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Purification of tropomyosin from *Saccharomyces cerevisiae* and identification of related proteins in *Schizosaccharomyces* and *Physarum*

(actin/yeast/microfilaments/cytoskeleton)

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ABSTRACT **Tropomyosin is a key component of the contractile systems found in muscle and nonmuscle cells of higher eukaryotes. Based on properties common to all tropomyosins, we have purified a protein from *Saccharomyces cerevisiae* that resembles tropomyosins from higher cells. The yeast protein remains soluble after heat treatment at 90°C, has an apparent polypeptide molecular weight of 33,000, an isoelectric point of 4.5, a Stokes radius of 3.5 nm, and a sedimentation coefficient of 2.6 S. It binds F-actin in a Mg^{2+}-dependent, KCl-modulated manner, up to a stoichiometry of about 1 polypeptide per 3.0 actin monomers. In all these properties it is very similar to tropomyosins from higher cells. Antigen-affinity-purified antibodies specifically recognize the M_r 33,000 polypeptide among total yeast proteins and crossreact with bovine brain tropomyosin. In addition, the antibodies specifically crossreact with heat-stable M_r 33,000 polypeptides in extracts of *Schizosaccharomyces pombe* and *Physarum polycephalum*. Our detection of tropomyosin in lower eukaryotes suggests that they might have contractile systems very similar to those found in higher organisms.**

Tropomyosin is a major component of the contractile apparatuses of skeletal and smooth muscles as well as of the microfilament arrangements of nonmuscle cells (reviewed in refs. 1 and 2). It is invariably associated with filamentous actin (F-actin), where it is generally believed to be involved in the Ca^{2+} regulation of the actomyosin interaction: in skeletal muscle it is complexed with troponin (3) and in smooth muscle it may also be involved in regulation in conjunction with caldesmon and calmodulin (reviewed in ref. 4). The similarity of the protein components of the contractile apparatus in smooth muscle with those present in an ordered pattern in stress fibers of nonmuscle cells (including myosin, actin, myosin light-chain kinase, caldesmon, and tropomyosin) has led to the notion that stress fibers may contain "contractile units" analogous to those found in smooth muscle (5). Therefore, in both muscle and nonmuscle cells, tropomyosin probably plays a pivotal role in the regulation of contraction of the actomyosin system.

We are interested in undertaking a combined genetic and biochemical analysis of the regulation of contraction, so we chose *Saccharomyces cerevisiae*, an organism in which the full power of genetics can be applied. It contains a single actin gene that predicts a protein sequence highly homologous to the nonmuscle actins of higher eukaryotes (6, 7), and this gene has been shown to be essential for viability (8); moreover, yeast contains myosin (9, 10). We therefore set out to determine whether yeast contains key regulatory proteins, in particular tropomyosin, and, if so, how the properties of yeast tropomyosin compare with those of higher cell tropomyosins. We chose tropomyosin not only because of its key role in regulation but also because tropomyosins as a group have distinct features that have been extensively studied at the functional, biochemical, and genetic levels in higher cells (1). To date, however, tropomyosins have only been clearly documented in higher multicellular organisms (2).

All tropomyosins so far isolated are highly α-helical, rod-shaped dimeric proteins. They are very acidic proteins that remain native after heating to 90°C and have the distinctive property of requiring a divalent cation in their interaction with F-actin. In this report we use these properties to isolate a protein from yeast extracts and show that it has many characteristics in common with typical nonmuscle cell tropomyosins. In addition, we show that immunologically and functionally related proteins are present in some other lower eukaryotes.

MATERIALS AND METHODS

Organisms and Culture Conditions. *S. cerevisiae* 20B-12 (*MATα, trp1, gal2, pep4-3*) from B. Tye (Cornell University) was used throughout and grown in YEPD medium to late logarithmic phase. *Schizosaccharomyces pombe* SP223 (*leu1-32, ade6-216, ura4*) from T. Fox (Cornell University) was grown in YEPD medium. The plasmodial and amoeboid forms of *Physarum polycephalum* (from Carolina Biological, Burlington, NC) were kindly grown and provided by D. Muscarella (Cornell University). *Acanthamoeba castellanii* was kindly provided by E. Korn (National Institutes of Health, Bethesda, MD) and grown as described (11). *Dictyostelium discoideum* strain AX3 was kindly provided by H. Lodish (Whitehead Institute for Biomedical Research, Cambridge, MA) and grown as described (12). *Chlamydomonas reinhardtii* was kindly grown and provided by K. Kindle (Cornell University).

