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Yeast actin filaments display ATP-dependent sliding movement over surfaces coated with rabbit muscle myosin

(motility assay/evolutionary conservation/protein function)

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ABSTRACT **The yeast *Saccharomyces cerevisiae* has been used to study the function of components of the actin cytoskeleton *in vivo*, mainly because it is easy to derive and characterize mutations affecting these proteins. In contrast, biochemical studies have generally used proteins derived from higher eukaryotes. We have devised a simple procedure to prepare, in high yield, homogeneous native actin from wild-type and *act1* mutant yeast. Using intensified video fluorescence microscopy, we found that actin filaments polymerized from these preparations exhibit ATP-dependent sliding movement over surfaces coated with rabbit skeletal muscle myosin. The rates of sliding movement of the wild-type and mutant yeast actins were each about half that of rabbit skeletal muscle actin under similar conditions. We conclude that over the large evolutionary distance between yeast and mammals there has been significant conservation of actin function, specifically the ability to be moved by interaction with myosin.**

The interaction of the ubiquitous eukaryotic proteins actin and myosin couples the hydrolysis of ATP to force production, driving muscle contraction and many other types of cell motility. After nearly 50 years of intense biochemical and structural analysis of actin–myosin interaction, the mechanisms of both chemomechanical coupling and regulation of actomyosin activity remain obscure. To enhance structure–function studies of proteins purified from mammalian muscle tissues, powerful biochemical techniques have been developed that provide true functional assays for actin–myosin interaction. The sliding movement of actin filaments over myosin-coated surfaces can be directly observed by fluorescence microscopy (1), reconstituting motility in a geometry where quantitative measurement of rates of movement (2) and magnitude of forces (3) can be made with purified proteins. However, lack of access to mutational analysis in studies with muscle proteins limits further progress. Bacterial expression of the cloned muscle actin and myosin genes has not yielded biochemical quantities of stable, soluble proteins. Although expression of modified muscle protein genes in both muscle and nonmuscle eukaryotic cells (4, 5) has been reported, no system has been established that offers both *in vivo* analysis of phenotypes and *in vitro* analysis of purified proteins.

A potentially more powerful approach is provided by analysis of altered forms of the nonmuscle actins and myosins of single-celled organisms expressed in their natural environments, such as is possible in *Dictyostelium discoideum* (6, 7) and *Saccharomyces cerevisiae* (8). The wild-type forms of these myosins and actins have been found by many criteria to be structurally and functionally interchangeable with their cognate muscle proteins. Studies in either slime mold or yeast have the potential to permit both analysis of *in vivo* effects of site-directed mutations and ready purification of the modified proteins, potentiating *in vitro* analysis. However, the well-developed classical and molecular genetic approaches available with yeast facilitate the identification of sequences mediating functional interactions between proteins.

In *Saccharomyces*, conventional actin is expressed from a single essential gene (9), *ACT1*, that encodes a 374-amino acid (42-kDa) sequence 87% identical to rabbit skeletal muscle actin (10, 11). Localization of actin in growing yeast to the bud neck and in cables pointing into the nascent bud (12, 13) suggest a role in myosin-based contraction and/or transport. Several myosin-related genes have been identified, including *MYO1*, the disruption of which results in a cell-division defect in the resulting mutant strain (14), and *MYO2*, an unconventional myosin required for proper secretory function (15). Purified yeast and muscle actins have similar biochemical properties. Yeast actin polymerizes to form stable filaments in high-ionic-strength buffer but remains monomeric at low ionic strength. Its polymerization is inhibited by DNase I and cytochalasins and promoted by phalloidin (16).

