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

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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 par-
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 gravityresponse 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 μE 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. M arder 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 10⁻³ 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 1993 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 caséin 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, Copyright © 2001 by Academic Press. All rights of reproduction in any form reserved.
MO) diluted 1:200 and 1:100, respectively, for that 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 Baluska 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
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 \( \mu \text{M} \) of latrunculin B induced maximal inhibition (Fig. 5A). The most prominent effect was detected in dark-grown hypocotyls, where 1 \( \mu \text{M} \) 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 \( \mu \text{M} \). 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. 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 cotyledones (D) in control seedlings (A, C, E) and seedlings treated with 1 \( \mu \text{M} \) 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 \( \mu \text{m} \).

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 \( \mu \text{M} \) 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 in the absence (three seedlings on the right side) of latrunculin B for 3 days. Bar = 7 mm.
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 coleoptile 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

<p>| TABLE 1 |</p>
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<th>Effects of Latrunculin B on Final Lengths and Volumes of Cortex Cells in Maize Roots</th>
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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.
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 (Baluska 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. 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).

**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; Poissner 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 \( \mu \)M) 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 (Thimmann and Biradivolu, 1994; Thimmann 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 Balusˇka 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 apex 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 interpret 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., Macheky and Insall, 1999; for plant cells see Volkman 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.

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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).

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