Properties of Actin from the Fission Yeast Schizosaccharomyces pombe and Interaction with Fission Yeast Profilin

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The fission yeast Schizosaccharomyces pombe serves as a model system for studying role of actin cytoskeleton, since it has simple actin cytoskeletons and is genetically tractable. In contrast, biochemical approaches using this organism are still developing; fission yeast actin has so far not been isolated in its native form and characterized, and therefore, biochemical assays of fission yeast actin-binding proteins (ABPs) or myosin have been performed using rabbit skeletal muscle actin that may interact with the fission yeast ABPs in a manner different from fission yeast actin. Here, we report a novel method for isolating functionally active actin from fission yeast cells. The highly purified fission yeast actin polymerized with kinetics somewhat different from those of muscle actin and forms filaments that are structurally indistinguishable from skeletal muscle actin filaments. The fission yeast actin was a significantly weaker activator of Mg2+-ATPase of HMM of skeletal muscle myosin than muscle actin. The fission yeast profilin Cdc3 suppressed polymerization of fission yeast actin more effectively than that of muscle actin and showed an affinity for fission yeast actin higher than for muscle actin. The establishment of purification of fission yeast actin will enable reconstruction of physiologically relevant interactions between the actin and fission yeast ABPs or myosins and contribute to clarification of function of actin cytoskeleton in various cellular activities.

The actin cytoskeleton is involved in many cellular motile processes, such as muscle contraction, migration of the cell, cytoplasmic streaming, organelle positioning, cell morphogenesis, and cytokinesis. In many cases, the actin cytoskeleton is dynamic and is constructed and destroyed during the cell cycle or in response to extracellular stimuli. The dynamic nature is important for functions of the actin cytoskeleton; many actin cytoskeletons are formed only when they are necessary for specific cellular activities to be exercised. For example, the contractile ring forms in the equatorial cortex of the cell only during cytokinesis, constricts the cell into two, and is no longer present when cytokinesis is completed. The dynamic nature is mainly attributed to a couple of factors. One is the intrinsic feature of actin itself (i.e. it polymerizes into filaments and depolymerizes responding to environmental conditions). The others are regulation of the assembly feature of actin by actin-modulating proteins and formation of three-dimensional structures of actin filaments by various actin-cross-linking proteins, activities of both of which may be further controlled by upstream signaling pathways. Therefore, analyses of both assembly properties of actin and actions of various actin-binding proteins are requisite for understanding the dynamic nature of each actin cytoskeleton.

The fission yeast Schizosaccharomyces pombe serves as a good model system for studying the actin cytoskeletal organization, since the cells have only three distinct F-actin structures: cortical patches located mainly at the growing cell tip; cables running longitudinally along the cell; and the contractile ring, which forms during mitosis (1, 2). These structures are constructed and destroyed during the cell cycle. Specific actin-binding proteins are necessary for formation and maintenance of these structures and localized to these structures, which are known by genetic studies using mutant cells and by localization studies.

Besides these studies, rigorous biochemical and biophysical analyses of properties of actin and interaction of ABPs with actin are necessary in order to elucidate physiological functions of these proteins. However, there has been no report on the isolation of functionally active fission yeast actin. Skeletal muscle actin has instead been used to investigate properties of fission yeast ABPs (3–6) and myosin (7), since it is easy to purify.

Fission yeast actin is 88–90% identical to skeletal muscle actin, nonmuscle β-actin, and budding yeast Saccharomyces cerevisiae actin over the whole deduced amino acid sequences. However, the differences among these acts, although they are small, may be significant in interaction of the actin with other proteins. For example, fission yeast actin has three serial negative charges (MEEEI−) like β-actin (MDDDI−), whereas skeletal muscle actin has four (MDEDET−) and budding yeast actin has only two (MDSEV−) in the N-terminal residues that are supposed to be involved in interaction with myosin and several ABPs (8–10). Actually, some nonmuscle ABPs have been reported to show significant species specificity to actin.
**Purification and Properties of Fission Yeast Actin**

*Acanthamoeba* or budding yeast profilin prevents polymerization of amoeba or yeast actin, respectively, but does not suppress polymerization of muscle actin at all (11, 12). Ezrin, one of the band 4.1 family proteins, has also been reported to interact specifically with β-actin but not with α (skeletal muscle)-actin filaments (13). Actophorin, an actin-depolymerizing factor (ADF) family protein of *Acanthamoeba*, depolymerizes muscle actin filaments more efficiently than *Acanthamoeba* actin (14). Therefore, it is necessary to investigate properties of fission yeast ABPs using fission yeast actin for correct understanding of interactions between actin and ABPs in fission yeast.

Pancreatic DNase I affinity chromatography, which exploits the stable and specific binding of DNase I to monomeric actin, may be the first choice to isolate nonmuscle actins, since it allows one to obtain actin with a high purity and a high yield by a single step purification (15, 16). However, we could not obtain polymerization-competent actin from fission yeast by this method. Here, we report a procedure for purification of fully functional actin from fission yeast and basic biochemicalcharacterizations of the purified actin. We also report that the fission yeast profilin Cdc3 showed a higher affinity for fission yeast actin than for muscle actin and reduced the extent of polymerization of fission yeast actin more potently than that of muscle actin.

