

Cofilin Is an Essential Component of the Yeast Cortical Cytoskeleton

Anne L. Moon, Paul A. Janmey,* K. Andrea Louie,† and David G. Drubin

Department of Molecular and Cell Biology, University of California, Berkeley, California 94720; * Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115; and † Genentech, Inc., South San Francisco, California 94080

Abstract. We have biochemically identified the *Saccharomyces cerevisiae* homologue of the mammalian actin binding protein cofilin. Cofilin and related proteins isolated from diverse organisms are low molecular weight proteins (15–20 kD) that possess several activities in vitro. All bind to monomeric actin and sever filaments, and some can stably associate with filaments. In this study, we demonstrate using viscosity, sedimentation, and actin assembly rate assays that yeast cofilin (16 kD) possesses all of these properties. Cloning and sequencing of the *S. cerevisiae* cofilin gene (*COFI*) revealed that yeast cofilin is 41% identical in amino acid sequence to mammalian cofilin and, surprisingly, has homology to a protein outside the family

of cofilin-like proteins. The NH₂-terminal 16 kD of Abplp, a 65-kD yeast protein identified by its ability to bind to actin filaments, is 23% identical to yeast cofilin. Immunofluorescence experiments showed that, like Abplp, cofilin is associated with the membrane actin cytoskeleton. A complete disruption of the *COFI* gene was created in diploid cells. Sporulation and tetrad analysis revealed that yeast cofilin has an essential function in vivo. Although Abplp shares sequence similarity with cofilin and has the same distribution as cofilin in the cell, multiple copies of the *ABPI* gene cannot compensate for the loss of cofilin. Thus, cofilin and Abplp are structurally related but functionally distinct components of the yeast membrane cytoskeleton.

MUSCLE contraction, cytokinesis, cell locomotion, and cytoplasmic streaming are among the numerous cellular processes that are mediated by the regulated interactions between actin and actin binding proteins in eukaryotic cells. Most of the actin binding proteins that function in these various processes can be grouped into a small number of classes based on biochemical activities and/or structural similarities (Stossel et al., 1985; Pollard and Cooper, 1986; Hartwig and Kwiatkowski, 1991; Vandekerckhove and Vancompernelle, 1992). Although yeast cells appear to exhibit a more modest variety of actin-based processes than more complex eukaryotes, representatives of most known classes of actin binding proteins have been identified in *S. cerevisiae* (Barnes et al., 1990). Genetic analyses in yeast have demonstrated that the activities of these actin binding proteins are important for proper actin function. Specifically, mutations in the genes that encode actin binding proteins (Liu and Bretscher, 1989a; Amatruda et al., 1990; Haarer et al., 1990; Adams et al., 1991; Johnston et al., 1991) often result in phenotypes similar to those of actin mutants (Novick and Botstein, 1985). It has been demonstrated that many of these yeast proteins, including capping protein, tropomyosin, profilin, and fimbrin, interact with actin in vitro much like their homologues from other organisms (Liu and Bretscher, 1989b; Haarer et al., 1990; Adams et al., 1991; Amatruda and Cooper, 1992). Because organisms with

very different uses for actin contain a seemingly similar arsenal of actin binding proteins, the wide variety of actin-based processes is likely to arise through the novel choreography of the activities of these ubiquitous proteins. It has not yet been possible to explain how the activities of actin binding proteins are collectively controlled and put to use to generate diverse biological phenomena.

The opportunity to apply genetic analysis in *S. cerevisiae* to the study of protein function in the context of a living cell provides incentive to identify the full battery of actin regulatory proteins from this organism. To date, members of the two families of actin filament severing proteins have not yet been identified in yeast. Both the gelsolin/villin/fragmin-type severing proteins and the smaller cofilin/destrin-type proteins appear to be widespread, having been found in vertebrates and simpler eukaryotes (reviewed by Vandekerckhove and Vancompernelle, 1992). Both classes of proteins possess activities in addition to filament severing. For example, members of the gelsolin family exhibit capping and sometimes nucleating and/or bundling activity (reviewed in Hartwig and Kwiatkowski, 1991). The small cofilin-type proteins are also multifunctional in vitro, displaying monomer binding and sometimes filament binding activities (see Hartwig and Kwiatkowski, 1991). Potential modes of regulation have been suggested by biochemical studies; the gelsolin-type protein activities are Ca²⁺ dependent, cofilin activity is affected by pH, and the activities of both types can be modulated by binding to phosphatidylinositol 4,5-bisphosphate (reviewed in Vandekerckhove, 1990).

Dr. Louie's present address is Applied Biosystems, Inc., Foster City, CA 94404.

In this study, we show that when yeast extracts are fractionated by conventional chromatography, several activities that increase or decrease the viscosity of an actin solution become apparent, demonstrating that yeast extracts contain a larger variety of actin regulatory activities than was previously recognized. This is of interest because the yeast *S. cerevisiae* is a simple, nonmotile organism, and it was not possible to predict whether yeast actin function would require the diverse repertoire of actin binding proteins characteristic of other cells. A protein that decreases the viscosity of an actin solution was purified to near-homogeneity and was shown to be the yeast homologue of the actin filament severing protein, cofilin. Yeast cofilin is a component of the cortical actin cytoskeleton, and the gene that encodes cofilin (*COF1*) is an essential gene. These results suggest that the essential role of cofilin involves regulation of actin assembly in the membrane cytoskeleton, a protein complex important for the polarized growth of yeast cells.

Materials and Methods

Plasmid and Strain Construction

All DNA manipulations were performed by standard techniques (Maniatis et al., 1982). Restriction endonucleases and other enzymes were obtained from either New England Biolabs (Beverly, MA) or Boehringer Mannheim Corp. (Indianapolis, IN), except for Taq DNA polymerase, which was obtained from Perkin-Elmer/Cetus (Norwalk, CT) and Sequenase (modified T7 DNA polymerase), which was obtained from United States Biochemical Corp. (Cleveland, OH).

The yeast strains used in this study are listed in Table I. Media were prepared, and standard genetic techniques of crossing, sporulation, and tetrad analysis were performed as described in Rose et al. (1990). Two *cof1* disruption mutants were constructed. An internal fragment of the *COF1* gene was used to create the partial disruption of *COF1* (*cof1-1::URA3*), which consists of two incomplete copies of *COF1*, one encoding a protein missing the COOH-terminal 19 amino acids (the 19 terminal codons are replaced with a single stop codon), and the other copy missing the upstream regulatory sequences and the first 46 codons. This was done by linearizing a pRS306-based vector (Sikorski and Hieter, 1989) containing a 270-bp polymerase chain reaction (PCR)¹ product of the *COF1* gene (region from nucleotides 655 to 888 delineated by PCR primers ALM2 and ALM4 in Fig. 5) by cutting with BglII, which recognizes a unique site within the 270-bp fragment (at residue 802) (see Fig. 5). DDY288 was transformed with this DNA by LiAc transformation (Ito et al., 1983) as modified by Schiestl and Gietz (1989), and heterozygous disruptants (DDY425) were recovered based on their Ura⁺ phenotype and confirmed by Southern hybridization. The complete disruption of *COF1* (*cof1-Δ::HIS3*) was created by transforming DDY426 with a 2.8-kb linear fragment composed of a 345-bp EcoRI-BglII fragment containing the first two codons of *COF1* and upstream regions, a 2-kb fragment containing the *HIS3* gene, and a 469-bp MnlI-SphI fragment containing the last eight codons of *COF1* and downstream sequence (see Fig. 5).

Purification of Yeast Cofilin

Yeast lysates were prepared from bricks of commercial Red Star baker's yeast (Universal Foods Corp., Oakland, CA) as previously described (Drubin et al., 1988). For cell lysis, each brick (~525 g of wet cells) was suspended in 450 ml lysis buffer (5 mM Hepes, pH 7.5, 1 mM EDTA, 20 mM KCl, 0.1% NP-40) before disrupting the cells. To minimize proteolysis, cell lysis and all subsequent chromatography steps were performed at 4°C and in the presence of protease inhibitors, all obtained from Sigma Chemical Co. (St. Louis, MO). Lysis buffer contained a 10⁻² dilution of ethanol protease inhibitor cocktail (100× = ethanol solution containing 0.1 M PMSF and 0.1 mg/ml phenanthroline) and a 10⁻³ dilution of aqueous pro-

tease inhibitor cocktail (1,000× = 0.5 mg/ml of antipain, leupeptin, pepstatin A, chymostatin, and aprotinin). Column buffers contained only the aqueous protease inhibitors. After a 16,000 g clearing spin, the lysate was centrifuged at 135,000 g for 120 min, yielding a high speed supernatant (HSS), our starting material.

All column resins were obtained from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). HSS was loaded on a 2.5 × 17.5-cm (85 ml) Q-Sepharose column equilibrated in lysis buffer. The column was washed with 8 column volumes of column buffer (10 mM Tris-HCl, pH 7.5, 0.5 mM EGTA, 20 mM KCl, 1 mM DTT) and eluted with a linear gradient of KCl (from 20 to 350 mM over 7.5 column volumes). The fractions containing a viscosity-decreasing activity that eluted from the column in 50–150 mM KCl were pooled. The proteins that precipitated between 35 and 85% saturation of ammonium sulfate were dissolved in a minimum volume of column buffer and dialyzed against two changes of column buffer. The ammonium sulfate precipitation step was used primarily to concentrate the protein for chromatography on a 2.6 × 100-cm (530 ml) Sephacryl S-100 gel filtration column. Three viscosity-decreasing activities were separated on this column. The first peak of activity eluted from 200 to 220 ml, the second from 230 to 250 ml, and the third from 260 to 290 ml. The fractions that contained the third peak of activity were pooled and contained a major protein with an apparent molecular mass of 18 kD. Additional purification was achieved by using fast protein liquid chromatography grade columns: a 5 × 50-mm (1 ml) Mono Q column and a 1 × 30-cm (24 ml) Superose 12 HR gel filtration column run using the Pharmacia fast protein liquid chromatography Chromatography Control System (Pharmacia LKB Biotechnology Inc.). Gel filtration was performed in column buffer, the Mono Q column was loaded in column buffer and eluted with a linear gradient of KCl from 20 to 350 mM. We estimate that by the final step (Mono Q with column buffer containing 10 mM Tris-HCl, pH 8.0), the 16-kD cofilin protein constitutes >94% of the protein as estimated by visual comparison with dilutions of Coomassie blue-stained standards.

Rabbit Muscle Actin Preparation

Acetone powder was prepared from rabbit muscle as previously described by Pardee and Spudich (1982). Actin was extracted, clarified, and polymerized, and tropomyosin was removed as described, but with a slightly modified buffer A (5 mM Tris 8.0, 0.2 mM CaCl₂, 0.2 mM ATP, 0.2 mM DTT, 0.02% Na azide). Filamentous actin (F-actin) was retrieved by centrifugation and dialyzed against three changes of buffer A over 2 d. F-actin was removed by centrifugation and aliquots of monomeric actin (globular, or G-actin) were quick-frozen in liquid nitrogen and stored at -80°C.

Falling Ball Viscometry Assay

Falling ball viscometry (Pollard, 1982) was applied as the primary assay to screen fractionated proteins for actin binding protein activity. Our apparatus consisted of a 100-μl capillary tube (Fisher Scientific Co., Pittsburgh, PA), a 12° ramp, and a 0.025-in.-diam stainless steel ball bearing (ABEK Inc., Bristol, CT). Rabbit muscle actin was used at a concentration of 4–6 μM. 10 μl of protein fraction, diluted protein fraction, or column buffer was added to the actin before initiation of polymerization. Actin was allowed to polymerize within the capillary tube for ~1 h at 23°C before viscosity measurements were taken.

Pyrene-labeled Actin Assembly

Pyrene-labeled actin was prepared by modifying the methods of Kouyama and Mihashi (1981). Briefly, 100 mg of polymerized actin was pelleted and homogenized in buffer P (10 mM Hepes, 7.4, 2 mM MgCl₂, 100 mM KCl, 0.5 mM ATP). 5.6 mg of *N*-(1-pyrenyl)iodoacetamide (Molecular Probes, Inc., Eugene, OR), previously dissolved in dimethyl formamide at a concentration of 14 mg/ml, was added with agitation. The solution was incubated at 4°C in the dark overnight. The labeled actin was dialyzed against three changes of buffer A for 24 h, and then F-actin and precipitated *N*-(1-pyrenyl)iodoacetamide were removed by centrifugation at 100,000 g for 2 h. Aliquots of pyrene-labeled G-actin were quick-frozen in liquid nitrogen and stored at -80°C.

