

Effects of Profilin and Profilactin on Actin Structure and Function in Living Cells

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Abstract. Previous studies have yielded conflicting results concerning the physiological role of profilin, a 12–15-kD actin- and phosphoinositide-binding protein, as a regulator of actin polymerization. We have addressed this question by directly microinjecting mammalian profilins, prepared either from an *E. coli* expression system or from bovine brain, into living normal rat kidney (NRK) cells. The microinjection causes a dose-dependent decrease in F-actin content, as indicated by staining with fluorescent phalloidin, and a dramatic reduction of actin and alpha-actinin along stress fibers. In addition, it has a strong inhibitory effect toward the extension of lamellipodia. However, the injection of profilin causes no detectable per-

turbation to the cell-substrate focal contact and no apparent depolymerization of filaments in either the non-lamellipodial circumferential band or the contractile ring of dividing cells. Furthermore, cytokinesis of injected cells occurs normally as in control cells. In contrast to pure profilin, high-affinity profilin-actin complexes from brain induce an increase in total cellular F-actin content and an enhanced ruffling activity, suggesting that the complex may dissociate readily in the cell and that there may be multiple states of profilin that differ in their ability to bind or release actin molecules. Our results indicate that profilin and profilactin can function as effective regulators for at least a subset of actin filaments in living cells.

THE polymerization of actin subunits plays an important role in the formation of many crucial cellular structures (for a recent review see Cooper, 1991). Although previous studies indicate that the reaction is under tight regulation, possibly involving the interaction of actin subunits with sequestration proteins (for review see Pollard and Cooper, 1986; Sanders and Wang, 1990), the detailed mechanism of regulation in vivo is still poorly understood.

Numerous proteins have been identified in non-muscle cells that can bind to actin monomers and inhibit their polymerization in vitro (Pollard and Cooper, 1986; Cooper, 1991; Hartwig and Kwiatkowski, 1991). The best characterized among these sequestration factors is profilin, a 12–15-kD protein found in a large variety of cells (Carlsson et al., 1976; Vandekerckhove et al., 1989; Haarer and Brown, 1990). In vitro, purified profilin binds actin monomers with a 1:1 stoichiometry and a dissociation constant of 1–10 μ M (DiNubile and Southwick, 1985; Pollard and Cooper, 1986). The effects of profilin on the rate and extent of actin polymerization have been attributed to the inability of the complex to associate with at least one end of the filament (Lal and Korn, 1985; Pollard and Cooper, 1986). In addition, transient capping of filaments by profilin and/or profilin-actin complexes has been suggested (Pollard and Cooper, 1986). It has also been shown that phosphatidylinositol-4,5-bisphosphate (PIP₂)¹ can interact with profilin and inhibit

profilin-actin interactions (Lassing and Lindberg, 1985; Machesky et al., 1990), thereby providing a potential mechanism for the regulation of profilin-mediated sequestration of actin subunits.

Despite the detailed biochemical analyses, many questions remain unanswered concerning the physiological role of profilin in vivo. First, although profilin is relatively abundant in non-muscle cells, its amount is not high enough to account for the sequestration of actin monomers in many cells examined (Lind et al., 1987). Second, during chemoattractant stimulation of neutrophils, the amount of actin released from profilin-actin complexes appears insufficient to account for the increase in actin polymerization (Southwick and Young, 1990). Third, the association between profilin and actin in vitro is of a relatively low affinity, whereas cells appear to contain an abundant quantity of high-affinity complexes, termed profilactin, that form through an unidentified mechanism (Carlsson et al., 1976). Such high-affinity complexes appear to polymerize relatively well onto the barbed end of actin filaments (Tilney et al., 1983), and are detectable in platelets only upon stimulation, when actin polymerization takes place (Lind et al., 1987). Fourth, recent studies indicate that profilin may serve the role of regulating the metabolism of phosphoinositides (Goldschmidt-Clermont et al., 1990; Vojtek et al., 1991). Since profilin can bind PIP₂ with a high affinity, it is possible that most profilin molecules may be associated with PIP₂ instead of actin in the cell (Machesky et al., 1990), and that profilin may interact with actin only as a means for controlling lipid turnover.

1. *Abbreviations used in this paper:* NRK, normal rat kidney; PIP₂, phosphatidylinositol-4,5-bisphosphate.