**MATERIALS AND METHODS**

**Strains and Cell Growth**—Cells of *S. pombe* strain JY1 h− (wild type) were grown in YPD (1% yeast extract, 2% bactopeptone, and 2% glucose) or YES (0.5% yeast extract, 3% glucose, 225 mg/liter adenine, histidine, leucine, uracil, and lysine hydrochloride) medium at 30 °C to give a density of 108 cells/ml. Cells were harvested and lysed by sonication in extraction buffer (0.1 mM CaCl₂, 1 mM NaN₃, 0.2 mM DTT, 0.5 mM ATP, 1 μg/ml pepstatin A, 10 mM Tris-HCl, pH 8.0). The following procedures were performed at temperatures between 0 and 4 °C unless otherwise noted. The cells were resuspended with the half-volume of 3 × T-buffer containing 1 mM phenylmethylsulfonyl fluoride and disrupted with glass beads using a Braun MSK homogenizer (Braun Instruments, Burlingame, CA). The homogenate was centrifuged successively at 12,000 × g for 30 min and at 100,000 × g for 1 h. The high speed supernatant (HSS) was sometimes frozen with liquid nitrogen and stored at −80 °C. Protamine sulfate was added to HSS to give 0.1% (w/v) to precipitate DNA and RNA, and then HSS was centrifuged at 20,000 × g for 20 min to remove them. Solid ammonium sulfate was dissolved in HSS to give 35% saturation. After 30 min, precipitates formed were collected by centrifugation at 20,000 × g for 20 min and resuspended in 0.15 M KCl-T-buffer. The suspension was swirled for 30 min and dialyzed overnight against the same buffer. The dialyzed suspension was centrifuged at 20,000 × g for 20 min, and the supernatant (AS fraction) was applied to a Mono Q HR 10/10 column (Amersham Biosciences) using a fast protein liquid chromatography system (Amersham Biosciences). The column was washed with 40 ml of 0.15 M KCl-T-buffer and eluted with 240 ml of a linear KCl gradient of 0.15–0.35 M in T-buffer at 2 ml/min. Actin was eluted at around 0.25 M KCl. Actin-containing fractions (MQ fraction) were pooled and concentrated at least 10-fold by ultrafiltration through a Centrilo CF 25 (Millipore, Bedford, MA). The concentrated MQ fraction was supplemented with 5 mM MgCl₂, 0.5 mM ATP, and 1 μg/ml pepstatin A. The solution was kept on ice overnight and then centrifuged at 300,000 × g for 1 h. The pellet containing polymerized actin was resuspended in a small volume of G-buffer (0.1 mM CaCl₂, 1 mM NaN₃, 0.2 mM DTT, 0.5 mM ATP, 1 μg/ml pepstatin A, 5 mM imidazole-HCl, pH 7.5) and homogenized with a Teflon-glass homogenizer. After a brief sonication, the suspension was dialyzed overnight against G-buffer. The dialyzed actin was centrifuged at 300,000 × g for 20 min. The supernatant (crude G-actin) was applied to a Sephadex G-150 (Amersham Biosciences) column (1.3 × 31 cm) and eluted with G-buffer at a rate of 2.4 ml/h. Fractions containing actin were pooled (GF fraction) and concentrated. The concentrated actin was again polymerized with 30 mM KCl and 3 mM MgCl₂ and depolymerized as above. The final supernatant was used as purified fission yeast G-actin within 4 days after purification.

**Preparation of Muscle and Recombinant Proteins**—Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt (17) and further purified by gel filtration using a Sephadex G-150 (Amersham Biosciences) column. The purified G-actin was concentrated and stored on ice. Chymotryptic HMM was prepared from rabbit skeletal muscle myosin as described by Okamoto et al. (18).

Recombinant Cdc3 was expressed in bacteria and purified as a glutathione S-transferase fusion protein. *E. coli* strain BL21 (Novagen) was transformed with pGEX-4T1-cdc3 (a gift from Dr. K. Nakano) and grown in LB medium (1% Tryptone, 0.5% NaCl, pH 7.5) at 30 °C. Expression of glutathione S-transferase-Cdc3 was induced for 5 h at 25 °C by the addition of 0.1 mM isopropyl-1-thio-β-D-galactopyranoside. Cells were harvested and lysed by sonication in extraction buffer (1% Triton X-100, 0.5 mM EDTA, 1 mM DTT, 1 μg/ml pepstatin A, 0.5 mM phenylmethylsulfonyl fluoride, 40 mM Tris-HCl, pH 7.5) on ice. The homogenate was clarified by centrifugation at 200,000 × g for 30 min and incubated with glutathione-Sepharose 4B beads (Amersham Biosciences) for 2 h at 4 °C with gentle swirling. The beads were washed with and resuspended in Tb buffer (0.15 M NaCl, 2.5 mM CaCl₂, 5 mM MgCl₂, 1 mM DTT, 50 mM Tris-HCl, pH 8.0) The suspension was supplemented with 5 units/ml of thrombin (Sigma) and gently swirled for 16 h at 4 °C. After thrombin was removed by benzamidine-Sepharose 6B (Amersham Biosciences), the eluted proteins were concentrated using a Centricon YM-10 (Millipore) and then applied to a Sephadex G-75 (Amersham Biosciences) column (1.4 × 28 cm) equilibrated with G-buffer.

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2 The abbreviations used are: ADF, actin-depolymerizing factor; ABPs, actin-binding proteins; DTT, dithiothreitol; eATP, 1,2′-ethenoadenosine 5′-triphosphate; HMM, heavy meromyosin; Lat-A, latrunculin A; HSS, high speed supernatant.

3 I. Mabuchi, unpublished observation.
Purified Cdc3 was concentrated up to about 200 μM and stored on ice or at −80 °C.

Electron Microscopy and Optical Diffraction—Samples were negatively stained with 1.5% uranyl acetate on carbon-coated Formvar grids and observed with a JEM1200EX electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV. Actin filaments were decorated with HMM according to Craig scope (JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV. Formvar grids and observed with a JEM1200EX electron micro-

Polymerization of Actins—Actins were stored on ice as concentrated (about 30 μM) Ca2+–G-actin. If necessary, they were converted to Mg2+-G-actin either by the addition of 0.1 volume of 10 × ME (1 mM MgCl2, 5 mM EGTA) or by dilution with 1 × MEI (0.1 mM MgCl2, 0.5 mM EGTA, 0.2 mM ATP, 0.5 mM DTT, 5 mM imidazole-HCl, pH 7.5), followed by preincubation for at least 2 min. The Mg2+-G-actin was subjected to polymerization within 20 min after the dilution. Polymerization was induced by the addition of salts to a G-actin solution in a cuvette followed by quick pipettings. It was monitored usually at 22 °C by light scattering measurement at 450 nm as previously described (21, 22) using an RF-540 spectrofluorophotometer (Shimadzu Corp., Kyoto, Japan).

