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Mutational Analysis of Yeast Profilin

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We have mutated two regions within the yeast profilin gene in an effort to functionally dissect the roles of actin and phosphatidylinositol 4,5-bisphosphate (PIP_2) binding in profilin function. A series of truncations was carried out at the C terminus of profilin, a region that has been implicated in actin binding. Removal of the last three amino acids nearly eliminated the ability of profilin to bind polyproline in vitro but had no dramatic in vivo effects. Thus, the extreme C terminus is implicated in polyproline binding, but the physiological relevance of this interaction is called into question. More extensive truncation, of up to eight amino acids, had in vivo effects of increasing severity and resulted in changes in conformation and expression level of the mutant profilins. However, the ability of these mutants to bind actin in vitro was not eliminated, suggesting that this region cannot be solely responsible for actin binding. We also mutagenized a region of profilin that we hypothesized might be involved in PIP_2 binding. Alteration of basic amino acids in this region produced mutant profilins that functioned well in vivo. Many of these mutants, however, were unable to suppress the loss of adenylate cyclase-associated protein (Cap/Srv2p [A. Vojtek, B. Haarer, J. Field, J. Gerst, T. D. Pollard, S. S. Brown, and M. Wigler, Cell 66:497–505, 1991]), indicating that a defect could be demonstrated in vivo. In vitro assays demonstrated that the inability to suppress loss of Cap/Srv2p correlated with a defect in the interaction with actin, independently of whether PIP_2 binding was reduced. Since our earlier studies of *Acanthamoeba* profilins suggested the importance of PIP_2 binding for suppression, we conclude that both activities are implicated and that an interplay between PIP_2 binding and actin binding may be important for profilin function.

Profilin is a low-molecular-size (12 to 15 kDa) protein that interacts with actin (16, 30). Profilin can also bind to the acidic phospholipid L-α-phosphatidylinositol 4,5-bisphosphate (PIP_2) and to a lesser extent, phosphatidylinositol monophosphate (23). It has been proposed that interaction with PIP_2 may regulate the availability of profilin for interaction with actin (23). Alternatively, profilin may be present to prevent the cleavage of PIP_2 by phospholipase Cγ1 (PLC); such inhibition can be overcome by phosphorylation of PLC (13, 14, 26), an event that can occur after stimulation of various growth factor receptor tyrosine kinases (39). Thus, activation of PLC by tyrosine kinase receptors could initiate signaling cascades by overcoming the profilin block, resulting in cleavage of PIP_2 into the second messengers inositol triphosphate and diacylglycerol.

In the yeast *Saccharomyces cerevisiae*, profilin is required (18) for the proper organization of the actin cytoskeleton into actin cables that generally run longitudinally through the cell and cortical actin spots that occur at regions of active growth (1, 22). Profilin, like actin, is required for proper maintenance of cell polarity, as exhibited by delocalized secretion of cell wall chitin and aberrant budding in cells lacking profilin (18). It has not been determined whether these phenotypes can be directly attributed to profilin's role as an actin-binding protein, to its ability to bind PIP_2, or perhaps to some as yet unidentified property of profilin. The recent finding that overexpression of profilin could suppress some of the defects associated with deletion of the gene encoding a 70-kDa adenylate cyclase-associated protein (*CAP* [38]; also known as *SRV2* [9]) provided interesting links between the signal transduction machinery and cytoskeletal maintenance and reorganization. Adenylate cyclase-associated protein (hereafter referred to as Cap/Srv2p, and not to be confused with actin-capping protein subunits, encoded by the *CAP1* and *CAP2* genes [3]) appears to be a bifunctional protein, apparently playing a role in the RAS-mediated activation of adenylate cyclase (however, see reference 40) and providing other functions that, when defective, result in abnormalities that are somewhat similar to the defects of a profilin-deficient strain (11, 18, 38). It is these latter defects that are suppressed by overexpression of profilin. We have found that the ability of two *Acanthamoeba* isoforms of profilin to suppress the latter defects of Cap/Srv2p correlates with their ability to bind PIP_2 (38). This finding suggests that Cap/Srv2p as well as profilin may interact with the signaling pathway that involves PIP_2 cleavage. Although little is known about PIP_2 signaling in yeast cells, the observation that PIP_2 is essential to growth (34) is an indication that it plays an important role. At our present level of knowledge, it is possible that both Cap/Srv2p and profilin are exerting effects on, or are being regulated by, actin and/or PIP_2 and related elements. To address these questions, and in an attempt to dissect the known properties of profilin, we have sought to specifically alter profilin's ability to interact with actin or PIP_2.

A likely candidate region for interaction with actin is found at the carboxy terminus of profilin. This region contains a sequence motif (hereafter referred to as LADYLIG) that is highly conserved among a subset of actin-binding proteins (35). In addition, chemical cross-linking experiments (36) have demonstrated a close interaction between actin and the homologous region of an *Acanthamoeba* profilin. The selection of potential PIP_2-binding sites rested

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on the observations that (i) multiple PIP_2 molecules could bind to individual profilin molecules (14), (ii) basic residues are likely candidates for interaction with acidic phosphate residues (29), (iii) yeast profilin exhibits a cluster of basic residues between amino acid positions 67 and 82, and (iv) residues 71 to 80 are highly conserved between yeast and *Acanthamoeba* profilins, and moreover, this region contains most of the basic residues whose positions are conserved.

In this report, we have altered these two regions of profilin by site-directed mutagenesis and have examined both in vivo effects and the ability of mutant profilins to bind actin and PIP_2 in vitro. We conclude that both actin and PIP_2 binding, perhaps working in a coordinated fashion, are relevant to profilin function.

MATERIALS AND METHODS

Reagents. Poly-L-proline (8,000 kDa), bovine serum albumin (BSA), and PIP_2 were obtained from Sigma Chemical Co. (St. Louis, Mo.); a site-directed mutagenesis kit and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (affinity purified) were obtained from Bio-Rad Laboratories (Richmond, Calif.); cyanogen bromide-activated Sepharose 4B was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.); Centricon microconcentrators were obtained from Amicon Corp. (Danvers, Mass.); Ultrafree MC low-binding Millipore PLTK filters (30,000-molecular-weight cutoff) were obtained from Millipore Corp. (Bedford, Mass.); DNase I and DNase I-Sepharose were obtained from Worthington Biochemical Corp. (Freehold, N.J.); yeast nitrogen base (without amino acids), yeast extract, Bacto Peptone, and Bacto Agar were obtained from Difco Laboratories (Detroit, Mich.); ^{125}I-labeled donkey anti-rabbit immunoglobulin was obtained from Amersham (Arlington Heights, Ill.).