The stable and specific binding of monomeric actin to pancreatic DNase I that results in inhibition of DNase activity (17) has been exploited in earlier purification protocols for yeast actin. Koteliansky *et al.* (18) performed anion-exchange chromatography of a yeast extract and pooled fractions that inhibited DNase I activity to isolate native actin of moderate purity. Water *et al.* (19) used DNase I affinity chromatography (17) to isolate actin from a yeast extract but found that elution with the denaturant guanidine hydrochloride led to irreversible loss of polymerizability. Using conventional chromatography and DNase inhibition as an assay, Greer and Schekman (16) purified biochemical quantities of homogeneous assembly-competent yeast actin. To obtain yeast actin for antibody production, Kilmartin and Adams (12) used formamide (20, 21) to elute actin from a DNase I column and achieved a single-step preparation of native actin from a whole-cell extract.

Random *in vitro* mutagenesis of *ACT1* and reintroduction of the mutant actin into yeast to replace the wild-type copy identified two independent point mutations that conferred a temperature-sensitive lethal phenotype *in vivo* (8). Subsequently, a site-directed mutagenesis approach has yielded a much wider library of viable actin mutants (K.F. Wertman, D.G.D., and D.B., unpublished results) with many conditional phenotypes. To make use of these mutants in biochemical studies, we needed a purification more adaptable to many small preparations than that of Greer and Scheckman (16) and

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Abbreviations: BSA, bovine serum albumin; CT-S1, chymotryptic myosin subfragment 1; DTT, dithiothreitol; Me_4RP, tetramethylrhodamine phalloidin; P-Mg.S1, papain/Mg^{2+} myosin subfragment 1.

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we needed additional biochemical assays for actin function. Here we report a motility assay that uses yeast actin purified by a simple procedure and purified rabbit muscle skeletal myosin.

MATERIALS AND METHODS

Muscle Proteins. Rabbit skeletal muscle actin was isolated by a modification (22) of the method of Pardee and Spudich (23). Myosin was isolated from rabbit back muscle (24). Chymotryptic myosin subfragment 1 (CT-S1) (25) and papain/Mg^{2+} subfragment 1 (P-Mg.S1) (2) were prepared as described. Total protein was measured by dye binding assay (26).

Yeast Strains and Cell Growth. Yeast strain DBY877 (*MATa his4-619*) was grown in YPD medium [1% yeast extract (Difco)/2% Bacto-peptone (Difco)/2% glucose] at 30°C in shaker flasks to stationary phase (OD_{600} 8–13). Yeast strain DBY1995 (*MATa his4-619 act1-3*), which expresses a temperature-sensitive mutant actin (8, 27), was grown in YPD medium at 22°C to stationary phase (OD_{600} 8–13). Cells were harvested by centrifugation, washed in cold 10 mM imidazole/HCl (pH 7.4), and recentrifuged. The second cell pellet was resuspended to 50% (wt/vol) in this buffer. This cell slurry was allowed to fall in large drops from a pipette into liquid N_2, rapidly freezing to form cell "sleet" that was stored at −20°C or −80°C.

Chromatography Materials. Formamide, deionized with AG501-X8 (Bio-Rad) and purged with N_2, was stored at 4°C or −20°C. DNase I (Sigma type IV) was coupled to Affi-Gel 10 (Bio-Rad) (28). In brief, 200 mg of DNase I was dissolved in 10 ml of coupling buffer (100 mM Hepes, pH 7.2/80 mM $CaCl_2$/1 mM phenylmethylsulfonyl fluoride), dialyzed against this buffer overnight at 4°C, mixed with 10 ml of washed Affi-Gel 10, and incubated 4 hr at 4°C. Excess reactive sites were blocked with 10 mM ethanolamine (pH 7.0) for 1 hr at 4°C. Column capacity was variable, but low relative to the amount of immobilized DNase I. Columns were stored in DNase column buffer [10 mM imidazole/HCl, pH 7.4/0.5 mM $CaCl_2$/0.5 mM ATP (Calbiochem)/1 mm dithiothreitol (DTT; Boehringer Mannheim ultrapure)] and gradually lost capacity over months. Others have found more satisfactory performance from other coupling methods (29) or commercially available DNase affinity media (30). TSK-gel DEAE 650M (Supelco) was packed into glass medium-pressure columns (Omnifit, Atlantic Beach, NY) run on a Waters 650 medium-pressure liquid chromatography system at 4°C.