Analytical Methods—SDS-PAGE was carried out according to Laemmli (23). Amounts of proteins in Coomassie Brilliant Blue-stained gels were estimated by densitometric analysis using Image J software (available on the World Wide Web; National Institutes of Health). Isolecicular focusing of proteins was performed as described by O’Farrell (24) with slight modifi-
cations. Anti-fission yeast actin, anti-fission yeast Arp3 (actin-related protein 3), and anti-Myo1 (fission yeast myosin-I) antibodies were those previously described (25, 26). Immunoreactive bands were visualized with the ECL reagents (Amersham Biosciences). Protein concentrations of crude samples were determined by the method of Lowry et al. (27) using bovine serum albumin as a standard. Concentrations of puri-
fied proteins were determined spectrometrically using extinction coefficients as follows: muscle or fission yeast actin, A290 = 0.633 mg−1 ml cm−1 (28); HMM, A280 = 0.647 mg−1 ml cm−1 (29); Cdc3, A280 = 1.63 mg−1 ml cm−1 (30). Concentration of purified fission yeast actin on an SDS-gel was also estimated by densitometry using muscle actin as a standard, which usually gave almost the same value as that obtained spectrometrically. Con-
ditions for ATPase assay of HMM were those described by Mabuchi (31). The inorganic phosphate was determined by a modified malachite green method according to Kodama et al. (32). The dissociation constant (Kd) of profilin (Cdc3) for fission yeast or muscle G-actin was determined according to Perelroizen et al. (33). G-actin (0–96 μM) in K buffer (0.1 mM CaCl2, 0.5 mM DTT, 0.2 mM ATP, 2 mM Tris-HCl, pH 7.5) was titrated with Cdc3 (200–290 μM) in K buffer. Increase in vol-
ume by successive addition of Cdc3 was always kept at less than 1.2%. Fluorescence of the solution was monitored at an excita-
tion wavelength of 295 nm and at an emission wavelength of 330 nm. Data were fitted to the equation, ΔF = Fobs − fA[A]tot − fP[P]tot = (fPA − fA − fP)P[A] using KaleidaGraph software (Synergy Software, Reading, PA), where Fobs is total observed fluorescence; fA, fP, and fPA are intrinsic fluorescence coefficients of free G-actin, free profilin, and profilin-actin complex, respectively; and [A]tot, [P]tot, and [PA] are concentrations of actin, profilin, and profilin-actin complex, respectively.

ATP Exchange Analysis—1, N′-ethenoadenosine 5′-triphos-
phate (eATP; Molecular Probes, Inc., Eugene, OR)-bound Ca2+-G-actin was prepared according to Lu and Pollard (30). In brief, G-actin (1 μM) in TG-buffer (0.1 mM CaCl2, 0.25 mM ATP, 0.5 mM DTT, 1 mM Na2SO4, 2 mM Tris-HCl, pH 8.0) was treated with Dowex-1 (X8 resin; Dow Chemical Co., Midland, MI) for 5 min on ice to remove free nucleotides. The Dowex-1 was pel-
leted by centrifugation at 2,000 × g for 30 s, and the supernatant (ATP-bound G-actin) was supplemented with 0.2 mM eATP and incubated for at least 4 h on ice. Immediately before measure-
ments, free eATP was removed with Dowex-1 as above, and the eATP-bound Ca2+-G-actin was diluted to 0.5 μM with TG-
buffer (TG-buffer without ATP) containing various concentra-
tions of Cdc3 (0–5 μM). After the addition of 0.2 mM ATP to the solution, the release of eATP from G-actin was monitored at 25 °C by the decrease in fluorescence at an excitation wave-
length of 360 nm and at an emission wavelength of 410 nm using an RF-5300PC spectrofluorophotometer (Shimadzu Corp.). The rate constant for dissociation of eATP from actin was determined by fitting the data to a single exponential func-
tion using a KaleidaGraph software.

Cosedimentation Assay—The binding affinity of HMM to actin was estimated by a cosedimentation experiment. HMM (0.5 μM) in assay buffer (25 mM KCl, 4 mM MgCl2, 0.5 mM EGTA, 0.5 mM DTT, 0.5 mg/ml bovine serum albumin, 1 μg/ml pepstatin A, 10 mM imidazole-HCl, pH 7.5) was mixed with various concentrations of actin-phallolidin complex (0–32 μM actin supplemented with a 1.1-fold molar excess of phallolidin (Sigma)), and the mixtures were incubated for 30 min at 25 °C. F-actin and bound HMM were pelleted by centrifugation at 300,000 × g for 10 min at 4 °C in a Hitachi RPI100AT3 rotor. The supernatants were subjected to SDS-PAGE. Amounts of HMM heavy chain remaining in the supernatants were deter-
mined by densitometry of Coomassie Brilliant Blue-stained gels and expressed as percentages of the amount of HMM in the control supernatant without F-actin. The cosedimentation assay was also used to examine the effect of Cdc3 on the extent of actin polymerization. G-actin (3 μM) was mixed with various concentrations of Cdc3 (0–9 μM) and polymerized at least for 16 h at 25 °C in 50 mM KCl and 1 mM MgCl2. The mixtures were centrifuged as above, and protein concentrations of the super-

Fluorescence Microscopy of Actin Filaments—Actin (3 μM) was polymerized at least for 16 h at 25 °C in the absence or presence of various concentrations of Cdc3 and diluted to 6.6 μM in FM buffer (50 mM KCl, 1 mM MgCl2, 0.5 mM EGTA, 10 mM DTT, 0.1% methylcellulose, 10 mM 1,4-diazabicyclo[2.2.2]octane (Sigma), 10 mM imidazole-HCl, pH 7.5) containing 0.8 μM BODIPY-FL-phallacidin (Molecular Probes). After incubation for 1 min, the solution was further diluted to 1:500 to 1:50 with FM buffer and applied to a coverslip coated with 0.01% (w/v) poly-l-lysine. Fluorescent images were acquired with a SPOT cooled charge-coupled device camera (Diagnostic Instruments, Sterling Heights, MI) using IPLab software (Scanalytics, Inc., Rockville, MD) on a Zeiss Axioskop fluorescence microscope.
Purification and Properties of Fission Yeast Actin