Yeast strains and growth conditions. *S. cerevisiae* diploid strain 22ABΔ1 (*MATa/MATα ura3/ura3 trp1/trp1 lys2/lys2 his3/his3 leu2/leu2 PFY1/pfy1::LEU2*) contains a complete deletion of one of its two copies of the profilin coding region as described previously (18). Wild-type and profilin deletion haploid strains (22ABΔ1-6A and 22ABΔ1-6D, respectively) are haploid segregants of 22ABΔ1. Profilin deletion haploid strains containing only plasmid-borne profilin genes or control plasmids were generated by transforming 22ABΔ1 with the appropriate plasmid and then sporulating the cells and performing tetrad dissection; the haploid progeny of interest were selected as being Leu^+ (marking the profilin deletion) and Ura^+ (from the plasmid-borne *URA3* gene). Note that this procedure also produces PFY^+ haploid segregants carrying plasmids, allowing us to determine that the mutant profilins had no apparent detrimental effects when overproduced. Multiple segregants from such crosses were examined for growth rate, morphology, and temperature sensitivity, and results were consistent for all segregants carrying a given plasmid. Thus, possible differences in genetic background do not grossly affect these properties.

Plasmid selection was maintained by growing cells in minimal medium (0.67% yeast nitrogen base, 2% glucose) supplemented with tryptophan, lysine, histidine, adenine, and leucine; strains were otherwise grown as described previously (18); growth was generally at 22°C.

DNA manipulations, yeast genetic techniques, and plasmid constructions. Standard procedures were used for recombinant DNA manipulation (31), *Escherichia coli* and yeast transformation (6, 25, 31), and yeast genetic manipulations (32).

Plasmid YEp420(PFY) (generously provided by A. Adams) consists of a 1.8-kbp *Bam*HI-*Hin*dIII fragment containing the complete profilin coding region inserted at the *Bam*HI-*Hin*dIII sites of YEp420 (25). Other parent plasmids used for maintenance of wild-type or mutant profilins were YEp102 (38) and YCp50 (25).

Nonsense mutations near the end of the profilin coding region were introduced by site-directed mutagenesis of single-stranded template from mp19(PFY) (consisting of a 1.8-kbp *Bam*HI-*Hin*dIII fragment containing the profilin gene in M13mp19), using the following mutagenic primers (each results in a TAA nonsense codon): TTGATTGGTTAAC AATACTAATTT (mutates codon 124 to make the -3PFY truncated profilin); GACTACTTGATTTAAGTTCAATACT AA (mutates codon 123 to make -4PFY); GGCTGACTACT AAATTGG (mutates codon 121 to make -6PFY); GCTGAC TAATTGATTG (mutates codon 120 to make -7PFY); and GCAATTGGCTTAATACTTG (mutates codon 119 to make -8PFY). In some cases, these primers were used for plaque hybridization to screen for mutations. Mutations were confirmed by sequencing; inserts with the appropriate mutations were shuttled to plasmids YCp50 and YEp102 as 1.8-kbp *Bam*HI-*Hin*dIII fragments.

The genes encoding *Acanthamoeba* profilins I and II were placed next to the yeast profilin promoter on high-copy-number vectors to create plasmids YEp(PFY-PI) and YEp-(PFY-PII) as described previously (38). In addition, these yeast-*Acanthamoeba* hybrids were also moved as *Eag*I-*Xho*I fragments into the YCp50 *Eag*I-*Sal*I sites to create the low-copy-number plasmids YCp(PFY-PI) and YCp(PFY-PII).

Purification of yeast actin and profilins. Yeast actin was prepared by DNase I affinity chromatography from strain DBY877 (*MATα his4*; provided by D. Botstein) and treated as described previously (18). Yeast profilins were prepared from profilin deletion strains harboring plasmid-borne wild-type or mutant profilin genes. Purification was by polyproline affinity chromatography as described previously (18) except that the bulk of mutant profilins 111, 112, 115, and 116 eluted with 3 M urea rather than 6 M urea, which is required to elute the bulk of wild-type and mutant 43 profilin (see Fig. 8).

Actin binding determined by DNase I-Sepharose batch adsorption. Actin binding was determined by batch adsorption to Sepharose-bound DNase I. Profilin-actin complexes in crude lysates bind, via the actin, to this resin with high affinity (7). Yeast strains carrying the appropriate mutant or wild-type profilin on a high-copy-number plasmid were grown in selective medium to late log phase and then harvested and lysed as described previously (18). Total protein lysates were partially clarified by spinning for 3 min in a microcentrifuge; supernatants were diluted to 2.5 μg/μl (for studies with truncated profilins and *Acanthamoeba* profilins expressed in yeast cells) or 1.25 μg/μl (for basic-residue mutant profilins) in lysis buffer (18). DNase I-Sepharose and BSA-Sepharose resins were washed several times in lysis buffer, with a final 1:1 suspension of resin in lysis buffer. Resin suspensions (40 μl) were mixed with 100 μl of diluted protein samples and incubated at 4°C with vigorous shaking for 60 to 75 min. Samples were then underlayered with 200 μl of 20% sucrose in lysis buffer and spun at 7,000 × *g* in a swinging-bucket microcentrifuge for 1 min. Western blots of the resin pellets and equivalent amounts of total protein were probed with anti-yeast profilin antiserum at 1:100 dilution; horseradish peroxidase-conju-

gated goat anti-rabbit secondary antibody was used at 1:250 dilution (see Fig. 2A).

The DNase I adsorption assay was also carried out with radioactive secondary antibody to quantitate binding (see Fig. 2B and 6). Because of large differences in expression levels of the truncated profilins, lysates from cells expressing wild-type profilin or -4PFY were diluted 4× and 2×, respectively, with lysate from a profilin deletion strain to adjust them to approximately the same profilin concentration as for lysates from strains expressing -6PFY or -7PFY (see Fig. 2B). It was not necessary to dilute lysates from basic-residue profilin mutants to normalize profilin ratios, as all were within a twofold range of one another. Duplicate Western blots were probed with affinity-purified anti-yeast profilin antibodies (1:100) and ^{125}I-labeled donkey anti-rabbit immunoglobulin G secondary antibody (1:100). Blots were examined by autoradiography; then bands were cut from the nitrocellulose filters (in 1-cm^2 sections) and analyzed by gamma counting (a blank portion of the nitrocellulose was used to determine background counts, which were subtracted from each sample). Serial dilutions of cell lysates and of antibodies were tested to determine conditions within the linear range of this assay.

Polyproline binding. Polyproline binding was determined by batch adsorption to polyproline-Sepharose (33) and was carried out essentially as for DNase I-Sepharose batch adsorption except that total protein was diluted to 1 μg/μl prior to mixing with polyproline-Sepharose resin; both pellet and supernatant fractions were analyzed. Poly-L-proline was coupled to Sepharose 4B as described previously (18).

PIP_2 binding. PIP_2 binding was assayed by using purified preparations of wild-type or mutant yeast profilins from strains overexpressing these profilins. As suggested by L. Machesky and T. Pollard, profilins were mixed with PIP_2 micelles (prepared by sonicating a freshly thawed aliquot of 1-mg/ml [~900 μM] PIP_2 in water) to a final concentration of 0.5 μM profilin and 100 μM PIP_2 in vesicle buffer (10 mM Tris [pH 7.5], 75 mM KCl, 0.5 mM dithiothreitol). Samples (150 μl) were incubated on ice 30 min, then layered on Millipore PLTK filters (30,000-molecular-weight cutoff), and spun at 2,000 × *g* in a swinging-bucket microcentrifuge for 90 to 120 s. Western blots (immunoblots) of filtrates from samples with and without PIP_2 and equivalent amounts of total protein were probed with 1:50 to 1:100 dilutions of crude or affinity-purified antiprofilin antibodies (18) and with a 1:250 dilution of horseradish peroxidase-conjugated goat anti-rabbit secondary antibody.

