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Probing the Plant Actin Cytoskeleton during Cytokinesis and Interphase by Profilin Microinjection

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We have examined the cytological effects of microinjecting recombinant birch profilin in dividing and interphase stamen hair cells of *Tradescantia virginiana*. Microinjection of profilin at anaphase and telophase led to a marked effect on cytokinesis; cell plate formation was often delayed, blocked, or completely inhibited. In addition, the initial appearance of the cell plate was wrinkled, thin, and sometimes fragmented. Injection of profilin at interphase caused a thinning or the collapse of cytoplasmic strands and a retardation or inhibition of cytoplasmic streaming in a dose-dependent manner. Confocal laser scanning microscopy of rhodamine–phalloidin staining in vivo revealed that high levels of microinjected profilin induced a degradation of the actin cytoskeleton in the phragmoplast, the perinuclear zone, and the cytoplasmic strands. However, some cortical actin filaments remained intact. The data demonstrate that profilin has the ability to act as a regulator of actin-dependent events and that centrally located actin filaments are more sensitive to microinjected profilin than are cortical actin filaments. These results add new evidence supporting the hypothesis that actin filaments play a crucial role in the formation of the cell plate and provide mechanical support for the cytoplasmic strands in interphase cells.

INTRODUCTION

Plant cells contain extensive arrays of actin filaments, which appear to participate in a variety of cellular functions, including cytoplasmic streaming (Salitz and Schmitz, 1989; Kuroda, 1990; Emons et al., 1991; Williamson, 1993; Staiger et al., 1994), nuclear positioning (Katsuta and Shibaoka, 1988; Lloyd, 1989; Cho and Wick, 1990; Staiger and Lloyd, 1991), and cell division (Schmit and Lambert, 1990; Lambert et al., 1991; Wick, 1991; Cleary et al., 1992; Hepler et al., 1993; Staehelin and Hepler, 1996). Of particular pertinence to our study is their participation in cell plate formation and alignment during cytokinesis. Microscopic investigations indicate that actin filaments are a major structural component of the cytokinetic apparatus. Actin filaments are distributed throughout the phragmoplast and aligned parallel to the palisade of microtubules (Kakimoto and Shibaoka, 1987; Schmit and Lambert, 1990; Zhang et al., 1993; Staehelin and Hepler, 1996). Although the microtubules of the phragmoplast initially arise through the coalescence of preexisting spindle–interzone elements, the actin filaments seem to originate de novo as two opposing groups, each initially associated with the surface of the re-forming daughter nuclei (Zhang et al., 1993; Valster and Hepler, 1997).

Although it is clear that actin filaments are associated with the phragmoplast, it is much less clear how they participate in cell plate formation. One possibility is that, together with a myosin motor, they cause the transport of Golgi vesicles, which contain cell wall precursors, to the edge of the developing cell plate. The observation that the actin filaments are oriented with their (+) ends proximal to the forming cell plate (Kakimoto and Shibaoka, 1988) is consistent with that view. Also, there is preliminary evidence that myosin is present in the phragmoplast (Parke et al., 1986). It is also possible that actin filaments participate in the alignment of the cell plate. This process is of particular importance in plants because morphogenesis (e.g., stomatal complex formation) may be in part dependent on the accurate placement of the new cross wall (Staehelin and Hepler, 1996). Evidence for a role of actin filaments in cell plate positioning comes from observations showing that cytochalasin can prevent proper alignment of the developing cell plate (Palevitz and Hepler, 1974; Palevitz, 1987; Schmit and Lambert, 1988; Mineyuki and Gunning, 1990). In addition, recent evidence reveals that actin filaments form bridging structures between the phragmoplast

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of the centrifugally expanding cell plate and cortical actin filaments (Valster and Hepler, 1997).

Although previous pharmalogical studies with cytochalasins have shown that actin filaments are required for normal cell plate formation (Palevitz and Hepler, 1974; Palevitz, 1987; Schmit and Lambert, 1988; Mineyuki and Gunning, 1990), the application of this reagent is likely to underestimate the importance of actin in cytokinesis. For example, cytochalasin does not completely depolymerize actin filaments, and in some systems, it may even promote polymerization and bundling of these cytoplasmic elements (Tang et al., 1989). On the other hand, microinjection of profilin has been shown to perturb more effectively plant actin filaments in vivo. The introduction of this protein into cells is thus likely to provide additional insights into the role that actin filaments play within the living cell, prompting this study on cell plate formation.

Profilin, which has been identified in a variety of plant cells (Valenta et al., 1992), is a small (12 to 17 kD) globular cytoplasmic protein that has the ability to bind monomeric actin, phosphoinositides, and poly-L-proline (Tanaka and Shibata, 1985; Machesky and Pollard, 1993). Of importance are the studies showing that it can promote or prevent actin nucleation or polymerization, depending on the profilin-to-actin ratio (Haarer and Brown, 1990; Aderem, 1992; Fechheimer and Zigmond, 1993; Theriot and Mitchison, 1993; Valenta et al., 1993; Ruhlandt et al., 1994; Sun et al., 1995; Vidali and Hepler, 1997). In this study, we have taken specific advantage of the depolymerization of actin filaments by artificially increasing the levels of profilin by using intracellular microinjection. This approach has already been demonstrated to be effective with living plant cells (Staiger et al., 1994).