For assembly experiments, actin was used at a concentration of 5 μM. Pyrene-labeled actin constituted 5–20% of total actin. Because some polymerization of actin occurs during the freezing of aliquots, thawed actin was spun at 312,000 g (90,000 rpm; TLA100 rotor; Beckman Instruments, Carlsbad, CA) for 40 min before assembly initiation to remove F-actin. Before initiation of polymerization, actin was stored in buffer A. After addition of column buffer (described above) and polymerization initiating

1. Abbreviations used in this paper: F-actin, filamentous actin; G-actin, globular actin (monomeric actin); HSS, high speed supernatant; PCR, polymerase chain reaction.

Table 1. Yeast Strains Used in This Study

Name	Genotype	Source
DDY177	<i>MATa, his4-619, act1-2</i>	2
DDY288	<i>MATa, ura3-52, his4-619, leu2-3,112</i> <i>MATα, ura3-52, +, +</i>	1
DDY425	<i>MATa, ura3-52, his4-619, leu2-3,112, cof1-1::URA3</i> <i>MATα, ura3-52, +, +, +</i>	1
DDY426	<i>MATa, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101</i> <i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, +</i>	1
DDY427	<i>MATa, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101, cof1-Δ1::HIS3</i> <i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, +, +</i>	1
DDY321	<i>MATa, ura3-52, his3-Δ200, leu2-3,112, abp1::LEU2</i>	3
Mapping strains:		
DDY131	<i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101</i>	2
DDY428	<i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101, COF1:URA3:COF1</i>	1
DDY480	<i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101, COF1:HIS3:COF1</i>	1
DDY429	<i>MATα, ura3-52, his3-Δ200, leu2-3,112, lys2-801, ade2-101, trp1-901, COF1:URA3:COF1</i>	1
DDY182	<i>MATa, ura3-52</i>	2
Y602	<i>MATa, ura3-52, his3-Δ200, lys2-801, ade2-101, trp1-901, spa2-Δ3::URA3</i>	4
Y609	<i>MATa, ura3-52, his3-Δ200, lys2-801, ade2-101, trp1-901, spa2-Δ2::TRP1</i>	4
DDY481	<i>MATa, ura3-52, his3-Δ200, lys2-801, ade2-101, trp1-901, spa2-Δ3::URA3, COF1:HIS3:COF1</i>	1,4

For each strain, the strain collection number, genotype, and source are indicated. Sources: 1, this study; 2, Botstein lab (Department of Genetics, Stanford University, Stanford, CA); 3, Drubin lab; 4, Snyder lab (Department of Biology, Yale University, New Haven, CT) (Gehring and Snyder, 1990).

salts, final conditions were 5 mM Tris 7.5, 0.5 mM ATP, 0.2 mM CaCl₂, 2 mM MgCl₂, 0.025 mM EGTA, 10 mM KCl, and 0.2 mM DTT. Fluorescence measurements were taken continuously over time using a Hitachi F-4010 fluorometer (Hitachi Scientific Instruments, Mountain View, CA) with a neutral density filter to minimize bleaching. Excitation wavelength was 355 nm and emission wavelength was 407 nm.

Pelleting Assay

Actin filament pelleting experiments were always done in parallel with the pyrene-labeled actin assembly experiments. Therefore, conditions are identical to those described above, with the pelleting experiments also containing pyrene-labeled actin. Polymerization proceeded for 2 h at 23°C. The filaments were then pelleted by centrifugation at 90,000 rpm in a TLA100 rotor (Beckman Instruments) ($k = 8.9, 312,000 g$) for 40 min at 23°C. For visualization on Coomassie blue-stained SDS-PAGE gels, it was necessary to TCA precipitate the proteins in the supernatant (Drubin et al., 1988). Samples were run on polyacrylamide gels, Coomassie stained, and analyzed using a Bio-Rad 620 video densitometer (Bio-Rad Laboratories, Richmond, CA).

G-actin Binding

A 1 × 30-cm Superose 12 HR gel filtration column (Pharmacia LKB Biotechnology Inc.) was equilibrated in buffer A (a buffer favoring depolymerization; see above), and cofilin or cofilin with approximately fivefold molar excess of actin was loaded on the column. Elution of cofilin alone peaks from 10 to 11 ml. If actin is present, the peak of cofilin elution is between 8 and 9 ml.

Antibodies

Antibodies against yeast cofilin were raised in three virgin female New Zealand white rabbits. The most pure preparations of cofilin were further purified by excision of Coomassie blue-stained bands from SDS-PAGE gels as described (Drubin et al., 1988). 50 μg of protein was used for each injection. Freund's complete adjuvant was used for the first immunization, and Freund's incomplete adjuvant was used for the following injections (days 21 and 36). Sera collected on day 48 showed reactivity toward purified

cofilin by immunoblotting, but was not of high enough titer to recognize the protein in a whole cell extract. An additional boost on day 78 raised the titer of sera collected on day 89 to a useful level. The antisera from two rabbits were affinity-purified using affinity columns prepared by coupling SDS-PAGE gel-purified yeast cofilin to CNBr-activated Sepharose (Pharmacia LKB Biotechnology Inc.) as previously described (Pfeffer et al., 1983). The affinity-purified antibodies from one rabbit (9515) recognized only one protein with an apparent molecular mass of 18 kD in immunoblots of whole cell extracts. Those from a second rabbit (9516) recognized the same 18-kD protein, but also a 50-kD protein. For immunofluorescence, affinity-purified antibodies from rabbit 9515 were used exclusively.

Immunofluorescence

Yeast cells were grown to early log phase in either YPD or supplemented synthetic media and were processed for immunofluorescence as previously described (Pringle et al., 1991). The cold methanol/acetone fixation step (Pringle et al., 1991) was required for both anti-actin and anti-cofilin reactivity. Affinity-purified anti-cofilin antibodies from rabbit 9515 were used at a dilution of 1:100. Affinity-purified rat anti-actin antibodies (kindly provided by J. Kilmartin, Medical Research Council Laboratory of Molecular Biology, Cambridge, England) were used at a dilution of 1:30. Detection was accomplished by applying fluorescein-labeled goat anti-rabbit and rhodamine-labeled goat anti-rat secondary antibodies (Cappel/Organon Teknika, Malvern, PA) at a dilution of 1:1,000. The secondary antibodies are species specific, as no staining was seen when rabbit primary antibodies were used with anti-rat secondary antibodies, or when rat primary antibodies were used with anti-rabbit secondary antibodies. Cells were viewed and photographed with a Zeiss Axioscope fluorescence microscope with a HB100 W/Z high pressure mercury lamp and a Zeiss 100X Plan-Neofluar oil immersion objective (Carl Zeiss, Thornwood, NY).

Peptide Sequencing and PCR Primer Design

Purified cofilin was subjected to proteolysis with endoproteinase Lys-C (Boehringer Mannheim Corp.) using an enzyme/substrate ratio of 1:50 for 2 h at 37°C in 50 mM Tris, pH 8.0. The resulting peptides were purified by HPLC chromatography using a Synchrom 2.1 × 10-cm C-18 column (Rainin Instrument Co., Woburn, MA) with 0.1% trifluoroacetic acid,

0–60% acetonitrile linear gradient. Three of the peptides (LysC#1, LysC#6, and LysC#7) were then amino terminal sequenced in a 470A gas phase sequencer (Applied Biosystems, Inc., Foster City, CA), and the amino acids were analyzed by a 120A on-line PTH analyzer (Applied Biosystems, Inc.). In one letter amino acid code, with uncertain residues in parenthesis, and with X representing unidentifiable residues, the sequences determined for the three peptides are as follows: LysC#1 = ETSTDPSYDAFLEK, LysC#6 = IVFFTWSPDTAPVR(E)K, LysC#7 = DALRRALNGVSTDVOGTDFSEVSYX(D)VLERVS(R)GAG. Underlined sequences were used to design degenerate PCR primers (see below).

Since Lys-C cuts after lysine residues, and since LysC#7 did not end in lysine, that peptide was assumed to be from the COOH terminus of cofilin. Therefore, sequences from LysC#7 were used to design the downstream PCR primer, ALM4 (5'CGGCTAGACTARAARTCNGTNCCTGNACRTC3'). The underlined sequence in LysC#1 was used to design upstream primers ALM2 (5'GGGGGATCCCTGAYCCNTCNTAYGAYGC3') and primer ALM3 (5'GGGGGATCCCTGAYCCNAGYTAYGAYGC3') but the primers use different codons for a serine residue. Sequences in LysC 6 were used to design an additional upstream primer, ALM1 (5'GGGGGATCCACAARATHGTNTTYTYACNTGG3'). Single underlined sequences represent XbaI and BamHI restriction endonuclease recognition sites and GC extensions included to facilitate cloning, and do not correspond to amino acids in the cofilin sequence. The double underlined sequence is a stop codon placed after the last amino acid encoded by the downstream primer. We did not bias our primer design by any codon usage criteria; the downstream primer was 1,024-fold degenerate and the upstream primers ranged from 64- to 384-fold degenerate (degeneracy created during synthesis by incorporating equimolar amounts of the following nucleotides at the positions indicated by: R = A, G; Y = C, T; H = A, C, T; N = A, G, C, T). These oligonucleotides were used to prime DNA synthesis on total yeast genomic DNA (S288C yeast strain background) following standard PCR protocols (Innis et al., 1990) using a DNA thermal cycler (Perkin-Elmer/Cetus). 270-bp (ALM2-ALM4) and 157-bp (ALM1-ALM4) PCR products were isolated from agarose gels and cloned using the restriction endonuclease sites designed into the primers. Sequencing of both products confirmed that each corresponds to a region of the gene that encodes cofilin. Since the 157-bp product is contained within the 270-bp product, only the latter product was used for further work.

DNA Sequence Analysis

ExoIII nuclease digestion was used to create deletion series for sequencing. Both strands of the *COFI* gene were sequenced by the dideoxynucleotide termination method using single-stranded DNA templates (Ausubel et al., 1989). Potential open reading frames and amino acid sequences were identified using the DNA Strider program (Commissariat à l'Energie Atomique, Gif-Sur-Yvette, France), while the position of the predicted intron was detected by visual analysis. Related proteins were identified by Hypercard Blastp (Apple Computer, Cupertino, CA) searches, but final alignments were done by visual inspection.