Purification of Proteins. Yeast tropomyosin was purified as described in *Results*. Bovine brain and chicken gizzard tropomyosins (13, 14) and rabbit skeletal muscle actin (15) were purified as described.

Immunological Techniques. Production of antibodies to bovine brain tropomyosin has been described (see, for instance, ref. 16). To generate antibodies to the yeast protein, purified M_r 33,000 protein (p33) was run in preparative $NaDodSO_4$/polyacrylamide gels, visualized by brief staining with Coomassie blue, excised, and electrophoretically eluted. About 0.2 mg of p33 in phosphate-buffered saline was mixed with an equal volume of Freund's complete adjuvant and injected into one rabbit. On days 21 and 42 the rabbit was given booster injections with about 30 μg of the protein in Freund's incomplete adjuvant. On day 54 the antiserum was tested by double diffusion against the purified protein and by immunoblot analysis against total yeast proteins. Affinity-

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Abbreviation: p33, polypeptide of apparent M_r 33,000.

purified antibodies were prepared by adsorption to purified p33 immobilized on Affi-Gel 15 (Bio-Rad) in borate buffer (0.1 M H_3BO_4/0.25 M $Na_2B_4O_7$/0.075 M NaCl, pH 8.4) and elution with 8 M urea in borate buffer. Immediately after elution, the antibodies were dialyzed into phosphate-buffered saline. Immunoblotting was performed essentially as described (17).

Other Techniques. F-actin cosedimentation assays were performed as described (14). In outline, all the proteins were separately dialyzed overnight into 100 mM KCl/0.5 mM $MgCl_2$/1 mM dithiothreitol/2 mM ATP/5 mM Tris·HCl, pH 7.6, and then mixed in appropriate amounts to give final actin concentrations of 0.1 mg/ml. Other proteins and additions were as described in the text. Samples (200 μl) were incubated at room temperature for 30 min and then the F-actin and its binding proteins were sedimented by centrifugation at 100,000 × *g* for 20 min at room temperature in a Beckman Airfuge. The pelleted and supernatant fractions were subjected to gel electrophoresis and the percentage of each protein in the two fractions was determined by densitometry of stained gels. $NaDodSO_4$ and isoelectric focusing gels were run as described (14). The sedimentation coefficient and Stokes radius of the yeast protein were determined by comparison with proteins of known values, as described previously (14). Amino acid analyses were performed in duplicate by HPLC on samples hydrolyzed *in vacuo* at 150°C for 1 hr as described (18).

RESULTS

Purification and Characterization of a Mg^{2+}-Dependent F-Actin-Binding Protein from Yeast. Preliminary experiments revealed that among the proteins present in a high-salt, heat-stable extract of *S. cerevisiae*, only a M_r 33,000 polypeptide (p33) could be detected that bound F-actin in a Mg^{2+}-dependent manner. Using this assay, we developed a method for its purification (Fig. 1), which is typical of that used for nonmuscle tropomyosins (see, for example, ref. 19). Yeast tropomyosin was routinely purified from 100 g (wet weight) of washed packed cells, which were resuspended in 150 ml of 0.3 M KCl/0.1 mM ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA)/0.5 mM $MgCl_2$/1 mM dithiothreitol/50 mM imidazole·HCl, pH 6.9/0.3 mM phenylmethylsulfonyl fluoride/0.5 mM benzamidine. The cells were broken by passage through a French pressure cell operated at >15,000 psi (103.5 MPa) and the lysate was immediately boiled for 10 min. After cooling to 4°C, the lysate was clarified at 12,000 × *g* for 20 min. The heat-soluble extract was made to 30% saturation in ammonium sulfate and clarified by centrifugation, and the ammonium sulfate concentration was increased to 65% saturation. This material was collected by centrifugation, dissolved and dialyzed into 0.2 M KCl/10 mM (KH_2PO_4/K_2HPO_4), pH 7.0/1 mM dithiothreitol, clarified by centrifugation, and applied to a 20-ml DE52 (Whatman) anion-exchange column. After the column was washed with 50 ml of this buffer, it was developed with a 200-ml gradient from 0.2 to 0.4 M KCl. The peak fractions of yeast tropomyosin, which were eluted at 0.3 M KCl, were loaded directly on a 7-ml hydroxylapatite column (HA-Ultrogel, LKB-Pharmacia), which was then washed with 10 ml of 1 M KCl/10 mM (KH_2PO_4/K_2HPO_4), pH 7.0/1 mM dithiothreitol and developed with a gradient from 10 to 200 mM phosphate. The purified protein was dialyzed into 0.5 mM dithiothreitol (pH 7.0) and concentrated by lyophilization. The protein was dissolved in a small volume of F-actin binding buffer and was stable in this buffer for at least 1 year at 4°C. We recovered a M_r 33,000 polypeptide and a variable minor M_r 20,000 polypeptide that was almost certainly a degradation product as it is immunologically related to p33 (data not shown). The yield of the protein was about 1 mg from 100 g of wet cells. This indicates that it is present in yeast at a level of at least 0.01% of the total cell protein.