Using stamen hair cells of *Tradescantia virginiana* as a model system, we examined both dividing and elongating interphase cells and found that an increase of the cellular concentration of profilin through microinjection profoundly perturbs cytokinesis. Cell architecture and cytoplasmic streaming are also affected in a dose-dependent and reversible manner. These events are coupled to a differential degradation of actin filament arrays.

RESULTS

Estimations Concerning Profilin Concentrations

To approximate the amount of injected profilin in our experiments, we combined data on the endogenous profilin concentration in Tradescantia stamen hair cells (2 to 8 μM; Staiger et al., 1994) with estimations on the increase in cell volume due to the pressure microinjection (1%; Zhang et al.,1990). Assuming that the vacuole occupies ~50% of the cell volume in mitotic cells and 80% in young expanding interphase cells, this means an increase in cytosolic volume of 2% in mitotic cells and 10% in interphase cells after microinjection. At a needle concentration of ~330 μM (i.e., 5 mg/mL), this means a two- to threefold increase of the cytoplasmic profilin concentration in mitotic cells and a five- to 15-fold increase in interphase cells. We emphasize that these calculations are rough estimations and are included to show that the microinjection of 330 μM profilin results in excess cytoplasmic profilin. From observations with microinjected fluorescein isothiocyanate–labeled profilin (data not shown), it became evident that the protein is confined to the cytosol of the injected stamen hair cell.

Microinjected Profilin Affects Progress of Cytokinesis

Excess profilin, microinjected during anaphase or telophase, has a profound (but not immediate) effect on cytokinesis in Tradescantia stamen hair cells. The effects vary from a marked delay in cytokinesis, with a normal or an aberrant young cell plate, to complete inhibition of cell plate formation. Normally, cell plate formation takes ~20 min in Tradescantia stamen hair cells (Hepler, 1985; Keifer et al., 1992; Wolniak and Larsen, 1995). A 10- to 100-min delay in completion of the cell plate occurred after profilin injection at late anaphase or early telophase, which was often accompanied by a wrinkled, thin, and sometimes fragmented appearance of the cell plate. A more striking effect was the complete inhibition of cytokinesis achieved at high levels of microinjected profilin. Figure 1A shows a time sequence of the inhibition of cytokinesis after microinjection with a needle containing 330 μM profilin. After injection, cell plate formation was initiated, as can be seen in the image taken at 30 min after injection, but the young cell plate remained fragile and failed to expand centrifugally. As membranes formed around the daughter nuclei, the incomplete cell plate disintegrated, resulting in a binucleate cell with aberrant cell architecture (Figure 1A; 240 min). Figure 1B shows the effect of BSA microinjected into an anaphase cell. The cell progressed to telophase and completed cytokinesis normally without a delay. When profilin was injected at telophase, cell plate formation came to a halt after several minutes. The already formed incomplete cell plate stayed in one piece in some instances or broke into several pieces in other instances. The remnants of the cell plate were dislocated to the cortical cytoplasm while chromosomes decondensed and nuclear envelopes were formed.

Rhodamine–phalloidin injection in control telophase cells shows actin filaments in the cortex, the central cytoplasm, and prominently throughout the phragmoplast (Figure 2C). In addition, we sometimes observed fluorescence associated with the growing cell plate, but we did not detect clear lateral connections with the side walls, as reported by Valster and Hepler (1997), who injected higher doses of fluorescently labeled phalloidin. Profilin injection at late anaphase followed by rhodamine–phalloidin injection at telophase showed that no phragmoplast actin filaments are present (Figures 2A and 2B). Some actin filaments in the cortical array persisted after