A direct way to probe the function of profilin is to manipulate, by genetic means or microinjection, the concentration of profilin in living cells and examine its effect on the organization and function of actin-containing structures. This approach has been used to show that the inhibition of profilin expression can cause changes in actin organization in the yeast *Saccharomyces cerevisiae* (Haarer et al., 1990). However, it is unclear whether the disruption is associated with a change in the level of actin polymerization. In the present study we have microinjected profilin prepared from an *Escherichia coli* expression system and from bovine brain into living normal rat kidney (NRK) cells. We demonstrate that the injection of profilin induces a dose-dependent decrease of total F-actin content and an inhibition of lamellipodial extension, while opposite effects are induced upon the injection of high-affinity profilactin complexes. Our results indicate that profilin and profilactin can indeed modulate the balance of actin polymerization in vivo. However, the mechanism may be more complicated than simple sequestration of actin monomers.

Materials and Methods

Preparation of Profilin and Profilactin

E. coli expressed spleen profilin was prepared according to Babcock and Rubenstein (1989). Bovine brain profilin was prepared with a procedure modified from that described by Kaiser et al. (1989). Briefly, fresh bovine brain was washed with cold PBS, trimmed to remove connective tissue, and homogenized with a blender (Waring Products; New Hartford, CT) in 3 vol of extraction buffer containing 1 mM EDTA, 0.5 mM DTT, 0.1 mM PMSF, 0.1 mM ATP, and 10 mM imidazole-HCl, pH 7.0. The homogenate was centrifuged in a rotor (model GSA; Sorvall Instruments, Newton, CT) at 12,000 rpm for 40 min, and then in a rotor (50.2 Ti; Beckman Instruments, Palo Alto, CA) at 35,000 rpm for 2 1/2 h. Supernatant was collected and loaded onto a 2.5 × 15 cm poly-proline (mol wt = 10,000–30,000; Sigma Chemical Co., St. Louis, MO)-conjugated Sepharose 4B (Pharmacia-LKB Biotechnology, Piscataway, NJ) affinity column that was pre-equilibrated with buffer A (10 mM Tris-HCl, 100 mM glycine, 100 mM NaCl, and 1 mM DTT, pH 7.8). The column was washed sequentially with 1,500 ml of buffer A and 400 ml of 3 M urea (Enzyme Grade; Bethesda Research Laboratories, Gaithersburg, MD) in buffer A. Profilin was then eluted with 300 ml of 8 M urea in buffer A. Fractions containing profilin were pooled, dialyzed extensively against buffer A, and concentrated by vacuum dialysis or by dialysis against aquacide II (Calbiochem-Behring Corp., San Diego, CA).

Profilactin was purified by a method modified from that of Rozycki et al. (1991). The procedure is similar to that for profilin, except that the affinity column was eluted with 400 ml of 30% (vol/vol) DMSO in buffer A without DTT following the initial wash with buffer A (without DTT). Fractions containing profilactin were pooled, dialyzed extensively against buffer A, and concentrated using the Centriprep concentrator (Amicon, Danvers, MA) with a molecular cutoff of 30 kD. This concentration step also removes free profilin that may be present in the preparation.

SDS-PAGE was performed on 5–20% gradient mini-gels (Matsudaira and Burgess, 1978). Protein concentrations were determined by the method of Lowry (1951). Purified profilin and profilactin were stored in liquid nitrogen. Before microinjection, aliquots were thawed, dialyzed against a buffer of 5 mM Tris-acetate, 0.2 mM DTT, pH 7.0, and clarified for 20 min in a rotor (42.2 Ti; Beckman Instruments) at 25,000 rpm. Stock solution of 30 mg/ml fluorescein dextran (70,000 D, lysine fixable; Molecular Probes, Eugene, OR), in 5 mM Tris-acetate, pH 7.0, was then added to obtain a final dextran concentration of 3 mg/ml. The solution was used within 5 d. Muscle actin was prepared by the method of Spudich and Watt (1971), and further purified by gel filtration in a G-150 column for some experiments. Column-purified actin yielded qualitatively similar results. However, because of the enhanced polymerizability and the difficulty in controlling the volume of microinjection, column-purified actin was not used for quantitative analysis.

Cell Culture and Microinjection

NRK cells (strain 52E; American Type Culture Collection, Rockville, MD) were cultured as described previously (Cao and Wang, 1990), and were used for microinjection 36–46 h after plating. Pressure for microinjection was provided by compressed air under the control of a custom-designed electronic regulator. The volume of microinjection was measured as described previously (Fishkind et al., 1991), and corresponded to ~8% of the cell volume. Both the volume of injection and the volume of the cell varied by 30% (SD). However, the variability of the concentration of injected molecules is likely to be smaller since during microinjection the volume delivered was judged based on the size and response of each cell.

For some experiments, pairs of sister cells were identified based on similar sizes and shapes. Cells at prometaphase were identified based on the appearance of condensed chromosomes under phase optics. Extension of lamellipodia was stimulated by wounding the monolayer, either by scratching the dish with a Pasteur pipet or by removing selected cells with a microneedle under a microscope.

