resembling bonsai plants as well as natural dwarfs induced via extremely severe environments (Körner *et al.*, 1989). Our present data suggest that both phytohormones and environmental factors converge on the actin cytoskeleton to modulate stimulus-responsive plant cell elongation. In fact, the actin cytoskeleton is extremely suitable to act as downstream effector of diverse signaling cascades (e.g., Machesky and Insall, 1999; for plant cells see Volkmann and Baluška, 1999; Staiger, 2000). The *ACT7* actin gene of *Arabidopsis*, which is strongly expressed in rapidly elongating cells, is the best candidate for such a function as *ACT7* expression is not only under control of phytohormones but also of diverse external stimuli (McDowell *et al.*, 1996).

In conclusion, long-term depolymerization of most F-actin via latrunculin B induces severe dwarfism in *Arabidopsis* and *Secale* seedlings because their cells do not elongate. Despite the fact that F-actin is essential for cell elongation, plant development, and morphogenesis proceed quite normally in the absence of F-actin. We suggest that the dynamic actin cytoskeleton acts downstream of phytohormones to tightly link diverse environmental inputs with rapid cell elongation.

ACKNOWLEDGMENTS

The research was supported by fellowships from the Alexander von Humboldt Foundation (Bonn, Germany) to J.J. and F.B. Moreover, F.B. receives partial support from the Slovak Academy of Sciences, Grant Agency Vega (Grant 6030), Bratislava, Slovakia. We gratefully acknowledge financial support to AGRAVIS by the Deutsche Agentur für Raumfahrtangelegenheiten (DARA, Bonn, Germany) and the Ministerium für Wissenschaft und Forschung (MWF, Düsseldorf, Germany).

Note added in proof. Part of the data cited as Xia *et al.*, unpublished data in Carlier *et al.* (1997), have been published recently by Ramachandran *et al.* (*Plant Physiol.* **124,** 1637–1647).

REFERENCES

Alessa, L., and Kropf, D. L. (1999). F-actin marks the rhizoid pole in living *Pelvetia compressa* zygotes. *Development* **126,** 201–209.

Ayscough, K. R. (1998a). Use of latrunculin-A, an actin monomer-binding drug. *In* "Methods in Enzymology: Molecular Motors and the Cytoskeleton" (R. B. Vallee, Ed.), Vol. 298, Part B, pp. 18–25. Academic Press, San Diego.

Ayscough, K. R. (1998b). *In vivo* functions of actin-binding proteins. *Curr. Opin. Cell Biol.* **10,** 102–111.

Ayscough, K. R., Stryker, J., Pokala, N., Sanders, M., Crews, P., and Drubin, D. G. (1997). High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin A. *J. Cell Biol.* **137,** 399–416.

Baluška, F., Barlow, P. W., and Kubica, Š. (1994). Importance of the post-mitotic "isodiametric" growth (PIG) region for growth and development of roots. *Plant Soil* **167,** 31–42.

Baluška, F., Barlow, P. W., and Volkmann, D. (2000a). Actin and myosin VIII in developing root cells. *In* "Actin: A Dynamic Framework for Multiple Plant Cell Functions" (C. J. Staiger, F. Baluška, D. Volkmann, and P. W. Barlow, Eds.), pp. 457–476. Kluwer Academic, Dordrecht, The Netherlands.

Baluška, F., Hauskrecht, M., Barlow, P. W., and Sievers, A. (1996a). Gravitropisms 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., Parker, J. S., and Barlow, P. W. (1992). Specific patterns of cortical and endoplasmic microtubules associated with cell growth and tissue differentiation in roots of maize (*Zea mays* L.). *J. Cell Sci.* **103,** 191–200.

Baluška, F., Parker, J. S., and Barlow, P. W. (1993a). The microtubular cytoskeleton in cells of cold-treated roots of maize (*Zea mays* L.) shows tissue-specific responses. *Protoplasma* **172,** 84–96.

Baluška, F., Parker, J. S., and Barlow, P. W. (1993b). A role for gibberellic acid in orienting microtubules and regulating cell growth polarity in the maize root cortex. *Planta* **191,** 149–157.