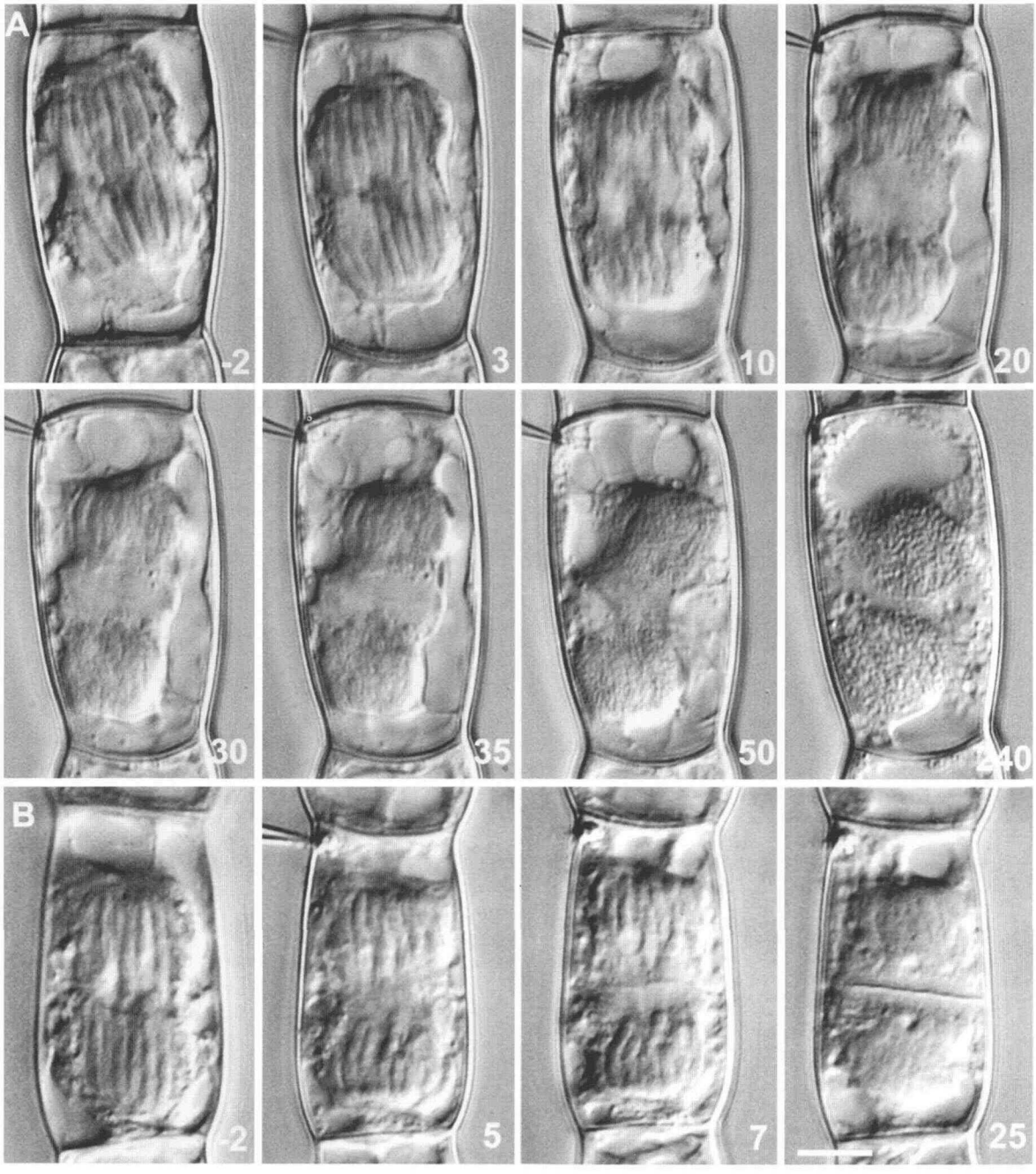

Figure 1. Time Lapse Sequence of the Effect of Microinjected Profilin on Cytokinesis in a Tradescantia Stamen Hair Cell.

(A) Microinjection of profilin (at needle concentration of 330 μM) in an anaphase cell. Chromosome separation continues (compare images taken after 3 and 20 min); at 30 min after injection, a fragile cell plate can be distinguished that fails to expand laterally and that completely disintegrates after 50 min. Chromosomes decondense, and nuclear envelopes are being formed around the two sets of chromosomes (see image taken 50 min after injection). After 240 min, the binucleate cell is still alive, although cytoplasmic strands and streaming have not resumed. Numbers indicate minutes before and after microinjection.

(B) Anaphase cell microinjected with a similar concentration of BSA. Even though the cell lost some turgor pressure (see image taken after 5 min), cell plate formation is initiated (see image taken after 7 min), and cytokinesis is completed without delay. Numbers indicate minutes before and after initiation of cell plate formation.

Bar in **(B)** = 10 μm for **(A)** and **(B)**.

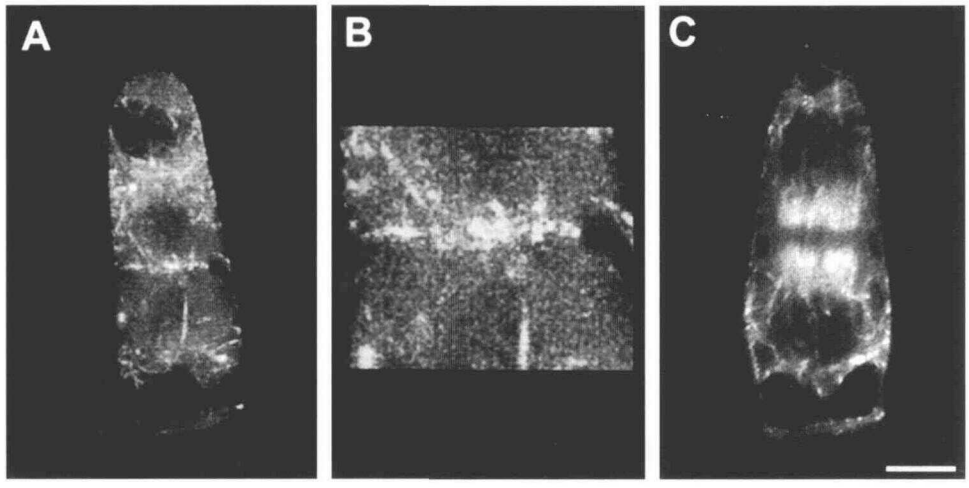

Figure 2. Distribution of Actin Filaments at Telophase in Control and Profilin-Treated Tradescantia Stamen Hair Cells.

