

Purification of Profilin from *Saccharomyces cerevisiae* And Analysis of Profilin-deficient Cells

B. K. Haarer,* S. H. Lillie,* A. E. M. Adams,‡ V. Magdolen,§ W. Bandlow,§ and S. S. Brown*

*Department of Anatomy and Cell Biology, The University of Michigan Medical School, Ann Arbor, Michigan 48109;

‡Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; and §Institute for Genetics and Microbiology, D 8000 Munich 19, Federal Republic of Germany

Abstract. We have isolated profilin from yeast (*Saccharomyces cerevisiae*) and have microsequenced a portion of the protein to confirm its identity; the region microsequenced agrees with the predicted amino acid sequence from a profilin gene recently isolated from *S. cerevisiae* (Magdolen, V., U. Oechsner, G. Müller, and W. Bandlow. 1988. *Mol. Cell. Biol.* 8:5108–5115). Yeast profilin resembles profilins from other organisms in molecular mass and in the ability to bind to polyproline, retard the rate of actin polymerization, and inhibit hydrolysis of ATP by mono-

meric actin.

Using strains that carry disruptions or deletions of the profilin gene, we have found that, under appropriate conditions, cells can survive without detectable profilin. Such cells grow slowly, are temperature sensitive, lose the normal ellipsoidal shape of yeast cells, often become multinucleate, and generally grow much larger than wild-type cells. In addition, these cells exhibit delocalized deposition of cell wall chitin and have dramatically altered actin distributions.

ACTIN is a highly conserved, ubiquitous eukaryotic protein that is important in cell shape, motility, cytokinesis, and intracellular movements of organelles (Weeds, 1982). Actin polymerization must be regulated, as actin filaments form at particular times and in discrete regions of the cell (e.g., Carlsson et al., 1979; Laub et al., 1981; Rao and Varani, 1982; Tilney et al., 1973). Indeed, a large number of actin-regulatory proteins have been identified and characterized biochemically (Pollard and Cooper, 1986; Stossel et al., 1985). One such protein, profilin, has been purified from several vertebrate tissues (Stossel et al., 1985) and unicellular organisms (*Acanthamoeba* [Reichstein and Korn, 1979], *Physarum* [Ozaki et al., 1983], and *Amoeba* [Sonobe et al., 1986]) and has been studied extensively in vitro (Stossel et al., 1985). Profilin forms a one-to-one complex with actin monomer and can thereby affect the rate and extent of actin polymerization. However, the effects of profilin may be more complicated than can be explained by simple sequestration of actin monomers (e.g., it may also affect one of the ends of the actin filament) and it is not easy to predict from its in vitro properties how profilin regulates actin in vivo (Pollard and Cooper, 1986). Therefore, genetic studies are needed to help elucidate the function(s) of profilin in vivo. For this reason, we have chosen to look for profilin

Preliminary reports of this work have been presented at the Fourth International Congress for Cell Biology in Montreal, Canada on 15 August 1988 and at the Joint Meeting of the American Society for Cell Biology and the American Society for Biochemistry and Molecular Biology in San Francisco, CA on 31 January 1989 and have appeared in abstract form (1988. *J. Cell Biol.* 107 [No. 6, pt. 3]: 103a [Abstr.]).

in the genetically tractable yeast, *Saccharomyces cerevisiae*. Studies of actin localization in wild-type yeast cells (Adams and Pringle, 1984; Kilmartin and Adams, 1984) and of the phenotypes of temperature-sensitive actin mutants (Shortle et al., 1984; Novick and Botstein, 1985) both suggest that actin in yeast is involved in directing secretion of cell wall components to regions of active growth.

We report here that we have succeeded in purifying profilin from yeast and that it is similar biochemically to profilins from other organisms. During the course of this work, isolation of a gene for yeast profilin was reported (Oechsner et al., 1987, 1988; Magdolen et al., 1988), and disruption of the gene showed it to be essential under the conditions used in the latter study. We have now expanded on the studies of profilin disruption and have found that, under different conditions, profilin is not essential, allowing us to study a more informative phenotype. We find that cells lacking profilin display a variety of abnormal characteristics, including aberrant cell size and shape, deposition of cell wall chitin, and actin localization.

Materials and Methods

Reagents

Poly-L-proline (8,000 kD), 4',6-diamidino-2-phenyl-indole (DAPI)¹ FITC-conjugated goat anti-rat IgG (affinity purified), and FITC-conjugated goat anti-rabbit IgG (affinity purified) were obtained from Sigma Chemical Co.

1. *Abbreviation used in this paper:* DAPI, 4',6-diamidino-2-phenyl-indole.

(St. Louis, MO); horseradish peroxidase-conjugated goat anti-rabbit IgG (affinity purified) was obtained from Bio-Rad Laboratories (Richmond, CA); cyanogen bromide-activated Sepharose 4B was obtained from Pharmacia Fine Chemicals (Piscataway, NJ); Centricon microconcentrators were obtained from Amicon Corp. (Danvers, MA); YOL1/34 anti-yeast tubulin monoclonal antibody was obtained from Accurate Chemical & Scientific Corp. (Westbury, NY); rhodamine-conjugated phalloidin was obtained from Molecular Probes Inc. (Eugene, OR); yeast extract and bacto-peptone were obtained from Difco Laboratories Inc. (Detroit, MI).

Yeast Strains and Growth Conditions

The following *S. cerevisiae* strains were used in this study: DBY877 (*MAT α his4*) and DBY1830 (*MAT α /MAT α ADE2/ade2 his3/his3 leu2/leu2 LYS2/lys2 trp1/trp1 ura3/ura3*), provided by D. Botstein (Genentech Inc., South San Francisco, Ca); BJ926 (*MAT α /MAT α TRP1/trp1 HIS1/his1 prc1-126/prc1-126 pep4-3/pep4-3 prb1-1122/prb1-1122 can1/can1 gal2/gal2*), provided by E. Jones (Carnegie Mellon University, Pittsburgh, PA); and PFYDO (*MAT α /MAT α ADE2/ade2 leu2/leu2 his4/his4 PFY/pfy::LEU2*; Magdolen et al., 1988). Yeast strain 1830/ppfy2 is a derivative of DBY1830 in which one of the copies of the profilin gene was disrupted by insertion of the *LEU2* gene, in the same manner as was done for PFYDO (Magdolen et al., 1988); Southern blot analysis (data not shown) confirmed disruption of the profilin gene, and the *LEU2* marker showed tight linkage to *ade2* (only 4 of 113 tetrads showed recombination between these markers), consistent with the previously reported genetic linkage between the *PFY* and *ADE2* loci (Magdolen et al., 1988).

Strains used for the purification of yeast actin and profilin were grown in liquid YEPD medium (1% yeast extract, 2% bacto-peptone, 2% glucose) as described below. Unless otherwise noted, strains used in the analysis of profilin deficiency were grown in the rich liquid medium YM-P (Lillie and Pringle, 1980); sporulation was accomplished by washing cells from stationary phase YM-P cultures with distilled water followed by a 10-fold dilution in SM-1 medium (1% potassium acetate, pH 6.7); dissected tetrads were incubated on YEPD plates (except where noted in text) at the indicated temperatures. For all media used in this study, the glucose was autoclaved separately unless otherwise noted.

Purification of Yeast Profilin

Yeast profilin was purified by polyproline affinity chromatography based on the method of Tanaka and Shibata (1985). The polyproline column was prepared by a modification of the method of Tuderman et al. (1975); 3 g of cyanogen bromide-activated Sepharose 4B was washed with 600 ml of 1 M HCl and then coupled overnight at 4°C to 200 mg polyproline in 15 ml water. The resin was then washed in 0.1 M Tris (pH 8) for 2 h followed by 0.1 M acetate buffer (pH 4), 0.5 M NaCl and then several changes of 0.1 M Tris (pH 8), 0.5 M NaCl. Efficiency of coupling was ~86%, as determined by A_{230} of the polyproline solution before and after the reaction.