Baluška, F., Salaj, J., Mathur, J., Braun, M., Jasper, F., Šamaj, J., Chua, N.-H., Barlow, P. W., and Volkmann, D. (2000b). Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-enriched F-actin meshworks accumulated within expansin-enriched bulges. *Dev. Biol.* **227,** 457–466.

Baluška, F., Vitha, S., Barlow, P. W., and Volkmann, D. (1997). Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: A major developmental switch occurs in the postmitotic transition region. *Eur. J. Cell Biol.* **72,** 113–121.

Baluška, F., Volkmann, D., and Barlow, P. W. (1996b). Specialized zones of development in roots: View from the cellular level. *Plant Physiol.* **112,** 3–4.

Barlow, P. W. (1994). Cell divisions in meristems and their contribution to organogenesis and plant form. *In* "Shape and Form in Plants and Fungi" (D. S. Ingram and A. Hudson, Eds.), pp. 169–193. Academic Press, London.

Barlow, P. W., and Rathfelder, E. L. (1985). Distribution and redistribution of extension growth along vertical and horizontal gravireacting maize roots. *Planta* **165,** 134–141.

Baskin, T. I., Cork, A., Williamson, R. E., and Gorst, J. R. (1995). *STUNTED PLANT 1*, a gene required for expansion in rapidly elongating but not in dividing cells and mediating root growth responses to applied cytokinin. *Plant Physiol.* **107,** 233–243.

Brown, S. S. (1999). Cooperation between microtubule- and actin-based motor proteins. *Annu. Rev. Cell Dev. Biol.* **15,** 63–80.

Carlier, M.-F., Laurent, V., Santolini, J., Melki, R., Didry, D., Xia, G.-X., Hong, Y., Chua, N.-H., and Pantaloni, D. (1997). Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: Implications in actin-based motility. *J. Cell Biol.* **136,** 1307–1323.

Cheng, J.-C., Lertpiriyapong, K., Wang, S., and Sung, Z. R. (2000). The role of the Arabidopsis *ELD1* gene in cell development and photomorphogenesis in darkness. *Plant Physiol.* **123,** 509–520.

Cho, S.-O., and Wick, S. M. (1990). Distribution and function of actin in the developing stomatal complex of winter rye (*Secale cereale* cv. Puma). *Protoplasma* **157,** 154–164.

Collings, D. A., Wasteneys, G. O., and Williamson, R. E. (1995). Cytochalasin rearranges cortical actin of the alga *Nitella* into short stable rods. *Plant Cell Physiol.* **36,** 765–772.

Cooper, J. A. (1987). Effects of cytochalasin and phalloidin on actin. *J. Cell Biol.* **105,** 1473–1478.

Cosgrove, D. (1987). Assembly and enlargement of the primary cell wall in plants. *Annu. Rev. Cell Dev. Biol.* **13,** 171–201.

Coué, M., Brenner, S. L., Spector, I., and Korn, E. D. (1987). Inhibition of actin polymerization by latrunculin A. *FEBS Lett.* **213,** 316–318.

Dawson-Day, M. J., and Millar, A. J. (1999). Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. *Plant J.* **17,** 63–71.

Devlin, P. F., Halliday, K. J., Harberd, N. P., and Whitelam, G. C. (1996). The rosette habit of *Arabidopsis thaliana* is dependent upon phytochrome action: Novel phytochromes control internode elongation and flowering time. *Plant J.* **10,** 1127–1134.

Edelmann, H. G., and Köhler, K. (1995). Auxin increases elastic wall properties in rye coleoptiles: Implications for the mechanism of wall loosening. *Physiol. Plant.* **93,** 85–92.

Foissner, I., and Wasteneys, G. O. (1997). A cytochalasin-sensitive actin filament meshwork is a prerequisite for local wound wall deposition in *Nitella* internodal cells. *Protoplasma* **200,** 17–30.

Gibbon, B. C., Kovar, D. R., and Staiger, C. J. (1999). Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell* **11,** 2349–2364.