FIGURE 1. Electrophoretic analysis of actin fractions during the purification. A, proteins at each purification step were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue. Lane M, molecular weight markers; lane 1, HSS (20 µg of protein); lane 2, AS fraction (20 µg); lane 3, MQ fraction (0.6 µg); lane 4, crude G-actin fraction (7 µg); lane 5, purified fission yeast actin (7 µg). The 42,000 band in lane M represents muscle actin. B, immunoblotting of the fractions shown in A using antibodies against _S. pombe_ actin. C, isoelectric focusing gels. Lane 1, muscle actin (1.5 µg); lane 2, purified fission yeast actin (1.5 µg); lane 3, mixture of muscle actin and fission yeast actin (1.5 µg each). D, protein composition of the flow-through fraction (9 µg) from Sephadex G-150 column chromatography analyzed by SDS-12.5% PAGE followed by Coomassie Brilliant Blue staining. Bands that reproducibly appeared in the fraction are numbered. Bands 5 and 6 could be separated on a 10% gel (inset). E, immunoblots of the fraction. Anti-Myo1, anti-Arp3, and anti-actin antibodies recognize bands 1, 3, and 4, respectively. The arrows in A and B indicate fission yeast actin. The numbers at the left of A and D indicate molecular weights x 10^-3. F, electron microscopy of fission yeast actin filaments polymerized in the presence of the flow-through fraction. The flow-through fraction (0.04 mg/ml) was mixed with fission yeast actin (0.12 mg/ml). A–C in the presence of 0.1 M KCl and 2 mM MgCl₂. The mixture was incubated for 30 min at 25 °C and then subjected to negative staining. Filaments were branched at an average angle of 70°. Bar, 100 nm.

received with a Plan Apochromat 63× oil lens (Carl Zeiss, Germany). Three to five images were analyzed for each Cdc3 concentration using Image J software, which allowed us to measure length of more than 800 filaments.

RESULTS

The protein composition of each fraction during purification of actin from the fission yeast _S. pombe_ is shown in Fig. 1A. Actin was identified with anti-actin antibodies in these fractions as a 42-kDa component (Fig. 1B). The content of actin in the total protein in HSS was about 1%, as estimated by densitometry of the gel. Fission yeast actin was finally purified to 96–99% homogeneity, and typically 0.3–0.5 mg of actin was obtained from 25 ml of packed cells (Table S1). Using quantitative immunoblotting, we estimated that wild-type cells had 8.7 µm actin/cytoplasm or 0.54 million molecules of actin/cell in midlog phase in YES medium (Fig. S1). By this estimation, the procedure described in this paper yielded as the purified actin about one-tenth of the whole actin in fission yeast cells. Fission yeast actin showed an isoelectric point slightly more basic than that of muscle actin (Fig. 1C).

We found that several proteins were reproducibly co-purified with actin even after the polymerization-depolymerization step (Fig. 1A, lane 4). On the following gel filtration column chromatography, however, 10 species of proteins eluted reproducibly as major components at the void volume (Fig. 1D, bands 1–10), which enabled effective separation of these proteins from G-actin. Apparent molecular weights of band 3, 5, 6, 7, 8, 9, and 10 components were close to the deduced molecular weights of seven subunits of fission yeast Arp2/3 complex (i.e., 47,000 (Arp3), 44,000 (Arp2), 42,000 (p41-Arc), 37,000 (p34-Arc), 20,000 (p21-Arc), 20,000 (p20-Arc), and 17,000 (p16-Arc). This protein composition agreed well with that of immunoprecipitated or affinity-purified fission yeast Arp2/3 complex (6, 25). Immunoblotting analyses revealed that bands 1, 3, and 4 corresponded to fission yeast myosin I, Arp3, and actin, respectively (Fig. 1E). Fission yeast actin filaments polymerized in the presence of the flow-through fraction showed branches at an average angle of 70 ± 8° (n = 34; Fig. 1F). Such branches were rarely observed in filaments polymerized from the purified actin as described below. These results suggested that the flow-through fraction contained the Arp2/3 complex as a major component, since the Arp2/3 complex forms branches of actin filaments (34).

Properties of Fission Yeast Actin—Actin polymerization was monitored by light scattering measurements. In the presence of 2 mM MgCl₂, fission yeast G-actin polymerized in a kinetics basically similar to that of muscle G-actin (Fig. 2A). However, the initial lag phase which has been considered to represent a nucleation phase was scarcely seen, and light scattering increased almost linearly and a little faster than muscle actin. Then the increase was slowed down but still continued at a slow rate (Fig. 2A, from 10 to 40 min). It took more than 3 h for light scattering from 4 µM polymerizing fission yeast actin to reach a plateau level (data not shown). On the other hand, muscle actin soon reached a steady state level. The characteristic polymerization kinetics of fission yeast actin was also observed for polymerization of Mg²⁺-G-actin induced by 2 mM MgCl₂ (data not shown).

In 0.1 M KCl, fission yeast Mg²⁺-G-actin polymerized as muscle Mg²⁺-G-actin did, but the polymerization took a longer time to reach a steady state (Fig. 2B, Mg). On the other hand, light scattering from fission yeast Ca²⁺-actin increased much more slowly than that from muscle Ca²⁺-actin, and the typical sigmoidal time course was not seen (Fig. 2B, Ca). Even after 16 h of incubation, the level of light scattering from the actin in 0.1 M KCl was about 70% of that of fission yeast Mg²⁺-actin polymerized with KCl (Fig. 2B, >12 h). The addition of 2 mM MgCl₂ during the slow polymerization accelerated the rate (Fig. 2B, Ca to Mg), and the final level of polymerization was the same as that of the Mg²⁺-actin. Similarly, the addition of 2 mM MgCl₂ to
fission yeast Ca^{2+}-actin, which had been incubated for 16 h in 0.1 M KCl, increased its light scattering level to that of the fission yeast Mg^{2+}-actin (data not shown). Polymerization of both fission yeast and muscle actins induced by the simultaneous addition of 0.1 M KCl and 2 mM MgCl_2 gave a similar kinetics to that by 2 mM MgCl_2 (data not shown).

To compare the speed of depolymerization of fission yeast and muscle actins, a molar excess of Lat-A, an actin monomer-sequestering agent (35), was added, and decrease in polymer concentration was monitored by light scattering. As shown in Fig. 2, C and D, both actins depolymerized gradually in the presence of Lat-A, although fission yeast actin depolymerized slightly more slowly than muscle actin. These actins should have depolymerized from both ends under the experimental conditions. Therefore, the observed rate should be proportional to the sum of the dissociation rate constants (k_−) at the two ends. Me_2SO, the solvent for Lat-A, did not affect the level of light scattering of the F-actin solutions (data not shown). The rates of depolymerization of both actins were the same in the presence of cytochalasin D, which caps the barbed end (36), suggesting that the k_− values at the pointed end of these actins were the same. Muscle actin was more sensitive to Lat-A at lower concentrations than fission yeast actin (Fig. 2D).