Two *S. cerevisiae* strains were used for purification of profilin: BJ926, a protease-deficient diploid (Figs. 2 and 4), and DBY877 (Fig. 3). Cells were grown to $3\text{--}5 \times 10^7$ cells/ml at 22–25°C in YEPD medium, collected from 1-liter cultures by centrifugation, and washed twice with distilled water at 4°C. The cell pellet was frozen in a dry-ice bath and stored at –70°C. Subsequent steps were carried out at 0–4°C. The pellet was thawed in 7 ml of lysis buffer (10 mM imidazole [pH 7.5], 0.1 mM CaCl₂, 0.2 mM DTT, 0.5 mM ATP) to which protease inhibitors were added immediately before use (35 μ l of a 100 mM stock of PMSF in isopropanol, 3.5 μ l of a 20 U/ml stock of aprotinin in water, 3.5 μ l of a 1 mg/ml stock of leupeptin in DMSO, 3.5 μ l of a 1 mg/ml stock of pepstatin in DMSO, and 3.5 μ l of a 1 mg/ml stock of tosylphenylalaninechloromethylketone in 95% ethanol). Cells were lysed by shaking for three 20-s periods with 30 g of 0.5-mm glass beads in a homogenizer (MSK; B. Braun Instruments, Burlingame, CA) cooled with CO₂. The cell lysate was pooled with several buffer rinses of the glass beads to give a final volume of ~15 ml. The cell lysate was divided into 5-ml aliquots, frozen in a dry-ice bath, and stored at –70°C. Cell lysate from 6 liters of cells was thawed, fresh protease inhibitors were added as described above, and the lysate was centrifuged for 1 h at ~100,000 g (40,000 rpm in a type 45 rotor [Beckman Instruments, Inc., Fullerton, CA]) to prepare a high-speed supernatant. The supernatant (~750 mg protein) was passed over a 10 ml polyproline column (which was stored between uses in 20 mM imidazole [pH 7.5], 150 mM NaCl, 0.5 mM ATP and was washed with this column buffer before each use). After the supernatant was loaded, the column was washed overnight with the column buffer (5–10 bed volumes), and profilin was eluted with 3 bed volumes of buffer containing 6 M urea. In later preparations, a 3 M urea wash was added before elution

since this wash helped to remove contaminants that were seen in some of the earlier preparations. (One such contaminant, of ~25 kD, was detected when a gel was overloaded with the profilin preparation used in Fig. 3 B.) Our results with yeast profilin differ from those of Tanaka and Shibata (1985), who were able to elute chick embryo profilin with 2 M urea. The peak profilin-containing fractions (as judged by protein determinations [Bradford, 1976]) were immediately diluted twofold in 2 mM Tris (pH 8), 0.2 mM DTT, 0.05 mM MgCl₂ and concentrated by ultrafiltration in microconcentrators (Centricon-10; Amicon Corp.). Several additional dilutions and concentrations were carried out to reduce the urea to ~1 mM. The final flow-through was saved for use as a control in assays. The profilin retained activity for at least 1 wk on ice. Typically 0.1–0.2 mg profilin was obtained from 6 liters of culture (~1,500 mg total protein).

Profilin Microsequencing

Starting with polyproline column-purified profilin, a peptide was obtained by cleavage at tryptophans using 2-(2-nitrophenylsulfonyl)-3-methyl-3-bromoindolenine (Ampe et al., 1985) and purified by high pressure liquid chromatography using a Beckman Instruments, Inc., model 342 apparatus and type 165 detector, a 300-Å pore C18 column (Brownlee Labs, Santa Clara, CA), and an aqueous acetonitrile gradient containing 0.1% trifluoroacetic acid. Approximately 600 pmol of this peptide was sequenced using a protein sequencer and analyzer (models 470 and 120A, respectively; Applied Biosystems, Inc., Foster City, CA).

Isolation of Yeast Actin

Actin was purified from yeast by the method of Zechel (1980) since Kilmartin and Adams (1984) have shown that polymerization-competent actin can be obtained from yeast in this way. All purification steps were performed at 0–4°C, except as noted. Cells were lysed as described above for preparation of profilin, except that the buffer used was one described by Zechel (1980) (2 mM Tris [pH 8], 0.2 mM CaCl₂, 0.2 mM DTT, 0.2 mM ATP), and cell lysates were made more dilute (25 ml from 1 liter of original culture). High-speed supernatants were prepared as described above except that cell lysate from 3–4 liters of original culture was adjusted to 10% formamide before centrifugation. High-speed supernatant was passed over a 2-ml DNase I-Sepharose (Cooper Biomedical, Inc., Malvern, PA) column, and protease inhibitors were added as described above to all column buffers except the 40% formamide buffer used to elute actin. The peak actin-containing fractions (as judged by protein determination [Bradford 1976]) were immediately diluted in G buffer (2 mM Tris [pH 8], 0.2 mM DTT, 0.05 mM MgCl₂, 0.1 mM ATP) and concentrated by ultrafiltration in a microconcentrator (Centricon 30; Amicon Corp.). The actin was diluted in G buffer and concentrated several more times to reduce the formamide to <0.1%.

There was considerable variability in the purity of the actin eluted from the DNase I column, as judged by examining samples on Coomassie-stained gels. One preparation of actin isolated from strain BJ926 appeared >99% pure; however, a number of preparations of actin from strain DBY877 were only ~50–80% pure. The purity could be improved by "cycling" the actin and re-passing it over the DNase I column. Actin is cycled between monomeric and filamentous forms by changing the salt concentration. To cycle to the filamentous form, monomer was clarified by centrifugation for 20 min at 30 psi in an airfuge (Beckman Instruments, Inc.) at room temperature, and 2.5 mM MgCl₂ was added. To cycle back to monomer, filaments were centrifuged as above, and the pellet was rinsed and sonicated (three 10-s bursts at power setting one on a micro-ultrasonic cell disrupter [Kontes Glass Co., Vineland, NJ] in 200 μ l 2 mM Tris (pH 8), 0.2 mM CaCl₂, 0.2 mM DTT, 0.2 mM ATP. The sonicate was reapplied to the DNase I column and left in contact with the column overnight. The column was washed with 10 ml of the same buffer after which the actin was eluted and treated by ultrafiltration as described above. It was then cycled additional rounds if necessary and stored in filamentous form. Taken together, these steps reduced the level of detectable contaminants to ~1% (see Fig. 3 B), with an ~55-kD polypeptide being the most prominent contaminant. Typically, 1–2 mg actin was obtained from 3 liters of culture (~750 mg total protein). This actin retained activity for at least 2 wk on ice, although the yield upon cycling did decrease with time.

Biochemical Assays

Actin polymerization was followed by low shear viscometry (Pollard, 1982). Although an assay that gave continuous monitoring of polymerization would have been more convenient, several assays of this sort were not

suitable. We did not use pyrene-labeled actin because pyrene interferes with the interaction between actin and profilin (for a recent discussion see Lee et al., 1988). Absorbance change at A_{232} (Spudich and Cooke, 1975) was unsatisfactory because we obtained a (variable) absorbance change with profilin in the absence of actin. Another important reason for using the low shear assay was that it required less protein than a number of other methods.