Results

Purification of a Yeast Actin Filament Severing Protein

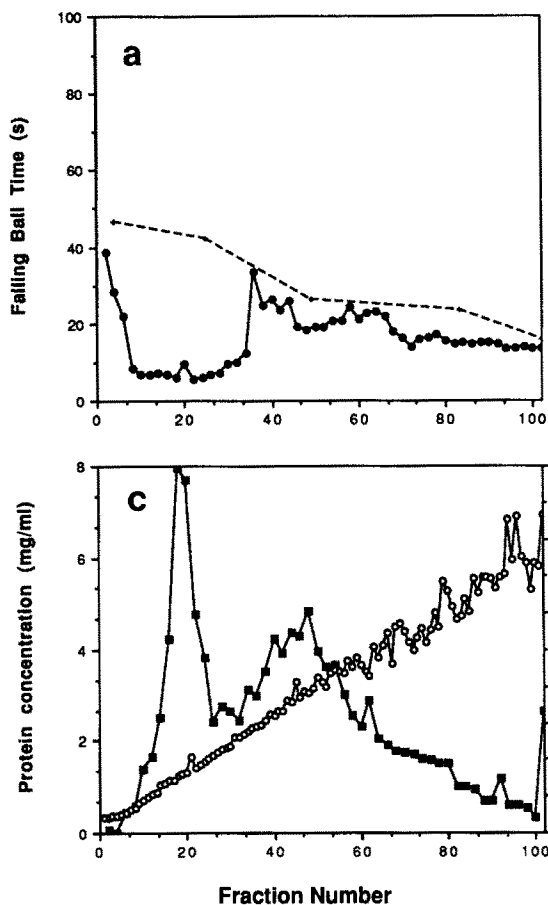
A solution of actin filaments possesses a viscosity that depends on the concentration, length, and crosslinking of the filaments. Actin binding proteins can alter these variables to create a more or less viscous solution or to form a more elastic gel. Falling ball viscometry (Pollard, 1982) has been successfully applied in the past to identify gelation proteins, capping protein, and a severing/monomer binding protein from *Acanthamoeba* (Isenberg et al., 1980; MacLean-Fletcher and Pollard, 1980; Cooper et al., 1986). We applied this assay to screen fractionated yeast proteins for activities that alter the low shear viscosity of actin solutions. Soluble yeast proteins were loaded on an anion exchange column (Q-Sepharose) and the flow-through from this column was loaded on a cation exchange column (S-Sepharose). Proteins were eluted using gradients of increasing salt concentration. Elution profiles of actin-interacting activities

as detected by the falling ball assay are shown in Fig. 1. The time that it takes for the ball to fall through the solution (falling ball time in Fig. 1, *a* and *b*) is related to the viscosity of the solution (Pollard, 1982). The dotted line shows the viscosity of actin alone; a decrease in viscosity occurs as the salt concentration in the column buffer is increased. When the proteins that elute from the Q-Sepharose column in 50–170 mM KCl are added to an actin assembly reaction, a decrease in viscosity is observed (Fig. 1 *a*). Under the conditions used in this study, 6 s corresponds to the maximum falling ball rate, as a solution of G-actin gives similar falling ball times. Because 6 s is the falling ball time measured in the peak shown in Fig. 1 *a*, dilutions of fractions in this region were performed to better quantitate the activities present in each fraction. Upon assaying the diluted fractions, the broad peak of activity separated into two peaks (not shown). A hint of this separation is evident in Fig. 1 *a*. In addition to this viscosity-decreasing activity, a viscosity-increasing activity was eluted from the S-Sepharose column (Fig. 1 *b*). From these experiments, it is apparent that there are several proteins present in yeast extracts that can be detected by their effects on actin viscosity.

We report the purification and characterization of a protein that has a viscosity-decreasing activity and elutes from the Q-Sepharose column (Fig. 1 *a*). This activity was selected for purification first because it cofractionates with an interesting additional activity; these fractions also cause an increase in the rate of actin assembly (see below). Subsequent purification revealed that a single protein with an apparent molecular mass of 18 kD was found to possess the dual properties of accelerating actin assembly and decreasing actin viscosity (Fig. 2, Table II). The Coomassie blue-stained gel presented in Fig. 2 shows the enrichment of this protein during our purification procedure (see Materials and Methods).

Both the falling ball viscometry assay and an assembly kinetics assay using pyrene-labeled actin (Cooper et al., 1983) were used to follow the two activities during the purification of the 18-kD protein (Table II). No activity is apparent in our starting material, the high speed supernatant; both activities appear only after the first fractionation step, the Q-Sepharose column. The Q-Sepharose column fractions that contained the viscosity-decreasing/assembly rate-increasing activity were pooled, the proteins were concentrated by ammonium sulfate precipitation, and then the precipitated proteins were fractionated on a Sephacryl S-100 gel filtration column. Three viscosity-decreasing activities were resolved on this column. For this study, only the fractions containing the third peak of activity that eluted from the column were pooled. The Sephacryl S-100 fractionation step actually increased the total amount of assembly rate-increasing activity (Table II, column 4), suggesting that an inhibitory activity was removed. An 18-kD protein was a major constituent after this step. During subsequent purification steps (see Materials and Methods), the 18-kD protein was enriched and the specific activity increased. The fact that the specific activity was still increasing during the final stages of our purification suggests that additional purification procedures could be used in the future to obtain even higher purity. However, neither cation exchange chromatography (S-Sepharose) nor hydrophobic chromatography (phenyl-Sepharose, octyl-Sepharose) were helpful for further purification of p18. We estimate that about eight minor contaminating proteins

Anion Exchange



Cation Exchange

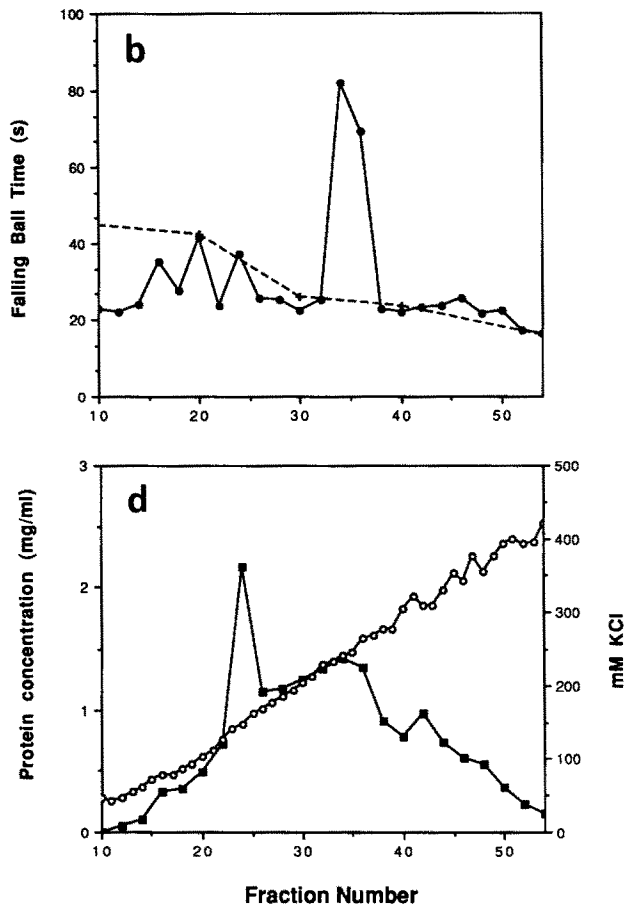


Figure 1. Fractionated yeast proteins affect actin viscosity. Elution profiles of activities from Q-Sepharose anion (*a* and *c*) and S-Sepharose cation (*b* and *d*) exchange columns are shown with fraction numbers plotted along the *x*-axes. Upper panels (*a* and *b*) show data from the falling ball viscometry assay (see Materials and Methods for details) and lower panels (*c* and *d*) plot protein and salt concentrations. ---+--- is falling ball time with salt concentrations indicated in lower panel, but with no yeast proteins added, ●— is falling ball time with yeast protein, —■— is protein concentration, and —○— is KCl concentration. Protein concentration was determined by the method of Lowry et al. (1951). Salt concentration was determined by conductivity compared with standards.

Table II. Purification Table of Yeast Cofilin

	Falling ball		Pyrene actin		Amount of cofilin	
	Total U	U/mg	Total U	U/mg	Total mg	%
HSS	—	—	—	—	31.8	0.3
Q-Sepharose	43,000	41	5,500	5.2		
35–85% cut	24,000	38	1,700	2.6		
Sepharcyl S-100	16,000	290	3,600	89		
Mono Q	4,100	400	3,800	370		
Superose 12 HR	4,200	860	1,700	355		
Mono Q 8.0	2,053	1,300	924	570	1.53	94

The activity present after each purification step (column 1) is displayed. For details on purification procedure, see Materials and Methods. Columns 2 and 3 show activity as determined by the falling ball assay. Falling ball assay units (U): 1 U = activity that would result from having 1 M of pure cofilin present for every 10 M of actin (assay uses 4.5 μ M actin and a 100- μ l volume). Columns 4 and 5 show activity as determined by pyrene actin assembly. Pyrene actin assay units (U): 1 U = activity that would result from having 1 M of pure cofilin present for every 10 M of actin (assay uses 5 μ M actin and a 200- μ l volume). Columns 6 and 7 show the amount of cofilin present in the HSS and most pure fractions. The amount of cofilin in the HSS was determined by running HSS on polyacrylamide gels, electroblotting onto nitrocellulose, and incubating with antibodies specific for cofilin. Antibody reactivity was detected using the Amersham ECL detection system (Amersham Corp., Arlington Heights, IL) and quantitated by densitometry. The amount of cofilin in the HSS was determined by comparison with pure cofilin standards treated the same way.

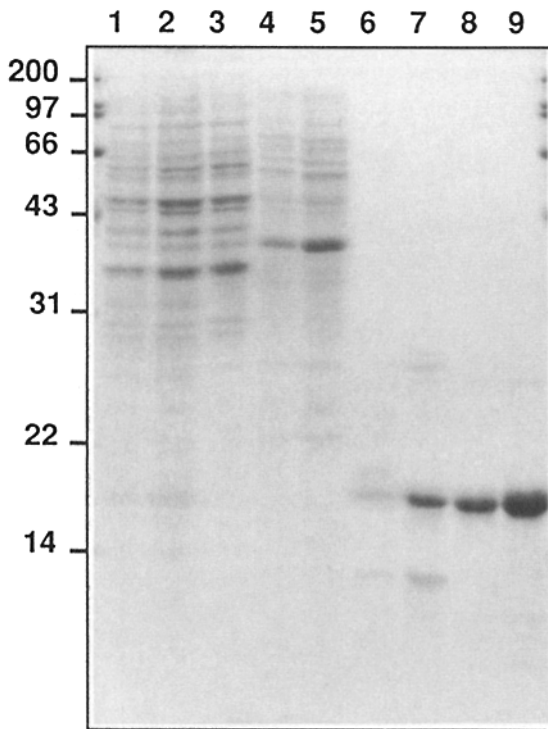


Figure 2. Purification of yeast cofilin. A Coomassie blue-stained SDS 13% polyacrylamide gel of pooled fractions from cofilin purification steps is shown (see Materials and Methods). 2.5 μ g of protein was loaded in each lane. Molecular mass standards are marked on the left in kilodaltons. Lane 1, crude lysate; lane 2, low speed supernatant; lane 3, high speed supernatant; lane 4, Q Sepharose; lane 5, 35–85% ammonium sulfate cut; lane 6, Sephacryl S-100 gel filtration; lane 7, Mono Q; lane 8, Superose 12 HR gel filtration; lane 9, Mono Q, pH 8.0.

account for \sim 6% of the total protein mass after the final step, as determined by comparison of staining of the contaminants to dilutions of proteins of known concentrations. To estimate the concentration of the 18-kD protein in the HSS, antibody reactivity on immunoblots of high speed supernatant was compared with reactivity toward known quantities of pure p18. Based on this value, \sim 5% of the 18-kD protein present in our starting material is recovered after our final purification step.

Microsequencing of peptides derived from the 18-kD protein revealed that it contains sequences similar to those found in a class of low molecular weight actin severing and monomer binding proteins. Cloning and sequencing of the gene encoding p18 confirmed that it belongs to this family of cofilin-like proteins (see below). This group includes the vertebrate cofilin (Nishida et al., 1984b) and ADF/detrin-type proteins (Bamburg et al., 1980; Nishida et al., 1984b), echinoderm depactin (Mabuchi, 1983), and *Acanthamoeba* actophorin (Cooper et al., 1986). These proteins all sever actin filaments and bind to actin monomers. In addition, cofilin can bind to actin filaments with a 1:1 molar ratio (Nishida et al., 1984b), while the other members of the protein family bind weakly (Koffer et al., 1988) or do not bind at all (Cooper et al., 1986). The biochemical similarities of the yeast protein to mammalian cofilin (see below) led us to

call the protein yeast cofilin and to name the gene that encodes the protein *COFI*.

Pyrene-labeled actin was used in an assembly assay to monitor the effects of yeast cofilin on actin polymerization over time (Cooper et al., 1983). Pyrene fluorescence increases when a pyrene-labeled actin subunit is incorporated into a filament (Fig. 3, *STD*). Yeast cofilin increases the rate of assembly (Fig. 3), but its effects are seen only in the late stage of actin polymerization, after the lag phase. This behavior can be explained by invoking a severing activity; when newly formed actin filaments are severed, new ends become available for subunit addition, which allows for more rapid actin assembly. The severing of actin filaments by *Acanthamoeba* actophorin has recently been confirmed by direct visual observation (Maciver et al., 1991).