To determine critical concentration (C_c) of these actins for polymerization, we plotted steady state levels of light scattering in the presence of both 0.1 M KCl and 2 mM MgCl_2 against total actin concentrations (37). The average C_c for fission yeast actin was 0.57 ± 0.06 μM (n = 3) in our experimental conditions, which was similar to that for muscle actin, 0.46 ± 0.08 μM under the same conditions, and to sea urchin egg actin, 0.52–0.60 μM under similar conditions.

Electron microscopic examination showed that polymerized fission yeast actin in 0.1 M KCl and 2 mM MgCl_2 formed beaded helical filaments. The filaments were very long and straight and had an average diameter of 6.2 ± 0.7 nm (n = 40, Fig. 3A), which was indistinguishable from those of muscle actin. These filaments bound HMM in the absence of ATP to show an arrowhead appearance (Fig. 3B). On the other hand, fission yeast Ca^{2+}-actin did not form filaments when 0.1 M KCl was added even after 16–24 h of incubation but formed
PARTICLES OF IRREGULAR SHAPES (Fig. 3C, LEFT) WITH AN AVERAGE LENGTH OF 15 \( \pm 3.1 \) nm AND AN AVERAGE THICKNESS OF 9.7 \( \pm 1.9 \) nm (\( n = 323 \)). It is noteworthy that besides these particles, those showing a ring structure were also present (Fig. 3C, ARROWS IN THE LEFT PANEL). These ring particles had an average outer diameter of 9.0 \( \pm 0.24 \) nm and an average inner diameter of 4.8 \( \pm 0.42 \) nm (\( n = 110 \); Fig. 3C, RIGHT PANELS) and comprised about 25% of the total particles. The ring particles were also observed when the actin was polymerized with \( \text{MgCl}_2 \), although the population was sparse (Fig. 3A). Filaments were observed after the addition of 2 mM \( \text{MgCl}_2 \) to fission yeast \( \text{Ca}^{2+} \)-actin even after a prolonged incubation in 0.1 M KCl (data not shown).

Upon the addition of 50 mM \( \text{MgCl}_2 \), fission yeast actin formed paracrystalline rods of filaments (Fig. 3D) as muscle actin, human platelet actin, \( \text{Dictyostelium} \) actin, and sea urchin egg actin do (37–40). To compare helical structures of fission yeast and muscle actin filaments, optical diffraction patterns were obtained from the images of the magnesium-paracrystals (Fig. 3E). In all of the diffraction patterns observed, the first and the sixth layer lines were detected as strong spots, which represent the helical periodicities of the double helix of the protofilaments and the genetic helix, respectively. The spacings of the first and the sixth layer lines were about 35 and 5.9 nm for both actsins (Table 1). The ratio

### TABLE 1

| Actin      | Spacing       | L6/L1  |
|------------|---------------|--------|
|            | L1  | L6    |       |
| S. pombe   | 35.2 \( \pm 0.4 \) | 5.87 \( \pm 0.04 \) | 6.00 \( \pm 0.08 \) |
| Muscle     | 35.4 \( \pm 0.4 \) | 5.92 \( \pm 0.04 \) | 5.98 \( \pm 0.08 \) |

Spacings of the diffraction pattern of actin paracrystals

L1 and L6 indicate the first and the sixth layer lines, respectively. Data are expressed as average \( \pm \) S.D. obtained by measuring the optical transforms of four individual \( \text{Mg}^{2+} \)-paracrystals of each actin.
of the spacings that represent the screw angle of the filament was about 6.0 for both actins, which indicates the helical symmetry of 13 subunits in six turns (41).

Activation of myosin Mg\(^{2+}\)-ATPase activity is one of the common properties of actin filaments. To evaluate this property, the Mg\(^{2+}\)-ATPase activity of rabbit HMM was measured in the presence or absence of fission yeast or muscle actin filaments. As shown in Fig. 4A, fission yeast actin activated the Mg\(^{2+}\)-ATPase activity less effectively than muscle actin did. The maximal activity in the presence of fission yeast actin was about 3-fold less than that in the presence of muscle actin (Table 2). The apparent affinities (K$_m$ values) of these actins for HMM were almost the same. We also estimated the binding constants of the two actins for HMM by cosedimentation experiment. Both actins formed a stoichiometric complex with HMM in the absence of ATP and were sedimented with HMM by centrifugation (Fig. 4B). The concentration of actin required for a half-maximal sedimentation of HMM (K$_{app}$) was 1.1 μM for fission yeast actin, which was 2.6-fold larger than that for muscle actin (0.42 μM; Fig. 4B, inset, and Table 2). When 2.2 mM ATP was added to the assay medium, HMM was recovered in the supernatant for both actins to the same extent (data not shown). These data indicate that the affinity of HMM to fission yeast actin is lower than that to muscle actin in the absence of ATP and that the interaction of both actins with HMM is ATP-sensitive.

**Interaction of Fission Yeast Profilin with Fission Yeast Actin**

We tested interaction of fission yeast profilin Cdc3 with fission yeast actin, because a species-specific effect or binding of profilin to actin has been reported for some organisms (11, 12, 42, 43). Even expression of budding yeast (S. cerevisiae) profilin Pfy1 has been reported to be incapable to suppress the temperature-sensitive phenotypes of cdc3-124 strain (44). Recombinant Cdc3 was successfully expressed in and purified from bacteria (Fig. 5A). We determined the K$_d$ of Cdc3 for fission yeast or muscle actin under low ionic strength conditions (0.1 mM CaCl$_2$, 0.5 mM DTT, 0.2 mM ATP, 2 mM Tris-HCl, pH 7.5) by measuring the quenching of tryptophan (probably Trp$_{356}$) fluorescence following binding of profilin to Ca\(^{2+}\)-G-actin (33). The addition of Cdc3 to fission yeast actin (Fig. 5B) or muscle actin (data not shown) caused quenching of the fluorescence emission at 330 nm. The K$_d$ values of Cdc3 for fission yeast and muscle G-actin were calculated to be 0.15 ± 0.09 μM (n = 6) and 1.3 ± 0.5 μM (n = 5), respectively.

The effect of Cdc3 on polymerization of actin was studied in the presence of both 50 mM KCl and 1 mM MgCl$_2$ (Fig. 5C). Cdc3 inhibited spontaneous polymerization of Mg\(^{2+}\)-G-actin either from fission yeast or from muscle in a concentration-dependent manner. A similar effect of Cdc3 was observed when Ca\(^{2+}\)-G-actin was polymerized by the addition of both 50 mM KCl and 1 mM MgCl$_2$ (data not shown).