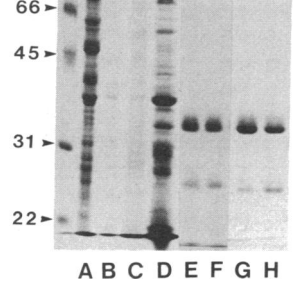

FIG. 1. Purification of yeast p33 as shown by $NaDodSO_4$/10% PAGE analysis of the polypeptides present at various stages in the purification of the protein. Lanes: A, total soluble yeast proteins; B, equivalent load of heat-soluble proteins; C, proteins precipitated by ammonium sulfate at 30% saturation; D, proteins precipitated by ammonium sulfate at 30–65% saturation, which were then dialyzed and applied to a DE52 anion-exchange column; E and F, peak fractions from the DE52 column, which were applied to a hydroxylapatite column; G and H, peak fraction from the hydroxylapatite column. Molecular weight standards ($M_r \times 10^{-3}$) are indicated at left. The same number of cells was used to generate the samples shown in lanes A and B, thus indicating that the vast majority of *S. cerevisiae* proteins are precipitated by the heat treatment.

The physical and chemical properties of the yeast protein were determined and compared with those of equine platelet tropomyosin (Tables 1 and 2). In terms of its polypeptide molecular weight (33,000), isoelectric point (4.5), and sedimentation coefficient (2.6 S), p33 is very similar to the higher cell tropomyosin. Its amino acid composition is similar to that of platelet tropomyosin in terms of acidic and basic amino acids and a low level of aromatic residues but is also significantly different (Table 2).

We have explored the interaction of the yeast p33 protein with F-actin by making use of an F-actin cosedimentation assay. Incubation of increasing amounts of p33 with F-actin resulted in a typical saturation curve with an estimated molar ratio of about one p33 polypeptide per 3.0 actin monomers near saturation. This is the same molar ratio found for the binding of platelet tropomyosin to F-actin (20). Use of an excess of the yeast p33 polypeptide in F-actin binding assays demonstrated that the yeast protein bound to F-actin in a Mg^{2+}-dependent manner, with optimal binding occurring above 2.5 mM Mg^{2+} (Fig. 2*A*). Binding to F-actin in the presence of Mg^{2+} was optimal in 50–150 mM KCl (Fig. 2*B*).

Table 1. Comparison of the physical properties of yeast p33 and equine platelet tropomyosin

Property	Yeast p33	Platelet tropomyosin*
Apparent subunit M_r†	33,000	30,000
Isoelectric point‡	4.5	4.5
Sedimentation coefficient§	2.6 S	2.7 S
Stokes radius¶	3.5 nm	ND

*Data from ref. 19. ND, not determined.
†Determined from $NaDodSO_4$/PAGE by comparison with standards.
‡Determined as described (14) by comigration with chicken gizzard tropomyosin in isoelectric focusing gels.
§Determined as described (14) by comparison with known proteins (bovine serum albumin, ovalbumin, and myoglobin).
¶Determined as described (14) by gel filtration on a Sephadex G-150 column calibrated with bovine serum albumin, aldolase, and catalase.

Table 2. Amino acid compositions of yeast p33 and equine platelet tropomyosin

Amino acid	mol/mol of polypeptide*	
	Yeast p33	Platelet tropomyosin
Asx	40	22
Glx	86	70
Ser	24	7
Gly	8	7
His	5	3
Arg	9	19
Thr	10	8
Ala	18	29
Pro	3	0
Tyr	4	3
Val	9	10
Met	2	5
Cys	ND	2
Ile	8	10
Leu	32	29
Phe	3	1
Lys	31	27

*Values were calculated by assuming a polypeptide molecular weight of 33,000 for the yeast protein and 30,000 for the platelet protein. Tryptophan and cysteine were not determined (ND). Data for platelet tropomyosin are from ref. 19.

Under all the conditions used, >90% of the actin sedimented, whereas a nondetectable amount of p33 sedimented on its own.