All of the other cofilin-like proteins show very similar effects on actin assembly, including the characteristic drop in total fluorescence intensity at steady state as increasing amounts of cofilin are added, and the initial overshoot of this final level (Mabuchi, 1983; Nishida et al., 1984a, b; Cooper et al., 1986). The drop in fluorescence intensity at steady state as the cofilin/actin ratio increases might represent the inhibition of actin polymerization by the monomer sequestering activity possessed by these proteins. However, the binding of proteins to pyrene-labeled actin subunits in a filament might also affect the fluorescence signal (Kouyama and Mihashi, 1981). The reasons behind the overshoot have not yet been determined. The behavior of yeast cofilin in these assembly experiments, as well as its viscosity-lowering effect, which may result from the lowering of average filament length, suggest that the severing activity of cofilin-like proteins is conserved in most, if not all, eukaryotic cells.

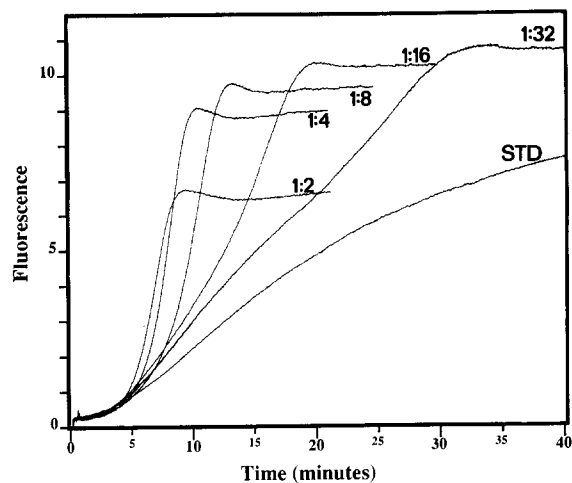


Figure 3. Yeast cofilin increases the rate of actin polymerization. A plot of fluorescence of pyrene-labeled actin over time is shown. Time is measured in minutes, with time 0 representing the point at which polymerization initiating salts are added. Fluorescence is in arbitrary units. *STD* is standard actin polymerization without cofilin. Standard actin assembly reactions (*STD*) reached steady state after 120 min. At that point, the final level of fluorescence of the standard was equal to the level reached in the plot labeled 1:32 at 40 min. For the other reactions, actin is polymerized in the presence of cofilin, with the relative amount of cofilin indicated as the molar ratio of cofilin/actin.

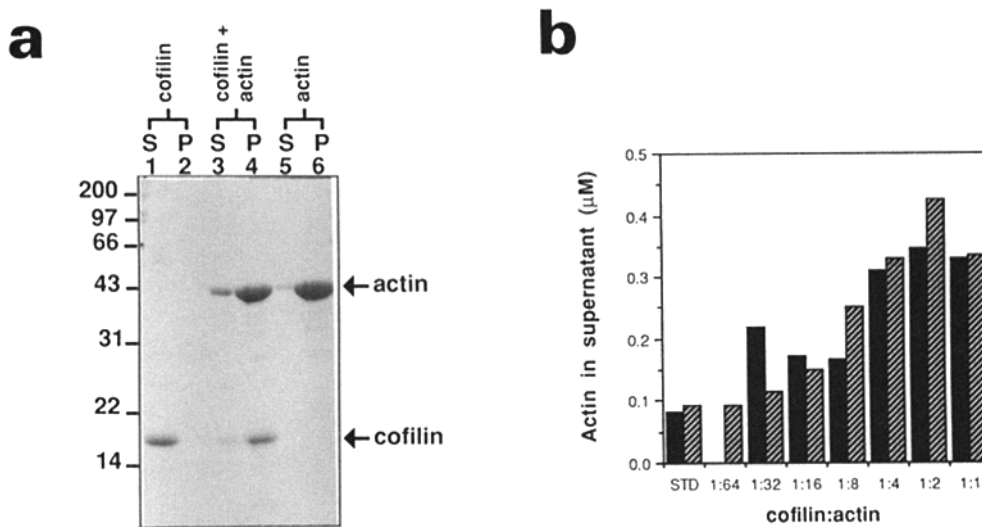


Figure 4. Copelleting of yeast cofilin with actin filaments. Solutions of cofilin alone, actin alone, or cofilin plus actin were incubated for 2 h in polymerization buffer and centrifuged (see Materials and Methods). (a) Cofilin binds stably to actin filaments. A Coomassie blue-stained SDS 13% polyacrylamide gel of proteins in the supernatants (S) or pellets (P) and of centrifuged samples is shown. Bracketed lanes contain equal molar amounts of supernatant and pellet from the same reaction. Lanes 1 and 2 are from a solution containing only cofilin. Lanes 3 and 4 are from a solution containing equal amounts of cofilin and actin. Lanes 5 and

6 are from a solution containing only actin. (b) Cofilin increases the amount of nonsedimentable actin. Gels similar to the one shown in a were run and stained, and bands were quantitated by densitometry (see Materials and Methods). Data from two independent trials are graphed here. Total actin in the reaction is 5 μM . The actin concentration (micromolar) in the supernatant is shown on the y-axis. Labels on the x-axis represent the relative amount of cofilin and actin in the reaction, expressed as a molar ratio of cofilin/actin. STD has no cofilin.

To determine whether yeast cofilin stably binds to actin filaments, actin and cofilin were incubated under assembly-inducing conditions, and the filaments were pelleted by centrifugation at 312,000 g for 40 min. Actin filaments >10 subunits in length will pellet under these conditions (Attri et al., 1991). While cofilin alone does not pellet (Fig. 4 a, lanes 1 and 2), it does copellet with actin filaments (Fig. 4 a, lanes 3 and 4). It is also apparent that cofilin affects the amount of actin that sediments. This can be seen by comparing lanes 3 and 5 in Fig. 4 a; more actin remains in the supernatant when cofilin is present. Although most of the actin is still pelleted, the amount left unsedimented is increased by the addition of cofilin (Fig. 4 b). Actin left in the supernatant might represent either sequestered monomers or very short filaments.

When observations from the pelleting and assembly rate (Fig. 3) experiments are compared, it becomes clear that the drop in fluorescence intensity of pyrene-actin at steady state caused by addition of cofilin cannot be accounted for completely by monomer sequestration. Fluorescence intensity is decreased by >40% when 2.5 μM of cofilin is added to the 5 μM actin solution (Fig. 3, plot labeled 1:2), suggesting that 2 μM of the actin would be present in the supernatant if monomer sequestration was the sole cause. However, <0.4 μM actin remains in the supernatant upon centrifugation (Fig. 4 b). In these experiments, only a portion of the actin is pyrene labeled. If cofilin preferentially sequestered pyrene-labeled actin, the fluorescence intensity would be dramatically affected without much effect on the total amount of polymerization. This does not appear to be the case, since changing the percentage of pyrene-labeled actin from 5 to 20% has no effect on the results (data not shown). Alternative causes for the decrease in fluorescence may be that binding of cofilin to the filaments quenches the fluorescence signal, or that pyrene-labeled actin subunits that are very near the ends of filaments fluoresce less than those incorporated

away from an end; therefore, distributing the same amount of polymerized actin in short filaments would cause a decrease in fluorescence intensity.

Although monomer sequestration does not account for the full magnitude of the characteristic drop in fluorescence that occurs when the cofilin-like proteins are added to F-actin (Mabuchi, 1983; Nishida et al., 1984b; Cooper et al., 1986), it has been demonstrated that all of these proteins bind to G-actin. To further document the affinity of yeast cofilin for monomeric actin, gel filtration chromatography of cofilin alone or cofilin with a fivefold molar excess of G-actin was performed. In the presence of G-actin, the retention time of cofilin is decreased (data not shown), adding further support to the conclusion that, like the other proteins in this family, yeast cofilin has an affinity for monomeric actin.

Molecular Cloning and Sequencing of COF1

Degenerate PCR primers designed after the 18-kD protein peptide sequences were used to amplify a 270-bp fragment from yeast genomic DNA (see Materials and Methods for peptide and primer sequences). The PCR product was sequenced to confirm that it is part of the gene that encodes the 18-kD protein. The cloned PCR fragment was then used to screen the YCp50-based library RB239 (Rose et al., 1987), and a plasmid containing the full-length *COF1* gene was obtained.

The nucleotide and predicted amino acid sequences of the *COF1* gene and protein are shown in Fig. 5. The hypothetical protein has a predicted molecular mass of 15.9 kD, similar to that determined for the purified protein (18 kD). The underlined amino acids match 63 of 66 residues determined by peptide sequencing. All three residues that differ (double underlined) were in regions where peptide sequencing was of low confidence (unidentifiable or uncertain assignments).

It is likely that the *COF1* gene contains a 179-bp intron.

EcoRI
1 GAATTCGCGCTGTATCCGGCGCCTATAAGCCGCTCCAATCGGTCACACCTGTATTCTAAA 60
61 GCTTCAACAAGCTTAAGACCAGGCATCAAACCTCTTTGGTAGAGGCTAATGGCCTTGA 120
121 AGCTCGAGAGAACATTCATAAGTTCATCAATCACTTATACTATATGCAGTACCTCTAAA 180
181 TTACCTAATATCCGGGTAATAAGAACAATATTCGGGTGACGCCGCCCTGTTTGATGACA 240
241 AAATTGGAAGAAAAGAGGAAAACAAGAAAAGACTGGTTAGCAACTACGTAAACTTCAGAT 300

BglII
301 TTCAAACATACATAAAACAAAAACTAACAAAAGAAGATGTCTAGATCTGGGTATGCTAA 360
1 M S R S G 5
361 ATTCATTTGTACTCCTGTGTAAACTATCAATTACTAACAATTTGACATGATGCTGTTTT 420
421 ATCTCGCTCTCTTATGTCCTTTCTTTCCCTTTTTAACATGGAACTTAAAAGGAATG 480
481 TGGGGGAAAAAATTCATAAGAAGCCAGGAAAATGTGAAGTTTCTGTGTAGTGTGCTGTT 540
6 V A V 8
541 GCTGATGAATCCCTTACCGCTTTCAATGACTTGAATTTGGGTAAAAAATACAAATTTATT 600
9 A D E S L T A F N D L K L G K K Y K F I 28
601 TTATTCGGATTGAACGATGCTAAAACCGAATCGTTGTCAAGGAAACCTCTACTGACCCA 660
29 L F G L N D A K T E I V V K E T S T D P 48
LysC#1
==ALM2====>
661 TCTTACGATGCCTTCTTAGAGAAATGCCAGAAAACGACTGTCTTTACGCCATTTACGAT 720
49 S Y D A F L E K L P E N D C L Y A I Y D 68
721 TTTGAATACGAAATTAATGGTAATGAAGGTAAGAGATCCAAGATTGTTTTCTCACTTGG 780
69 F E Y E I N G N E G K R S K I V F F T W 88
LysC#6

BglII
781 TCTCCAGACACTGCTCCAGTCAAGATCTAAGATGGTCTATGCATCCTCCAAGGATGCCTTA 840
89 S P D T A P V R S K M V Y A S S K D A L 108
LysC#7
<=====ALM4=====

841 AGAAGAGCCTTAAACGGTGTCTCTACCAGTGTTCAGGTAAGTACTGATTTTTCCGAAGTTTCT 900
109 R R A L N G V S T D V Q G T D F S E V S 128

MnlI
901 TACGATTTCTGTTTTGGAAAGAGTCAGCAGAGGCGCTGGTTCTCATTAAAGGTAATGATT 960
129 Y D S V L E R V S R G A G S H * 143

961 CACAAATGACAACAATCTTCAAGAAAATGAAAATGTATATCTATATAAAATCGTCAT 1020
1021 ATATATACAGAATACATGTATAGAATTATTATAATTATAATTTCTCTTCGCAACTCTAT 1080
1081 CTAAAACACAATACTTAGATTTAAGGTCCCCTACACCCCGGCTAGTGGGCATTGTTTTTG 1140

BalI
1141 CACCCATATTTGTAATTCATAATTCAGAACGAAATCTGGCCATGTAGATCAAGAAAAG 1200
1201 ATACAAGGTCATCGAACCAGGCAGCAATGATTTTTCTATAATCTTAAAGTGAAGCATG 1260
1261 ACGACCATAGCTCGTTGAATATGCATAGAACGCTTCTGTTTTAACGTGGCTGATATCTT 1320
1321 TAACAAAGGCTTTTAATATAAAATTACCACACACTGAAAAAAAAGACCATTGGAATCCA 1380

SphI
1381 ACGCTGTGCTGGCATGC

Figure 5. Sequence of the *COFI* gene. The nucleotide sequence of a 1.4-kb *EcoRI*-*SphI* fragment that contains the *COFI* gene is shown. Deduced amino acid sequence is shown below in one letter amino acid code. Double underlined nucleotides indicate potential TATA sequences. Conserved sequence motifs of yeast introns are single underlined (Woolford, 1989). Restriction enzyme sites used for construction of deletion mutants are indicated. Positions of primers used for PCR are depicted (5'====>3'). Underlined amino acids correspond to sequences derived from analysis of peptides from a LysC digest of yeast cofilin. These sequence data are available from EMBL under accession number Z14971.