Fixation and Fluorescent Staining

For the staining of whole cells with fluorescent phalloidin or alpha-actinin antibodies, cells were rinsed with warm PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, and 2 mM MgCl₂, pH 6.9; Schliwa and van Blerkom, 1981) and fixed for 10 min with 4% formaldehyde (16% stock solution, EM grade; Electron Microscopy Sciences, Fort Washington, PA) in PHEM buffer, pH 6.1. After rinsing twice with PHEM buffer, cells were extracted with acetone at –20°C for 5 min. Staining with rhodamine phalloidin (Sigma Chemical Co.) or mAbs against alpha-actinin (Sigma Chemical Co.) was performed as described previously (Fishkind et al., 1991). Primary antibodies were used at a 1:50 dilution, and rhodamine-conjugated secondary antibodies (Tago, Burlingame, CA) were used at a 1:100 dilution.

A different fixation method was used for the preservation of lamellipodia (Small, 1981). Cells were rinsed with a warm buffer of 137 mM NaCl, 5 mM KCl, 1.1 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 2 mM MgCl₂, 2 mM EGTA, 5 mM Pipes, and 5.5 mM glucose, pH 6.1, and fixed and extracted with 0.5% glutaraldehyde, 0.3% Triton X-100 (Fisher Scientific, Pittsburgh, PA) in the same buffer for 1 min. Cells were then postfixed with 3% glutaraldehyde for 10 min and treated with 0.05% NaBH₄ for 5 min, before staining with rhodamine phalloidin.

Microscopy and Image Processing

All observations were performed with a Zeiss IM35 or Axiovert inverted microscope, using a 100×/NA 1.30 Neofluar, a 40×/NA 1.0 Apochromat, a 25×/NA 0.8 Neofluar, or a 40× Achromat phase objective. A 100 W quartz halogen lamp was used as the light source for epi-illumination. Interference reflection microscopy was performed by removing the barrier filter from the fluorescein fluorescence filter set and closing down the illumination field diaphragm.

Fluorescence images were acquired with a cooled CCD camera (Star I; Photometrics, Tucson, AZ), and phase images of living cells were recorded with an ISIT camera under reduced illumination (Dage-MTI, Michigan City, IN). Fluorescence images were processed by background subtraction. Integrated intensities in defined areas were obtained with a graphics tablet (GTCO, Rockville, MD) as described previously (McKenna et al., 1985).

Results

Effects of Profilin on the Polymerization State of Actin

We have microinjected *E. coli*-expressed profilin (Babcock and Rubenstein, 1989), or brain profilin (Fig. 1 c), at concentrations between 3.5 and 22 mg/ml into interphase NRK cells. After 40–60 min of incubation, injected cells showed no apparent retraction or gross changes in phase morphology, although the lamellipodia were greatly reduced in size or became undetectable (discussed later). However, after fixation and staining of actin filaments with rhodamine phalloidin, injected cells showed a dramatic reduction in the intensity of fluorescence as compared to neighboring unin-

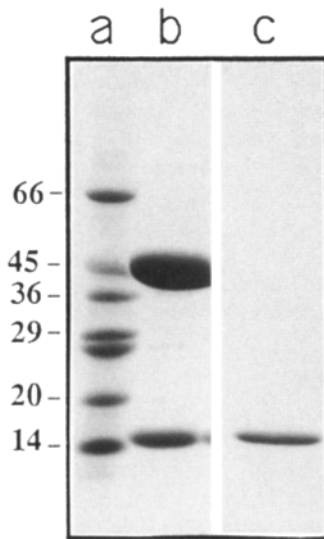


Figure 1. SDS-PAGE of profilin and profilactin prepared from bovine brain. Lane *b* shows fractions pooled from the poly-proline column upon the elution with 30% DMSO, and subsequently passed through a Centriprep-30 concentrator. Two bands with molecular weights of 43,000 and 15,000, respectively, are visible. Lane *c* shows pooled fractions from the poly-proline affinity column eluted with 8 M urea. The single visible band has an apparent molecular weight of 15,000. Lane *a* shows molecular weight standards.