(A) Actin filament distribution in a profilin-treated telophase cell. Profilin (330 μM needle concentration) was injected at late anaphase. After injection, the cell proceeded to telophase, and cell plate formation was initiated but then came to a halt after ~15 min. Profilin injection resulted in the disappearance of the dense array of actin filaments in the phragmoplast, as shown by a second injection of rhodamine–phalloidin made at telophase.

(B) Detail of **(A)** showing only a few remnants of actin filament bundles at the site of the cell plate after injection of profilin.

(C) Control telophase cell microinjected with rhodamine–phalloidin showing actin filaments in the phragmoplast. A dense array of actin filaments, oriented perpendicular to the expanding cell plate, is present at either side of the cell plate.

Bar in **(C)** = 10 μm for **(A)** and **(C)**. Bar in **(C)** = 3 μm for **(B)**.

profilin treatment, and some filaments were also observed associated with the forming cell plate (Figures 2A and 2B).

Table 1 shows the observed effects of a total of 62 microinjections of different profilin concentrations in anaphase or telophase cells. Although a small percentage completed cytokinesis normally, the majority of profilin-injected cells showed either a delay in cytokinesis or incomplete cell plate formation. In the most severe case, cytokinesis was completely inhibited.

Table 1. Injection of Profilin at Anaphase or Telophase in Tradescantia Stamen Hair Cells: Effect on Cell Plate Formation

Profilin Needle Concentration (μM)	Phase at Injection	No. of Cells	Normal Progression	Abnormal Progression[a]
Control	Anaphase	6	6	0
	Telophase	3	3	0
50 to 100	Anaphase	7	1	6
	Telophase	6	0	6
100 to 200	Anaphase	7	1	6
	Telophase	6	0	6
200 to 330	Anaphase	20	2	18
	Telophase	7	0	7

[a] The cell plate formation was delayed or partly or completely inhibited.

Microinjected Profilin Affects Cytoplasmic Streaming and Cell Architecture

The microinjection of profilin in elongating interphase Tradescantia stamen hair cells resulted in an effect on cytoplasmic streaming and cell architecture in a dose-dependent manner. Table 2 shows the results of 48 injections with different profilin concentrations in elongating interphase cells. The lowest dose of microinjected profilin (needle concentration at ~50 μM) caused only stagnation of cytoplasmic streaming in the proximity of the injection site. At the highest dose (needle concentration at ~330 μM), cytoplasmic streaming was perturbed in the entire cell; both stagnation of cytoplasmic streaming and Brownian-like movement of organelles were observed. In addition, cytoplasmic strands, including perinuclear cytoplasmic strands, became thinner and were eventually completely incorporated into the cortical cytoplasm, as illustrated in Figure 3A. Similar results have been reported by Staiger et al. (1994), who injected profilin into mature interphase cells of *T. blossfeldiana*. As a consequence of the disruption of the cytoplasmic strands,

Table 2. Injection of Profilin at Interphase in Tradescantia Stamen Hair Cells: Effect on Cytoplasmic Streaming

Profilin Needle Concentration (μM)	No. of Cells	Recovery within 10 min	Thinning of Cytoplasmic Strands	Collapse of Strands
Control	14	14	0	0
50 to 100	4	1	3	0
100 to 200	8	0	6	2
200 to 330	22	0	2	20

the nucleus lost its anchorage and translocated to the cell periphery. Some organelle movement in the cortical cytoplasm could still be detected. In general, cells recovered from the profilin microinjections, and cytoplasmic streaming resumed after a lapse of time varying from 20 min (after low-dose microinjections) to >5 hr (after high-dose microinjections). Controls, which included interphase cells that had been microinjected with substances such as water, injection buffer, BSA, or BSA–fluorescein isothiocyanate, responded to the microinjection process by a prompt transient perturbation of cytoplasmic streaming. However, cytoplasmic strands did not break, and streaming rapidly resumed; the microinjected cells completely recovered within 10 min (Figure 3B). The transient perturbance of the cytoplasmic streaming in control cells seemed to be more related to the pressure wave caused by injection than to the concentration or type of injected control substance.

To visualize the structure of the array of actin filaments, cells were injected with a low dose of rhodamine–phalloidin. In the central cytoplasm of young control interphase cells, bundles of actin filaments were present in the cytoplasmic strands and formed a network encaging the nucleus (Figures 4D and 4E). In addition, a cortical network of actin filaments was present in control interphase cells (Figure 4C). Rhodamine–phalloidin injection in cells preinjected with profilin revealed that bundles of centrally and some cortically located cytoplasmic actin filaments had broken down (cf. Figures 4B and 4E). Figure 4B is a median focal plane view of the same cell as shown in Figure 4A. This cell does not have actin filaments in the central cytoplasm. However, in contrast to the published results by Staiger et al. (1993), who used mature interphase cells, some cortical actin filaments were still present (Figure 4A), and a diffuse fluorescence could be observed throughout the cytoplasm (Figure 4B). In some instances, cortical actin filaments clustered around the profilin injection site (data not shown), as has been observed previously by Staiger et al. (1994).