DNase I Inhibition Assay. A modification of the method of Blikstad *et al.* (31) was used to measure actin inhibition of DNase I activity. Salmon sperm DNA (Sigma type III) was dissolved in DNase assay buffer (10 mM imidazole/HCl, pH 7.4/4 mM $MgSO_4$/1 mM $CaCl_2$) and stored at −80°C. DNA aliquots were thawed, diluted to 50 μg/ml in assay buffer, and equilibrated to 22°C. Pancreatic DNase I (Worthington) was dissolved in assay buffer and diluted to 50 μg/ml before each experiment. To begin each assay, 0.75 μg of DNase and a known volume of an actin-containing solution were combined and assay buffer was added to reach a final volume of 25 μl. This solution was incubated >5 min at 22°C. Then 975 μl of DNA solution was added and the solution was mixed and transferred to a cuvette. After 10–15 sec, the A_{260} was measured at 0.5-sec intervals for 20 sec (model 8452A; Hewlett–Packard), and the data were subjected to linear regression analysis. Percent inhibition was derived from the relative rates of increase in absorbance with and without added actin. DNase inhibition activity of 1 unit/ml was defined as the inhibition due to purified yeast actin at 1 μg/ml.

Binding Assay. The binding of CT-S1 to actin was measured by centrifugation and assay of myosin ATPase activity remaining in the supernatant. Centrifugation of 150-μl aliquots of 4 μM skeletal muscle actin–phalloidin complex for 6 min at 100,000 rpm in a TLA100 rotor (Beckman–Spinco) was found to pellet >95% of protein, whereas centrifugation of CT-S1 under the same conditions pelleted little or no protein even after 15 min. Each binding reaction mixture contained 9 μg of CT-S1 in 150 μl of binding buffer [25 mM imidazole/HCl, pH 7.4/25 mM KCl/4 mM $MgCl_2$/0.5 mM EGTA/1 mM DTT/0.067% bovine serum albumin (BSA; Sigma A7511)]. For binding to the rabbit actin–phalloidin complex, 0–2 μM actin was used, while for binding to yeast actin, 0–7.9 μM actin was used. Reaction mixtures were incubated 60 min on ice before centrifugation at 4°C for 10 min at 100,000 rpm. The K^+-EDTA ATPase activity remaining in the supernatant was determined.

ATPase Assays. The K^+-EDTA ATPase activity of CT-S1 was measured in K-ATPase buffer (25 mM imidazole/HCl, pH 7.4/0.6 M KCl/25 mM EDTA/1 mM ATP/1 mM DTT) at 30°C. Actin activation of P-Mg.S1 ATPase was measured in Mg-ATPase buffer (25 mM imidazole/HCl, pH 7.4/25 mM KCl/4 mM $MgCl_2$/1 mM EGTA/1 mM DTT) at 30°C with 1 mM ATP, 0.05% BSA, and 0–20 μM yeast or rabbit skeletal muscle actin. For rabbit actin, 0.75 μg of P-Mg.S1 was used in each reaction. For yeast actin, 6 μg of P-Mg.S1 was used. Release of the γ-[^{32}P]phosphate from ATP was measured by precipitation of phosphomolybdate (32).

Motility Assays. Motility was assayed essentially as described (22), in a modified motility assay buffer (10 mM imidazole/HCl, pH 7.4/4 mM $MgCl_2$/1 mM EGTA/2 mM DTT). In brief, P-Mg.S1, diluted in motility assay buffer, was applied to nitrocellulose-coated coverslips in a flow cell and allowed to bind. Excess P-Mg.S1 was eluted with buffer containing 0.05% BSA. Tetramethylrhodamine phalloidin (Me_4RP; Molecular Probes)-labeled yeast or rabbit skeletal muscle actin filaments were diluted to 10 nM monomer in BSA-containing buffer and allowed to bind to the P-Mg.S1-coated surface. Free actin filaments were washed away with BSA-containing buffer with an enzymatic oxygen scavenging system (3) and then the same buffer with 2 mM ATP was introduced into the flow cell. The flow cell was equilibrated to 30°C on the microscope stage. Sliding movement of actin filaments was observed by intensified video epifluorescence microscopy (1) and recorded on video tape. The sliding speed of actin filaments was measured from the video record with a video tracking program (33).