Actin binding determined by viscometry. Actin polymerization (rolling-ball viscosity) measurements were performed as described previously (18) except that the concentrations of actin and profilins were 2 and 2.85 μM, respectively. Protein concentrations were determined by the method of Bradford (5), using BSA as a standard.

Immunoblots and cell staining. Proteins were separated on 0.75-mm sodium dodecyl sulfate (SDS)–12% polyacrylamide gels with 5% stacking layers, blotted to nitrocellulose, and probed with antibodies essentially as described previously (17, 24). To further reduce background, 2.5% BSA and 0.5% Tween 20 were added to the blocking solution when radioactive secondary antibody was used. Culture supernatants for anti-*Acanthamoeba* profilin monoclonal antibodies PI.2 (recognizes profilins I and II with equal affinity [20]) and PII.1 (specific for profilin II) were generously provided by D. Kaiser and T. Pollard and were used undiluted on immunoblots. Staining of nuclei, microtubules, actin, and cell wall chitin was done as described previously (18).

RESULTS

Overexpression of yeast profilin. We introduced mutated profilin genes (described below) into a profilin deletion strain to see whether they could substitute for the missing wild-type gene. Mutant genes were introduced either on high- or low-copy-number plasmids to vary the expression level. These experiments were first carried out with the wild-type gene to investigate the effects of overexpression of normal profilin. Western blotting suggests that the presence of the wild-type profilin gene on a high-copy-number plasmid results in ≥10-fold overproduction of immunoreactive profilin (data not shown). We were unable to detect any effect of profilin overexpression on growth rate, microtubule or nuclear localization, chitin distribution, or general cell size and shape. Actin appearance and localization were nearly normal except for a small increase in the number of actin bars (Table 1). This is a characteristic of profilin deletion cells and is probably due to plasmid loss in these strains. These observations support those of Magdolen et al. (27), who also detect little or no effect of profilin overexpression on otherwise wild-type cells.

Truncation of profilin. To examine the role of the C-terminal region of profilin, which has been implicated in actin binding, we created a series of C-terminal truncations by 3′ exonucleolytic cleavage. These truncations indicated that the ability to complement a profilin gene deletion was lost somewhere between removal of three and eight amino acids (data not shown). Therefore, we introduced nonsense mutations at specific codons to truncate profilin by three to eight amino acids (designated -3PFY to -8PFY; Fig. 1). Expression of profilins lacking the C-terminal three or four amino acids complemented most defects (18) of profilin deletion mutants. Profilin deletion cells carrying -3PFY or -4PFY have normal growth rates (as judged by colony size [Fig. 1] and by growth in liquid medium [Table 1]), morphology, and cell wall chitin distribution and are not temperature sensitive. Actin localized properly, although both mutants show a reduction in the intensity of staining with rhodamine-phalloidin and anti-actin antibodies and in localization of actin spots (percent actin clusters; Table 1), indicating minor perturbations in the actin cytoskeleton.

Expression of profilin lacking six to eight amino acid residues complemented fewer of the profilin deletion defects; these cells showed loss of visible actin cables, increase in cell size, and temperature sensitivity (data not shown). The growth rate is slower than wild type (Table 1; Fig. 1) and is dependent on the copy number of the mutated profilin genes (Fig. 1). However, these more severely truncated profilins still provide some profilin function, as they (like the less severely truncated profilins) prevent the formation of the abnormal actin bars seen in profilin deletion cells and reduce the loss in clustering of actin spots. Also, the growth rates are faster than that of deletion cells (Fig. 1; Table 1).

Upon examination of the truncated profilins, we noted abnormalities in their abundance and migration on Western blots. Truncation of six to eight residues increased the apparent molecular weight of profilin on SDS-polyacrylamide gels (Fig. 2A). We treated wild-type profilin with carboxypeptidase A to remove C-terminal residues and found that this gave the same shift in apparent molecular weight as the truncations did (data not shown). This result indicates that the upward shift is likely to be due to a change in folding, which is maintained even in the presence of SDS, rather than translation reading through the inserted nonsense codon. These observations raise the possibility that the

TABLE 1. Actin patterns in cells expressing C-terminally truncated profilins

Type of profilin expressed	Haploid strain	% Actin bars[a]	% Actin clusters[b]	Doubling time[c] (h) in synthetic medium
Wild type	22ABΔ1-6A	0[d]	83	ND[e]
None	22ABΔ1-6D	76[f]	38[f]	ND
-3PFY	[22ABΔ1 + YEp(-3PFY)]-1C[g]	9[h]	66	3.9
-4PFY	[22ABΔ1 + YEp(-4PFY)]-2B[g]	8[h]	66	3.6
-6PFY	[22ABΔ1 + YEp(-6PFY)]-2B[g]	6[h]	51	4.8
-7PFY	[22ABΔ1 + YEp(-7PFY)]-3C[g]	11[h]	60	5.4
-8PFY	[22ABΔ1 + YEp(-8PFY)]-1C[g]	2[h]	65	4.5
Wild type	[22ABΔ1 + YEp420(PFY)]-2B[g]	6[h]	83	3.7
None	[22ABΔ1 + YCp50]-6C[g]	ND	ND	6.9

[a] Actin bars are abnormal accumulations of actin seen in profilin null cells (18); these were visualized by using rabbit anti-yeast actin primary antibody; unless otherwise specified, $n = 200$.

[b] Actin clusters are concentrations of cortical actin spots that occur at regions of active growth (1); these were visualized by using rhodamine-conjugated phalloidin; unless otherwise specified, $n = 100$.

[c] Based on optical density at 660 nm. In general, the values are consistent with the qualitative assessment of growth rates (based on colony size) shown in Fig. 1; a minor exception is the strain carrying -8PFY. We believe that the data in Fig. 1 are more reliable, as they are based on scoring multiple segregants.

[d] $n = 500$.

[e] ND, not determined.

[f] $N = 50$.

[g] Haploid profilin deletion strains carrying a control plasmid (YCp50) or wild-type or truncated profilins on high-copy-number vectors (see Materials and Methods).

[h] The occasional bars are probably due to plasmid loss, resulting in a profilin deletion cell. Plasmid loss occurs at low frequency even under conditions that select for plasmid retention (4).

phenotypic effects resulting from truncation of six or more residues may result from a conformational change in profilin rather than a specific effect of the loss of those residues.

We also noted that successive removal of C-terminal residues reduced the level of profilin detected (compare totals in Fig. 2A). We do not know whether this is due to a reduction in the synthesis, a reduction in staining intensity, or more likely, to increased lability of the truncated profilins. However, a reduction in the amount of profilin cannot by itself account for the reduced ability to complement a profilin deletion mutation, as even the cells carrying the most extensive truncation on a high-copy-number plasmid produce as much profilin as is present in wild-type cells, quantitated by probing Western blots using radioactive secondary antibody (data not shown).