The low shear assay was performed as follows. The rate at which a steel ball (Specialty Ball Co., Rocky Hill, CT; 0.025 inch diameter) rolled through 50 μ l of sample in a 100 μ l capillary tube (Corning Glass Works, Corning, NY) at an angle of 10° was measured approximately every 30 s during polymerization at room temperature (23°C). Readings were discarded if the movement of the ball was jerky instead of smooth. The 0-min time point was obtained by averaging several readings taken before the addition of $MgCl_2$ to initiate polymerization. Actin was cycled (see above) within 1 d of its use in the low shear assay to remove any molecules that had lost the ability to polymerize or depolymerize.

Hydrolysis of ATP by monomeric actin was followed by release of radioactive phosphate (Tobacman and Korn, 1982). Actin (24 μ M; 1 mg/ml) was cycled (see above) and equilibrated overnight at 0°C in G buffer adjusted to 260 mM ATP, including 80 μ Ci/ml [γ - ^{32}P]ATP. It was then mixed with various concentrations of profilin and incubated at 30°C. At each time point, 5- μ l aliquots were assayed for release of inorganic phosphate: the phosphate was selectively precipitated (Sugino and Miyoshi, 1964); precipitates were collected on filters (GF/C; Whatman Inc., Clifton, NJ); and filters were counted in a scintillation counter (LS9000; Beckman Instruments, Inc.) in 2 ml of aqueous counting solution (Amersham Corp., Arlington Heights, IL).

Gel Electrophoresis and Protein Determinations

Standard SDS-12% polyacrylamide gels (Laemmli, 1970) were used. These were stained with Coomassie blue or used for immunoblotting (Towbin et al., 1979). Actin concentration was calculated from A_{290} (Gordon et al., 1976) and/or by the method of Bradford (1976). The values obtained spectrophotometrically agreed with those obtained by the method of Bradford (1976), as we had observed previously for *Dictyostelium* and rabbit muscle actin (data not shown). Profilin concentration was determined using the method of Lowry et al. (1951) as modified by Peterson (1977). BSA was used as a standard in protein determinations.

DNA Manipulations, Yeast Genetic Techniques, and Plasmid Construction

Standard procedures were used for recombinant DNA manipulation (Maniatis et al., 1982), *Escherichia coli* and yeast transformation (Maniatis et al., 1982; Bruschi et al., 1987), and yeast genetic manipulations (Sherman et al., 1986).

Plasmid pSK-HBL was constructed as follows (see Fig. 1): (a) a 450-bp Ssp I-Hind III fragment, which occurs 78 bp downstream from the profilin gene termination codon, was inserted into the Eco RV and Hind III sites within the polylinker region of pBluescript SK(+) (Stratagene, La Jolla, CA) to create plasmid pSK-H; (b) a 590-bp Bam HI-Ssp I fragment, which occurs 119 bp upstream from the profilin gene initiation codon, was inserted into the Bam HI and Sma I sites of pSK-H to create plasmid pSK-HB; (c) the 4-kbp Pst I *LEU2*-containing fragment from YEp13 was inserted at the Pst I site (between the first two inserts) of pSK-HB to form pSK-HBL. The source of profilin region DNA was the plasmid pBHP1700, which contains the entire yeast profilin (*PFY*) gene on a 1.8-kbp Bam HI-Hind III fragment inserted in pUC19 (see Magdolen et al., 1988).

Antibody Production and Purification and Immunoblots

Actin- and profilin-specific antibodies were obtained by injecting rabbits with purified fractions of yeast actin and yeast profilin, respectively. Polyclonal sera were affinity purified (Lillie and Brown, 1987; Haarer and Pringle, 1987) using nitrocellulose strips containing, respectively, purified yeast actin and *trpE*:profilin fusion protein (produced from a derivative of the fusion vector pATH11 in which a portion of the profilin gene from a Sal I site [see Fig. 1] through the end of the coding region was joined to the *E. coli trpE* gene at a Sal I site in pATH11; this construction lacks the 15 amino-terminal amino acids of profilin). Proteins were separated on SDS-polyacrylamide gels, blotted, and probed with antibody essentially as described previously (Lillie and Brown, 1987; Haarer and Pringle, 1987). Blots were

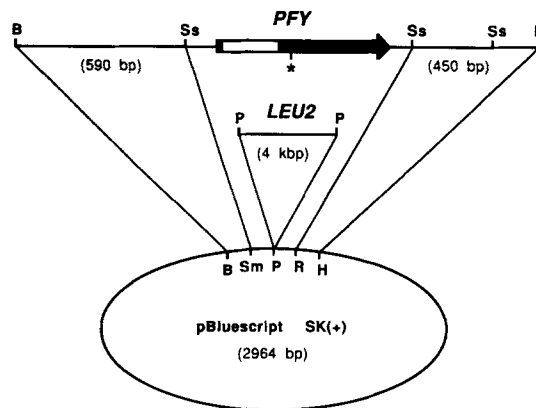


Figure 1. Construction of plasmid pSK-HBL. See Materials and Methods for details. The top line shows the 1.8-kbp *PFY*-containing Bam HI-Hind III fragment of pBHP1700; the middle line shows the 4-kbp *LEU2*-containing Pst I fragment of YEp13; the lower section is plasmid pBluescript SK(+) (the 36-bp Bam HI-Hind III region of the multiple cloning site is expanded for clarity); DNAs are not drawn to scale. Restriction endonuclease abbreviations are as follows: B, Bam HI; H, Hind III; P, Pst I; R, Eco RV; Sm, Sma I; Ss, Ssp I. The dark arrow in the top line represents the *PFY* coding region with the intron indicated as an open box. The asterisk within the *PFY* coding region denotes the location of the Sal I site used in the construction of the *trpE*:*PFY* fusion described in Materials and Methods.

stained with Ponceau S (Lillie and Brown, 1987) to assess relative amounts of protein transferred.

Indirect Immunofluorescence Microscopy

Immunofluorescence microscopy was performed essentially as described previously (Adams and Pringle, 1984). Microtubules were visualized using the anti-yeast tubulin monoclonal antibody YOL1/34 (Kilmartin and Adams, 1984) at 1:100 dilution with FITC-conjugated goat anti-rat IgG secondary antibody at 1:640 dilution. Actin was visualized using either rhodamine-conjugated phalloidin (Kilmartin and Adams, 1984; Adams and Pringle, 1984) or affinity-purified rabbit anti-yeast actin antibodies at 1:10 dilution with FITC-conjugated goat anti-rabbit IgG secondary antibody at 1:40 dilution. For anti-actin staining, it was necessary to treat the yeast cells with methanol at -20°C for 6 min followed by 30 s in acetone at -20°C before incubation with primary antibody (see Drubin et al., 1988; Pringle et al., 1989). Cell wall chitin was visualized using Calcofluor as described previously (Sloat and Pringle, 1978; Haarer and Pringle, 1987). Nuclei were stained by adding DAPI to the mounting medium (Kilmartin and Adams, 1984).

Results

Biochemical Activities of Yeast Profilin

We have used polyproline chromatography (Tanaka and Shibata, 1985) to purify a yeast protein the size of profilin (Fig. 2). We found that the NH₂-terminal 19 amino acids of a peptide produced by cleavage of this protein at tryptophan was identical to amino acids 4-22 encoded by the yeast profilin gene (Magdolen et al., 1988), confirming the identity of the protein.

Like other profilins, yeast profilin retards the rate of yeast actin polymerization (Fig. 3). Yeast profilin also inhibits the rate of ATP hydrolysis by monomeric yeast actin (Fig. 4). The magnitudes of these effects are similar to those observed for *Acanthamoeba* (Reichstein and Korn, 1979; Tobacman and Korn, 1982) under somewhat similar conditions.