RESULTS

Preparation of Yeast Cell Extract. An actin-rich crude cell extract was prepared by lysis in a low-ionic-strength buffer. Frozen yeast suspension (50 g of DBY877 "sleet") was melted in cool 10 mM imidazole/HCl (pH 7.4), and the cells were centrifuged to a pellet. The pellet was resuspended to a 50% (wt/vol) cell suspension in ice-cold lysis buffer [10 mM imidazole/HCl, pH 7.4/0.5 mM $CaCl_2$/0.5 mM ATP with 1:200 protease inhibitor mixture (leupeptin, 1.6 mg/ml; pepstatin, 0.8 mg/ml; chymostatin, 0.2 mg/ml; aprotinin, 0.2 mg/ml; soybean trypsin inhibitor, 0.8 mg/ml; suspension in ethanol and water, stored frozen at −20°C)]. Glass beads (1-mm diameter, 50 ml) were washed with lysis buffer and cooled to 0°C. The cell slurry and glass beads were transferred to a glass-bead disruption device (Biospec Products, Bartlesville, OK; 75-ml cup), the chamber was topped off with lysis buffer, and 0.2 M phenylmethylsulfonyl fluoride in ethanol was added to 1 mM final concentration. The chamber was jacketed with an ice/salt/water bath (−5°C). The cells were disrupted with 30 cycles of 30-sec agitation and 90-sec cooling interval, resulting in >99% lysed cells as judged by

phase-contrast microscopy. The lysate was cool (<4°C) when recovered from the disruptor. (Warming during lysis resulted in markedly reduced yields.) The slurry of cell lysate and glass beads was filtered through nylon cloth and the retained beads were washed with lysis buffer. The extract was then centrifuged 60 min at 50,000 rpm in a Ti70 rotor (Beckman) at 4°C. The high-speed supernatant (80 ml) contained 655 mg of protein and 2200 units of DNase I inhibition activity.

DNase I Affinity Chromatography of Crude Extract. DNase I affinity chromatography was found to give an impressive single-step purification of the crude extract. The Affi-Gel DNase I affinity column (2.5 × 3 cm) and the Sephadex G-25 (Pharmacia LKB) column (2.5 × 40 cm) were equilibrated with DNase column buffer at 4°C. Flow was maintained at 0.5 ml/min by a peristaltic pump. The high-speed supernatant was diluted with 80 ml of column buffer and applied to the DNase I column. Unbound protein was eluted with several volumes of column buffer. Assay of the flowthrough showed that all DNase-inhibiting activity had been retained. Following the method of Zechel (20), the column was washed with 20 ml each of 10% formamide in column buffer, 10% formamide/0.2 M NH_4Cl in column buffer, and then column buffer alone. The outlet of the DNase column was then connected to the inlet of the G-25 column and the DNase column was eluted with 20 ml of 50% formamide in column buffer and washed with 20 ml of column buffer. The DNase column was bypassed and the elution of the G-25 column continued. Protein-containing fractions eluted from the G-25 column were pooled (Fig. 1). The DNase/G-25 column pool (22.5 ml) contained 2.9 mg of protein and 3400 units. This single step resulted in apparently complete recovery of the loaded actin and a purification of >200-fold.

Ion-Exchange Chromatography. Purification and concentration of the DNase I column pool was performed by medium-pressure anion-exchange chromatography. A DEAE 650M column (0.6 × 12 cm) was equilibrated with DNase column buffer and the DNase I affinity column/G-25 pool was loaded at 1 ml/min and washed at 1 ml/min with column buffer. No protein was detected in the flowthrough. Elution with a 15-ml linear gradient of 0–400 mM KCl in column buffer gave a single peak of protein at 200 mM KCl (Fig. 1 *b* and *c*). At this point, the total purification was calculated to be >200-fold with a yield approaching 100% and a net concentration of nearly 100-fold (Table 1).