We next tested the ability of truncated profilins to bind actin in the DNase I-Sepharose assay. Even the most severely truncated profilins were able to bind actin (Fig. 2A; compare lanes D and B for each mutant). However, the decreasing amounts of total profilin made it difficult to assess to what degree this binding might be affected. Therefore, we

FIG. 1. Carboxy-terminal truncation of profilin by nonsense codon mutagenesis. The last 10 amino acid residues of profilin are shown on the top line. The second line lists the designations for truncated profilins and indicates the number of missing amino acids. The ability of each truncated gene, when present on plasmid YEp102 (high copy) or YCp50 (low copy), to complement a profilin deletion is indicated as follows: ++, wild-type growth rate; −, profilin deletion growth rate; + and +/−, intermediate growth rates. These relative growth rates were determined by observation of segregant growth on YEPD plates after dissection of tetrads (see Materials and Methods) and thus may partially reflect spore germination rates as well.

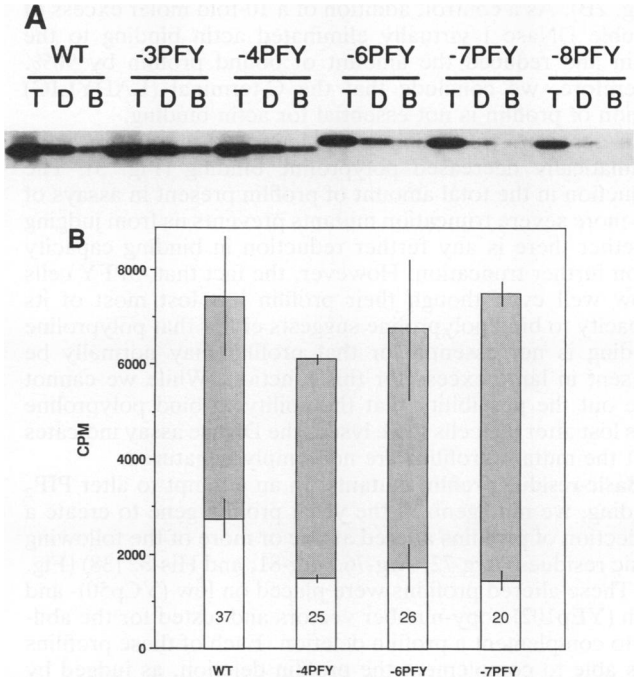

FIG. 2. DNase I-batch adsorption assay of truncated profilins. Shown are assays of strains (see Table 1) expressing wild-type profilin (WT) or mutant profilins lacking three to eight C-terminal amino acids (-3PFY to -8PFY); in each case, profilins were expressed from high-copy-number plasmids. (A) Western blot of total proteins (lanes T) and proteins adsorbed to DNase I-Sepharose (lanes D) or BSA-Sepharose as a control (lanes B). (B) Quantitation of DNase I-batch adsorption data obtained by using a ^{125}I-labeled secondary antibody. The open portion of each box represents the amount of profilin bound to DNase I-Sepharose (values indicate percent profilin bound); open plus stippled regions represent total input profilin in the assay. Lines are ranges of values from two sets of samples. All truncated profilins (including -3PFY and -8PFY, which were not assayed in this experiment) were assayed in duplicate in at least two separate experiments, and all show similar binding.

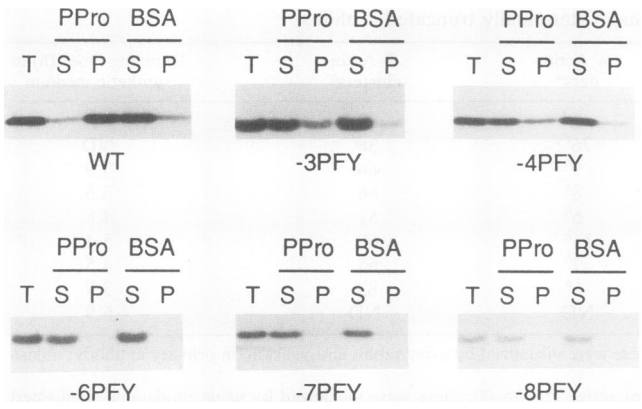

FIG. 3. Polyproline binding of truncated profilins. Western blots of total protein (lanes T) and supernatant (lanes S) and pellet (lanes P) fractions from polyproline (PPro) and control (BSA) batch adsorption assays were probed with anti-yeast profilin antibodies. Strains used were the same as for Fig. 2.

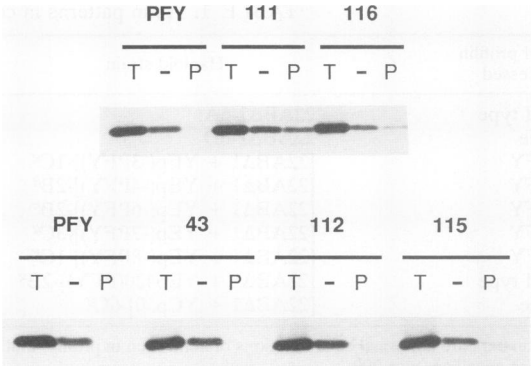

FIG. 5. PIP_2 binding of wild-type and basic-residue mutant profilins. Western blot of total input profilin (lanes T) and profilin that can pass through a 30,000-molecular-weight cutoff filter in the absence (lanes −) or presence (lanes P) of PIP_2, using wild-type (PFY) or basic-residue mutant (43, 111, 112, 115, and 116) profilins (for mutant strain designations, refer to Fig. 4). Only the 12- to 15-kDa regions of the immunoblots are presented.

adjusted samples to the same actin/profilin ratio and quantitated binding by using radioactive secondary antibody. This assay revealed that the truncated profilins show only a modest reduction in binding relative to wild-type profilin (Fig. 2B). As a control, addition of a 10-fold molar excess of soluble DNase I virtually eliminated actin binding to the resin and reduced the amount of bound profilin by 90%. Therefore, we conclude that the C-terminal (LADYLIG) region of profilin is not essential for actin binding.

In contrast, truncation of as few as three amino acids dramatically decreased polyproline binding (Fig. 3). The reduction in the total amount of profilin present in assays of the more severe truncation mutants prevents us from judging whether there is any further reduction in binding capacity upon further truncation. However, the fact that -3PFY cells grow well even though their profilin has lost most of its capacity to bind polyproline suggests either that polyproline binding is not essential or that profilin may normally be present in large excess for this function. While we cannot rule out the possibility that the ability to bind polyproline was lost after the cells were lysed, the DNase assay indicates that the mutant profilins are not simply denatured.