This conclusion is supported by the presence of consensus TACTAAC and splice donor/acceptor sequences found in yeast introns (Woolford, 1989). Utilization of the splice junction predicted by these sequences would allow for the production of a protein whose NH₂ terminus is similar in sequence to other members of the family of cofilin-like proteins (Fig. 6). The NH₂-terminal methionine was assigned to the first ATG upstream of the predicted upstream splice site. A stop codon (TAA) is found in the same reading frame, four codons upstream of this ATG, with no intervening ATG sequences. Protein sequencing could not be used to confirm the sequence of this region because the NH₂ terminus of the

protein was blocked. Therefore, until cDNA sequence is obtained to confirm the splice junction, we leave open the possibility that an ATG within the predicted intron (dotted underline) may be used as the initiator codon. Use of this ATG would result in a protein that is 13 amino acids longer than that produced by a spliced mRNA (MWGKKFIRSQENVKFLCS . . . instead of MSRSG . . .). Based on the arguments of favorable nucleotide sequence context for splicing and of homology to proteins in the cofilin-like protein family, Fig. 5 presents the most likely sequence of yeast cofilin.

Fig. 6 presents an alignment of yeast Cofilin to one cofilin (mouse), one destrin/ADF type protein (pig), and depactin

YEAST COFILIN: MSRSGVAVADESLTAFNDLRKLGK-----KYKFLHFGLNDAKTEIVVK- 42
MOUSE COFILIN: MA-SGVAVSDGVIKVNDMKVRKSSSTPEEVKRRKKAFLFCLSEDKKNIIEE 51
PIG DESTRIN: MA-SGVAVADEVCRIFDYDMKVRKCSSTPEEIKRKKKAVLFLCLASADKCCITVEE 51
STARFISH DEPACTIN: PQ-SGTAI-DENVK--EETRAFKMDQS-KVKVPWMLLEIVQNDR-IDVVKV 46

Y. COFILIN: -----ETSDPSYDAFLLEKLPENDCLYAIYDFEYEINGNEGKRSKI^{FF}WSPD 91
COFILIN: GKEILVGDVGGTIVDDP-YTTFVVKM^{LP}DKDRYALYDATYETK--ESRKEDELVIFWAP 107
DESTRIN: GKEILVGDVGVITIDP-EKH^{LV}GM^{LP}EKDRYALYDASFEETK--ESRKEELMIFLWAP 107
DEPACTIN: TKKAGPSDNLETLREE-LKQREVYFVLDYEPSEEKRAKH-NIPKGS-TYPLTCFWSME 102

Y. COFILIN: TAPVRSKMVYASSKDALRRALNGVSTDVGGTDFSEVSYD-VLERVSRGAGSH 143
COFILIN: NAPLKSKM^{VY}ASSKDAIKKK^{LT}GKHELDQANCYEEV^KDRCTLA^EKGGSLIVISLEGKPL 166
DESTRIN: LAPLKSKM^{VY}ASSKDAIKKK^{FG}GKHECQANGPEDLN^{RAC}-IA^EKGGSLIVAFEGCPV 165
DEPACTIN: TANIKLKM^{VY}SS^{TV}GTLLKSASTTK^{TY}LEAHD^{DD}LS^{EE}EA-IGDKIKNF 150

indicate amino acid number. Previous studies with mammalian and starfish proteins have identified the overlined regions as potential actin-interacting regions based on crosslinking (*open bars*) or synthetic peptide studies (*solid bars*). Murine cofilin (Moriyama et al., 1990a), porcine destrin (Moriyama et al., 1990b), and starfish depactin (Takagi et al., 1988) sequences were obtained from Genbank.

(starfish). Black shading shows residues that are identical to corresponding residues in yeast cofilin, and grey shading indicates conservative replacements. Overlined sequences correspond to regions implicated in actin binding by previous crosslinking (*open bars*) (Sutoh and Mabuchi, 1989; Yonezawa et al., 1991b) and peptide binding experiments (*solid bars*) (Yonezawa et al., 1989, 1991b) (see Discussion). Yeast cofilin is most similar to the mammalian cofilin protein, being 41% identical and 63% similar in amino acid sequence. The corresponding values for destrin are 37%/59%, and for depactin they are 22%/42%. Yeast cofilin is somewhat smaller than the mammalian and starfish proteins. To compensate for this, two major gaps were introduced to optimize the alignments.

Yeast Cofilin Is Similar to the NH₂ Terminus of Abplp

Abplp is a 65-kD yeast actin binding protein discovered by its ability to bind to actin filaments (Drubin et al., 1988).

It contains an SH3 domain (*src* homology 3) at its COOH terminus (Drubin et al., 1990). This ~50 amino acid motif is found in many nonreceptor tyrosine kinases; cytoskeletal proteins including Abplp, myosin I, and α -spectrin; and another yeast morphogenetic protein, Bemlp (Koch et al., 1991; Chenevert et al., 1992). The NH₂ terminus of Abplp is homologous to yeast cofilin (Fig. 7). The proteins share 23% identity and 41% similarity over the entire 16-kD region, with the COOH-terminal portion of cofilin showing stronger homology to Abplp (Fig. 7 b). Particularly strong homology is found in a region that corresponds to a potential actin binding region of mammalian cofilin (overlined sequence) (Yonezawa et al., 1991b). This is the first identification of homology between cofilin and a protein outside of the immediate family of cofilin-like proteins.

Genomic Mapping

Chromosome blot analysis (Gerring et al., 1991) revealed

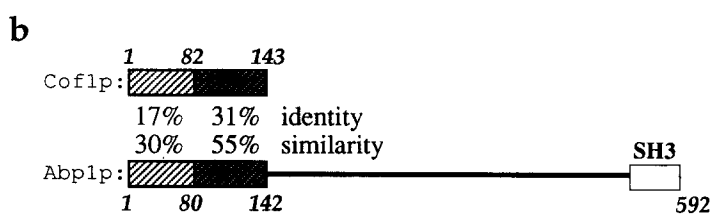
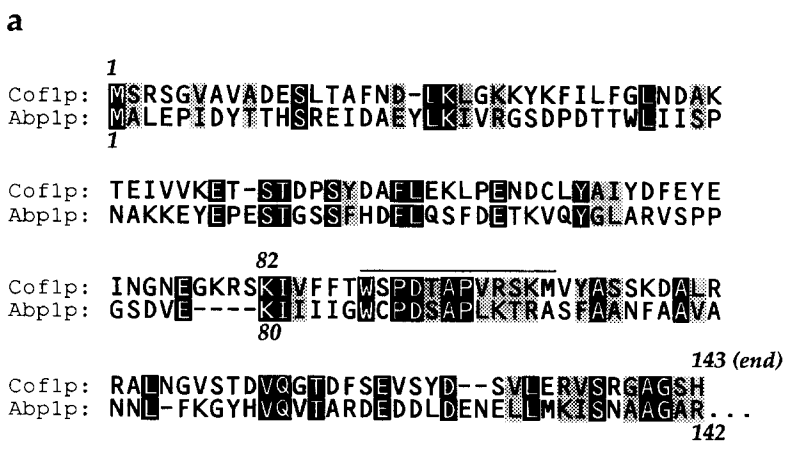


Figure 7. Yeast cofilin is similar to the NH₂-terminus of the yeast actin binding protein Abplp. (a) The entire yeast cofilin sequence (*upper lines*) is compared with the NH₂-terminal 142 amino acids of Abplp. Black shading indicates identity and grey indicates similar residues (see legend to Fig. 6). Overlining designates potential actin binding region. (b) Schematic diagram of the cofilin and Abplp proteins. Shaded regions show homology, with percent identity and percent similarities indicated. Open box in the Abplp gene represents SH3 domain (Drubin et al., 1990).

Table III. Genetic Mapping of COF1

	PD	NPD	TT	cM
<i>COF1-SPA2</i>	169	0	27	<i>COF1-SPA2</i> = 7 cM
<i>SPA2-LEU2</i>	51	53	57	
<i>SPA2-TRP1</i>	42	52	26	<i>SPA2-CENXII</i> = 12 cM
<i>COF1-LEU2</i>	60	67	86	
<i>COF1-TRP1</i>	38	44	38	<i>COF1-CENXII</i> = 15 cM

Tetrad analysis results and calculated genetic map distances in centimorgans are shown. *COF1-SPA2* distance was calculated using the formula $[50 \times (TT + 6NPD)] / \text{total}$ using data derived from crosses between Y602 \times DDY428 (35 tetrads), Y609 \times DDY429 (41 tetrads), Y602 \times DDY480 (92 tetrads), and DDY481 \times DDY131 (28 tetrads). Each of the crosses gave similar results. Centromere linkage was determined by using the *LEU2* gene (5 cM away from *CENIII*) and the *TRP1* gene (0.8 cM away from *CENIV*) (Mortimer et al., 1989). Distances from *COF1* and *SPA2* to the chromosome XII centromere were estimated using the formula $[(50 \times TT) / \text{total}]$, less the distance of *LEU2* (5 cM) or of *TRP1* (0.8 cM) from their respective centromeres. Data for *COF1-CENXII* and *SPA2-CENXII* were derived from crosses between Y609 \times DDY429 (41 tetrads), Y602 \times DDY480 (92 tetrads), and DDY481 \times DDY131 (28 tetrads). Additional data for *COF1-CENXII* were derived from the cross DDY182 \times DDY428 (52 tetrads). The distances calculated above predict an ordering of *CENXII-SPA2-COF1*, and this was confirmed by analysis of data from the crosses Y602 \times DDY480 and DDY481 \times DDY131, which create diploids heterozygous for *leu2*, *trp1*, *COF1* (*HIS3*), and *SPA2* (*URA3*). Our data place *SPA2* 12 cM away from *CENXII*, closer than the published distance of 21 cM (Snyder, 1989).

that *COF1* is on chromosome XII. When the filters of the ordered yeast library (Olson et al., 1986) were probed, we found that *COF1* is present on clone 4354. This clone spans the region 34–43 kb from the left end of chromosome XII. Comparison of the *COF1* restriction map to that of clone 4354 more precisely places *COF1* in the region 35.5–40 kb from the end. Genetic mapping agrees with the gene order determined by physical mapping; *COF1* maps 7 cM away from *SPA2*, which was previously known to be on the left arm of chromosome XII. *SPA2* lies between the centromere and *COF1* (Table III).