DNase/DEAE-Purified Rabbit Skeletal Muscle Actin. To compare conventionally prepared actin with that prepared by the described method, we performed DNase and DEAE chromatography on rabbit skeletal muscle actin. Rabbit skeletal muscle actin (22) was dialyzed against DNase column buffer to prepare a solution of monomeric actin. This was applied to the DNase I affinity column and eluted as described above. The DNase/G-25 pool was applied to the DEAE column and eluted as described above. The fractions that eluted near 200 mM KCl were viscous. The repurified actin was "cycled" by sequential depolymerization and repolymerization (23) and stored on ice. DNase/DEAE-purified skeletal muscle actin was indistinguishable from conventionally purified actin in its ability to activate myosin P-Mg.S1 Mg^{2+}-ATPase and in its rate of sliding over P-Mg.S1-coated surfaces (data not shown). This actin was remarkably stable, maintaining its viscosity when stored on ice for many months.

Polymerization of DNase/DEAE-Purified Yeast Actin. Even brief contact of actin with formamide may result in loss of polymerizability due to denaturation (20). However, yeast actin purified by DNase affinity and DEAE chromatography was found to be assembly-competent. The DEAE pool was dialyzed against depolymerization buffer (2.5 mM imidazole/HCl, pH 7.4/0.5 mM $CaCl_2$/0.5 mM ATP/0.5 mM DTT) overnight at 4°C, recovered from dialysis (DEAE pool, 1.0 mg/ml) and centrifuged 15 min at 100,000 rpm in a TL100.3 rotor (Beckman; DEAE pool supernatant, 1.0 mg/ml). Addition of 1/19th volume of 20× polymerization buffer (450 mM imidazole/HCl, pH 7.4/500 mM KCl/80 mM $MgCl_2$/20 mM EGTA) resulted in a rapid increase in solution viscosity. Polymerized actin was stored on ice and its viscosity remained stable for several months.