Gray, W. M., Östin, A., Sandberg, G., Romano, C. P., and Estelle, M. (1998). High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **95,** 7197–7202.

Gupta, G. D., and Heath, I. B. (1997). Actin disruption by latrunculin B causes turgor-related changes in tip growth of *Saprolegnia ferax* hyphae. *Fungal Genet. Biol.* **21,** 64–75.

Holliday, L. S., Lu, M., Lee, B. S., Nelson, R. D., Solivan, S., Zhang, L., and Gluck, S. L. (2000). The amino-terminal domain of the B subunit of vacuolar H^+-ATPase contains a filamentous actin binding site. *J. Biol. Chem.* **275,** 32331–32337.

Hwang, J.-U., Eun, S.-O., and Lee, Y. (2000). Structure and function of actin filaments in mature guard cells. *In* "Actin: A Dynamic Framework for Multiple Plant Cell Functions" (C. J. Staiger, F. Baluška, D. Volkmann, and P. W. Barlow, Eds.), pp. 427–436. Kluwer Academic, Dordrecht, The Netherlands.

Hwang, J.-U., Suh, S., Kim, J., and Lee, Y. (1997). Actin filaments modulate both stomatal opening and inward K^+-channel activities in guard cells of *Vicia faba* L. *Plant Physiol.* **115,** 335–342.

Ishikawa, H., and Evans, M. L. (1992). Induction of curvature in maize roots by calcium or by thigmostimulation. Role of the postmitotic isodiametric growth zone. *Plant Physiol.* **100,** 762–768.

Ishikawa, H., and Evans, M. L. (1993). The role of the distal elongation zone in the response of maize roots to auxin and gravity. *Plant Physiol.* **102,** 1203–1210.

Ivanov, V. B., and Maximov, V. N. (1999). The change in the relative rate of cell elongation along the root meristem and the apical region of the elongation zone. *Russ. J. Plant Physiol.* **46,** 73–82.

Jasik, J., Salajová, T., and Salaj, J. (1995). Developmental anatomy and ultrastructure of early somatic embryos in European black pine (*Pinus nigra* Arn.). *Protoplasma* **185,** 205–211.

Jones, A. M., Im, K.-H., Savka, M. A., Wu, M.-J., DeWitt, N. G., Shillito, R., and Binns, A. N. (1998). Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein 1. *Science* **282,** 1114–1117.

Jürgens, G. (1996). Cell division and morphogenesis in angiosperm embryogenesis. *Semin. Cell Dev. Biol.* **7,** 867–872.

Jürgens, G., Grebe, M., and Steinmann, T. (1997). Establishment of cell polarity during early plant development. *Curr. Opin. Cell Biol.* **9,** 849–852.

Kandasamy, M. K., and Meagher, R. B. (1999). Actin–organelle interaction: Association with chloroplast in *Arabidopsis* leaf mesophyll cells. *Cell Motil. Cytoskeleton* **44,** 110–118.

Knoblich, J. A., Jan, L. Y., and Jan, Y. N. (1997). The N terminus of the *Drosophila* Numb protein directs membrane association and actin-dependent asymmetric localization. *Proc. Natl. Acad. Sci. USA* **94,** 13005–13010.

Körner, Ch., Pelaez Menendez-Riedl, S., and John, P. C. L. (1989). Why are bonsai plants small? A consideration of cell size. *Aust. J. Plant Physiol.* **16,** 443–448.

Lancelle, S. A., and Hepler, P. K. (1988). Cytochalasin-induced ultrastructural alterations in *Nicotiana* pollen tubes. *Protoplasma Suppl.* **2,** 65–75.

Lee, B. S., Gluck, S. L., and Holliday, L. S. (1999). Interaction between vacuolar H^+-ATPase and microfilaments during osteoclast activation. *J. Biol. Chem.* **274,** 29164–29171.

Lüthen, H., Bigdon, M., and Böttger, M. (1990). Re-examination of the acid growth theory of auxin action. *Plant Physiol.* **93,** 931–939.

Machesky, L. M., and Insall, R. H. (1999). Signaling to actin dynamics. *J. Cell Biol.* **146,** 267–272.