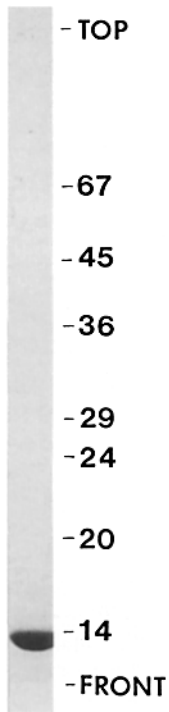


Figure 2. Gel of purified profilin. Approximately 6 μg of purified yeast profilin were run on an SDS-polyacrylamide gel. Sizes of molecular mass standards (in kilodaltons) are noted next to the gel.

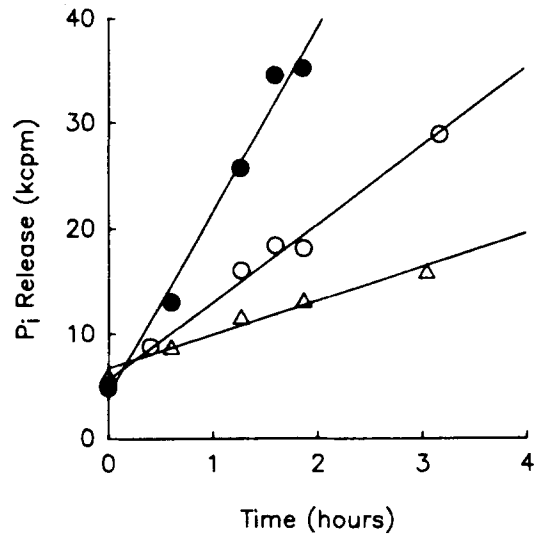


Figure 4. Effect of profilin on hydrolysis of ATP by monomeric actin. Equivalent volumes of profilin or control buffer (as in Fig. 3) were added to monomeric actin (final concentration 2.4 μM) that had been equilibrated with radioactive ATP to give final concentrations of 0 μM (\bullet), 4.8 μM (0.06 mg/ml; \circ), or 9.6 μM (0.12 mg/ml; Δ) profilin. The mixtures were assayed for radioactive phosphate release as a function of time. 6 kcpm represented hydrolysis of 1 mol ATP/mol actin. Lines were generated by linear regression analysis.

Examination of Profilin Disruptants

Previously, we reported that the profilin gene was essential for spore germination at 30°C (as determined by dissection of tetrads from the profilin disruption heterozygote, strain PFYDO, in which the profilin gene was disrupted at codon 54 by insertion of the *LEU2* gene; Magdolen et al., 1988).

Upon examination of a similar disruption heterozygote (strain 1830/ppfyV2; see Materials and Methods), we found that third and fourth (*Leu*⁺) segregants were able to germinate and grow, albeit slowly, when incubated on YEPD plates at 16, 22, or 30°C (these slow-growing segregants grew best at

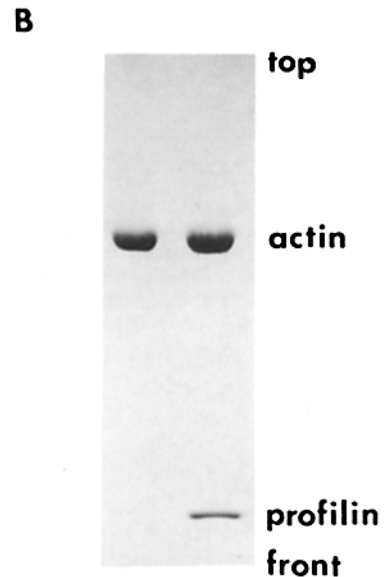
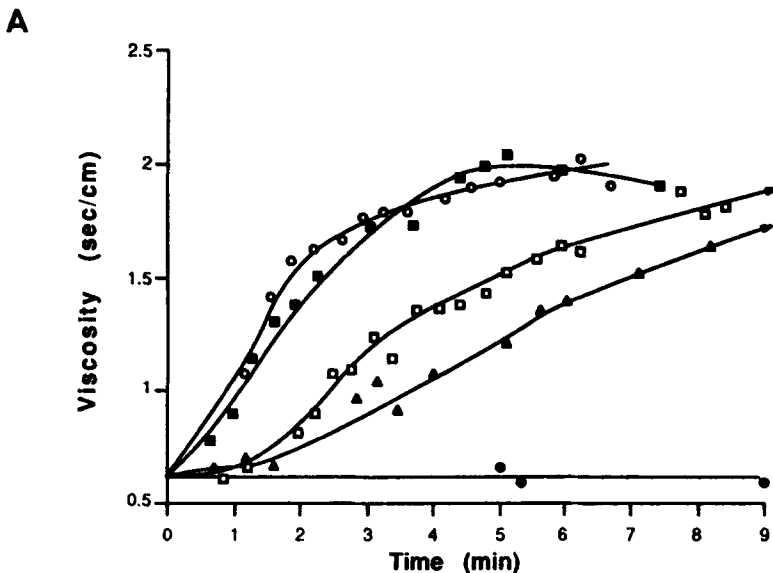


Figure 3. (A) Effect of profilin on the rate of actin polymerization. Equivalent volumes of profilin or control buffer (microconcentrator flow-through; see Materials and Methods) were added to monomeric actin (final concentration 6 μM or 0.25 mg/ml) in G buffer to give final concentrations of 0 μM (\circ), 3 μM (0.037 mg/ml; \blacksquare), 4.5 μM (0.055 mg/ml; \square), and 6 μM (0.074 mg/ml; \blacktriangle) profilin. As a control, actin was omitted from a sample containing 6 μM profilin (\bullet). Polymerization was initiated by the addition of 5 mM MgCl_2 at 0 min and assayed by low shear viscometry. (B) Gel of actin with and without equimolar profilin. After being used in polymerization assays, mixtures identical to those in A were run on an SDS-polyacrylamide gel. This panel demonstrates the purity of the actin and shows that the profilin preparation is not affecting polymerization by detectably proteolyzing actin.

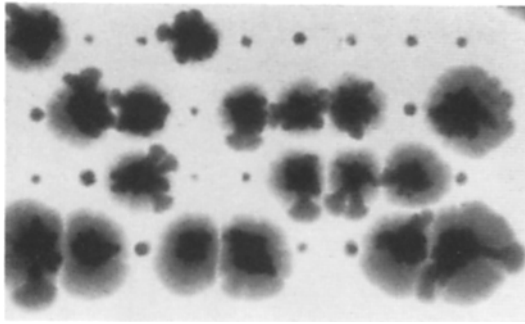


Figure 5. Dissection slab of spores of a profilin disruption heterozygote. Shown are the spore progeny of nine tetrads from strain 1830/ppfyV2 after 13 d at 22°C on a plate containing 1% yeast extract, 2% glucose.

22°C [Fig. 5] and significantly slower at 16 and 30°C [data not shown]. Incubation at 36°C prevented the growth of segregants carrying the disruption; these segregants formed only two to four large cells that eventually lysed. In addition, slow-growing segregants that arose at 22°C were unable to grow when shifted to 36°C, indicating that it is not simply germination that is temperature sensitive. Interestingly, the addition of 1 M sorbitol to the medium to provide osmotic support did not rescue cells at 36°C and even prevented germination of profilin disruptants at 16 and 22°C. Similarly, Novick and Botstein (1985) found that increased osmotic strength lowered the restrictive temperature of warm-sensitive actin mutants.