In addition to the effect that microinjected profilin had on cytoplasmic streaming and cell architecture, profilin retarded wound plug or wound wall formation at the microinjection site. Cells injected with control substances rapidly formed a prominent wound plug at the microinjection site, which allowed the needle to be removed within 15 min after microinjection. In contrast, profilin-microinjected cells showed delayed wound plug formation.

DISCUSSION

Effects of Microinjected Birch Profilin in Tradescantia Stamen Hair Cells

Artificial elevation of the profilin concentration by microinjection in Tradescantia stamen hair cells during late anaphase or telophase profoundly affects the process of cytokinesis: cell plate formation is delayed, blocked, or completely inhibited. In addition, our results show that the microinjection of profilin in elongating young interphase cells affects cell architecture and cytoplasmic streaming. This is similar to the effect microinjected profilin has in mature *T. blossfeldiana* stamen hair cells, as reported by Staiger et al. (1994). These effects of microinjected profilin are closely related to the degradation of the actin cytoskeleton, which preferentially occurs in the central part of the cell, that is, in the cytoplasmic strands, the perinuclear region, and the phragmoplast region. In our study, cortical actin filaments were more resistant to elevated profilin concentrations than those reported in the study by Staiger et al. (1994). Possibly, the sensitivity to perturbation of the cytoskeleton is different among the two species (*T. virginiana* versus *T. blossfeldiana*) and among cells at different stages of maturity (young, still elongating cells versus fully expanded cells), as proposed earlier by Salitz and Schmitz (1989). The aberrant cell plate formation and positioning in cells injected with profilin during anaphase or telophase suggest a specific function of the actin cytoskeleton at cytokinesis. This view is further supported by the delay of cytokinesis and the improper positioning of the cell plate in experiments using cytochalasins (Palevitz and Hepler, 1974; Schmit and Lambert, 1988; Mineyuki and Gunning, 1990).

A number of working mechanisms can be hypothesized for the functioning of phragmoplast actin filaments. First, actin filaments may be responsible for consolidation of the phragmoplast during cell plate growth, thus providing structural support. Second, phragmoplast actin filaments may function to guide cell plate and phragmoplast growth, thereby contributing to the orientation and anchoring of the cell plate at the proper division site. This option presumes a physical connection consisting of actin filaments between the phragmoplast and the predetermined division site in the cell cortex, which has been detected in both tobacco BY-2 culture cells (Kakimoto and Shibaoka, 1987) and, recently, in Tradescantia stamen hair cells (Valster and Hepler, 1997). Third, phragmoplast actin filaments may account for the transport of vesicles to the growing cell plate. This idea is consistent with observations from heavy meromyosin decoration showing that the actin filaments are oriented with their (+) ends proximal to the cell plate in isolated BY-2 cell phragmoplasts