Indirect Immunolocalization of Cofilin to Actin Structures in the Cell

Actin forms two types of structures in yeast cells: actin cables that run through the cytoplasm, and actin patches that are located at the cell cortex at or near sites of cell surface growth (Adams and Pringle, 1984). Both the cortical actin and the actin in the cables are known to be F-actin structures because they stain with fluorochrome-conjugated derivatives of the mushroom toxin phalloidin, which binds only to F-actin. Since yeast cofilin binds to F-actin in vitro (Fig. 4), it was important to determine if this is also the case in vivo. Polyclonal antisera against purified cofilin were raised in rabbits. When affinity-purified against cofilin, the antibodies from rabbit 9515 recognized only one band of apparent molecular mass 18 kD on an immunoblot of whole cell extract (Fig. 8, lane 2). When immunofluorescence with anti-cofilin antibodies was performed on fixed yeast cells, general cytoplasmic staining and brightly staining "dots" located primarily in the bud or presumptive bud were seen (Fig. 9, *b* and *d*). DAPI staining (data not shown) indicates that the dark regions correspond to the nucleus, indicating nuclear exclusion (see Discussion for significance). In cells double-labeled for actin, the cofilin dots colocalize with the cortical actin structures, but cofilin is not seen in association with the

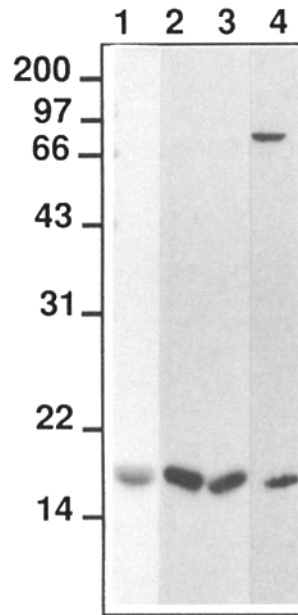


Figure 8. Affinity-purified antibodies recognize a single protein species in yeast whole cell extracts. Lane 1, Coomassie-stained gel of purified yeast cofilin; lanes 2 and 3, immunoblot using affinity-purified antibodies from rabbit 9515; whole cell extract was loaded in each lane from the following strains: wild-type (DDY-288) (2) and diploid with one truncated copy of *COF1* (*COF1/cof1-1::URA3*) (DDY425) (3); lane 4, Immunoblot of whole cell extract using antibodies to both Cofilin and Abplp. Abplp runs on SDS-PAGE gels as an 85-kD protein.

actin cables found in the mother cell (Fig. 9). It is possible that the cytoplasmic staining might obscure a weak signal from the cytoplasmic actin cables.

Interestingly, cofilin distribution is identical to that of Abplp (Drubin et al., 1988, 1990), with which it shares sequence similarity (Fig. 7). Because of this similarity, it was important to demonstrate that the anti-cofilin antibodies do not recognize Abplp. Immunoblots reveal only one 18-kD protein that reacts with anti-cofilin antibodies (Fig. 8, lane 2). To confirm that high molecular weight proteins were transferred to the membrane, another lane was probed with both anti-cofilin and anti-Abplp antibodies (Drubin et al., 1988) (Fig. 8, lane 4). In addition, when immunofluorescence experiments were repeated on *abpl* null mutants (DDY321), anti-cofilin antibodies still recognized the cortical patches and cytoplasm.

Abnormal actin structures arise in cells with altered cytoskeletal components. Thick actin "bars" form in *act1-2* cells at the permissive temperature (Novick and Botstein, 1985). Similar structures form in profilin mutants (Haarer et al., 1990), occasionally in tropomyosin mutants (Liu and Bretscher, 1989a), and in cells that overexpress Abplp (Drubin et al., 1988). Although cofilin could not be detected in association with wild-type actin cables, yeast cofilin is a component of the abnormal actin bars found in the *act1-2* mutant (Fig. 10). Double labeling with anti-actin antibodies confirms that the bars containing cofilin are actin bars (data not shown). These bars are usually found near the nucleus, but do not appear to be intranuclear. The bars do not stain with phalloidin, indicating that they might not be composed of normal actin filaments. Abplp also associates with these aberrant actin structures, but not with wild-type cables (Drubin et al., 1988).

COF1 Is an Essential Gene

To determine the effect of loss of cofilin function on the cell, two types of *COF1* gene disruptions were created. One disruption resulted in a truncation of the last 19 amino acids of

anti-actin

anti-Cof1p

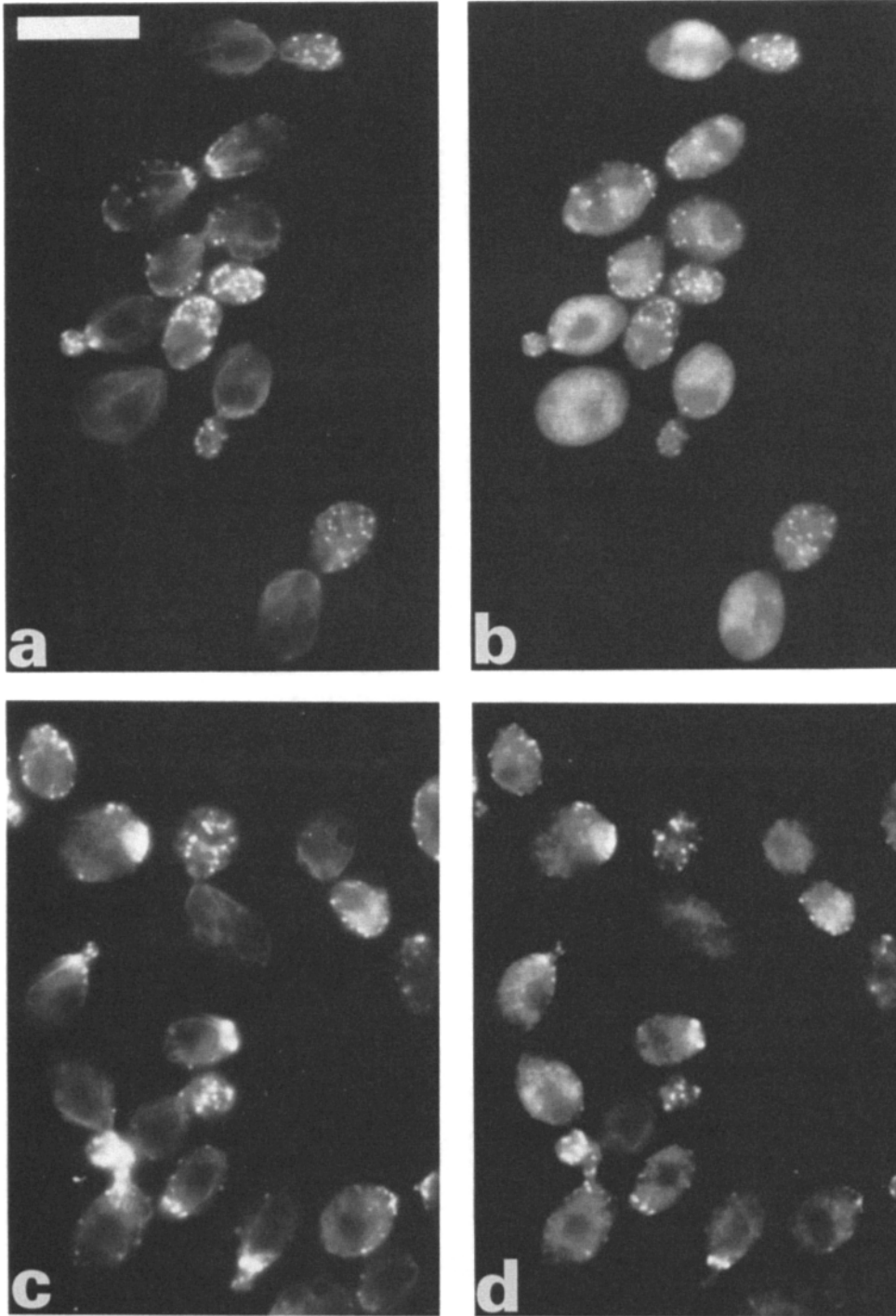


Figure 9. Cofilin localizes to cortical actin structures in the yeast cell. Wild-type cells (DDY 288) were processed for immunofluorescence using affinity-purified anti-yeast actin and anti-yeast cofilin antibodies as described in Materials and Methods. *a* and *c* show fluorescein staining of actin structures. *b* and *d* show rhodamine staining of cofilin structures. Bar is 10 μ m.

cofilin (*cofl-1::URA3*) and another removed all but the first two and last eight codons of *COFI* (*cofl- Δ 1::HIS3*) (see Materials and Methods for details). The disruptions were constructed by homologous recombination in diploid strains, and transformants were recovered based on Ura⁺ or His⁺ phenotypes. Three Ura⁺ and five His⁺ isolates were sporulated and tetrads were dissected. In every case but one, only

two of four spores were viable (Fig. 11), and the two live spores never carried the marker for the disruptions. Southern hybridizations confirmed that all strains contained one copy of *cofl-1::URA3* (DDY425) or *cofl- Δ 1::HIS3* (DDY427) except for the single His⁺ isolate, which yielded four viable spores. The spores that did not produce colonies were analyzed by microscopy. The *cofl-1::URA3* spores sometimes

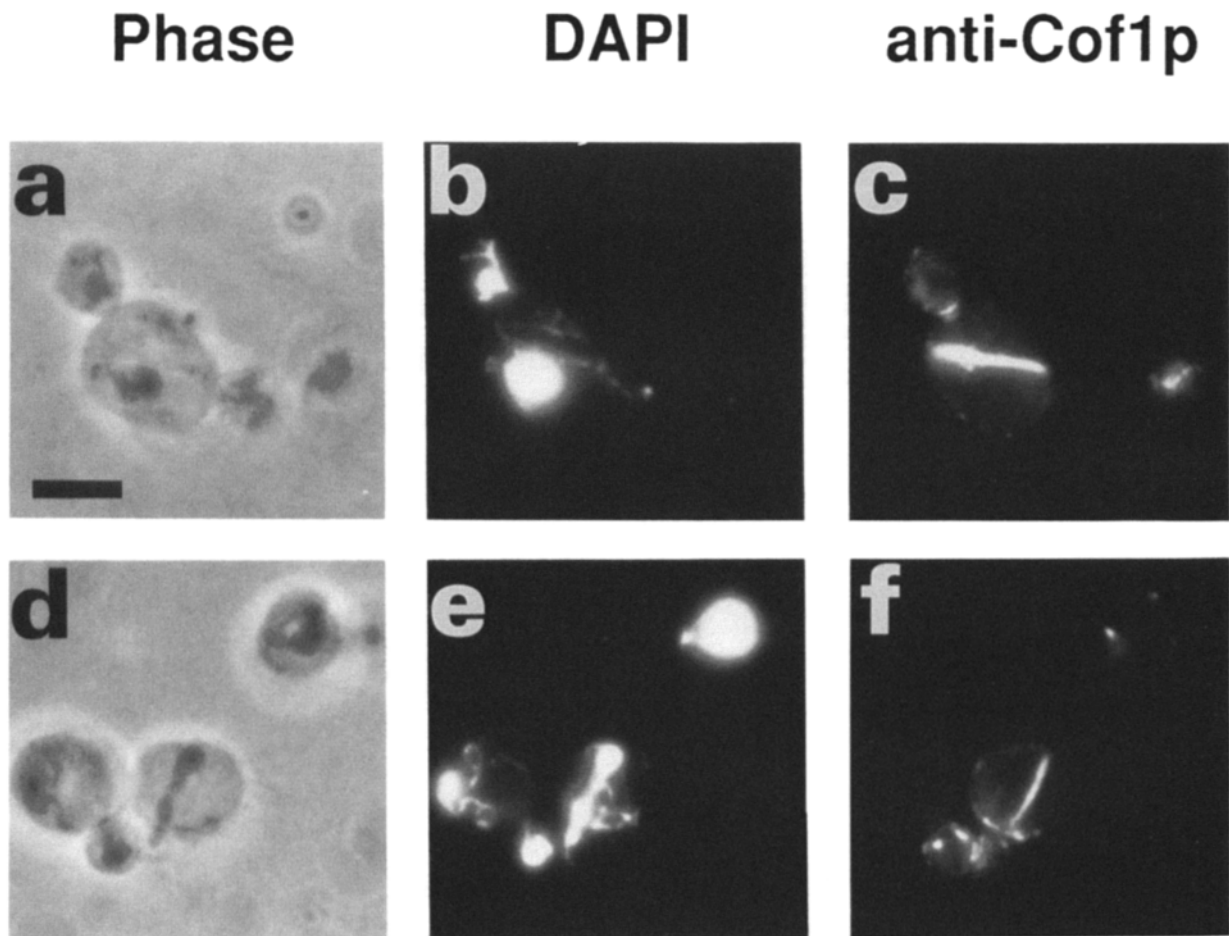


Figure 10. Cofilin is a component of abnormal actin structures that form in *actl-2* mutants. *actl-2* cells (DDY177) were grown at the permissive temperature of 25°C, conditions under which most cells contain actin bars, and the cells were processed for immunofluorescence using affinity-purified anti-yeast cofilin antibodies and a fluorescein-labeled secondary antibody. *a* and *d* show phase-contrast pictures of cells, *b* and *e* show DAPI staining of DNA, and *c* and *f* show fluorescein staining of cofilin structures. Bar is 5 μ m.

germinated and divided once or twice. Cells with the complete disruption (*cofl- Δ 1::HIS3*) never divided. However, these cells sometimes formed projections, which might reflect attempts at budding.