Further examination of the profilin disruptant, strain PFYDO, used to demonstrate essentiality of profilin in our previous study (Magdolen et al., 1988), showed that it too yielded many surviving third and fourth spores at 22 and 30°C but not at 36°C. These slow-growing, profilin-disrupted cells are phenotypically similar to those arising from 1830/ppfyV2. Since in our previous study, PFYDO was grown in 1% yeast extract, 2% glucose instead of YM-P medium (see Materials and Methods), we examined the effects of different growth media on the ability of profilin disruptants to grow. However, we found that growth (before sporulation) in 1% yeast extract, 2% glucose with or without 2% bactopectone and with or without autoclaving the glucose separately from the other components followed by sporulation in liquid SM-1 medium did not affect spore germination and growth at the temperatures examined (22 and 30°C).

In our previous study (Magdolen et al., 1988), PFYDO was sporulated on solid sporulation medium (plates containing potassium acetate); this resulted in tetrads that contained two normal spores and two spores that appeared shrunken and that failed to germinate. In contrast, sporulation in liquid SM-1 medium produces four phenotypically normal spores. Therefore, conditions of sporulation may be responsible for the lack of germination of profilin-deficient cells observed previously (Magdolen et al., 1988).

During the course of these studies, we dissected tetrads from a 1-mo-old SM-1 culture of 1830/ppfyV2, and incubated the spores on YEPD plates at 30°C. Interestingly, no more than two segregants per tetrad grew (these segregants were *Leu*⁻, grew at wild-type rates, and formed normal colonies). The third and fourth spores failed to bud and eventually lysed. Thus, the age of the sporulated culture may

also affect the ability of profilin-disrupted cells to germinate and grow.

Complete Deletion of the Profilin Gene

The profilin disruptants could have residual profilin function from remaining NH₂-terminal sequences. Therefore, we deleted the entire profilin coding region. This deletion was performed in a strain designed to eliminate possible heterozygous suppressors of the profilin disruption (since a heterozygous suppressor was found to be responsible for the viability of clathrin-deficient yeast [Lemmon and Jones, 1987; cf. Payne and Schekman, 1985]). First, we took the two wild-type (PFY⁺) segregants from a tetrad of 1830/ppfyV2 having four surviving spores; if a heterozygous suppressor were present (either linked or unlinked to the profilin gene), it should be present in the profilin-deficient cells only. These two wild-type (putative *Sup*⁻) segregants were then mated to form a diploid, which was transformed with the 5-kb Bam HI–Hind III fragment from plasmid pSK-HBL (Fig. 1; see Materials and Methods) to replace one copy of the profilin gene with the *LEU2* gene (this construction removes the entire profilin coding region as well as 119 bp of upstream and 78 bp of downstream noncoding sequence). The transformant analyzed was found to be stably *Leu*⁺ (suggesting that integration had occurred), and Southern blot analysis revealed the expected bands for a proper gene replacement event (data not shown). Tetrad analysis of this strain gave results indistinguishable from those of 1830/ppfyV2 when examined at 16, 22, 30, and 36°C. Thus, possible expression of a truncated profilin does not account for the growth of profilin-disrupted cells. Furthermore, we conclude that the growth of profilin-deficient cells was not due to a heterozygous suppressor.

Characterization of Profilin-deficient Cells

Segregants from 1830/ppfyV2 were examined for the presence of profilin by probing blots of total protein from these strains with yeast profilin-specific antibodies (Fig. 6). Whereas *Leu*⁻ segregants contained immunoreactive profilin (Fig. 6, lane 3), 11 of 11 *Leu*⁺, *Ade*⁻ segregants examined showed no detectable bands in the region of the blot probed (from ~18 kD down to the dye front; Fig. 6, lanes 1 and 2). Probing with anti-actin antibodies showed that there is no gross alter-

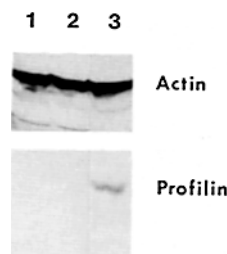
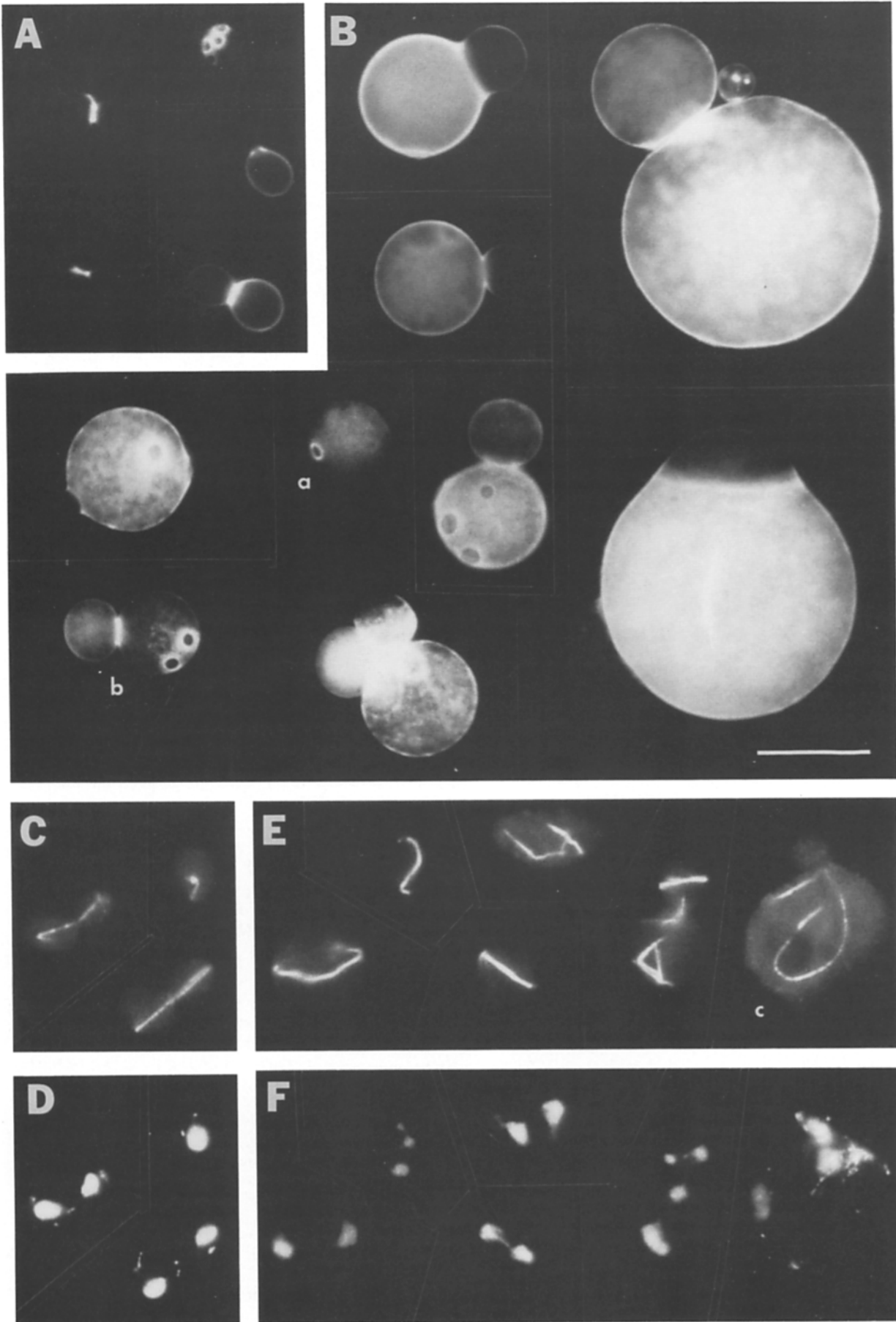


Figure 6. Immunoblot of total proteins from profilin-deficient and wild-type cells, probed with anti-profilin and anti-actin antibodies. Total protein from two different profilin-disrupted (*pfy::LEU2*) segregants of 1830/ppfyV2 (lanes 1 and 2) and a “wild-type” (PFY⁺, *Leu*⁻) segregant of 1830/ppfyV2 (lane 3). The lower panel contains the region of the blot from ~18 kD down to the dye front and was probed with affinity-purified anti-profilin antibodies at 1:20 dilution; the upper panel contains a region of the blot centered around 42 kD and was probed with anti-actin antibodies at 1:500 dilution. Secondary antibody for each was horseradish peroxidase-conjugated goat anti-rabbit IgG at 1:200 dilution. Ponceau S staining of the blot demonstrated that approximately equal amounts of protein were loaded in each lane.