We also tested the effect of Cdc3 on the exchange rate of ATP from muscle or fission yeast G-actin. In the absence of Cdc3, muscle Ca\(^{2+}\)-G-actin-bound ATP exchanged with ATP according to first order kinetics with a dissociation rate constant (k$_{-\text{ATP}}$) of 0.0038 ± 0.0008 s$^{-1}$ (Fig. 5D, n = 6). Cdc3 increased the k$_{-\text{ATP}}$ by 4.4-fold (0.017 ± 0.004 s$^{-1}$). On the other hand, fission yeast Ca\(^{2+}\)-G-actin-bound ATP exchanged with ATP with a k$_{-\text{ATP}}$ of 0.013 ± 0.002 s$^{-1}$ (n = 6), which was promoted by Cdc3 only by 1.3-fold (0.016 ± 0.003 s$^{-1}$).

We next examined the effect of Cdc3 on actin filaments at a steady state by cosedimentation experiments and direct observation of fluorescently labeled filaments. Cdc3 reduced the amount of sedimented actin in a concentration-dependent manner (Fig. 6A). The amount of fission yeast actin recovered in the supernatant was nearly proportional to the concentration of Cdc3, whereas muscle actin was hardly recovered in the supernatant.

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**TABLE 2**

Parameters of actin-HMM interaction

| Actin     | K$_m$ (μM) | V$_{max}$ (μM mg$^{-1}$ min$^{-1}$) | V$_{max}$/K$_m$ (s$^{-1}$) | V$_{max}$/K$_m$ (μM$^{-1}$ s$^{-1}$) | Relative efficiency | K$_{app}$ (μM) |
|-----------|------------|-----------------------------------|---------------------------|-------------------------------------|--------------------|----------------|
| F. pombe  | 6.3        | 0.34                              | 0.97                      | 0.15                                | 1                  | 1.1            |
| Muscle    | 7.9        | 1.2                               | 3.3                       | 0.41                                | 2.7                | 0.42           |

$^a$ The concentration of actin that gave a half-saturation of the actin-HMM binding in the absence of ATP calculated from the double-reciprocal plot of the data shown in Fig. 4B (inset).

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**FIGURE 4. Activation of Mg\(^{2+}\)-ATPase activity of HMM by fission yeast and muscle actin.** A, double-reciprocal plots of HMM Mg\(^{2+}\)-ATPase activity by fission yeast actin (circles) and by muscle actin (squares). The reaction mixture contained 0.08 μM HMM with or without 1–12 μM fission yeast or muscle F-actin (see "Materials and Methods"). The reaction mixture was supplemented with 2 mM ATP to start the reaction and incubated at 25°C for 20 min. Liberated phosphates were measured as described under "Materials and Methods." B, cosedimentation of HMM with F-actin. The mixture of HMM (0.48 μM) and F-actin (0.4–3.2 μM actin) stabilized with phalloidin was centrifuged in the absence ATP. The amount of HMM heavy chain in the supernatant was quantified by densitometric analysis. The average values obtained from three independent experiments are shown in A and B, respectively.
at low concentrations of Cdc3. Cdc3 did not practically cosediment with both actin filaments. Fission yeast actin filaments as well as muscle actin filaments were clearly visualized by labeling with BODIPY-phallacidin (Fig. 6B). On the other hand, rhodamine-phalloidin labeling of fission yeast actin filaments was not stable, and the fluorescence was rapidly lost from the filaments (data not shown), as shown for budding yeast actin filaments (45, 46). The length distributions of both actin filaments seemed to be exponential (Fig. 6C). Cdc3 increased the percentage of short filaments (less than 5 μm) of both fission yeast and muscle actin over 2-fold. The total number of filaments tended to decrease dependent on the concentration of Cdc3, which is consistent with the results of cosedimentation experiments of actins and Cdc3. The increase of short actin filaments and decrease in density of filaments in the presence of Cdc3 were also confirmed by electron microscopic observation (data not shown).

**DISCUSSION**

We purified the fission yeast actin, which was competent to polymerize for the first time and investigated its biochemical properties. The highly purified fission yeast actin showed an apparent molecular weight of 42,000 on an SDS-gel, which was almost identical to that of muscle actin, and showed an isoelectric point slightly more basic than that of muscle actin. These results agreed well with the deduced molecular weight and isoelectric point of Act1, 41,762 and 5.31, respectively, and those of muscle actin, 41,817 and 5.23, respectively. Moreover, the results imply that there is only one polymerizable actin species in fission yeast, which is consistent with the report that *S. pombe* has only one actin gene (47).

The current method of purification of fission yeast actin included sedimentation of F-actin. Therefore, it was expected that some F-actin-binding proteins were present in crude fractions. Actually, the Arp2/3 complex was usually contained in the crude G-actin fraction. Moreover, there were three more components in the fraction in addition to the conserved seven subunits of Arp2/3 complex, which eluted together with the Arp2/3 complex at the void volume in the following gel filtration chromatography; they were band 1 (Myo1p), band 2, and band 4 (actin). It has already been shown that the tail portion of recombinant Myo1 binds to the Arp2/3 complex and stimulates its actin-nucleating and -branching activities in vitro (6, 48).
Presence of Myo1 in the Arp2/3 complex fraction would suggest persistent associations of these proteins \textit{in vivo}. On the other hand, the actin present in the void fraction may be actin oligomers that were not removed by the ultracentrifugation step. The nature of the band 2 protein is still unknown.

One reason for the preferential co-purification of the Arp2/3 complex may be that the complex would be more abundant than other actin-binding proteins in \textit{S. pombe} cells. This speculation may be reasonable, since the cortical F-actin patches to which the Arp2/3 complex faithfully localizes (25, 49, 50) are the major F-actin structures in interphase cells (1, 2). On the other hand, other known F-actin-binding proteins, such as type-II myosins Myo2 and Myo3, fimbrin Fim1,  \(\alpha\)-actinin Ain1, coronin Crn1, and tropomyosin Cdc8, were hardly detected, as judged by Coomassie Brilliant Blue staining of SDS-gels.