Mattsson, J., Sung, Z. R., and Berleth, T. (1999). Responses of plant vascular systems to auxin transport inhibition. *Development* **126,** 2979–2991.

McDowell, J. M., An, Y.-Q., Huang, S., McKinney, E. C., and Meagher, R. B. (1996). The *Arabidopsis* ACT7 actin gene is expressed in rapidly developing tissues and responds to several external stimuli. *Plant Physiol.* **111,** 699–711.

Meagher, R. B., McKinney, E. C., and Kandasamy, M. K. (1999a). Isovariant dynamics expand and buffer the responses of complex systems: The diverse plant actin gene family. *Plant Cell* **11,** 995–1005.

Meagher, R. B., McKinney, E. C., and Kandasamy, M. K. (2000). The significance of diversity in the plant actin gene family: Studies in *Arabidopsis*. *In* "Actin: A Dynamic Framework for Multiple Plant Cell Functions" (C. J. Staiger, F. Baluška, D. Volkmann, and P. W. Barlow, Eds.), pp. 3–27. Kluwer Academic, Dordrecht, The Netherlands.

Meagher, R. B., McKinney, E. C., and Vitale, A. V. (1999b). The evolution of new structures: Clues from plant cytoskeletal genes. *TIG* **15,** 278–284.

Miller, D. D., de Ruijter, N. C. A., Bisseling, T., and Emons, A. M. C. (1999). The role of actin in root hair morphogenesis: Studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. *Plant J.* **17,** 141–154.

Morton, W. M., Ayscough, K., and McLaughlin, P. J. (2000). Latrunculin alters the actin-monomer subunit interface to prevent polymerization. *Nat. Cell Biol.* **2,** 377–378.

Németh, K., *et al.* (1998). Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. *Genes Dev.* **12,** 3059–3073.

Nick, P. (1999). Signals, motors, morphogenesis—The cytoskeleton in plant development. *Plant Biol.* **1,** 169–179.

Okada, K., and Shimura, Y. (1990). Reversible root tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. *Science* **250,** 274–276.

Ovecka, M., Baluška, F., Nadubinská, M., and Volkmann, D. (2000). Actomyosin and exocytosis inhibitors alter root hair morphology in *Poa annua* L. *Biológia* **55,** 105–114.

Palevitz, B. A. (1980). Comparative effects of phalloidin and cytochalasin B on motility and morphogenesis in *Allium*. *Can. J. Bot.* **58,** 773–785.

Palevitz, B. A. (1988). Cytochalasin-induced reorganization of actin in *Allium* root cells. *Cell Motil. Cytoskeleton* **9,** 283–298.

Reichelt, S., Knight, A. E., Hodge, T. P., Baluška, F., Šamaj, J., Volkmann, D., and Kendrick-Jones, J. (1999). Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. *Plant J.* **19,** 555–569.

Salajová, T., Jasik, J., Kormuták, A., Salaj, J., and Hakman, I. (1996). Embryogenic culture initiation and somatic embryo development in hybrid firs (*Abies alba* × *Abies cephalonica*, and *Abies alba* × *Abies numidica*). *Plant Cell Rep.* **15,** 527–530.

Salchert, K., Bhalerao, R., Koncz-Kálmán, Zs., and Koncz, Cs. (1998). Control of cell elongation and stress responses by steroid hormones and carbon catabolic repression in plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **353,** 1517–1520.

Schatten, G., Schatten, H., Spector, I., Cline, C., Paweletz, N., Simerly, C., and Petzelt, C. (1986). Latrunculin inhibits the microfilament-mediated processes during fertilization, cleavage and early development in sea urchins and mice. *Exp. Cell Res.* **166,** 191–208.

Schumacher, K., Vafeados, D., McCarthy, M., Sze, H., Wilkins, T., and Chory, J. (1999). The *Arabidopsis det3* mutant reveals a central role for the vacuolar H^+-ATP-ase in plant growth and development. *Genes Dev.* **13,** 3259–3270.