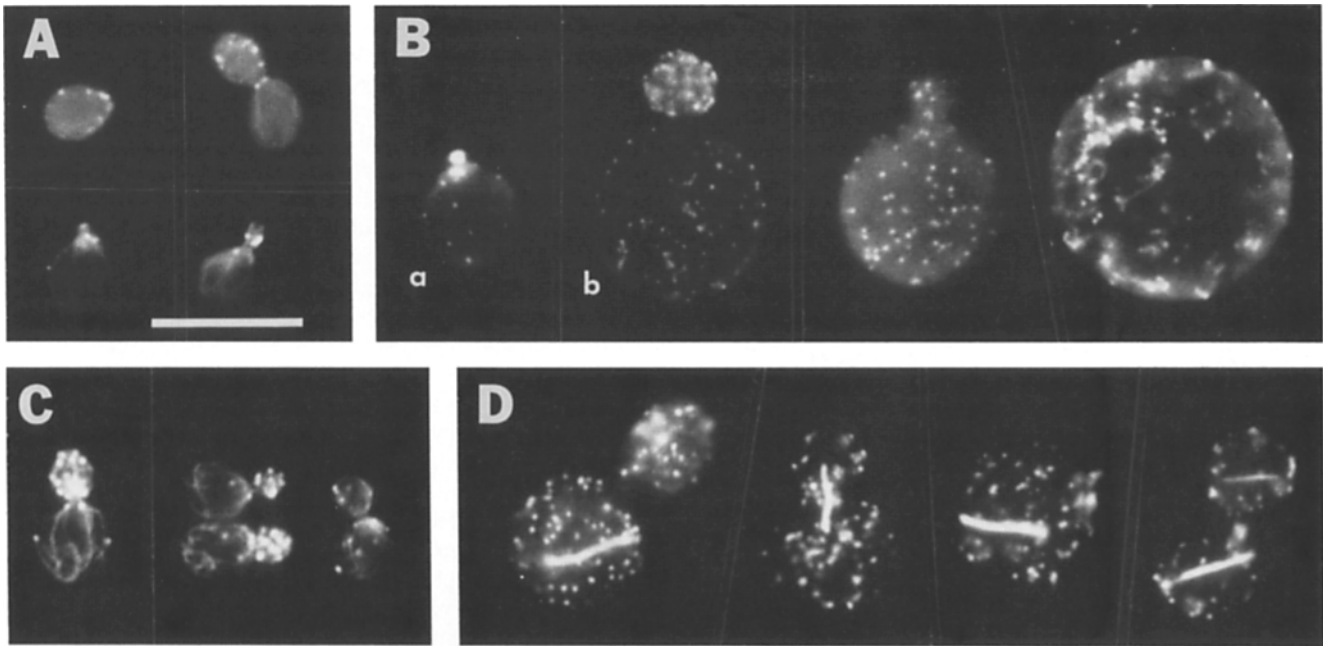


Figure 8. Actin staining in wild-type and profilin-deficient cells. Wild-type (*A* and *C*) and profilin-deficient (*B* and *D*) cells stained with rhodamine-conjugated phalloidin (*A* and *B*) or anti-yeast actin antibodies (*C* and *D*). Strains are the same as in Fig. 7. Individual cells are labeled for reference in the text. Bar, 10 μ m.

ation in actin levels in profilin-deficient cells (Fig. 6). Although these results show that the disruptants lack a complete profilin protein, we cannot rule out a truncated protein that is not recognized by our antibody preparation.

Cells lacking immunoreactive profilin display a variety of aberrant characteristics. At 22°C they grow with a doubling time approximately three times that of a comparable wild-type strain (a PFY⁺, Leu⁻ segregant of 1830/ppfyV2). The cells lose the ellipsoidal shape of normal yeast cells, becoming round, and display a wide range of sizes; some cells from late log phase cultures were estimated to be more than 50 times the volume of normal haploid cells (compare cells of Fig. 7, *A* with *B*), though most of the cells do not get that large.

Yeast cells deposit a ring of chitin in the cell wall through which the bud emerges (Pringle and Hartwell, 1981); the chitin ring persists on the mother cell after budding (see Fig. 7 *A*). Staining of cell wall chitin in profilin-deficient cells (Fig. 7 *B*) shows a marked delocalization of chitin deposition. This delocalization is quite heterogeneous and appears to be roughly correlated with cell size (and, thus, perhaps age). Although some of these cells are able to form nearly normal chitin rings (Fig. 7 *B*, cells *a* and *b*), the usual unipolar budding pattern of haploid cells (Streiblová, 1970) often appears disrupted (Fig. 7 *B*, cell *b*).

The above findings suggested that cell growth was continuing in the absence of efficient cell division (budding). Thus, we asked if the nuclear division cycle were also disrupted.

Nuclear localization (as visualized by DAPI; Fig. 7 *F*) and microtubule staining (Fig. 7 *E*) demonstrate that nuclear division continues in the absence of normal budding, resulting in multinucleate cells (cf. PFY⁺ cells of Fig. 7, *C* and *D*). 10–20% of the unbudded cells are binucleate and occasional cells contain more than two nuclei. The nuclei of uninucleate budded cells were often positioned away from the neck region in contrast to wild-type cells, where nuclei are generally at or near the mother bud neck. In addition, microtubules in profilin-deficient cells often take on an abnormal appearance, sometimes meandering throughout the cell (Fig. 7 *E*, cell *c*), whether or not a bud is present. Therefore, although the nuclear division cycle continues, perturbations in the actin cytoskeleton may be directly or indirectly affecting the proper positioning of nuclei and microtubules.

Actin Localization in Profilin-deficient Cells

Actin in wild-type cells (Fig. 8, *A* and *C*) occurs in the form of cables, generally running longitudinally through the cell, and as cortical spots, which, in budding cells, occur almost exclusively on the bud (Kilmartin and Adams, 1984; Adams and Pringle, 1984; Novick and Botstein, 1985; Drubin et al., 1988). The distribution of actin in profilin-deficient haploid cells was examined by staining cells with rhodamine-conjugated phalloidin (Fig. 8 *B*) and with anti-yeast actin antibodies (Fig. 8 *D*). In most of these cells, phalloidin staining shows a random distribution of cortical actin spots and an ab-

Figure 7. Chitin, microtubule, and nuclear distribution in wild-type and profilin-deficient cells. Wild-type (*A* and *C*) and profilin-deficient (*B* and *E*) cells stained with Calcofluor (*A* and *B*) or anti-tubulin antibodies (*C* and *E*). (*D* and *F*) DAPI staining of the same cells shown in *C* and *E*, respectively. Cells in *B*, *E*, and *F* are from several different profilin-deficient segregants of 1830/ppfyV2; each segregant demonstrated the same heterogeneous morphologies. Cells in *A*, *C*, and *D* are from PFY⁺, Leu⁻ (“wild type”) segregants of 1830/ppfyV2. Individual cells are labeled for reference in the text. Bar, 10 μ m.

sence of detectable actin cables (Fig. 8 B). Some profilin-deficient cells, however, do maintain an asymmetric distribution of cortical spots as seen in Fig. 8 B, cell *a*, and to a lesser extent in cell *b*. Staining with anti-yeast actin antibodies (Fig. 8 D) reveals a somewhat different actin distribution. As with phalloidin, most cells show a random distribution of cortical spots and an absence of normal actin cables. However, actin-specific antibodies also identify thick actin bars within most profilin-deficient cells (Fig. 8 D), generally with only one actin bar per cell.