Basic-residue profilin mutants. In an attempt to alter PIP_2 binding, we mutagenized the yeast profilin gene to create a collection of profilins altered at one or more of the following basic residues: Arg-72, Arg-76, Arg-81, and His-82 (38) (Fig. 4). These altered profilins were placed on low (YCp50)- and high (YEp102)-copy-number vectors and tested for the ability to complement a profilin deletion. Each of these profilins was able to complement the profilin deletion, as judged by

STRAIN	R_{72}	R_{76}	R_{81}	H_{82}
111	E			
124	G		K	
121	G	G	K	
116		E		
112		G		
115		G	K	
119		G	G	
43			G	
126		G	K	D

FIG. 4. Altered amino acids in basic-residue mutant profilins.

growth in liquid medium (see Fig. 7B) and by relative colony size on YEPD plates (see also reference 38), even when the mutant gene was expressed from a low-copy-number plasmid. Microscopic examination demonstrated that profilin deletion strains suppressed by any of these constructs were normal with respect to actin and tubulin localization, chitin deposition, and polarity of budding (data not shown).

It was previously shown that as with wild-type profilin, overexpression of two of the basic-residue mutant profilins (strains 112 and 116; Fig. 4) partially suppresses defects associated with mutation of the gene encoding adenylate cyclase-associated protein (Cap/Srv2p), while overexpression of profilins 111, 115, 119, or 124 does not (38). To further characterize the basic-residue mutants and to help explain these observations, we tested a subset of the basic-residue mutants for the ability to interact with PIP_2. Microfiltration assays (Fig. 5) revealed that profilin 111 reproducibly showed the most dramatic loss in PIP_2 binding. Profilin 116 appeared to bind PIP_2 slightly less well than wild-type profilin in two separate experiments, whereas the others (profilins 43, 112, and 115) could not be reliably distinguished from wild type in multiple experiments. Thus, there is no obvious correlation between loss of *cap/srv2* suppression and loss of PIP_2 binding; although suppressors (profilins 112 and 116) bind PIP_2 reasonably well, nonsuppressors may (profilin 111) or may not (profilin 115) show a defect.

All of the basic-residue mutant profilins appeared to bind actin approximately as well as wild type in the DNase I-Sepharose batch adsorption assay (Fig. 6). In contrast to these results, defects were seen in a viscometric assay (Fig. 7A) for profilin-actin interaction. In that assay, suppressor profilin 112 was less defective than profilins 115 (a nonsuppressor) and 43. Similar results were obtained in other experiments (not shown), in which profilin 116 (the other suppressor) was less defective than profilin 111 (a nonsuppressor).

Given the apparent discrepancy between the DNase and viscometry assays, we wished to determine whether some mutant profilins were simply more denatured in the latter assay (profilin must be purified for this assay, so there may be more opportunity for denaturation). Therefore, we examined the viscometry samples for the ability to bind actin in the DNase assay. All of these profilins were bound to

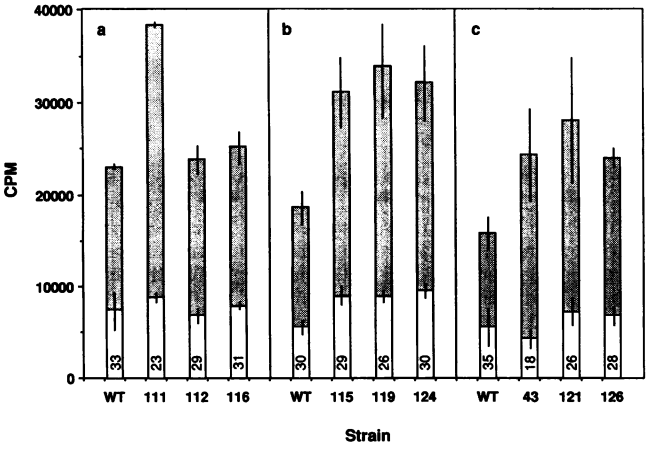

FIG. 6. DNase I-batch adsorption assay of basic-residue mutant profilins. Binding of mutant profilins to DNase I-Sepharose was quantitated by using a ^{125}I-labeled secondary antibody; data are presented in the same format as for Fig. 2B. a, b, and c indicate separate nitrocellulose transfers, each with an internal wild-type profilin control (WT). Wild-type and mutant profilins were expressed from high-copy-number plasmids. For mutant strain designations, refer to Fig. 4.

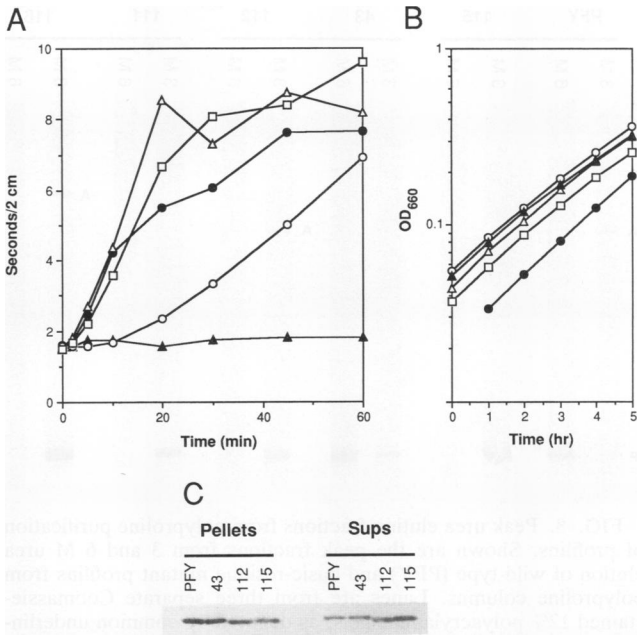

FIG. 7. Ability of basic-residue mutants to retard actin polymerization. (A) Viscometry assay. Profilin (2.85 μM) was added to 2 μM actin, and $MgCl_2$ was added at 0 min to polymerize the actin. Viscosity was determined at the time points shown by rolling-ball viscometry; duplicate curves were performed and were in close agreement (data not shown). Closed circles, no added profilin; closed triangles, wild-type profilin; open circles, profilin 112; open squares, profilin 115; open triangles, profilin 43. (B) Growth curves for strains overexpressing the profilins assayed in panel A. Cells were grown in liquid medium, and the optical density at 660 nm (OD_{660}) was recorded. Symbols are the same as in panel A except that closed circles represent the growth rate of a wild-type strain (DBY877) that is not overexpressing profilin. (C) DNase I-Sepharose assay of the viscometry samples shown in panel A. Pellets, amount of profilin in each sample that associates with the resin; Sups, amounts of profilins that remain in the supernatant fraction (diluted threefold relative to the pellets). Strains used were the same as for Fig. 5 and 6.

approximately the same extent (Fig. 7C), indicating that viscometry is more sensitive or assays a different aspect of actin association, rather than being more denaturing. Goldschmidt-Clermont et al. (15) have previously documented such disparities between binding and polymerization assays, which they attribute to the complex nature of profilin's effects on actin polymerization. Thus, the DNase assay may be a better indicator of binding, while viscometry may make subtle effects more detectable. Independent evidence for differences in actin binding among the mutant profilins was obtained from their behavior on polyproline columns. Wild-type profilin and the two suppressor profilins all cause a substoichiometric amount of actin to be retained on the column, whereas the other basic-residue mutant profilins do not (Fig. 8). We also noted that while the basic-residue mutant profilins efficiently bind polyproline, some can be eluted at lower urea concentrations than others. This property did not appear to correlate with defects in either PIP_2 or actin interaction (Table 2).