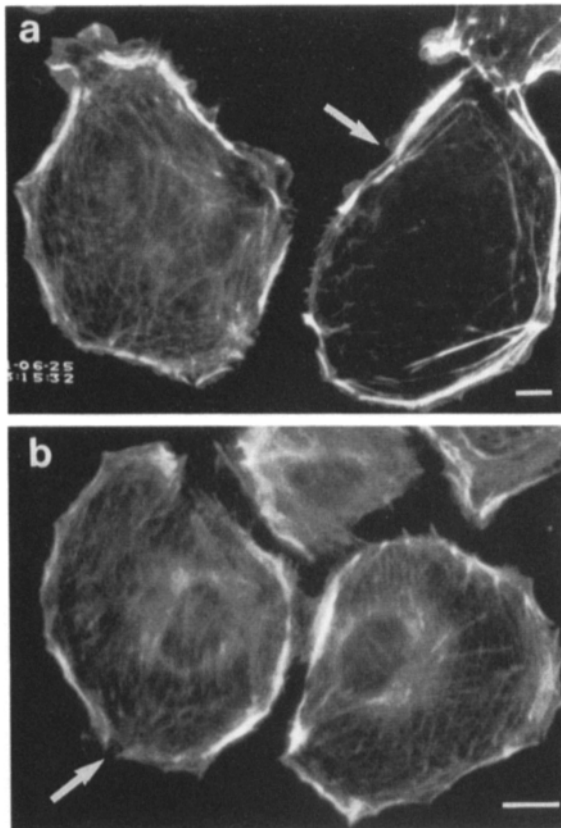


Figure 2. Disassembly of actin filaments following the microinjection of profilin. Pairs of sister cells were identified, and one member was microinjected with a mixture of fluorescein dextran (3 mg/ml) and brain profilin at a concentration of 7 mg/ml (*a*, arrow). Cells were fixed and stained with rhodamine phalloidin 40–60 min after microinjection. Injected profilin induced a dramatic decrease in the content of filamentous actin in the interior region of the cell but no apparent change or a slight increase along the periphery. Microinjection of dextran-containing buffer alone induced no apparent change in the intensity or pattern of phalloidin staining (*b*, arrow). Bar, 5 μ m.

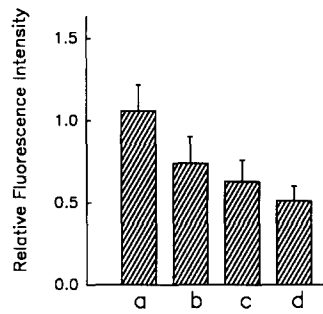


Figure 3. Dependence of F-actin content on the microinjection of profilin. Pairs of sister cells were identified, and one member was microinjected with carrier solution (*a*), 3.5 mg/ml brain profilin (*b*), 7.0 mg/ml brain profilin (*c*), or 22 mg/ml *E. coli*-expressed profilin (*d*). Cells were fixed and stained with rhodamine phalloidin 40–60 min after microinjection. Effects on actin assembly were obtained by calculating the ratio of total fluorescence intensity of injected cells relative to uninjected sister cells. Each point was obtained from 15 pairs. Vertical bars indicate the SEM.

jected cells (Fig. 2 *a*), whereas buffer-injected cells showed no apparent decrease in fluorescence intensity (Fig. 2 *b*). Interestingly, actin filaments along the circumferential region appeared to be resistant to the injection of profilin, even when those in the more interior regions had diminished to a very low level. Similar effects were observed with *E. coli* expressed profilin and with brain profilin.

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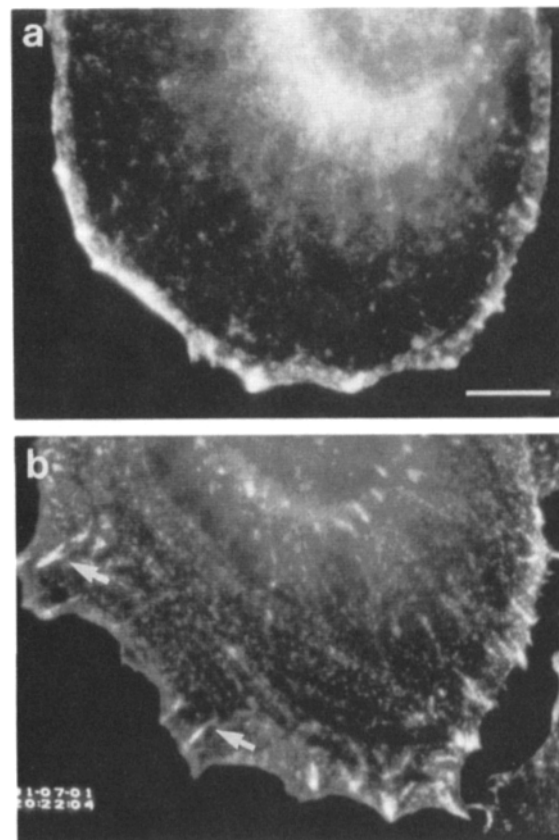


Figure 4. Effects of profilin on the organization of alpha-actinin. A cell was microinjected with 7 mg/ml brain profilin, incubated for 40 min, and stained with a mAb against alpha-actinin (*a*). Numerous small aggregates were detectable and were concentrated along the cell periphery. Uninjected cells (*b*) showed the typical linear array of punctate structures along stress fibers and elongated adhesion plaques (arrows). Bar, 10 μ m.