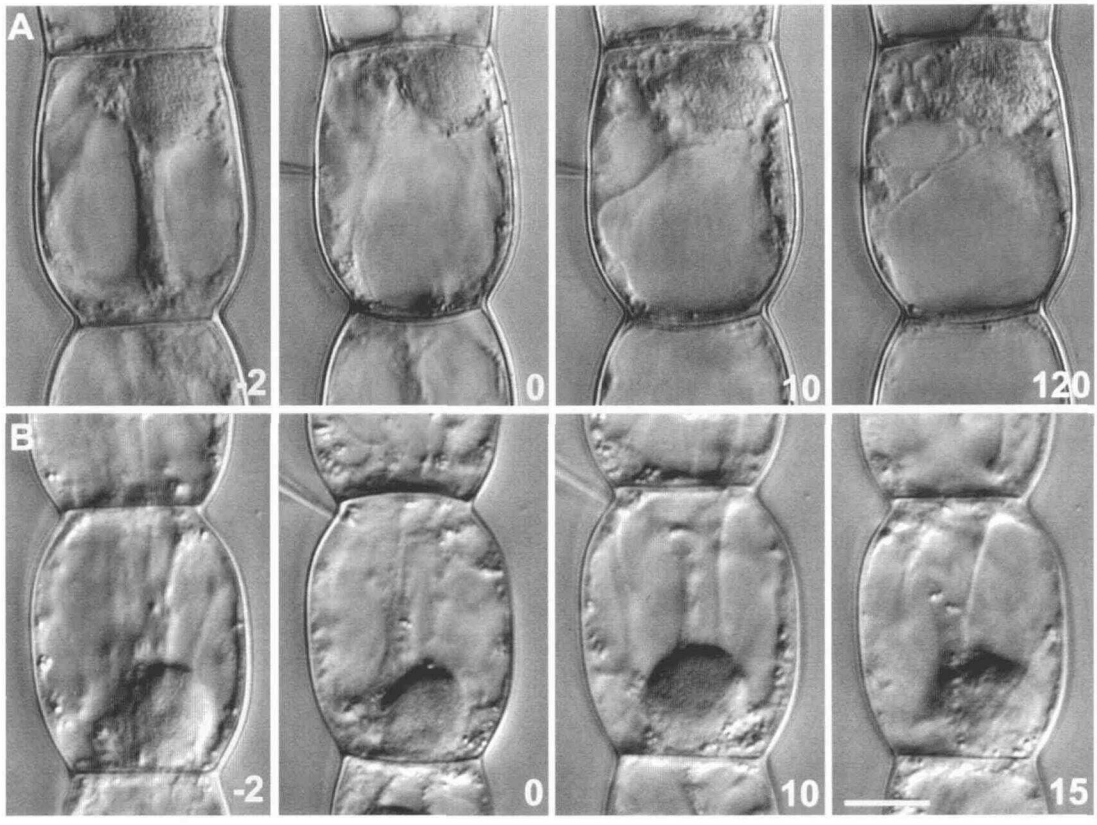

Figure 3. Time Lapse Sequence of the Effect of Microinjected Profilin in an Elongating Interphase Tradescantia Stamen Hair Cell.

(A) Microinjection of profilin (330 μM needle concentration) in an interphase cell. Before injection (−2), prominent cytoplasmic strands in which vigorous cytoplasmic streaming occurs can be seen. Right after microinjection (0), cytoplasmic strands are somewhat affected as a direct result of pressure changes. However, organelle movement in the remaining strands is still present at 0 min. After 10 min, cytoplasmic strands have broken down and cytoplasmic streaming has stopped. After 2 hr, reestablishment of cytoplasmic strands and cytoplasmic streaming has not occurred, although the cell is still alive (as indicated by non-Brownian organelle movement). Numbers indicate minutes before and after microinjection.

(B) Interphase cell microinjected with a similar concentration of BSA. After microinjection, a brief disruption of cytoplasmic streaming occurs. However, cytoplasmic strands do not break down, and within 10 min the cell is completely recovered. Numbers indicate minutes before and after microinjection.

Bar in **(B)** = 10 μm for **(A)** and **(B)**.

(Kakimoto and Shibaoka, 1988). It is also in agreement with immunofluorescence data showing the presence of myosin in the phragmoplast region of dividing onion root tip cells (Parke et al., 1986). Fourth, actin filaments might be involved in the process of vesicle fusion at the cell plate.

The inhibition of cytoplasmic streaming shown in this study and earlier by Staiger et al. (1994) appears to be a direct consequence of the specific degradation of cytoplasmic actin filaments; cortical actin filaments are less affected by elevated profilin concentrations in the Tradescantia stamen hair cells used in this study. The profilin-induced destruction of the cytoplasmic strands and the concomitant translocation of the nucleus at interphase to the cell periphery indicate a direct role of the actin cytoskeleton in maintaining cell architecture. Our observations thus confirm the view of Grabski and Schindler (1995) that the actin cytoskeleton provides an elastic scaffold for the plant cell.

Finally, our observations indicate that profilin markedly retards the ability of a plant cell impaled with a micropipette to form a wound plug. When these results are considered together with the recently reported actin dependency of wound wall formation in characean internodal cells (Foissner et al., 1996), it becomes attractive to postulate a role for the actin cytoskeleton in wound plug formation in Tradescantia stamen hair cells. It is conceivable that actin filaments direct delivery of vesicles containing cell wall polysaccharides or perhaps interfere with docking and/or fusion of vesicles with the plasma membrane, which is necessary for normal wound recovery.

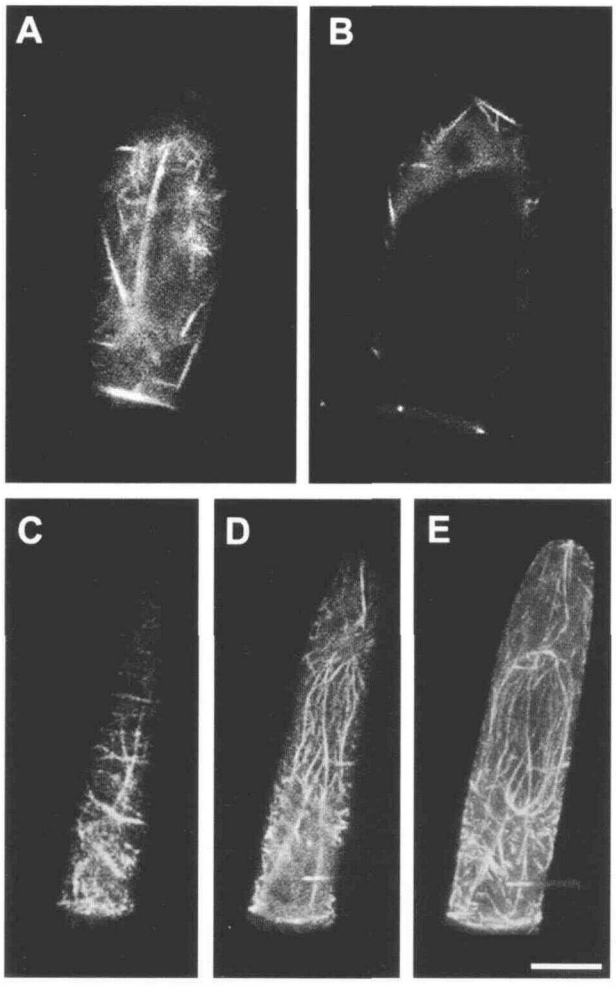

Figure 4. Distribution of Actin Filaments in Profilin-Treated and Control Interphase Tradescantia Stamen Hair Cells.