Expression of *Acanthamoeba* profilins in yeast cells. We have investigated the role of expression level in the relative ability of two isoforms of *Acanthamoeba* profilin to suppress deletion of the yeast profilin gene or *CAP/SRV2*. As reported previously (38), expression of *Acanthamoeba* profilin I or II from high-copy-number vectors is able to suppress a deletion of the yeast profilin gene. We have also examined suppression by these profilins when expressed from low-copy-number vectors and have found that YCp(PFY-PI), which contains the profilin I coding region, provides intermediate levels of suppression, while YCp(PFY-PII) has little or no effect on the profilin deletion phenotype (data not shown). These results are consistent with our previous report that YEp(PFY-PI) affords slightly better suppression than YEp(PFY-PII) at elevated temperatures (38). The relative ability of these constructs to suppress a profilin deletion may be due to relative expression levels, as profilin I is present at a higher level (Fig. 9; compare lanes 1 and 4). DNase I-batch adsorption assays (Fig. 9) indicate that profilins I and II have a low (compare with Fig. 2A) but detectable (compare the D and B lanes in Fig. 9) affinity for yeast actin. This may explain why expression of these profilins from high-copy-number plasmids is required for suppression of the yeast profilin gene deletion.

In contrast to suppression of a yeast profilin gene deletion, YEp(PFY-PII) is able to suppress *cap/srv2*, while YEp(PFY-PI) is not (38), leading to the conclusion that this suppression is not due to relative abundance. Thus, our original hypothesis, that the difference in *cap/srv2* suppression may be due to differences in the affinities of the two *Acanthamoeba* profilin isoforms for PIP_2, still stands.

DISCUSSION

Profilin has been shown to interact in vitro with several molecules, including actin, PIP_2, and polyproline. To investigate the contribution of these interactions to profilin function, we have used site-directed mutagenesis to study two different regions of profilin (a summary of our findings is provided in Table 2). First, we have altered basic residues in a highly conserved region that we postulated might be involved in PIP_2 binding. We found that substituting an acidic residue at one position (Arg-72 to a Glu) had a

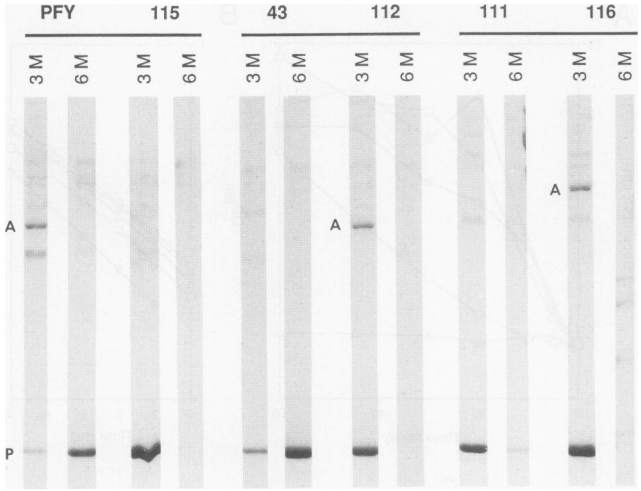

FIG. 8. Peak urea elution fractions from polyproline purification of profilins. Shown are the peak fractions from 3 and 6 M urea elution of wild-type (PFY) and basic-residue mutant profilins from polyproline columns. Lanes are from three separate Coomassie-stained 12% polyacrylamide gels, as denoted by common underlining; profilin bands were used to align lanes, but note that separation is greater in the 111-116 gel. P, profilin bands; A, actin bands in 3 M urea fractions from purification of wild-type, 112, and 116 profilins. The apparent bands in the profilin 116 6 M urea fraction are from spillover of molecular weight markers in an adjacent lane. Strains used were the same as for Fig. 5 and 6.

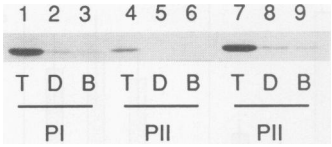

FIG. 9. DNase I-batch adsorption assay of *Acanthamoeba* profilins I and II expressed in yeast cells. Western blots were made of total protein (T) and protein adsorbed to DNase I-Sepharose (D) or BSA-Sepharose (B). Lanes 1 to 3 and 4 to 9 are from yeast strains harboring plasmids YEp(PFY-PI) and YEp(PFY-PII), respectively, which contain the *Acanthamoeba* profilin I (PI) and profilin II (PII) genes linked to the yeast profilin gene promoter (32). Lanes 1 to 6 were probed with monoclonal antibody PI.2 (recognizes profilins I and II with equal affinity); lanes 7 to 9 were probed with monoclonal antibody PII.1 (specific for profilin II).

substantial effect on PIP_2 binding, but a similar change at a nearby residue (Arg-76 to a Glu) had less of an effect. Other changes, which did not introduce a negative charge, had undetectable effects on PIP_2 binding. These findings are consistent with the hypothesis that charge in this region is involved in PIP_2 binding. Unexpectedly, these mutations also affect actin binding. Both Arg-72 and Arg-81 appear important for actin interaction, whereas altering Arg-76 has less effect. The fact that a given mutation can have different effects on these two binding properties, taken together with the fact that all of the basic-residue mutant profilins bind polyproline, argues that these mutations are having local rather than global effects. The three-dimensional structure of *Acanthamoeba* profilin (obtained by nuclear magnetic resonance spectroscopy [37]) does not immediately provide an explanation of our results but does not rule out that they could be due to local effects. Further discussion of the structural implications of our mutations is forthcoming in a paper on the X-ray crystal structure of *Acanthamoeba* profilin (2). While it does not appear that the same region of profilin is involved in both PIP_2 and actin binding (perhaps in contrast to other actin-binding proteins [19, 29, 41]), the probable binding faces are adjacent (37). Thus, steric effects may explain the observation (23) that PIP_2 causes dissociation of actin-profilin complexes, and the mechanism of action of profilin might involve switching between binding actin or PIP_2.

These findings also further our understanding of the mechanism of suppression of *cap/srv2* (see the introduction). We have found that suppression is lost when the interaction with actin is most affected, regardless of whether PIP_2 binding is also reduced. This correlation leads us to suggest that profilin might suppress by substituting for Cap/Srv2p in binding to actin. This fits with the identification by Gieselmann and Mann (12) of an actin-binding protein (ASP-56) that shows significant homology to Cap/Srv2p and with the

TABLE 2. Summary of wild-type and mutant profilin properties

Profilin type	ΔPFY suppression	ΔCAP suppression[a]	PIP_2 binding[b]	Actin binding			Polyproline binding[b,c]
				By viscometry[b]	By DNase	On polyproline[b]	
Wild type	+	+	+	+	+	+	++
43	+	ND[d]	+	−	+	−	++
111	+	−	−	−	+	−	+
112	+	+	+	+/−	+	+	+
115	+	−	+	−	+	−	+
116	+	+	+	+/−	+	+	+
119, 124	+	−	ND	ND	+	ND	ND
121, 126	+	ND	ND	ND	+	ND	ND
-3, -4PFY	+	ND	ND	ND	+	ND	−
-6, -7, -8PFY	+/−	ND	ND	ND	+	ND	−
PI	+	−	+[e]	ND	+/−	ND	ND
PII	+	+	++[e]	ND	+/−	ND	ND

[a] From Vojtek et al. (38).
[b] A minus sign does not necessarily indicate a total lack of activity.
[c] ++, 6 M urea is required to elute the bulk of profilin from a polyproline column; +, the profilin can be eluted with 3 M urea.
[d] ND, not determined.
[e] Extrapolated from data of Machesky et al. (26) and therefore not directly comparable with other data in this column.

finding that Cap/Srv2p can coimmunoprecipitate with actin (10).