Discussion

Phenotypic analysis of profilin-deficient yeast cells indicates that profilin affects actin function *in vivo*, consistent with *in vitro* observations. A number of the defects are similar to those seen in *act1* mutant cells, which carry a conditional mutation in the single yeast actin gene (Novick and Botstein, 1985). These similarities, discussed below, include the larger and rounder morphology, slow growth, osmotic sensitivity, and abnormalities in chitin and actin localization. It has been concluded from these and other phenotypes (Novick and Botstein, 1985), as well as from the localization of actin in wild-type cells (Kilmartin and Adams, 1984; Adams and Pringle, 1984), that one function of actin is to direct secretion to regions of active growth (largely confined to the bud; see Adams and Pringle [1984] and references cited therein). However, at least some of these phenotypes may be relatively nonspecific (Novick et al., 1989). While it seems quite likely that the phenotypes of profilin-deficient cells result from the loss of actin-profilin interaction, we remain alert to the possibility that profilin has effects independent of its interactions with actin.

Actin is found in both cortical spots and cables in wild-type cells. Profilin-deficient mutants, temperature-sensitive actin mutants (Novick and Botstein, 1985), tropomyosin-deficient mutants (Liu and Bretscher, 1989), and some *sac* (suppressor of actin) mutants (Novick et al., 1989) lack detectable actin cables, show a delocalization of actin spots, and, in some cases, display actin bars. These effects may result from improper regulation of actin assembly or from loss of actin stabilization by associated proteins. Since actin bar formation also results from overexpression of an 85-kD actin-binding protein (Drubin et al., 1988), a balance among various actin-binding proteins and actin may be involved. Our finding that the actin bars stain with antibody but not phalloidin suggests that these structures may not be composed of typical actin filaments, as phalloidin binds specifically to filamentous actin (Estes et al., 1981). The observation that tropomyosin, which binds filamentous actin, is not detectable in the bars of the *act1-2* mutant (Liu and Bretscher, 1989) is consistent with this suggestion. The fact that the 85-kD protein was isolated by affinity to filamentous actin but nonetheless localizes to actin bars but not cables (Drubin et al., 1988) may suggest that this protein can interact with nonfilamentous actin. An alternative hypothesis is that tropomyosin and phalloidin binding, but not 85-kD protein binding, are somehow blocked in the bars. Finally, it is possible that subtle differences exist in the bars seen in these different cases.

The delocalization of cell wall chitin and increase in cell size seen in profilin-deficient cells are phenotypes also as-

sociated with defects in the actin gene and in the genes of several putative actin-associated proteins (Novick and Botstein, 1985; Novick et al., 1989) and when the 85-kD actin-binding protein is overproduced (Drubin et al., 1988). Another class of mutants, typified by the temperature-sensitive cell division-cycle mutant, *cdc24* (Sloat et al., 1981), also shows an increase in cell size and random deposition of cell wall chitin and is unable to bud at restrictive temperature. Sloat et al. (1981) attributed these phenotypes to the failure of polarized secretion and the inability to properly organize the budding site. Thus, the delocalization of cell wall chitin and dramatic increase in cell size of profilin-deficient cells suggest a breakdown in the targeting of secretion and perhaps in the selection and organization of the budding site due to lack of actin regulation by profilin.

Profilin-deficient cells are unable to grow at high temperatures. One possibility consistent with this finding is that actin is more susceptible to denaturation at higher temperatures, increasing the importance of stabilization of actin monomer by profilin. It is possible that the temperature sensitivity of the actin mutants reported by Shortle et al. (1984) is a related phenomenon; the mutations in the actin gene may exacerbate the postulated thermal lability of actin. We have preliminary evidence consistent with this possibility; actin isolated from *act1-3* mutant cells (*act1-3* is equivalent to *act1-1* [Shortle et al., 1984]) shows a time-dependent loss in the ability to polymerize (relative to wild-type actin) when incubated at 37°C (data not shown).

We have found conditions that allow yeast cells to survive in the absence of profilin. These observations are compatible with our previous report (Magdolen et al., 1988) in which we found that profilin was essential for spore germination, as composition of the sporulation medium and age of the sporulated culture can affect the ability to germinate. (The latter observation is not unprecedented; for related examples see Johnson et al. [1985] and Finley et al. [1987].) Such a phenomenon may reflect a threshold of profilin necessary for proper spore formation or germination; even low levels of protein turnover in spores lacking the profilin gene could reduce the level of residual profilin below this threshold.

We also addressed the possibility that a simple suppressor was responsible for the growth of cells lacking profilin. While it appears that no such heterozygous suppressor is present, there may be a homozygous suppressor present in the genetic background of the strains used in this study. However, since this arrangement would have to exist in at least two genetically distinct backgrounds, it would be inappropriate to postulate a suppressor unless a genetic configuration is identified in which the profilin deletion is not "suppressed" (for related discussions see Technical Comments of Lemmon and Jones [1988] and Schekman and Payne [1988]).

To address the question of whether profilin from yeast behaves like other profilins, we have purified yeast profilin and studied its interaction with yeast actin. We have shown that yeast profilin retards the assembly of yeast actin and the hydrolysis of ATP by actin monomer in a manner quantitatively similar to that of *Acanthamoeba* profilin, suggesting that the affinity of profilin for actin is in the same range as reported for *Acanthamoeba* and other organisms (2–10 μM; Pollard and Cooper, 1986). In addition, we have found that yeast profilin binds to polyproline, a property exhibited by mammalian profilins (Tanaka and Shibata, 1985; Lind et al.,

1987; Lindberg et al., 1988). Thus, many of the conclusions that have been drawn about the mode of action of profilin in other organisms may well be valid for yeast. For instance, biochemical studies indicate that profilin cannot be solely responsible for the high actin monomer concentration in cells, leading to the speculation that profilin acts in concert with other regulatory proteins. This might explain our finding that profilin is beneficial but not essential in yeast. The physiological role of profilin's inhibition of actin-mediated ATP hydrolysis is not known; molecular genetic studies may allow us to shed some light on the significance of this interaction.

Our observations that profilin can be isolated from yeast in a native form whose activity we can assay, and that viable cells lacking profilin can be obtained, set the stage for biochemical as well as in vivo studies of mutant profilins and their interaction with actin. In addition, further studies may reveal important functions of profilin that are independent of its association with actin.

We thank E. Jones and D. Botstein for yeast strains used in these studies and J. Pringle, D. Botstein, and D. Drubin for helpful comments.

This work was supported by National Institutes of Health grants GM34967 to S. S. Brown and GM21253 to D. Botstein; a grant by the Deutsche Forschungsgemeinschaft to W. Bandlow; and postdoctoral stipends to B. K. Haarer from The University of Michigan National Institutes of Health Training Grant in Developmental Biology (HD07274) and from National Institutes of Health grant GM12981. A. E. M. Adams is a Burroughs Wellcome Fund Fellow of the Life Sciences Research Foundation.

Received for publication 8 August 1988 and in revised form 15 September 1989.