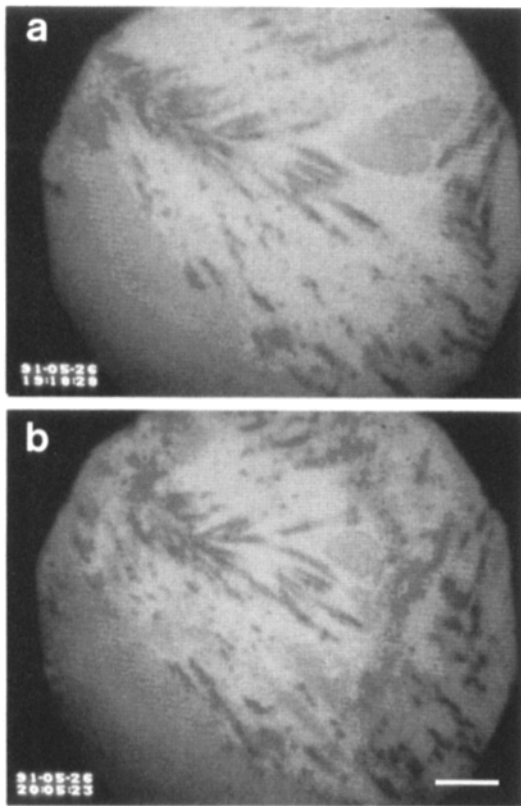


Figure 5. Effects of microinjected profilin on the integrity of cell-substrate adhesions. A cell was injected with 7 mg/ml brain profilin and observed continuously with interference reflection microscopy, which reveals focal adhesions as dark streaks or patches. Although there were localized increases and decreases in adhesion before (a) and 47 min (b) after the microinjection, overall no significant disruption of focal adhesions was observed. Bar, 5 μ m.

Profilin-induced depolymerization of actin was quantified by comparing integrated intensities of rhodamine phalloidin staining in profilin-injected cells with those in uninjected sister cells. 15 pairs of cells were evaluated at each (needle) concentration of profilin at 0, 3.5, 7.0, and 22 mg/ml. As shown in Fig. 3, cells injected with buffer alone showed an identical average intensity as uninjected cells. Profilin induced a dose-dependent decrease in the amount of actin filaments, with a depolymerization of 37% observed upon the injection of 7.0 mg/ml profilin.

Effects of Profilin on Stress Fibers and Adhesion Plaques

After the injection of high concentrations of profilin (≥ 7 mg/ml), stress fibers showed a dramatic reduction in the intensity of phalloidin staining (Fig. 2 a; 89% among 56 cells observed; the remaining 11% showed a discernible but lower degree of reduction). Immunofluorescence staining of alpha-actinin indicated a concomitant disruption of the organization of alpha-actinin (Fig. 4 a). While control cells showed the typical linear array of punctate structures along stress fibers and elongated adhesion plaques (Fig. 4 b; *arrows*), injected cells contained predominantly small aggregates that concentrated near the cell's periphery (Fig. 4 a).

Cell-substrate adhesions were further examined with interference reflection microscopy, which reveals focal contacts as dark streaks. Surprisingly, despite the extensive disruption of actin and alpha-actinin along stress fibers and adhesion plaques, no detectable change was observed in the adhesion of cells with the substrate (100% among five cells injected with 7 mg/ml profilin; Fig. 5). The resistance of focal contacts may account for the lack of retraction of cells after the injection of profilin.

Effects of Profilin on Lamellipodia Protrusion and on Cytokinesis

To determine whether an increase in profilin concentration can interfere with the assembly of actin-containing structures, we examined two actin-based activities, cytoplasmic protrusion and cytokinesis, in cells microinjected with profilin. Cells within a confluent monolayer were microinjected with profilin. After incubation for 40 min, cytoplasmic protrusion was induced by removing neighboring cells with a microneedle. As shown in Fig. 6, profilin at 7 mg/ml exerted a strong inhibitory effect on the formation of lamellipodia (100% among 10 cells observed). Injected cells showed either no lamellipodia or lamellipodia with a greatly reduced size.