The present results do not address the question of whether PIP_2 binding is also involved in *cap/srv2* suppression, as both suppressor profilins bind PIP_2 reasonably well. Our previous results with *Acanthamoeba* profilins (38) indicated that PIP_2 binding is important. In that study, the basic isoform of *Acanthamoeba* profilin (profilin II) could suppress Cap/Srv2p defects, while the acidic isoform (profilin I) could not. Profilin II binds PIP_2 at least 10 times more strongly than profilin I does (26), but both bind to *Acanthamoeba* actin equally well (21). Our results (Fig. 9) suggest that both also bind yeast actin to roughly the same degree, although neither binds as well as yeast profilin does. We have also examined expression levels of the *Acanthamoeba* profilins in yeast cells and find that the suppressor (profilin II) is actually expressed at a lower level than the nonsuppressor (profilin I) is; thus, *cap/srv2* suppression is not simply a function of expression level. Taken together, these findings suggest that both PIP_2 and actin binding may be involved in *cap/srv2* suppression and, by extension, that both of these in vitro properties of profilin are used in vivo. It is appealing to suggest that profilin and Cap/Srv2p can each serve as a link between signaling pathways that involve PIP_2 and responses of the actin cytoskeleton.

We have also altered a second region of profilin, a highly conserved sequence near the C terminus that has been implicated in actin binding (see the introduction). We have deleted most of this region by truncation. Contrary to expectation, the region does not appear to be essential for actin binding, as these profilins continue to show activity in the DNase assay and prevent the formation of actin bars in a profilin deletion background. However, these experiments do not rule out the possibility that this C-terminal region participates in the interaction with actin, especially since we have seen that the DNase assay may not be the most sensitive assay for actin interaction. We have also shown that the C terminus is important for conformation and stability. Our results are consistent with those of Eichinger et al. (8), who found that a similar deletion in severin might reduce but did not eliminate actin binding, and those of McLaughlin et al. (28), who have recently reported the structure of a segment of gelsolin complexed with actin. A comparison with that structure predicts that our most severe truncation of profilin would remove the last turn of an actin-binding α helix but would not remove residues that make contact with actin.

It was also interesting to note that the last three residues of profilin are important for polyproline binding in vitro. While we cannot rule out that the residual binding activity is adequate for in vivo function, it is also possible that polyproline binding does not correspond to a physiologically relevant function of profilin.

There are several considerations that are important to the comparison of in vitro and in vivo data. One is expression level; mutant proteins may work less well in vivo because less of the protein is present rather than because of any specific change that has been made. Therefore, we have quantitated expression levels of mutant profilins on Western blots by using a radioactive secondary antibody. Truncation does appear to make profilin less stable; it can be seen in Fig. 2A that progressively less profilin is present with increasing truncation. However, this does not appear to account for the deleterious in vivo effects, as even the most truncated profilin, when expressed from a high-copy-number plasmid, is present at a roughly wild-type level. We postulate that in vivo effects may instead result from the apparent conformation change in profilin and/or to some effect on the interaction with actin or other components that have not been identified.

The basic residue mutants, on the other hand, do not appear to be less stable (Fig. 6). On the contrary, *cap/srv2* nonsuppressor profilins are apparently overexpressed (less than twofold) relative to wild-type and suppressor profilins on high-copy-number plasmids. Perhaps a somewhat higher copy number of plasmid is selected for to compensate for the defect(s) in these profilins (even though no obvious defects are seen in cells presumably expressing less mutant profilin, i.e., with low-copy-number plasmids).

Because we are studying the mutant profilins expressed from plasmids, we have to take into account effects of overexpression as well as underexpression of profilin. We were initially surprised to find that overexpression of wild-type profilin had no phenotypic effects, as we anticipated that overexpression might cause sequestration of too much actin or PIP_2. We have also found that overexpression of any of the mutant profilins in this study has no phenotypic effects (see Materials and Methods). Although we can conclude that overexpression is not having negative effects, the possibility remains that it may mask partial defects in some of the mutant profilins. This is a likely explanation in the case of mutant 111. Although this mutation can suppress profilin gene deletion when present on either high- or low-copy number plasmids, gene replacement leads to morphological abnormalities and a growth rate that is intermediate between wild-type and deletant rates (unpublished results). Overexpression may also mask phenotypic effects in other basic residue mutants, especially those that cannot suppress *cap/srv2* and are therefore demonstrably defective in an in vivo function. Overexpression also diminishes phenotypic effects in the case of the truncation mutants (Fig. 1). Similarly, overexpression may allow *Acanthamoeba* profilins to function in yeast cells, despite their poor interaction with yeast actin (Fig. 9). Interestingly, yeast profilin may be constitutively overexpressed in the sense that a deletion heterozygote, presumably with half the normal level of profilin, shows no phenotypic defects (data not shown).

Another issue is that a defect seen in vitro may not necessarily occur in vivo. Perhaps the mutant protein is more easily denatured during in vitro handling or is stabilized by in vivo conditions (including interaction with other proteins). For example, we cannot be certain that the more severely truncated profilins, which appear to have an altered conformation in vitro, actually have this alteration in vivo. However, the close correlation between conformation change and altered morphology supports the idea that the conformation change may be present in vivo and may be causal. The ability of basic-residue mutant profilin genes to suppress profilin gene deletion might have led one to suspect that the defects seen were in vitro artifacts, except for the fact that some mutants could not suppress *CAP/SRV2* deletion, confirming that defects also existed in vivo. The discrepancy may instead be due to overexpression, as discussed above.

In conclusion, our studies of two regions of profilin indicate that one region is important for interaction with polyproline but not solely responsible for binding actin and that the other region is important for interaction with PIP_2 and actin. These studies combined with studies of *cap/srv2* suppression suggest that an interplay between actin and PIP_2 binding may be important in profilin function. We are currently using these profilin mutants to genetically identify

other proteins that may be involved in profilin function and to elucidate profilin's intriguing relationship to Cap/Srv2p.