To further analyze the *cofl-1::URA3* truncation, in which a stop codon replaces the last 19 codons, extracts from heterozygous diploid cells were prepared and analyzed by immunoblotting with anti-cofilin antibodies. There is no higher mobility species of cofilin detected in these strains (Fig. 8, lane 3). Therefore, either the truncation creates a protein that is rapidly degraded, or the last 19 amino acids of yeast cofilin possess all of the epitopes recognized by our polyclonal antibodies.

The homology between cofilin and Abplp (Fig. 7), as well as their identical subcellular location, suggests that the two proteins might play similar roles in the cell. Although the single copy of *ABPL* present in wild-type cells cannot compensate for the loss of cofilin (Fig. 11), we speculate that overproduction of Abplp might suppress *cofl- Δ 1* lethality. The strain DDY427, a diploid heterozygous for a *COFL* disruption, was transformed with pRB1201, a multicopy vector containing a copy of the *ABPL* gene (Drubin et al., 1988). Sporulation and tetrad dissection gave results identical to

those in Fig. 11. Apparently, although Abplp contains sequences similar to cofilin, the functions of the two proteins are not redundant.

Discussion

Although *S. cerevisiae* is a simple eukaryote, the yeast actin cytoskeleton requires many types of actin regulatory proteins to function efficiently. Motors, filament bundling, filament stabilizing, monomer sequestering, and capping proteins have been identified, and each contributes to the proper functioning of actin in facilitating spatially directed cell growth. We wished to determine whether two other types of widely distributed actin binding proteins, the severing and gelation proteins, are utilized by yeast. The activities of these proteins are regarded as important for the cytoplasmic gel-sol transitions that are associated with cell locomotion. We provide the first evidence for the presence of both types of proteins in this nonmotile cell. There are at least three actin viscosity-decreasing activities in yeast extracts. The isolation and subsequent characterization of a cofilin-like protein as the agent of one of these activities confirms that yeast contain at least one severing protein. The other activities may

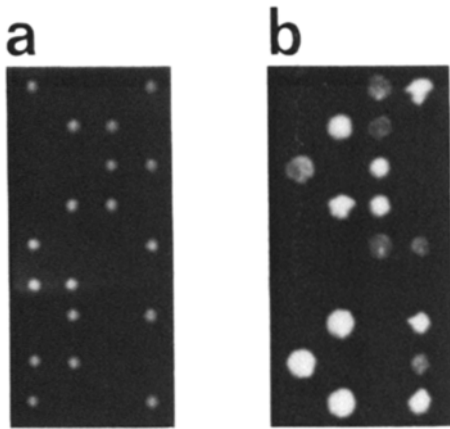


Figure 11. *COF1* is an essential gene. Four spores from tetrads were micromanipulated in rows on a YPD agar plate and grown at 25°C. (a) Tetrad dissection of DDD425 (*COF1/cof1-1::URA3*, a partial truncation). (b) Tetrad dissection of DDD427 (*COF1/cof1-Δ1::HIS3*, a full disruption).

be due to additional severing proteins, such as a gelsolin-type protein, sequestering proteins, or nucleating proteins (Isenberg et al., 1980). There is also a viscosity-increasing activity that binds to and elutes from a cation exchange column. This suggests the presence of a protein that can crosslink filaments into actin networks. Therefore, our work expands the possible arsenal of yeast actin binding proteins to include severing and gelation proteins. The presence of most types of actin binding proteins in yeast shows that even apparently simple actin function requires sophisticated regulation by numerous actin binding proteins.

Yeast cofilin is only 41% identical to mammalian cofilin, but retains remarkably similar biochemical activities. Comparison of the cofilin-like proteins from evolutionarily distant organisms may help to identify residues that are required for activity (Fig. 6). The regions corresponding to yeast cofilin residues 4–11 and 88–109 have the highest degree of similarity when the sequences of four proteins (yeast cofilin, mammalian cofilin, mammalian destrin, and starfish depactin) are compared. Biochemical studies also suggest that these two regions interact with actin. Residues of mammalian cofilin located within the highly conserved region corresponding to yeast cofilin residues 88–109 can be cross-linked to actin (Yonezawa et al., 1991b). In addition, the dodecapeptide WAPECAPLKSKM designed after sequences in this region of porcine cofilin has actin-depolymerizing activity (Yonezawa et al., 1991b). Site-directed mutagenesis of mammalian cofilin and analysis *in vitro* showed that specific point mutations of residues in this region can cause a marked decrease in actin binding ability (Moriyama et al., 1992). It is noteworthy that this stretch of residues is not only highly conserved among the cofilin-like proteins, but also in Abplp (Fig. 7, overlined sequence). Another peptide, DAIKKKL, corresponding to a region of mammalian cofilin that follows the WAPECAPLKSKM sequence, also has actin binding activity (Yonezawa et al., 1989). This sequence is not as well conserved in depactin or Abplp as it is in the cofilins and in destrin. The functional significance of these differences remains to be determined. Residues in the NH₂-terminal re-

gion of depactin (depactin residues 1–20) can also be cross-linked to actin (Sutoh and Mabuchi, 1989). Although no synthetic peptide or mutagenesis studies have yet been undertaken to further probe the interaction of this region with actin, the homology among the cofilin-like proteins' NH₂ termini indicates that this region might also be functionally important. The site-directed mutagenesis of residues in the NH₂ terminal, WAPECAPLKSKM, and DAIKKKL regions of yeast cofilin followed by gene replacement will help to determine how important these conserved residues are for cofilin's essential function *in vivo*.

In agreement with predictions based on its biochemical properties, cofilin was demonstrated by immunofluorescence to colocalize with F-actin structures in the cell. In addition, the possibility that cofilin might interact with G-actin *in vivo* is suggested by the cytoplasmic cofilin staining that is observed by immunofluorescence. This staining is absent from the nuclear region. Because a 16-kD protein should be below the exclusion limit of the nuclear pore (reviewed in Dingwall and Laskey, 1986), cofilin might be excluded from the nucleus because it associates with both F- and G-actin in the cytoplasm. Yeast cofilin associates with cortical F-actin, but not with cytoplasmic actin cables (Fig. 9). Yeast tropomyosin has the opposite distribution, associating only with the cytoplasmic cables (Liu and Bretscher, 1989a). It is perhaps relevant that mammalian tropomyosin and the mammalian cofilin-like proteins compete for binding to actin filaments *in vitro* (Bernstein and Bamburg, 1982; Nishida et al., 1984b, 1985).

Mammalian cofilin is distributed throughout the cytoplasm of mouse fibroblasts, is absent from stress fibers, and is concentrated in F-actin-rich ruffling membranes (Yonezawa et al., 1987). This localization is reminiscent of yeast cofilin distribution throughout the cytoplasm and in the cortical actin structures, but not on the cytoplasmic cables. Moreover, when cultured fibroblasts are exposed to an isotonic NaCl solution, stress fibers disappear and shorter actin rods form (Iida and Yahara, 1986). Like the bars formed in *act1-2* mutants, these rods do not stain with phalloidin, but are recognized by anti-cofilin antibodies (Nishida et al., 1987). Yeast and mammalian cofilin have similar activities *in vitro*, and their analogous subcellular localizations indicate that the cellular roles of the proteins might also be conserved. However, there are conditions under which the localizations of mammalian and yeast cofilins are not similar. Upon heat shock or treatment with 10% DMSO, abnormal actin bars similar to the cytoplasmic bars described above are found in the nucleus of mammalian cells (Nishida et al., 1987). Residues 26–34 of the mammalian protein (P---KRRKK) resemble the SV-40 large T-antigen nuclear localization signal (Matsuzaki et al., 1988). Mutation of the sequence to P---KTLKK prevents the nuclear accumulation of cofilin (Iida et al., 1992). The corresponding region of yeast cofilin contains the sequence GKYYK, but this does not appear to function as a nuclear localization signal. We have never seen yeast cofilin in the nucleus, even upon subjecting cells to heat shock (data not shown). Dephosphorylation of mammalian cofilin accompanies its nuclear accumulation upon heat shock or DMSO treatment (Ohta et al., 1989). It has been suggested (Ohta et al., 1989) that phosphorylation of Ser²⁴, which is a potential Ca²⁺/calmodulin-dependent protein kinase II target and is two residues upstream of the putative

nuclear localization signal P--KKRKK, might somehow prevent recognition of that sequence as a nuclear localization signal. The sequence of yeast cofilin does not reveal any potential phosphorylation sites in this region that might prevent nuclear accumulation. When mouse fibroblastic cells cultured under normal conditions are stained for cofilin, some nuclear localization is also seen (Yonezawa et al., 1987). These observations suggest that the mammalian protein might have roles in the nucleus that the yeast protein does not have.

We show that the entire cofilin sequence is similar to the NH₂-terminal 16 kD of Abplp. Yeast cofilin localization is also identical to that of Abplp. Despite these similarities, the presence of *ABPI* on a multicopy plasmid cannot compensate for the loss of cofilin, suggesting that the two proteins do not have redundant functions in the cell. Since loss of Abplp has no mutant phenotype, we cannot perform the reciprocal experiment. Like cofilin, we know that Abplp binds to actin filaments; it is now important to determine whether Abplp has severing activity and/or monomer binding activity. The configuration of Abplp with an NH₂-terminal cofilin domain and a COOH-terminal SH3 domain is analogous to myosin I, which has an NH₂-terminal motor domain with a COOH-terminal SH3 domain (Drubin et al., 1990). The SH3 domain of the Abl nonreceptor tyrosine kinase binds to a guanosine triphosphatase-activating-like protein (Cicchetti et al., 1992). Similar interactions involving SH3 domains may provide a linkage between signal transduction proteins and cytoskeletal proteins.

While the potential cofilin-like activity of Abplp might be controlled in part by its SH3 domain, other means of regulating cofilin activity in vivo are suggested by experiments done in vitro. The interaction of mammalian cofilin with actin is influenced in vitro by both pH (Yonezawa et al., 1985) and phospholipid binding (Yonezawa et al., 1990). Mammalian cofilin is also phosphorylated in vivo; this modification has not been demonstrated to influence cofilin's actin binding activity or cellular distribution (Ohta et al., 1989). Our preliminary results indicate that the phospholipid inhibition of cofilin's activity is conserved in yeast. This is consistent with peptide studies that suggest that the highly conserved region containing the WAPECAPLKSKM sequence is important for both actin and phospholipid binding (Yonezawa et al., 1991a). Phosphatidylinositol 4,5-bisphosphate has been implicated in the regulation of several actin binding proteins, including the monomer sequestering protein profilin (Lassing and Lindberg, 1985) and the actin severing/nucleating protein gelsolin (Janmey and Stossel, 1987). Compared with profilin and gelsolin, cofilin binds with nearly equal affinity to a larger range of phospholipids (phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 4-monophosphate, and phosphatidylinositol) (Yonezawa et al., 1990). The ability to differentially regulate proteins with overlapping biochemical activities may be important for cellular coordination of actin cytoskeleton dynamics.

Cofilin is an essential component of the yeast cortical actin cytoskeleton. Capping protein, fimbrin, and Abplp have previously been shown to be present in the yeast membrane cytoskeleton (Drubin et al., 1988; Amatruda and Cooper, 1992). The membrane cytoskeleton of yeast dynamically redistributes to determine the sites of cell surface growth. Regulation of the multiple actin binding activities at the cell

cortex is likely to be central to the ability of a yeast cell to develop different morphologies in response to cell cycle signals, nutrient limitation (Gimeno et al., 1992), or pheromone gradients (Jackson and Hartwell, 1990).

We thank Linda Riles for her help in the physical mapping of *COFI*. We thank Matthew Welch, Nate Machin, and Katayoun Chamany for helpful comments on the manuscript.

This work was supported by grants to D. Drubin from the National Institute of General Medical Sciences (GM-42759) and the Searle Scholars Program/The Chicago Community Trust, and by a grant to P. A. Janmey from the United States Public Health Service (AR-38910).

Received for publication 10 August 1992 and in revised form 30 September 1992.