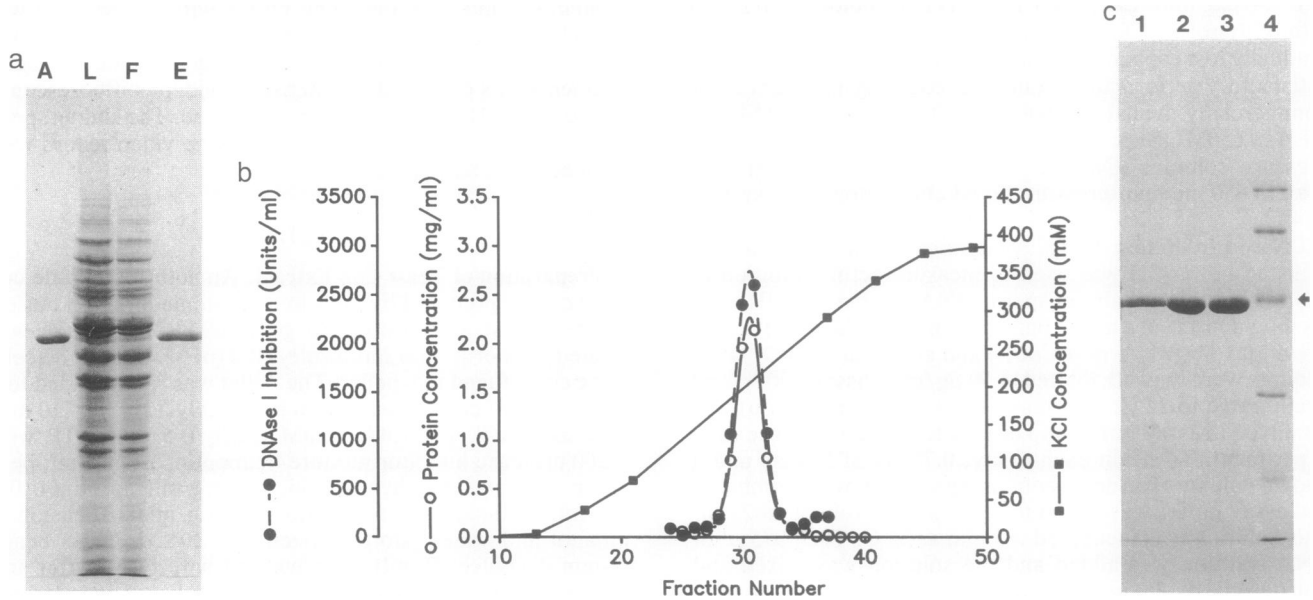

FIG. 1. DNase I affinity and anion-exchange column chromatography of a yeast cell extract. (*a*) SDS/12.5% PAGE of protein fractions stained with Coomassie brilliant blue R. Lane A, rabbit skeletal muscle actin, conventionally purified (22); lane L, high-speed supernatant of yeast lysate loaded onto DNase I column; lane F, DNase I column flowthrough; lane E, 50% formamide-eluted DNase column pool. (*b*) DEAE column profile, showing correspondence between total protein (○) and DNase I inhibition activity (●) eluted in fractions near 200 mM KCl. (*c*) SDS/12.5% PAGE of protein fractions stained with Coomassie brilliant blue R: Lane 1, DNase I column pool/DEAE column load; lane 2, 200 mM KCl-eluted DEAE column pool; lane 3, DEAE column pool after cycling by polymerization and depolymerization; lane 4, electrophoretic standards (Bio-Rad) with arrow indicating ovalbumin (45 kDa).

Table 1. Purification of wild-type actin from yeast

Fraction	Volume, ml	Protein, mg	DNase I inhibition, units	Yield, %	Purification, fold
HSS	80	655	2200	—	1
DNase pool	22.5	2.9	3400	100	200
DEAE pool	2.0	2.9	3500	100	>200

The high-speed supernatant (HSS) of the crude cell extract, taken as the starting material, contained nearly all of the actin released by cell lysis, as judged by Western immunoblot analysis (data not shown).

To remove any nonpolymerizable species, actin was "cycled" by polymerization/depolymerization. A 1.0-ml aliquot of the polymerized DEAE pool (0.93 mg/ml) was centrifuged 30 min at 100,000 rpm in a TL100.3 rotor and the supernatant (1.0 ml, 0.17 mg/ml) was drawn off. The pellet was washed and soaked in depolymerization buffer, resuspended to 0.2 ml, dialyzed overnight at 4°C against this buffer, and then recentrifuged. To polymerize the actin supernatant, 1/19th volume of 20× polymerization buffer was added (final volume, 0.21 ml; final protein concentration, 4.3 mg/ml). The yield of cycled actin was 95%.

Binding of Yeast Actin to Myosin Subfragment 1 and Activation of ATPase. Consistent with the results of Greer and Schekman (16), we found that yeast actin is a significantly weaker activator of rabbit skeletal muscle myosin Mg^{2+}-ATPase than skeletal muscle actin, using activation of P-Mg.S1 Mg^{2+}-ATPase (Fig. 2 *Upper*). We examined the basis for this difference by measuring the relative binding affinity of CT-S1 for the two actins. A binding assay performed in the absence of ATP revealed a remarkably lower apparent "rigor" affinity of CT-S1 for yeast actin than for skeletal muscle actin (Fig. 2 *Lower*). CT-S1 formed a stoichiometric complex with skeletal muscle actin filaments [stabilized with a molar excess of phalloidin (Calbiochem)] even at 0.2 μM actin monomer, whereas binding to yeast actin was insignificant below 1 μM actin.