References

- 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.
- Ampe, C., J. Vandekerckhove, S. L. Brenner, L. Tobacman, and E. D. Korn. 1985. The amino acid sequence of *Acanthamoeba* profilin. *J. Biol. Chem.* 260:834-840.
- 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.
- 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.
- Carlsson, L., F. Markey, I. Blikstad, T. Persson, and U. Lindberg. 1979. Reorganization of actin in platelets stimulated by thrombin as measured by the DNase I inhibition assay. *Proc. Natl. Acad. Sci. USA.* 76:6376-6380.
- Drubin, D. G., K. G. Miller, and D. Botstein. 1988. Yeast actin-binding proteins: evidence for a role in morphogenesis. *J. Cell Biol.* 107:2551-2561.
- Estes, J. E., L. A. Selden, and L. C. Gershman. 1981. Mechanism of action of phalloidin on the polymerization of muscle actin. *Biochemistry.* 20:708-712.
- Finley, D., E. Özkaynak, and A. Varshavsky. 1987. The yeast polyubiquitin gene is essential for resistance to high temperature, starvation, and other stresses. *Cell.* 48:1035-1046.
- Gordon, D. J., Y.-Z. Yang, and E. D. Korn. 1976. Polymerization of *Acanthamoeba* actin. *J. Biol. Chem.* 251:7474-7479.
- 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.
- Johnson, L. M., M. Snyder, L. M. S. Chang, R. W. Davis, and J. L. Campbell. 1985. Isolation of the gene encoding yeast DNA polymerase I. *Cell.* 43:369-377.
- 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.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
- Laub, F., M. Kaplan, and C. Gitler. 1981. Actin polymerization accompanies Thy-1-capping on mouse thymocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 124:35-38.
- Lee, S., M. Li, and T. D. Pollard. 1988. Evaluation of the binding of *Acanthamoeba* profilin to pyrene-labeled actin by fluorescence enhancement. *Anal. Biochem.* 168:148-155.
- Lemmon, S. K., and E. W. Jones. 1987. Clathrin requirement for normal growth of yeast. *Science (Wash. DC)* 238:504-509.
- Lemmon, S. K., and E. W. Jones. 1988. Technical comments. *Science (Wash. DC)* 239:919.
- Lillie, S. H., and S. S. Brown. 1987. Artfactual immunofluorescent labelling in yeast, demonstrated by affinity purification of antibody. *Yeast.* 3:63-70.
- Lillie, S. H., and J. R. Pringle. 1980. Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. *J. Bacteriol.* 143:1384-1394.
- Lind, S. E., P. A. Janmey, C. Chaponnier, T.-J. Herbert, and T. P. Stossel. 1987. Reversible binding of actin to gelsolin and profilin in human platelet extracts. *J. Cell Biol.* 105:833-842.
- Lindberg, U., C. E. Schutt, E. Hellsten, A.-C. Tjäder, and T. Hult. 1988. The use of poly(L-proline)-Sepharose in the isolation of profilin and profilactin complexes. *Biochim. Biophys. Acta.* 967:391-400.
- Liu, H., and A. Bretscher. 1989. Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell.* 57:233-242.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Magdolen, V., U. Oechsner, G. Müller, and W. Bandlow. 1988. The intron-containing gene for yeast profilin (PFY) encodes a vital function. *Mol. Cell. Biol.* 8:5108-5115.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
- Novick, P., and D. Botstein. 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell.* 40:405-416.
- Novick, P., B. C. Osmond, and D. Botstein. 1989. Suppressors of yeast actin mutations. *Genetics.* 121:659-674.
- Oechsner, U., V. Magdolen, and W. Bandlow. 1987. The cDNA and deduced amino acid sequence of profilin from *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 15:9078.
- Oechsner, U., V. Magdolen, and W. Bandlow. 1988. Corrigendum. *Nucleic Acids Res.* 16:1232.
- Ozaki, K., H. Sugino, T. Hasegawa, S. Takahashi, and S. Hatano. 1983. Isolation and characterization of *Physarum* profilin. *J. Biochem.* 93:295-298.
- Payne, G. S., and R. Schekman. 1985. A test of clathrin function in protein secretion and cell growth. *Science (Wash. DC)* 230:1009-1014.
- Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem.* 83:346-356.
- Pollard, T. D. 1982. A falling ball apparatus to measure filament cross-linking. *Methods Cell Biol.* 24:301-311.
- 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.
- Pringle, J. R., and L. H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. In *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 97-142.
- Pringle, J. R., R. A. Preston, A. E. M. Adams, T. Stearns, D. G. Drubin, B. K. Haarer, and E. W. Jones. 1989. Fluorescence microscopy methods for yeast. *Methods Cell Biol.* 31:357-435.
- Rao, K. M. K., and J. Varani. 1982. Actin polymerization induced by chemotactic peptide and concanavalin A in rat neutrophils. *J. Immunol.* 129:1605-1607.
- Reichstein, E., and E. D. Korn. 1979. *Acanthamoeba* profilin—a protein of low molecular weight from *Acanthamoeba castellanii* that inhibits actin nucleation. *J. Biol. Chem.* 254:6174-6179.
- Schekman, R., and G. Payne. 1988. Technical comments. *Science (Wash. DC)* 239:919.
- Sherman, F., G. R. Fink, and J. B. Hicks. 1986. *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 186 pp.
- Shortle, D., P. Novick, and D. Botstein. 1984. Construction and genetic characterization of temperature-sensitive mutant alleles of the yeast actin gene. *Proc. Natl. Acad. Sci. USA.* 81:4889-4893.
- Sloat, B. F., and J. R. Pringle. 1978. A mutant of yeast defective in cellular morphogenesis. *Science (Wash. DC)* 200:1171-1173.
- Sloat, B. F., A. Adams, and J. R. Pringle. 1981. Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. *J. Cell Biol.* 89:395-405.
- Sonobe, S., S. Takahashi, S. Hatano, and K. Kuroda. 1986. Phosphorylation of *Amoeba* G-actin and its effect on actin polymerization. *J. Biol. Chem.* 261:14837-14843.
- Spudich, J. A., and R. Cooke. 1975. Supramolecular forms of actin from amoebae of *Dictyostelium discoideum*. *J. Biol. Chem.* 250:7485-7491.
- Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner. 1985. Nonmuscle actin-binding proteins. *Annu. Rev. Cell Biol.* 1:353-402.
- Streiblová, E. 1970. Study of scar formation in the life cycle of heterothallic *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 16:827-831.
- Sugino, Y., and Y. Miyoshi. 1964. The specific precipitation of orthophosphate and some biochemical applications. *J. Biol. Chem.* 239:2360-2364.

- 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.
- Tilney, L. G., S. Hatano, H. Ishikawa, and M. S. Mooseker. 1973. The polymerization of actin: its role in the generation of the acrosomal process of certain echinoderm sperm. *J. Cell Biol.* 59:109-126.
- Tobacman, L. S., and E. D. Korn. 1982. The regulation of actin polymerization and the inhibition of monomeric actin ATPase activity by *Acanthamoeba* profilin. *J. Biol. Chem.* 257:4166-4170.
- Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
- Tuderman, L., E.-R. Kuutti, and K. I. Kivirikko. 1975. An affinity-column procedure using poly(L-proline) for the purification of prolyl hydroxylase. *Eur. J. Biochem.* 52:9-16.
- Weeds, A. 1982. Actin-binding proteins—regulators of cell architecture and motility. *Nature (Lond.)*. 296:811-816.
- Zechel, K. 1980. Isolation of polymerization-competent cytoplasmic actin by affinity chromatography on immobilized DNAse I using formamide as eluant. *Eur. J. Biochem.* 110:343-348.