The effects of profilin on cytokinesis were examined by injecting prometaphase cells with 10 mg/ml profilin and examining the progress of cytokinesis and the distribution of actin filaments. Profilin-injected cells showed no detectable inhibition of mitosis or cytokinesis (100% among six cells observed). Anaphase onset was observed at 26.5 ± 2.8 min after nuclear envelope breakdown, similar to that obtained with uninjected cells (27.1 ± 4.4 min). The time between the

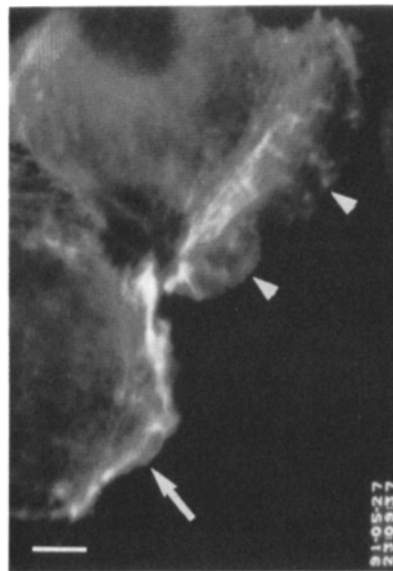


Figure 6. Inhibition of lamellipodia protrusion by the microinjection of profilin. A cell was microinjected with 7 mg/ml brain profilin and incubated for 40 min. Neighboring cells were then carefully removed with a microneedle to stimulate the formation of lamellipodia. After incubation for another 20 min, cells were fixed and stained with rhodamine phalloidin. No lamellipodium was observed in the injected cell (*arrow*), whereas an uninjected neighboring cell formed prominent lamellipodia (*arrowheads*). Bar, 10 μ m.

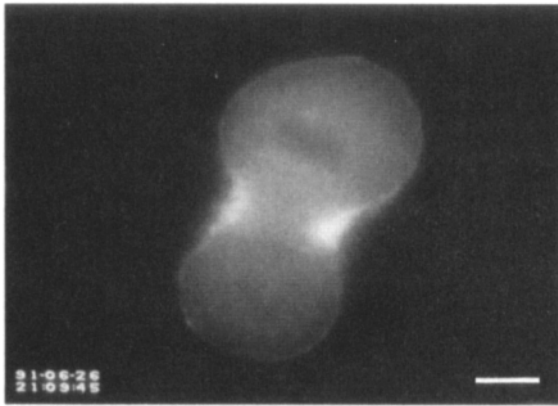


Figure 7. Lack of effects of microinjected profilin on the distribution of actin filaments during cytokinesis. A cell was microinjected with 10 mg/ml brain profilin at prometaphase and was incubated on the microscope stage until mid-cytokinesis and then fixed and stained with rhodamine phalloidin. The fluorescence shows the typical distribution of actin filaments along the cortex and in the cleavage furrow. Bar, 5 μ m.

onset of anaphase and mid-cytokinesis was 4.8 ± 0.8 min for controls and 4.3 ± 0.6 min for profilin-injected cells. Moreover, control and profilin-injected cells exhibited a similar localization of actin in the cleavage furrow (Fig. 7; Cao and Wang, 1990).

Effects of Profilactin on Actin-containing Structures

To test whether the effects of profilin were related to its interactions with endogenous actin, we have performed similar microinjections with high-affinity profilactin complexes purified from bovine brain. SDS-PAGE of the complex revealed two bands with molecular weights of 43,000 and 15,000, respectively (Fig. 1 b). Densitometry of the gel indicated an apparent actin/profilin molar ratio of 1:1. Non-denaturing gel electrophoresis showed no detectable free actin (not shown). Unlike purified actin, which became very difficult to microinject at concentrations >8 mg/ml because of the rapid polymerization at the tip of the needle, the complex maintained a low viscosity at concentrations as high as 40 mg/ml.

After the microinjection of profilactin at concentrations between 4.5–36 mg/ml, cells showed no decrease in the amount of actin filaments as compared to uninjected sister cells (Fig. 8). To the contrary, there was a large increase in the intensity of phalloidin staining (Fig. 8). Quantitative analysis indicated a $41 \pm 14\%$ (SD) increase in phalloidin staining upon the microinjection of 4.5 mg/ml profilactin (Fig. 9). However, a similar increase was observed after the injection of an equivalent molar concentration of muscle G-actin (3.4 mg/ml; Fig. 9). These results are consistent with an extensive release of actin from the complex.

To determine the effects of profilactin on cell motility, cells injected with 36 mg/ml profilactin, which corresponds to ~ 9 mg/ml profilin and ~ 27 mg/ml actin, were induced to form protrusion by removing neighboring cells. Microinjected cells consistently showed an increase in the width of lamellipodia and an increase in ruffling activity, detected as transient phase-dark lines in the lamellipodia (100% among five cells observed; Fig. 10). Such effects were particularly strik-