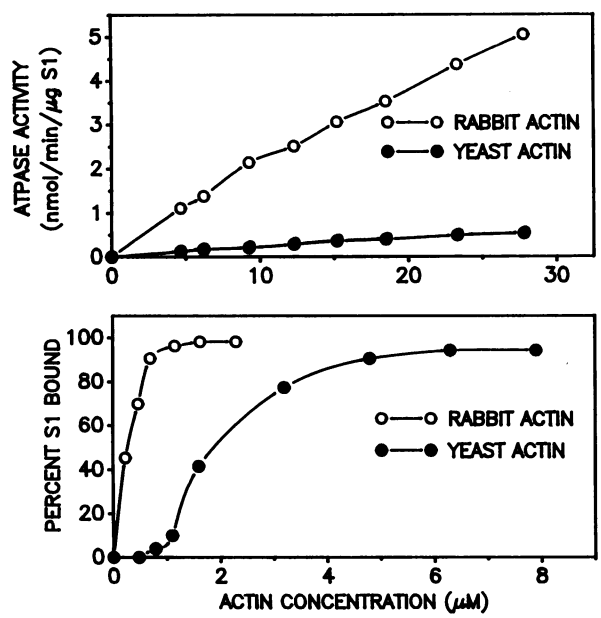

FIG. 2. Activation of myosin ATPase and myosin binding distinguish yeast actin from its cognate in rabbit skeletal muscle. (*Upper*) Actin-stimulated Mg^{2+}-ATPase activity of rabbit skeletal muscle P-Mg.S1 at a series of yeast and skeletal muscle actin concentrations. (*Lower*) Binding of CT-S1 to yeast actin or skeletal muscle actin–phalloidin complex in the absence of ATP. Bound CT-S1 was measured from depletion of actin-independent myosin ATPase after centrifugation to pellet actin filaments.

Fluorescent Actin Filaments. To fluorescently label the purified yeast actin filaments, Me_4RP was mixed at a slight molar excess with actin (1 μM monomer) in motility assay buffer and incubated at 0°C (22). When the labeled actin filaments were diluted to ≈10 nM actin monomer, individual actin filaments could be imaged by rhodamine fluorescence in intensified video epifluorescence microscopy. The Me_4RP-labeled actin filaments were uniformly bright and varied between <1 μM and >10 μm in length. In comparison to similarly labeled skeletal muscle actin filaments, yeast actin appeared to be less rigid, with filaments often demonstrating sharp bends and tightly wound loops without fracture. The stability of yeast actin filaments under fluorescence illumination with respect to photobleaching and filament fragmentation was similar to that of Me_4RP-labeled skeletal muscle actin. However, independent of illumination, when the labeled filaments were diluted into buffer, their fluorescence gradually decreased over a period of minutes, suggesting a markedly lower affinity for phalloidin than that of skeletal muscle actin. The rate of loss of fluorescence from yeast actin filaments increased with increasing temperature.

Actin Sliding Movement over Myosin-Coated Surfaces. In the *in vitro* motility assay, Me_4RP-labeled yeast actin filaments moved on skeletal muscle myosin-coated surfaces in an ATP-dependent manner. When P-Mg.S1 was applied to a nitrocellulose surface and Me_4RP-labeled yeast actin was diluted and introduced into the flow cell, actin filaments bound to the myosin-coated surface along their lengths in a stable rigor association. When 2 mM ATP was infused, actin filaments immediately began sliding over the surface.

The movement of yeast actin filaments over P-Mg.S1-coated surfaces was distinct from that of skeletal muscle actin filaments. At 22°C, only a fraction of the yeast actin filaments that had bound to the surface were observed to undergo sliding movement when ATP was infused, and the moving filaments exhibited intermittent sliding interrupted by periods of binding to the surface. Such behavior is characteristic of the movement of skeletal muscle actin only at lower temperatures (<15°C) or in very low-ionic-strength buffers. At 30°C, though nearly all yeast actin filaments exhibited smooth sliding movement, the sliding speed of yeast actin was significantly slower than that of skeletal muscle actin. While DNase/DEAE-purified skeletal muscle actin moved over P-Mg.S1 at 0.95 ± 0.10 μm/sec, filaments of wild-type yeast actin moved at only 0.46 ± 0.06 μm/sec (mean ± SD).