Fission yeast actin spontaneously polymerized under physiological Mg\(^{2+}\) concentrations in a manner similar to muscle actin, although there were some differences. First, fission yeast actin nucleated more rapidly than muscle actin did, as previously observed for polymerization of budding yeast actin (51, 52). We also observed that upon the addition of both 50 mM KCl and 1 mM MgCl\(_2\), fission yeast Ca\(^{2+}\)-G-actin and Mg\(^{2+}\)-G-actin nucleated at almost the same rate (Fig. 5C, left), whereas muscle Mg\(^{2+}\)-G-actin nucleated significantly faster than muscle Ca\(^{2+}\)-G-actin (Fig. 5C, right). This observation suggests that fission yeast Ca\(^{2+}\)-actin would be converted to Mg\(^{2+}\)-actin more readily than muscle Ca\(^{2+}\)-actin is.

Second, polymerization of fission yeast actin seemed to have occurred through two steps: the initial rapid phase and the following slow phase. The rapid phase may represent monomer addition to nuclei or ends of filaments. The slow phase was not seen for muscle actin. It may represent annealing of filaments, the number of which was possibly large.

**FIGURE 6. Effects of Cdc3 on actin polymerization at steady state.** A, dependence of the amounts of sedimentable F-actin at steady state on the concentration of Cdc3. Fission yeast or muscle Mg\(^{2+}\)-G-actin (3 \(\mu\)M) was incubated with various concentrations of Cdc3 (0–9 \(\mu\)M) for more than 16 h in both 50 mM KCl and 1 mM MgCl\(_2\), and the solutions were centrifuged at 300,000 \(\times\) g for 10 min. The supernatants and the pellets were subjected to SDS-PAGE, and the relative contents of actin in the supernatants were quantified by densitometric analysis of a Coomassie Brilliant Blue-stained gel. The values are expressed as the average \(\pm\) S.D. obtained from three or four independent experiments for fission yeast or muscle actin, respectively. Typical examples of the gels are shown. L, loading control (bovine serum albumin and muscle actin). Numbers at the tops of lanes indicate the concentration of Cdc3 added. A and P, bands of actin and Cdc3, respectively. B, fluorescence micrographs of F-actin. Fission yeast or muscle Mg\(^{2+}\)-G-actin (3 \(\mu\)M) was polymerized for 16 h at 25 °C in the absence (Control) or presence of 3 \(\mu\)M Cdc3 (+Cdc3) in both 50 mM KCl and 1 mM MgCl\(_2\), and the filaments were labeled with BODIPY-phallacidin. Samples were diluted and applied to coverslips coated with poly-L-lysine. Bar, 5 \(\mu\)M. C, length distribution of actin filaments (3 \(\mu\)M Mg\(^{2+}\)-G-actin) polymerized with buffer (open bars) or 3 \(\mu\)M Cdc3 (shaded bars) as described in B. The median lengths are shown in the graphs.
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due to polymerization from many nuclei. The slow phase was not observed when polymerization of fission yeast actin was monitored by fluorescence change of a trace amount of pyrene-labeled muscle actin (53), which was added to the fission yeast actin; the polymerization soon reached a plateau following the rapid phase (data not shown). This observation supported the idea that the second phase corresponded to annealing of filaments, because the increase in fluorescence of pyrene actin is proportional only to polymer weight concentration and seems to be insensitive to length or thickness of filaments (54).

To exclude the possibility that the purified preparation of fission yeast actin might have contained short oligomers that did not sediment by ultracentrifugation following the final polymerization-depolymerization step and might have served as nuclei upon polymerization, we purified fission yeast actin without the final polymerization-depolymerization step to avoid inclusion of actin oligomers. We pooled only the peak fraction eluted from the gel filtration column (Fig. S2A), which did not contain oligomers. The actin in this fraction was pure enough (Fig. S2B), although the yield was low. The G-actin still showed faster nucleation than muscle actin and biphasic polymerization kinetics (Fig. S2C), which would indicate that these properties are characteristic of fission yeast actin.

Dissociation rate constants at the two ends of actin filaments are intrinsic properties of actin, and they mainly determine the speed of depolymerization, whereas that of polymerization varies depending on the concentration of polymerizable monomers. It was expected that fission yeast actin would depolymerize faster than muscle actin, because actin cytoskeletons in fission yeast cells are highly dynamic structures, whereas muscle thin filaments are structurally static. This is the first report that compared directly the rate of depolymerization of muscle and nonmuscle actins. However, fission yeast actin was revealed to depolymerize at a rate similar to that of muscle actin. It has been recently reported that the actin-depolymerizing factor Adf1 accelerates depolymerization of actin filaments in living fission yeast cells (55). The function of this protein may explain the instability of actin filaments in nonmuscle cells.

The $C_C$ of fission yeast actin for polymerization was nearly equal to that of muscle actin and comparable with those of budding yeast (56), maize pollen (57), sea urchin egg (37), and Dictyostelium (58) actins. The reasonable value of the $C_C$ excludes the possibility of some contaminants that can cap the ends of filaments and thereby affect the apparent $C_C$.

Electron microscopy revealed structural similarities of fission yeast actin filament to other actin filaments. The fission yeast actin filament was indistinguishable from actin filaments from skeletal muscle (41) and other species (54, 59, 60) in terms of their general appearance, thickness of individual filaments, the helical parameters of the filaments, and the arrowhead appearance when HMM was attached. The pitches of the right-handed double helix of two parallel protofilaments and the diameters of filaments were almost identical for these actins.

On the other hand, it has been reported by three-dimensional reconstruction that budding yeast F-actin shows weaker contacts between the two long pitch helical strands than muscle actin does (61). Therefore, a three-dimensional analysis on fission yeast actin with a higher resolution will be necessary in the future.

In 0.1 M KCl, fission yeast Ca$^{2+}$-actin polymerized into filaments that seemed to change into filaments only after the addition of Mg$^{2+}$. This feature is quite different from animal actins, which polymerize into filaments by the addition of 0.1 M KCl. This is also different from that of budding yeast Ca$^{2+}$-G-actin, which does not show an increase in light scattering in the presence of KCl (52). The structural transition from the clusters into filaments seems to be worth investigating for further understanding of the role of Mg$^{2+}$ in the formation of nuclei of actin polymerization.