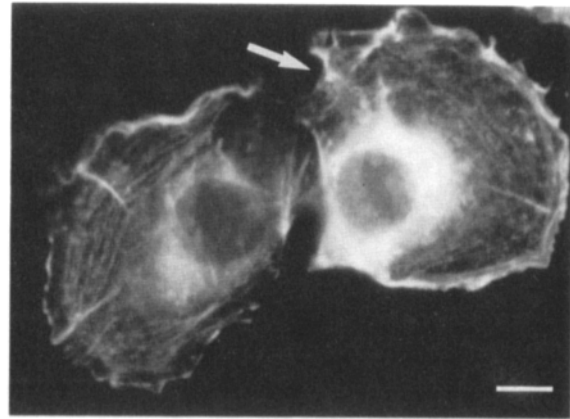


Figure 8. Increases in the content of filamentous actin following the microinjection of profilactin complexes. One member of a pair of sister cells was microinjected with 36 mg/ml profilactin (arrow). Cells were fixed and stained with rhodamine phalloidin 40–60 min after microinjection. The injected cell shows a dramatic increase in intensity compared to the uninjected sister cell to its left. Bar, 5 μ m.

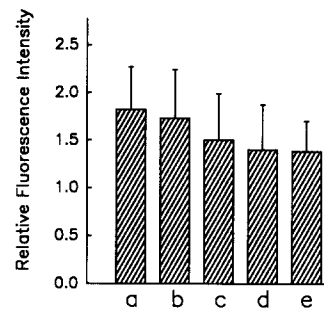


Figure 9. Dependence of F-actin content on the microinjection of profilactin. Pairs of sister cells were identified, and one member was microinjected with 36 mg/ml (a), 18 mg/ml (b), 9.0 mg/ml (c), 4.5 mg/ml (d), of brain profilactin, or 3.4 mg/ml muscle G-actin (e). After 40–60-min incubation, cells were fixed and stained with rhodamine phalloidin. The ratio of fluorescence intensity in injected and uninjected sister cells indicates that F-actin content is increased by the microinjection of either profilactin or muscle G-actin. Vertical bars indicate SEM.

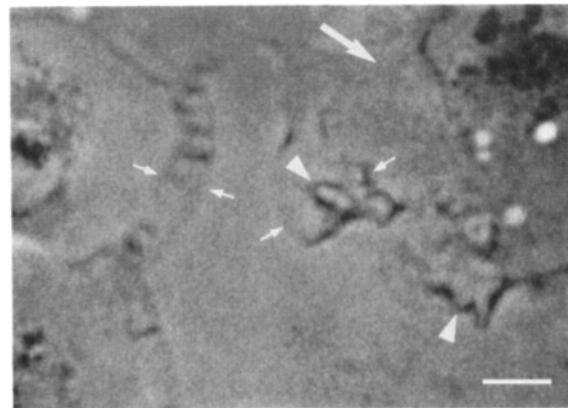


Figure 10. Stimulation of cytoplasmic protrusion and ruffling by the microinjection of profilactin complexes. A cell was microinjected with 36 mg/ml profilactin and incubated for 40 min before observation (large arrow). The formation of lamellipodia was stimulated by removing neighboring cells with a microneedle. The size of lamellipodia in injected cell is larger than that in the control cell (small arrows). In addition, the injection induced an increase in ruffles, which appeared as phase-dark lines (arrowheads). Bar, 10 μ m.

ing when sequences of images were viewed in rapid succession as motion pictures. The stimulation of lamellipodia became undetectable at lower concentrations of the complex (6.5 mg/ml).

Discussion

To probe the physiological function of profilin, we have induced an increase in its intracellular concentration by microinjection and observed the effects on cell structure and function. An average of 8% cell volume at a profilin concentration of 230–1,500 μM was delivered into interphase and mitotic cells. This would cause an increase of profilin concentration by 19 to 120 μM , 60–400% over its endogenous level assuming an intracellular profilin concentration of 30 μM (Goldschmidt-Clermont et al., 1991).

Effects of Profilin on Actin Filaments

Our results clearly indicate that profilin can induce the disassembly of a significant fraction of actin filaments. The simplest mechanism for the depolymerization involves the direct binding of profilin to actin subunits, which probably undergo continuous on-off reactions at the ends of filaments (Kreis et al., 1982). Subunits that come off the filaments would be sequestered by profilin and become unable to reincorporate into filaments.

Assuming an intracellular F-actin concentration of 100 μM , a 37% reduction upon the injection of 7 mg/ml profilin would correspond to the disassembly of ~ 37 μM actin filaments, close to the concentration of exogenous profilin introduced (8% of 470 μM). Thus it is possible that injected profilin may bind actin subunits more efficiently than predicted by the low-affinity binding *in vitro*. However, without a definitive understanding of the state of cytoplasmic actin and the concentration of actin filaments *in vivo*, it is difficult to obtain a precise value for the intracellular affinity.