Purification and Motility of Yeast Mutant Actin. Temperature-sensitive lethal mutant actin was purified, polymerized, labeled with Me_4RP, and then assayed for motility at a temperature that is nonpermissive for growth of the mutant strain. Actin was isolated from DBY1995 (*act1-3*) actin mutant cells under the conditions described above, then cycled and stored as filamentous actin, with all manipulations at 0–22°C. Me_4RP-labeled mutant actin was indistinguishable from similarly prepared wild-type actin with respect to properties observed by fluorescence microscopy such as filament length distribution and extent of polymerization. The sliding movement of Me_4RP-labeled mutant actin filaments over P-Mg.S1-coated nitrocellulose films was assayed at 30°C. No

depolymerization of the labeled mutant actin filaments at this temperature was observed. The quality and speed (0.49 ± 0.05 μm/sec) of sliding at 30°C of the mutant actin were very similar to those of the wild-type actin, suggesting that the lethal phenotype of the mutant actin is not due to loss of function of polymerized actin.

DISCUSSION

We have sought to transform a genetically simple model system for the study of actin–myosin interaction, the single conventional actin gene of the yeast *S. cerevisiae*, into a tractable system for *in vitro* structure–function analysis as well. To establish the biochemical arm of our studies, we have developed a simple scheme to purify yeast actin and have examined its properties in several functional assays that probe its interaction with myosin. By DNase I affinity and anion-exchange chromatography, wild-type and conditional-lethal mutant actin could be purified to homogeneity and recovered quantitatively in native, assembly-competent form. The assayed properties of the mutant actin, its assembly at 4°C and motility at 30°C, were indistinguishable from those of wild-type. Yeast actin could substitute for skeletal muscle actin in a wide range of assays that measure actin–myosin interaction. Binding assays, ATPase assays, and motility assays each report significant conservation of function between yeast actin and skeletal muscle actin in their interaction with skeletal muscle myosin. With respect to motility, we were surprised to observe sliding movement at all, considering the evolutionary distance between *Saccharomyces* and vertebrates and the obvious differences in actin function and regulation between yeast cells and vertebrate skeletal muscle cells. Sites of divergence between the yeast actin sequence and the canonical actin sequence, that of rabbit muscle actin, most likely identify amino acids that are relatively subtle determinants of actin structure.

Despite the fundamental similarities, we confirmed previously reported differences between yeast and skeletal muscle actin in their interaction with skeletal muscle myosin. We extended these findings to show both lower affinity of muscle myosin for yeast than for muscle actin filaments in the absence of ATP and slower motility of yeast than muscle actin filaments over myosin in the presence of ATP. The sliding movement of yeast actin over skeletal muscle myosin was only half as fast as that of skeletal muscle actin. Most likely, the observed differences between yeast and skeletal muscle actins are due to one or more of the 44 amino acids that differ between their sequences. One site with several nonconservative substitutions, the extreme N terminus, is less acidic in yeast than in skeletal muscle actin. Schwyter *et al.* (34) found that proteolytic cleavage of skeletal muscle actin within a boundary between the N terminus and the actin "core" strongly affects actin sliding movement over skeletal muscle myosin, producing a form of actin with motility properties very similar to those of yeast actin. Sutoh *et al.* (7) found that replacing N-terminal aspartates of *Dictyostelium* actin with histidine residues resulted in a similar change in the motility properties of this actin. These and other data suggest that the close association of the actin N terminus with a site at the junction between two subdomains in the myosin head reflects an important functional interaction. As suggested for cleaved actin (34), it may be that binding of yeast actin to muscle myosin is neither stable nor stiff enough to transmit a mechanical impulse through the actin–myosin junction, uncoupling the system so that ATP hydrolysis becomes mechanically unproductive.

Our results suggest that specific models for the structural basis of both functional conservation and divergence between yeast actin and other actins can be tested directly. Both genetic tools for manipulation of the yeast actin gene and cell biological techniques for analyzing the effects of such mutations on the cell are now available. We have complemented these with a biochemical approach. The combination of a simple purification method with practical functional assays should facilitate *in vitro* studies of mutant actins expressed in yeast.

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