References

- Adams, A. E., D. Botstein, and D. G. Drubin. 1991. Requirement of yeast fimbrin for actin organization and morphogenesis in vivo. *Nature (Lond.)* 354:404-408.
- Adams, A. E. M., and J. R. Pringle. 1984. Relationship of actin and tubulin distribution in wild-type and morphogenetic mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* 98:934-945.
- Amatruda, J. F., and J. A. Cooper. 1992. Purification, characterization, and immunofluorescence localization of *Saccharomyces cerevisiae* capping protein. *J. Cell Biol.* 117:1067-1076.
- 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 (Lond.)* 344:352-354.
- Attri, A. K., M. S. Lewis, and E. D. Korn. 1991. The formation of actin oligomers studied by analytical ultracentrifugation. *J. Biol. Chem.* 266: 6815-6824.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1989. *Short Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York. 208-228.
- Bamburg, J. R., H. E. Harris, and A. G. Weeds. 1980. Partial purification and characterization of an actin depolymerizing factor from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 121:178-182.
- Barnes, G., D. G. Drubin, and T. Stearns. 1990. The cytoskeleton of *Saccharomyces cerevisiae*. *Curr. Opin. Cell Biol.* 2:109-115.
- Bernstein, B. W., and J. R. Bamburg. 1982. Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). *Cell Motil.* 2:1-8.
- Chenevert, J., K. Corrado, A. Bender, J. Pringle, and I. Herskowitz. 1992. A yeast gene (*BEM1*) necessary for cell polarization whose product contains two SH3 domains. *Nature (Lond.)* 356:77-79.
- Cicchetti, P., B. J. Mayer, G. Thiel, and D. Baltimore. 1992. Identification of a protein that binds to the SH3 region of Abl and is similar to Bcr and GAP- ρ . *Science (Wash. DC)* 257:803-806.
- Cooper, J. A., J. D. Blum, R. J. Williams, and T. D. Pollard. 1986. Purification and characterization of actophorin, a new 15,000-dalton actin-binding protein from *Acanthamoeba castellanii*. *J. Biol. Chem.* 261:477-485.
- Cooper, J. A., S. B. Walker, and T. D. Pollard. 1983. Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization. *J. Muscle Res. Cell Motil.* 4:253-262.
- Dingwall, C., and R. A. Laskey. 1986. Protein import into the cell nucleus. *Annu. Rev. Cell Biol.* 2:367-390.
- 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.
- Drubin, D. G., J. Mulholland, Z. Zhimin, and D. Botstein. 1990. Homology of a yeast actin-binding protein to signal transduction proteins and myosin-I. *Nature (Lond.)* 343:288-290.
- Gehring, S., and M. Snyder. 1990. The *SPA2* gene of *Saccharomyces cerevisiae* is important for pheromone-induced morphogenesis and efficient mating. *J. Cell Biol.* 111:1451-1464.
- Gerring, S. L., C. Connelly, and P. Hieter. 1991. Positional mapping of genes by chromosome blotting and chromosome fragmentation. *Methods Enzymol.* 194:57-77.
- Gimeno, C. J., P. O. Ljungdahl, C. A. Styles, and G. R. Fink. 1992. Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* 68:1077-1090.
- Haarer, B. K., S. H. Lillie, A. E. 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.
- Hartwig, J. H., and D. J. Kwiatkowski. 1991. Actin-binding proteins. *Curr. Opin. Cell Biol.* 3:87-97.
- Iida, K., and I. Yahara. 1986. Reversible induction of actin rods in mouse C3H-2K cells by incubation in salt buffers and by treatment with non-ionic detergents. *Exp. Cell Res.* 164:492-506.

- Iida, K., S. Matsumoto, and I. Yahara. 1992. The KKRRK sequence is involved in heat shock-induced nuclear translocation of the 18-kDa actin-binding protein, cofilin. *Cell Struct. Funct.* 17:39-46.
- Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. 1990. PCR Protocols. Academic Press, Inc., San Diego. 3-12
- Isenberg, G., U. Aebi, and T. D. Pollard. 1980. A novel actin binding protein from *Acanthamoeba* which regulates actin filament polymerization and interactions. *Nature (Lond.)*. 288:455-459.
- Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163-168.
- Jackson, C. L., and L. H. Hartwell. 1990. Courtship in *S. cerevisiae*: both cell types choose mating partners by responding to the strongest pheromone signal. *Cell*. 63:1039-1051.
- Janmey, P. A., and T. P. Stossel. 1987. Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. *Nature (Lond.)*. 325:362-364.
- Johnston, G. C., J. A. Prendergast, and R. A. Singer. 1991. The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. *J. Cell Biol.* 113:539-551.
- Koch, C. A., D. Anderson, M. F. Moran, C. Ellis, and T. Pawson. 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science (Wash. DC)*. 252:668-674.
- Koffer, A., A. J. Edgar, and J. R. Bamburg. 1988. Identification of two species of actin depolymerizing factor in cultures of BHK cells. *J. Muscle Res. Cell Motil.* 9:320-328.
- Kouyama, T., and K. Mihashi. 1981. Fluorimetry study of N-(1-pyrenyl)-iodoacetamide-labeled F-actin. *Eur. J. Biochem.* 114:33-38.
- Lassing, I., and U. Lindberg. 1985. Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. *Nature (Lond.)*. 314:472-474.
- Liu, H. P., and A. Bretscher. 1989a. Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell*. 57:233-242.
- Liu, H. P., and A. Bretscher. 1989b. Purification of tropomyosin from *Saccharomyces cerevisiae* and identification of related proteins in *Schizosaccharomyces* and *Physarum*. *Proc. Natl. Acad. Sci. USA*. 86:90-93.
- 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.
- Mabuchi, I. 1983. An actin-depolymerizing protein (depactin) from starfish oocytes: properties and interaction with actin. *J. Cell Biol.* 97:1612-1621.
- Maciver, S. K., H. G. Zot, and T. D. Pollard. 1991. Characterization of actin filament severing by actophorin from *Acanthamoeba castellanii*. *J. Cell. Biol.* 115:1611-1620.
- MacLean-Fletcher, S. D., and T. D. Pollard. 1980. Viscometric analysis of the gelation of *Acanthamoeba* extracts and purification of two gelation factors. *J. Cell Biol.* 85:414-428.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Matsuzaki, F., S. Matsumoto, I. Yahara, N. Yonezawa, E. Nishida, and H. Sakai. 1988. Cloning and characterization of porcine brain cofilin cDNA. Cofilin contains the nuclear transport signal sequence. *J. Biol. Chem.* 263:11564-11568.
- Moriyama, K., S. Matsumoto, E. Nishida, H. Sakai, and I. Yahara. 1990a. Nucleotide sequence of mouse cofilin cDNA. *Nucleic Acids Res.* 18:3053.
- Moriyama, K., E. Nishida, N. Yonezawa, H. Sakai, S. Matsumoto, K. Iida, and I. Yahara. 1990b. Destrin, a mammalian actin-depolymerizing protein, is closely related to cofilin. Cloning and expression of porcine brain destrin cDNA. *J. Biol. Chem.* 265:5768-5773.
- 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.
- Mortimer, R. K., D. Schild, C. R. Contopoulou, and J. A. Kans. 1989. Genetic map of *Saccharomyces cerevisiae*, edition 10. *Yeast*. 5:321-403.
- Nishida, E., K. Iida, N. Yonezawa, S. Koyasu, I. Yahara, and H. Sakai. 1987. Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc. Natl. Acad. Sci. USA*. 84:5262-5266.
- Nishida, E., S. Maekawa, E. Muneyuki, and H. Sakai. 1984a. Action of a 19K protein from porcine brain on actin polymerization: a new functional class of actin-binding proteins. *J. Biochem.* 95:387-398.
- Nishida, E., S. Maekawa, and H. Sakai. 1984b. Cofilin, a protein in porcine brain that binds to actin filaments and inhibits their interactions with myosin and tropomyosin. *Biochemistry*. 23:5307-5313.
- Nishida, E., E. Muneyuki, S. Maekawa, Y. Ohta, and H. Sakai. 1985. An actin-depolymerizing protein (destrin) from porcine kidney. Its action on F-actin containing or lacking tropomyosin. *Biochemistry*. 24:6624-6630.
- Novick, P., and D. Botstein. 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell*. 40:405-416.
- Ohta, Y., E. Nishida, H. Sakai, and E. Miyamoto. 1989. Dephosphorylation of cofilin accompanies heat shock-induced nuclear accumulation of cofilin. *J. Biol. Chem.* 264:16143-16148.
- Olson, M. V., J. E. Dutchik, M. Y. Graham, G. M. Brodeur, C. Helms, M. Frank, M. MacCollin, R. Scheinman, and T. Frank. 1986. Random-clone strategy for genomic restriction mapping in yeast. *Proc. Natl. Acad. Sci. USA*. 83:7826-7830.
- Pardee, J. D., and J. A. Spudich. 1982. Purification of muscle actin. *Methods Enzymol.* 85:164-181.
- Pfeffer, S. R., D. G. Drubin, and R. B. Kelly. 1983. Identification of three coated vesicle components as alpha- and beta-tubulin linked to a phosphorylated 50,000-dalton polypeptide. *J. Cell Biol.* 97:40-47.
- 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., A. E. Adams, D. G. Drubin, and B. K. Haarer. 1991. Immunofluorescence methods for yeast. *Methods Enzymol.* 194:565-602.
- Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink. 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. *Gene (Amst.)*. 60:237-243.
- Rose, M. D., F. Winston, and P. Hieter. 1990. Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 198 pp.
- Schiestl, R. H., and R. D. Gietz. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* 16:339-346.
- Sikorski, R. S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*. 122:19-27.
- Snyder, M. 1989. The SPA2 protein of yeast localizes to sites of cell growth. *J. Cell Biol.* 108:1419-1429.
- Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, H. L. Southwick, H. L. Yin, and K. S. Saner. 1985. Nonmuscle actin binding proteins. *Annu. Rev. Cell Biol.* 1:353-402.
- Sutoh, K., and I. Mabuchi. 1989. End-label fingerprintings show that an N-terminal segment of depactin participates in interaction with actin. *Biochemistry*. 28:102-106.
- Takagi, T., K. Konishi, and I. Mabuchi. 1988. Amino acid sequence of starfish oocyte depactin. *J. Biol. Chem.* 263:3097-3102.
- Vandekerckhove, J. 1990. Actin-binding proteins. *Curr. Opin. Cell Biol.* 2:41-50.
- Vandekerckhove, J., and K. Vancompernelle. 1992. Structural relationships of actin-binding proteins. *Curr. Opin. Cell Biol.* 4:36-42.
- Woolford, J. L. J. 1989. Nuclear pre-mRNA splicing in yeast. *Yeast*. 5:439-457.
- Yonezawa, N., E. Nishida, and H. Sakai. 1985. pH control of actin polymerization by cofilin. *J. Biol. Chem.* 260:14410-14412.
- Yonezawa, N., E. Nishida, S. Koyasu, S. Maekawa, Y. Ohta, I. Yahara, and H. Sakai. 1987. Distribution among tissues and intracellular localization of cofilin, a 21kDa actin-binding protein. *Cell Struct. Funct.* 12:443-452.
- Yonezawa, N., E. Nishida, M. Ohba, M. Seki, H. Kumagai, and H. Sakai. 1989. An actin-interacting heptapeptide in the cofilin sequence. *Eur. J. Biochem.* 183:235-238.
- Yonezawa, N., E. Nishida, K. Iida, I. Yahara, and H. Sakai. 1990. Inhibition of the interactions of cofilin, destrin, and deoxyribonuclease I with actin by phosphoinositides. *J. Biol. Chem.* 265:8382-8386.
- Yonezawa, N., Y. Homma, I. Yahara, H. Sakai, and E. Nishida. 1991a. A short sequence responsible for both phosphoinositide binding and actin binding activities of cofilin. *J. Biol. Chem.* 266:17218-17221.
- Yonezawa, N., E. Nishida, K. Iida, H. Kumagai, I. Yahara, and H. Sakai. 1991b. Inhibition of actin polymerization by a synthetic dodecapeptide patterned on the sequence around the actin-binding site of cofilin. *J. Biol. Chem.* 266:10485-10489.