Immunological Relationship of Yeast p33, Bovine Brain Tropomyosin, and Proteins Present in Other Lower Eukaryotes. On immunoblots, no crossreaction was detected between an antibody to bovine brain (nonmuscle) tropomyosin and yeast p33 (Fig. 3*B*). However, antibodies generated to yeast p33 and affinity-purified on immobilized antigen recognize only this protein in total yeast extracts and also crossreact with bovine brain tropomyosin (Fig. 3*C*), indicating at least some immunological relatedness between these proteins. Interestingly, the antibodies to the yeast protein do not recognize chicken gizzard (smooth muscle) tropomyosin, whereas the antibodies to bovine brain tropomyosin do.

Affinity-purified antibodies to the *S. cerevisiae* protein were used to search for immunologically related proteins in other lower eukaryotes. High-salt extracts of each organism were prepared and boiled, and the heat-soluble proteins were concentrated and subjected to gel electrophoresis and immunoblotting with the antibody to the *S. cerevisiae* protein p33.

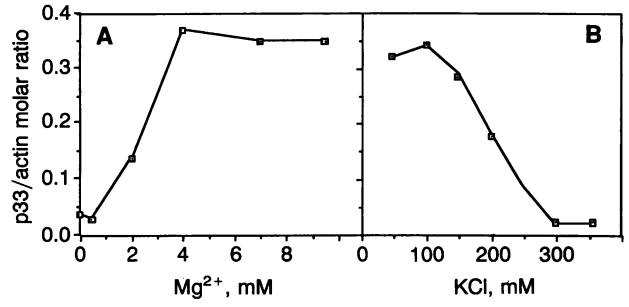

FIG. 2. Effect of Mg^{2+} and KCl on the interaction of yeast p33 with F-actin. (*A*) Binding of the yeast protein to F-actin as a function of Mg^{2+} concentration, determined by F-actin cosedimentation assay. All tubes contained p33 at 0.05 mg/ml and actin at 0.1 mg/ml. Results are expressed as a polypeptide molar ratio as determined by densitometry of Coomassie blue-stained gels. (*B*) Similar experiment in which the KCl concentration was varied; all tubes contained 8 mM $MgCl_2$.

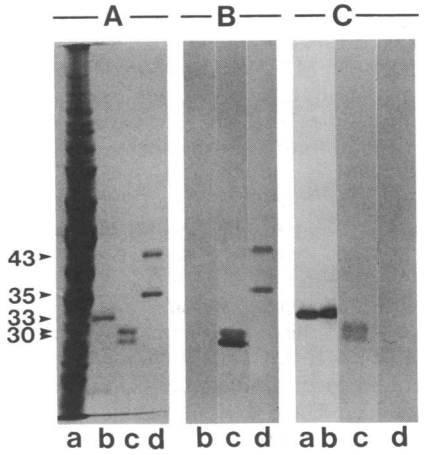

FIG. 3. Immunological characterization of yeast p33. (*A*) Stained $NaDodSO_4$/10% polyacrylamide gel. Lanes a, total yeast proteins; b, purified yeast p33; c, bovine brain tropomyosin (a doublet with apparent $M_r \approx 30,000$); d, chicken gizzard tropomyosin (polypeptides with apparent M_r of 35,000 and 43,000). (*B*) Immunoblot of the proteins in lanes b–d probed with antibody to bovine brain tropomyosin. (*C*) Immunoblot of the proteins in lanes a–d probed with an antigen-affinity-purified antibody to yeast p33. Apparent polypeptide molecular weights ($M_r \times 10^{-3}$) are shown at left.

Immunologically related proteins of apparent M_r 33,000 were found in *Sch. pombe* and the amoeboid phase of *P. polycephalum* (Fig. 4, lanes A–C and a–c). To test whether these were functionally similar to the *S. cerevisiae* protein, the ability of the *Physarum* immunoreactive species to bind F-actin was determined by the F-actin cosedimentation assay. The resulting supernatants and pellets were subjected to gel electrophoresis and the locations of the immunoreactive species