(A) and **(B)** Microinjection of rhodamine–phalloidin in a profilin-treated cell. Cytoplasmic streaming was vigorous before injection but slowed down in the minutes after microinjection of profilin (330 μM needle concentration; injected at 0 min). At 10 min, a second injection of rhodamine–phalloidin was performed. **(A)** The cortical network of actin filaments appeared to be less dense than in control cells, but some thick bundles are still clearly visible in this region at 24 min. Cytoplasmic streaming has ceased completely. **(B)** Only a few actin filaments can be seen in the median focal plane of view (26 min).

(C) to **(E)** Different focal planes of a control cell microinjected with rhodamine–phalloidin. **(C)** shows transversely oriented actin filaments in the cortical region of this narrow tip cell. **(D)** is a subcortical focal plane view showing an extensive array of longitudinally oriented actin filaments throughout the cytoplasm. **(E)** is a median focal plane view showing the abundance of actin filaments in the central cytoplasm. A network of actin filaments encages the nucleus.

Bar in **(E)** = 10 μm for **(A)** to **(E)**.

Mechanism of Action of Profilin

Although the degree of sequence conservation between profilins from distant phylogenetic sources and various tissues is poor (Staiger et al., 1993; Rozycki et al., 1994), the protein has retained a high degree of functional and structural (Federov et al., 1997) similarity through evolution and development (Schutt et al., 1993; Cedergren-Zeppezauer et al., 1994; Giehl et al., 1994). The similarity in structure and folding of profilins from birch pollen, mammals, and amoebae indicates a conserved basic mechanism of action (Federov et al., 1997). Also, the finding that birch and mammalian profilins can substitute for each other's actions when overexpressed in animal cells (Rothkegel et al., 1996) and in Dictyostelium (Karakesisoglou et al., 1996) emphasizes the conservative character of the action mechanism of profilin and may account for the effectiveness of birch pollen profilin to inhibit cellular processes in stamen hair cells of Tradescantia. Our analysis and earlier observations of the loss of actin filaments in the central cytoplasm after profilin microinjection (Staiger et al., 1994) are in agreement with the observation that, at high concentrations, free profilin sequesters monomeric actin to form a profilin–actin complex, thereby shifting the equilibrium toward depolymerization of actin filaments (Carlsson et al., 1977; Cao et al., 1992; Schutt et al., 1993; Valenta et al., 1993; Giehl et al., 1994; Ruhlandt et al., 1994).

In this study, as has been reported by Staiger et al. (1994), we occasionally observed the formation of aggregates of actin filaments at the profilin injection site. However, in contrast to Staiger et al. (1994), who report the virtually complete destruction of the native actin filament network at needle concentrations >200 μM, we routinely observed a preferential persistance of the cortical actin filaments. Similarly, an increase in density of cortical actin filaments—as opposed to centrally located actin filaments—has been reported in Chinese hamster ovary cells (Finkel et al., 1994) and fibroblasts (Giuliano and Taylor, 1994) in which the profilin-to-actin ratio had been manipulated. Possibly, a great deal of the injected profilin pool is immobilized by phosphoinositides present in the plasma membrane (Goldschmidt-Clermont et al., 1990), which cause a lowering of the concentration in the proximity of the plasma membrane. At low concentrations, profilin pushes the equilibrium from ADP–actin to ATP–actin, which adds more efficiently to the barbed end of actin filaments (Hartwig and Kwiatkowski, 1991; Aderem, 1992; Goldsmidt-Clermont et al., 1992). Promoted actin polymerization near the plasma membrane may result in the persistence of the cortical actin filaments and the aggregation of actin filaments around the profilin injection site.

Together, our results provide new evidence that underscores the importance of actin filaments in the process of cytokinesis and the maintenance of cell architecture in dividing and interphase Tradescantia stamen hair cells. In addition, our results support the hypothesis that profilin is able to

act as a regulator of actin polymerization in Tradescantia stamen hair cells.

METHODS

Plant Material

Plants (*Tradescantia virginiana*) were grown in growth chambers with either an 18-hr photoperiod at 25°C or a 16-hr photoperiod (45 W/m^2 light intensity at 20°C and 70% relative humidity) and an 8-hr dark period (18°C and 65% relative humidity). Stamen hairs with dividing and still-elongating cells in the apical region were collected from immature, unopened flower buds with a length of ~5 mm. Following the procedure described by Cleary et al. (1992) and Jürgens et al. (1994), we dissected and immobilized stamen hairs in a thin layer of 1% low-temperature gelling agarose (grade VII; Sigma; or No. 44415; British Drug House) in culture medium (5 mM Hepes, 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$, pH 7.0) and 0.02% Triton X-100 (British Drug House). The layer of agarose containing the stamen hair cells was solidified by cooling at 5°C for 15 sec and flooded with culture medium.