The particle structures of Ca$^{2+}$-actin in 0.1 M KCl contained a ring structure. We consider that this ring structure is made of fission yeast actin, since the purity of the purified actin preparation was more than 96%, whereas the ring comprised 25% of the total particles. It has been reported that chaperonin containing TCP-1 (CCT), a representative eukaryotic type II chaperonin, forms a ring structure (reviewed in Ref. 62), and it is conserved in fission yeast (products of cct1–cct8 genes). However, it is not likely that the present ring is composed of the chaperonin, since 1) we could not detect a 60-kDa component that represents the CCT subunit in the purified actin preparation; 2) although the ring structure appeared to decrease in number in 2 mM MgCl$_2$, the CCT ring seems to be stable in the presence of 1–5 mM MgCl$_2$ (e.g. Ref. 63); 3) we could not detect in our actin images a barrel-like structure with four stripes, which is characteristic of a side view of the CCT ring (63, 64); 4) the average diameter of the ring structure (9 nm) was smaller than that of the CCT ring (15 nm) (64–66).

The intrinsic nucleotide exchange rate on fission yeast G-actin was 3.4 times faster than that of muscle G-actin, which was similar to the intrinsic rate on budding yeast G-actin (67). It has been reported that the nucleotide binding cleft in budding yeast actin seems to open more widely than in muscle actin, which may account for the faster nucleotide exchange in budding yeast actin than in muscle actin (61). Thus, again, a detailed structural analysis of fission yeast G-actin would be necessary in order to interpret its property on the structural basis.

Fission yeast actin activated the Mg$^{2+}$-ATPase activity of skeletal muscle HMM, but it was less effective than muscle actin. This may be explained by the species specificity in the actin-myosin interaction, which has been shown for other hybrid actomyosin complexes (56, 68). The fission yeast actin had a $K_m$ value for HMM similar to that of muscle actin but showed an approximately 3-fold lower $V_{max}$ value than that for muscle actin. Thus, the low activation of HMM by yeast actin may mainly be due to its lesser ability to activate myosin to release inorganic phosphate (69). This result is different from the results reported for activation of HMM by budding yeast (56) or Dictyostelium actin (68), which has a higher $K_m$ for HMM and gives the same $V_{max}$ compared with muscle actin. Binding properties of HMM to actins were more directly examined by cosedimentation assays. HMM showed an affinity for fission yeast actin lower than for muscle actin in the absence of ATP. However, HMM was effectively released from both F-actins in the presence of excess ATP, which suggests that the
release of myosin-ATP complex from these F-actins induced by ATP (69) occurs in the same manner.

Fission yeast profilin Cdc3 inhibited nucleation of fission yeast and muscle actins and decreased the extent of polymerization of both actins in a concentration-dependent manner, as previously described for Physarum profilin (70). Moreover, we showed for the first time that profilin significantly shortened the average length of actin filaments at steady state. These functions of Cdc3 seemed apparently consistent with the effect of overproduction of Cdc3 in vivo, which resulted in complete disappearance of F-actin structures from cells (44).

The species-specific function of Cdc3 was clearly observed as a more potent effect to increase the amount of nonsedimentable fission yeast actin than muscle actin and its significantly lower \( K_f \) value for fission yeast actin than for muscle actin measured under low salt conditions. The \( K_f \) of Cdc3 for muscle actin (1.3 \( \mu \)M) was different from the value previously reported (0.21 \( \mu \)M) (30). It may be due to the differences of the assay conditions to obtain the \( K_f \).

Moreover, Cdc3 was a weak accelerator of nucleotide exchange on fission yeast G-actin. This contrasted with the 3-fold acceleration of exchange rate on muscle G-actin (this report) (30). Thus, the role of Cdc3 in the nucleotide exchange activity of fission yeast actin may not be important in fission yeast cells. It has been reported that this activity is essential for viability of fission yeast cells (30). The reason for the discrepancy is not clear at the moment.

Taken together, the action of Cdc3 on actin polymerization in vitro and the effect of Cdc3 overproduction in the cell can be attributed solely to its stoichiometric sequestration of G-actin, which consequently reduces the concentration of nucleation- and polymerization-competent G-actin. On the other hand, cdc3 mutants were not able to form the contractile ring during mitosis, which suggests a function of Cdc3 in the local actin assembly (44). Considering that Cdc12, a yeast actin isoform that is required for formation of the contractile ring and localizes to the ring (4, 71), can elongate muscle actin filaments in the presence of Cdc3 but not in its absence in vitro (4), Cdc3 may function as a cofactor of Cdc12 in assembly of the contractile ring actin filaments at the medial cortex during late mitosis. On the other hand, Cdc3 may prevent spontaneous polymerization of actin as an actin-sequestering protein in cytoplasm during interphase when Cdc12 is possibly inactive. To further understand the functions of Cdc12 and Cdc3, it is necessary to investigate the action of Cdc12 on fission yeast actin in the presence or absence of Cdc3.

As previously reported on budding yeast F-actin (45, 46), rhodamine-phalloidin showed a low binding affinity to fission yeast F-actin, although it binds to the actin structures in fixed S. pombe cells for some reason. Nonlabeled phalloidin can stabilize fission yeast F-actin only when used at concentrations 100–1000 times higher than those used for fluorescence microscopy (data not shown). The reduced affinities of rhodamine-phalloidin for the two yeast F-actins might be caused by the change of one or two amino acids in residues 198–201 of actins (muscle, YSFV; budding yeast, YSFS; fission yeast, YTSF), which are reportedly involved in the binding of phalloidin or rhodamine-phalloidin to muscle actin (72). On the other hand, BODIPY-phallacidin stained both fission yeast and muscle F-actins to a similar extent. This may be because the two fluorescently labeled phalloidins would attach to different sites on F-actins and/or in different manners.

The method for purification of fission yeast actin reported here enables us to obtain the highly purified actin capable of polymerization. The availability of the purified fission yeast actin will facilitate in vitro reconstitutions of physiologically meaningful interactions between the actin and fission yeast ABPs, which will provide genuine insights into their cellular functions. Since the method can also be applied to purify mutant fission yeast actins if they can polymerize, it will enable us to relate the phenotypes of the mutant strains to the properties of the mutant actins. These future studies will contribute to understanding the function of actin cytoskeleton in eukaryotic cells, since fission yeast is the excellent model organism. The Arp2/3 complex accompanied by myosin-I can also be isolated, which would facilitate more detailed structural and functional examinations of the S. pombe Arp2/3 complex. Moreover, it is expected that by an F-actin affinity column chromatography using fission yeast actin, we may be able to find novel ABPs and/or myosins that would specifically interact with fission yeast actin filaments.

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