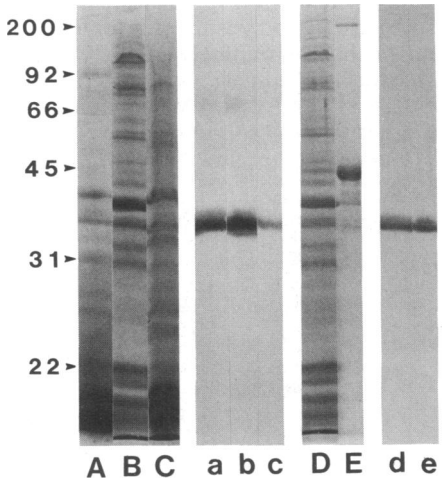

FIG. 4. *Sch. pombe* and *P. polycephalum* contain M_r 33,000 polypeptides that are immunologically related to the *Saccharomyces cerevisiae* M_r 33,000 protein p33. High-salt, heat-soluble extracts of *S. cerevisiae* (lanes A and a), *Sch. pombe* (lanes B and b), and the amoeboid phase of *P. physarum* (lanes C and c) were run in duplicate $NaDodSO_4$/12% polyacrylamide gels. One set was stained with Coomassie blue (lanes A–C) and the other set was transferred to nitrocellulose and probed with affinity-purified antibodies to the *S. cerevisiae* protein p33. In a separate experiment, heat-soluble proteins from the amoeboid phase of *P. physarum* were dialyzed into F-actin binding buffer containing 6 mM $MgCl_2$, mixed with F-actin, and sedimented by high-speed centrifugation, and samples were prepared for electrophoresis. Lanes D and E, Coomassie-stained gels of the supernatant and pellet fractions; lanes d and e, the corresponding immunoblots probed with antibody to the *S. cerevisiae* protein. Positions of molecular weight markers ($M_r \times 10^{-3}$) are indicated at left.

were determined by immunoblotting (Fig. 4, lanes D, E, d, and e). Under the conditions used, about 50% of the immunologically related peptide sedimented with F-actin, whereas none sedimented on its own.

DISCUSSION

We describe the identification of a protein from *S. cerevisiae* that has many properties in common with higher cell nonmuscle tropomyosins, but also some notable differences. All tropomyosins so far characterized migrate with an anomalously high apparent molecular weight in urea/NaDodSO$_4$ gels, although the basis for this effect is not known. Surprisingly, the yeast p33 protein does not show this property (data not shown). In preliminary experiments we have not observed a stimulation of the skeletal muscle actomyosin ATPase by the yeast protein, as is found for smooth muscle tropomyosin (21). However, the physical properties of the yeast protein and the way in which it binds to F-actin in a saturable, Mg^{2+}-dependent manner are very characteristic of a typical tropomyosin (see, for example, ref. 20). One surprising feature of the amino acid composition was the presence, albeit low, of proline residues.

Tropomyosins contain >90% α-helix and are typically devoid of helix-breaking proline residues. Results to be presented elsewhere on the protein sequence derived from the DNA sequence indicate no proline residues; the apparent presence of these residues in our sample must be due to contamination. The biochemical data therefore strongly suggest that the *S. cerevisiae* protein is closely related to higher cell tropomyosins, and immunological data indicate a relationship between the yeast protein and higher cell nonmuscle tropomyosins. Our recent molecular genetic experiments reveal that *S. cerevisiae* contains a single gene for this protein, and the DNA sequence predicts a polypeptide sequence showing significant homology to tropomyosins (unpublished data). Moreover, localization of the protein by immunofluorescence microscopy revealed that it colocalizes with actin filaments in yeast cells (unpublished data). We believe these results provide overwhelming evidence that the yeast protein is tropomyosin. It is present in yeast as at least 0.01% of the total cell protein, which is about the level expected, as actin is present at about 0.1% of the total protein (22).

Lower eukaryotes have been used extensively to study the actomyosin contractile system. Extensive characterization of actin and myosin from *Acanthamoeba, Dictyostelium,* and *Physarum* has revealed that these proteins are remarkably similar to their higher cell counterparts (for review, see ref. 23). We have not been able to find any report describing the presence of tropomyosin in yeasts or in these organisms. Our demonstration that *S. cerevisiae* contains tropomyosin and that related proteins are present in *Sch. pombe* and the amoeboid phase of *P. polycephalum* suggests that tropomyosins may be present in other lower eukaryotes. In preliminary experiments we have been unable to detect a protein immunologically related to yeast tropomyosin in *Acanthamoeba, Dictyostelium,* or *Chlamydomonas* or, surprisingly, in the plasmodial phase of *P. physarum*. However, this may simply be a problem of immunological crossreactivity, or low abundance of the proteins, rather than a true absence of tropomyosin from these organisms.

The presence of actin (6–8), myosin (9, 10), and now tropomyosin in *S. cerevisiae* indicates that this organism may have a contractile system similar to that of higher cells. It may therefore provide an ideal system in which to undertake a molecular genetic approach to investigate the mechanism and regulation of contraction.

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