It is also possible that the depolymerization of actin may be induced indirectly by the formation of excess profilin-PIP₂ complexes, which have been shown to inhibit the turnover of PIP₂ by phospholipase C and may affect actin polymerization indirectly through changes in protein kinase C activities and/or calcium concentration (Goldschmidt-Clermont et al., 1990). However, this mechanism cannot easily explain the results with profilactin, which maintains the ability to form profilin-PIP₂ complexes (and release bound actin; Lassing and Lindberg, 1985) but lacks any depolymerization effect (Fig. 8).

Effects of Profilactin on Actin Filaments

One surprising observation is that high-affinity profilactin complexes can induce a significant increase in the amount of actin filaments in microinjected cells. Based on the results of profilin and on the apparent high-affinity interaction in the complex, one might expect the microinjection of profilactin to cause no significant change in the amount of actin filaments. The increase in actin filaments throughout the cell, similar to that induced by the injection of unpolymerized actin, suggests that the complex may dissociate easily in the cytoplasm. This raises the possibility that there may be multiple states of profilin, possibly regulated by posttranslational modification, that differ in their ability to associate

with or dissociate from actin in the cell. As suggested by Goldschmidt-Clermont et al. (1991), under some conditions profilin may even be able to stimulate the assembly of actin filaments.

Additional questions arise upon the scrutiny of the quantitative results. For example, the injection of either 4.5 mg/ml profilactin or 3.4 mg/ml actin at 8% cell volume should cause a net increase in actin by ~ 7 μM . Even if the injected actin polymerizes completely, it should cause no more than a 7% increase in actin filaments assuming an intracellular F-actin concentration of 100 μM . However, both injected profilactin and actin induced a >30% increase in the amount of actin filaments. One possibility is that free actin subunits may be able to stimulate the polymerization of endogenous sequestered actin subunits. However, it is also possible that the amount of intracellular actin polymers that can bind phalloidin may be lower than commonly envisaged.

Differential Sensitivity of Actin Structures to Profilin

An interesting observation is that different populations of actin filaments appear to have different sensitivities to the increase in profilin concentration. While actin filaments in lamellipodia and stress fibers are highly sensitive, those in circumferential belts and in the contractile ring show no apparent disruption. Our results contrast with those after the microinjection of two extracellular actin-depolymerization factors, DNase I, and serum vitamin D-binding protein. Unlike profilin, both were shown to induce drastic cell retraction at estimated intracellular concentrations >6–12 μM (Sanger et al., 1990). We have confirmed the effects of the vitamin D-binding protein under our experimental conditions. Extensive retraction was observed in 38% of the cells injected with 5 μM vitamin D-binding protein, which induces a limited (17%) depolymerization of actin filaments. In contrast, no such retraction was observed upon a 24% actin depolymerization induced by 19 μM profilin. Thus the difference between profilin and vitamin D-binding protein cannot be explained by a difference in binding affinity. More likely, the effects on cell morphology of DNase I and vitamin D-binding protein are caused by a non-discriminative disassembly of actin filaments throughout the cell, or by additional activities such as the capping of filament ends (Pollard and Cooper, 1986).

The differential sensitivity of actin filaments is probably related to the differential distribution of various actin-binding proteins in the cell. At least some of those proteins, such as tropomyosin, are known to localize in specific regions (Lazarides, 1976). Thus the stability of actin filaments may be achieved simply through the inhibition of on-off reactions at filament ends. However, a more interesting hypothesis is that profilin-actin complexes may be able to polymerize, at least to some extent, onto a subpopulation of actin filaments. This hypothesis of differential regulation would suggest that there may be multiple actin monomer-binding proteins, each directed toward the regulation of a specific set of actin filament structures. A particular form of complex may be inactive for polymerization onto certain filaments but active toward other structures. This could explain the presence of multiple actin sequestration proteins in the cell and the insufficient amount of any individual protein alone to account for the extent of sequestration. Moreover,

these proteins may respond to different second messengers and allow differential regulation of the assembly and disassembly of different structures. Consistent with this hypothesis, we have recently observed a similar depolymerization of actin filaments upon the microinjection of thymosin beta 4 (Sanders et al., 1992), a small peptide that can inhibit actin assembly in vitro (Safer et al., 1990, 1991). However, unlike profilin, thymosin beta 4 does not cause the preferential disruption of the extension of lamellipodia.

The present observations clearly demonstrate the ability of profilin to regulate the polymerization of actin in living cells. Although the results are qualitatively consistent with the ability of profilin to sequester a fraction of actin subunits, considerable complexities are indicated by the differential response of different structures and the different effects of profilin and profilactin. Numerous important questions, such as the relationship among various sequestering mechanisms, the effects of different actin-binding proteins on sequestered complexes, the mechanism for the formation of the high-affinity profilactin complexes, and the biological activities of the profilactin, must be addressed in the future.

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