Sharp, R. E., Silk, W. K., and Hsiao, T. C. (1988). Growth of the maize primary root at low water potentials. Spatial distribution of expansive growth. *Plant Physiol.* **87,** 50–57.

Spector, I., Schochet, N. R., Blasberger, D., and Kashman, Y. (1989). Latrunculins—Novel marine macrolides that disrupt microfilament organization and affect cell growth: I. Comparison with cytochalasin D. *Cell Motil. Cytoskeleton* **13,** 127–144.

Spector, I., Shochet, N. R., Kashman, Y., and Groweiss, A. (1983). Latrunculins: Novel marine toxins that disrupt microfilament organization in cultured cells. *Science* **219,** 493–495.

Staiger, C. J. (2000). Signaling to the actin cytoskeleton in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51,** 257–288.

Takahashi, H. (1997). Hydrotropism: The current state of our knowledge. *J. Plant Res.* **110,** 163–169.

Thimann, K. V., and Biradivolu, R. (1994). Actin and the elongation of plant cells. II. The role of divalent ions. *Protoplasma* **183,** 5–9.

Thimann, K. V., Reese, K., and Nachmias, V. T. (1992). Actin and the elongation of plant cells. *Protoplasma* **171,** 153–166.

Valster, A. H., and Hepler, P. K. (1997). Caffeine inhibition of cytokinesis: Effect on the phragmoplast cytoskeleton in living *Tradescantia* stamen hair cells. *Protoplasma* **196,** 155–166.

Vitha, S., Baluška, F., Braun, M., Šamaj, J., Volkmann, D., and Barlow, P. W. (2000a). Comparison of cryofixation and aldehyde fixation for plant actin immunocytochemistry: Aldehydes do not destroy F-actin. *Histochem. J.* **32,** 457–466.

Vitha, S., Baluška, F., Jasik, J., Volkmann, D., and Barlow, P. W. (2000b). Steedman's wax for F-actin visualization. *In* "Actin: A Dynamic Framework for Multiple Plant Cell Functions" (C. J. Staiger, F. Baluška, D. Volkmann, and P. W. Barlow, Eds.), pp. 619–636. Kluwer Academic, Dordrecht, The Netherlands.

Volkmann, D., and Baluška, F. (1999). The actin cytoskeleton in plants: From transport networks to signaling networks. *Microsc. Res. Tech.* **47,** 135–154.

Wada, A., Fukuda, M., Mishima, M., and Nishida, W. (1998). Nuclear export of actin: A novel mechanism regulating the subcellular localization of a major cytoskeletal protein. *EMBO J.* **17,** 1635–1641.

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

Waller, F., Wang, Q.-Y., and Nick, P. (2000). Actin and signal-controlled cell elongation in coleoptiles. *In* "Actin: A Dynamic Framework for Multiple Plant Cell Functions" (C. J. Staiger, F. Baluška, D. Volkmann, P. W. Barlow, Eds.), pp. 477–496. Kluwer Academic, Dordrecht, The Netherlands.

Wang, Q.-Y., and Nick, P. (1998). The auxin response of actin is altered in the rice mutant *Yin-Yang. Protoplasma* **204,** 22–33.

Whitelam, G. C., Patel, S., and Devlin, P. F. (1998). Phytochromes and photomorphogenesis in *Arabidopsis*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **353,** 1445–1453.

Williamson, R. E. (1978). Cytochalasin B stabilises the sub-cortical actin bundles of *Chara* against a solution of low ionic strength. *Cytobiologie* **18,** 107–113.

Williamson, R. E., and Hurley, U. A. (1986). Growth and regrowth of actin bundles in *Chara:* Bundle assembly by mechanisms differing in sensitivity to cytochalasin. *J. Cell Sci.* **85,** 21–32.

Zonia, L., Tupý, J., and Staiger, C. J. (1999). Unique actin and microtubule arrays co-ordinate the differentiation of microspores to mature pollen in *Nicotiana tabacum*. *J. Exp. Bot.* **50,** 581–594.

Received for publication August 30, 2000
Revised November 9, 2000
Accepted November 11, 2000
Published online January 24, 2001