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REFERENCES

1. **Adams, A. E. M., and J. R. Pringle.** 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98:**934–945.
2. **Almo, S. (Albert Einstein College of Medicine).** Personal communication.
3. **Amatruda, J. F., J. F. Cannon, K. Tatchell, C. Hug, and J. A. Cooper.** 1990. Disruption of the actin cytoskeleton in yeast capping protein mutants. Nature (London) **344:**352–354.
4. **Botstein, D., and R. W. Davis.** 1982. Principles and practice of recombinant DNA research with yeast, p. 607–636. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Bruschi, C. V., A. R. Comer, and G. A. Howe.** 1987. Specificity of DNA uptake during whole cell transformation of *S. cerevisiae*. Yeast **3:**131–137.
7. **Carlsson, L., L.-E. Nystrom, I. Sundkvist, F. Markey, and U. Lindberg.** 1976. Profilin, a low-molecular weight protein controlling actin polymerisability, p. 39–49. *In* S. V. Perry et al. (ed.), Contractile systems in non-muscle tissues. Elsevier/North-Holland Biomedical Press, Amsterdam.
8. **Eichinger, L., A. A. Noegel, and M. Schleicher.** 1991. Domain structure in actin-binding proteins: expression and functional characterization of truncated severin. J. Cell Biol. **112:**665–676.
9. **Fedor-Chaiken, M., R. J. Deschenes, and J. R. Broach.** 1990. *SRV2*, a gene required for *RAS* activation of adenylate cyclase in yeast. Cell **61:**329–340.
10. **Field, J. (Columbia University).** Personal communication.
11. **Gerst, J. E., K. Ferguson, A. Vojtek, M. Wigler, and J. Field.** 1991. CAP is a bifunctional component of the *Saccharomyces cerevisiae* adenylyl cyclase complex. Mol. Cell. Biol. **11:**1248–1257.
12. **Gieselmann, R., and K. Mann.** 1992. ASP-56, a new actin sequestering protein from pig platelets with homology to CAP, an adenylate cyclase-associated protein from yeast. FEBS Lett. **298:**149–153.
13. **Goldschmidt-Clermont, P. J., J. W. Kim, L. M. Machesky, S. G. Rhee, and T. D. Pollard.** 1991. Regulation of phospholipase C-γ1 by profilin and tyrosine phosphorylation. Science **251:**1231–1233.
14. **Goldschmidt-Clermont, P. J., L. M. Machesky, J. J. Baldassare, and T. D. Pollard.** 1990. The actin-binding protein profilin binds to PIP_2 and inhibits its hydrolysis by phospholipase C. Science **247:**1575–1578.
15. **Goldschmidt-Clermont, P. J., L. M. Machesky, S. K. Doberstein, and T. D. Pollard.** 1991. Mechanism of the interaction of human platelet profilin with actin. J. Cell Biol. **113:**1081–1089.
16. **Haarer, B. K., and S. S. Brown.** 1990. Structure and function of profilin. Cell Motil. Cytoskel. **17:**71–74.
17. **Haarer, B. K., and J. R. Pringle.** 1987. Immunofluorescence localization of the *Saccharomyces cerevisiae CDC12* gene product to the vicinity of the 10-nm filaments in the mother-bud neck. Mol. Cell. Biol. **7:**3678–3687.
18. **Haarer, B. K., S. H. Lillie, A. E. M. Adams, V. Magdolen, W. Bandlow, and S. S. Brown.** 1990. Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. J. Cell Biol. **110:**105–114.
19. **Janmey, P. A., J. Lamb, P. G. Allen, and P. T. Matsudaira.** 1992. Phosphoinositide-binding peptides derived from the sequences of gelsolin and villin. J. Biol. Chem. **267:**11818–11823.
20. **Kaiser, D. A. (Johns Hopkins University).** Personal communication.
21. **Kaiser, D. A., M. Sato, R. F. Ebert, and T. D. Pollard.** 1986. Purification and characterization of two isoforms of *Acanthamoeba* profilin. J. Cell Biol. **102:**221–226.
22. **Kilmartin, J. V., and A. E. M. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98:**922–933.
23. **Lassing, I., and U. Lindberg.** 1985. Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. Nature (London) **314:**472–474.
24. **Lillie, S. H., and S. S. Brown.** 1987. Artifactual immunofluorescent labelling in yeast, demonstrated by affinity purification of antibody. Yeast **3:**63–70.
25. **Ma, H., S. Kunes, P. Schatz, and D. Botstein.** 1987. Plasmid construction by homologous recombination in yeast. Gene **58:**201–216.
26. **Machesky, L. M., P. J. Goldschmidt-Clermont, and T. D. Pollard.** 1990. The affinities of human platelet and *Acanthamoeba* profilin isoforms for polyphosphoinositides account for their relative abilities to inhibit phospholipase C. Cell Regul. **1:**937–950.
27. **Magdolen, V., D. G. Drubin, G. Mages, and W. Bandlow.** 1993. High levels of profilin suppress the lethality caused by overproduction of actin in yeast cells. FEBS Lett. **316:**41–47.
28. **McLaughlin, P. J., J. T. Gooch, H.-G. Mannherz, and A. G. Weeds.** 1993. Structure of gelsolin segment 1-actin complex and the mechanism of filament severing. Nature (London) **364:**685–692.
29. **Moriyama, K., N. Yonezawa, H. Sakai, I. Yahara, and E. Nishida.** 1992. Mutational analysis of an actin-binding site of cofilin and characterization of chimeric proteins between cofilin and destrin. J. Biol. Chem. **267:**7240–7244.
30. **Pollard, T. D., and J. A. Cooper.** 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu. Rev. Biochem. **55:**987–1035.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Tanaka, M., and H. Shibata.** 1985. Poly(L-proline)-binding proteins from chick embryos are a profilin and a profilactin. Eur. J. Biochem. **151:**291–297.
34. **Uno, I., K. Fukami, H. Kato, T. Takenawa, and T. Ishikawa.** 1988. Essential role for phosphatidylinositol 4,5-bisphosphate in yeast cell proliferation. Nature (London) **333:**188–190.
35. **Vandekerckhove, J.** 1989. Structural principles of actin-binding proteins. Curr. Opin. Cell Biol. **1:**15–22.
36. **Vandekerckhove, J. S., D. A. Kaiser, and T. D. Pollard.** 1989. *Acanthamoeba* actin and profilin can be cross-linked between glutamic acid 364 of actin and lysine 115 of profilin. J. Cell Biol. **109:**619–626.
37. **Vinson, V. K., S. J. Archer, E. E. Lattman, T. D. Pollard, and D. A. Torchia.** 1993. Three-dimensional solution structure of *Acanthamoeba* profilin-I. J. Cell Biol. **122:**1277–1283.

38. **Vojtek, A., B. Haarer, J. Field, J. Gerst, T. D. Pollard, S. S. Brown, and M. Wigler.** 1991. Evidence for a functional link between profilin and CAP in the yeast *S. cerevisiae*. Cell **66:**497–505.
39. **Wahl, M., and G. Carpenter.** 1991. Selective phospholipase C activation. Bioessays **13:**107–113.
40. **Wang, J., N. Suzuki, Y. Nishida, and T. Kataoka.** 1993. Analysis of the function of the 70-kilodalton cyclase-associated protein (CAP) by using mutants of yeast adenylyl cyclase defective in CAP binding. Mol. Cell. Biol. **13:**4087–4097.
41. **Yonezawa, N., Y. Homma, I. Yahara, H. Sakai, and E. Nishida.** 1991. A short sequence responsible for both phosphoinositide binding and actin binding activities of cofilin. J. Biol. Chem. **266:**17218–17221.