Preparation of Profilin, Rhodamine–Phalloidin, and Control Solutions

Lyophilized recombinant birch pollen profilin was prepared as indicated by Valenta et al. (1991). Studies performed by other researchers with similar batches of profilin have shown this material to be functional (e.g., Valenta et al., 1993; Giehl et al., 1994). Small aliquots of profilin of known weight were resuspended in water or in injection buffer (5 mM Tris-acetate buffer and 0.2 mM DTT, pH 7.5), kept on ice, and stored in aliquots of various concentrations at −80°C for use within 4 months or at −20°C for use within a few days. Before use, the protein was rapidly thawed, centrifuged for 5 to 10 min at 10,000 or 12,000 rpm, and kept on ice for microinjection on the same day. Some of the microinjections were done with fluorescein isothiocyanate–labeled profilin. For in vivo labeling of actin filaments, a 5-μL aliquot of cold (−20°C) 6.6 μM rhodamine–phalloidin (Molecular Probes, Eugene, OR), dissolved in methanol, was dried down until almost all of the methanol was evaporated and then taken up in 4 μL of injection buffer (5 mM Tris-acetate buffer and 0.2 mM DTT, pH 7.5) to obtain an aqueous solution of ~8 to 9 μM rhodamine–phalloidin. Injected at this concentration, most cells remained alive, and some completed cytokinesis normally. The solution was sonicated for 5 min and centrifuged for 5 min at 6000 rpm. The solution was kept on ice in the dark for direct use. Filtered deionized water, injection buffer, or 5 mg/mL BSA (same weight-to-volume ratio as for the highest concentration of profilin) in deionized water was used for control injections.

Microinjection

The microinjection experiments were conducted, with similar results, by three people in two laboratories with different experimental setups. A detailed description of the microinjection process and equipment used was published in Zhang et al. (1990). In short, needles were pulled from borosilicate capillaries with filament (World Precision Instruments, Sarasota, FL, or Clark Electromedical Instruments, Reading, UK) by using a vertical (model 700D; David Kopf Instruments, Tujunga, CA) or a horizontal pipette puller (C.F. Palmer, London, UK). The needles were back-filled with the experimental solutions and water and mounted to a pressure injector consisting of a microneedle holder (Eppendorf, Madison, WI) and a 0.2-mL micrometer syringe (Gilmont Instruments, Barrington, IL) connected via a water-filled fine polyethylene tubing. The position of the pressure injector was controlled by hydraulic micromanipulators (models MO-103-R, MO-303, MO-22, and MN-2; Narashige Scientific Instruments, Tokyo, Japan).

The experiments included a series of single injections of control solutions (deionized water, BSA, or injection buffer) or profilin (at different needle concentrations) in young, still-elongating interphase cells or cells at various stages of mitosis. Experiments with the actin cytoskeleton included control and treatment injections in 245 cells. Control injections consisted of either single injections of rhodamine–phalloidin or double injections of distilled (deionized) filtered water followed by rhodamine–phalloidin injection. Treatment injections were profilin injections (mostly at 330 μM needle concentration), followed by a rhodamine–phalloidin injection. The time lapse between the first injection of control substance or profilin and the second injection of rhodamine–phalloidin was between 10 and 30 min. The rate of successful double injections was <10%; therefore, the number of reliable records is limited.

Microscopy

Microinjections and observations were performed with inverted microscopes (a modified model IM-35; Zeiss, Oberkochen, Germany; and a Diaphot model; Nikon Europe, Badhoevedorp, The Netherlands) equipped with high numerical aperture objectives and Nomarski differential interference contrast and fluorescence filter sets. Images of the cellular organization and organelle behavior were collected with a video camera (model DAGE-MTI-65; Vidicon, Michigan City, IL) and a VHS-HQ video cassette recorder (model HS-359 UR; Mitsubishi, Cypress, CA). The differential interference contrast images were processed with the image analysis systems Image 1 (Universal Imaging Corporation, West Chester, PA) or Adobe Photoshop (Adobe Systems Incorporated, Mountain View, CA). Most transmission microscopy micrographs were taken from a Trinitron (Sony, Tokyo, Japan) monitor, using Kodak Technical Pan films (100 ASA). Fluorescence imaging of the rhodamine–phalloidin–stained actin filaments was performed with a Bio-Rad MRC-600 (Hemel-Hempstead, UK) confocal laser scanning microscope equipped with an argon–krypton laser. Observations were made by using the 568-nm wavelength excitation line of the laser and a YHS filter set (585-nm dichroic mirror, 585-nm-long pass filter). Optical sections were collected with a moderately closed pinhole (setting 3 or 4), using high or maximum gain settings and 3 to 5 Kalman averages. For final display, image contrast stretching and low band smoothing (s3a) were performed.

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