Tropomyosin-dependent Filament Formation by a Polymerization-defective Mutant Yeast Actin (V266G,L267G)*

Kuo-Kuang Wen, Bing Kuang, and Peter A. Rubenstein‡
From the Department of Biochemistry, University of Iowa College of Medicine, Iowa City, Iowa 52242

A major function of tropomyosin (TPM) in nonmuscle cells may be stabilization of F-actin by binding longitudinally along the actin filament axis. However, no clear evidence exists in vitro that TPM can significantly affect the critical concentration of actin. We previously made a polymerization-defective mutant actin, GG (V266G, L267G). This actin will not polymerize alone at 25 °C but will in the presence of phalloidin or beryllium fluoride. With beryllium fluoride, but not phalloidin, this polymerization rescue is cold-sensitive. We show here that GG-actin polymerizability was restored by cardiac tropomyosin and yeast TPM1 and TPM2 at 25 °C with rescue efficiency inversely proportional to TPM length (TPM2 > TPM1 > cardiac tropomyosin), indicating the importance of the ends in polymerization rescue. In the presence of TPM, the apparent critical concentration of actin is 5.5 μM, 10–15-fold higher than that of wild type actin but well below that of the GG-actin alone (>20 μM). Non N-acetylated TPMs did not rescue GG-actin polymerization. The TPMs did not prevent cold-induced depolymerization of GG F-actin. TPM-dependent GG-actin polymerization did not occur at temperatures below 20 °C. Polymerization rescue may depend initially on the capture of unstable GG-F-actin oligomers by the TPM, resulting in the strengthening of actin monomer- monomer contacts along the filament axis.

Tropomyosins are a family of highly conserved eukaryotic actin-binding proteins with molecular masses ranging from 19 kDa for yeast TPM2 (1) to 40 kDa for rat fibroblast TPM1 (2). Tropomyosins possess a dimeric α-helical coiled-coil structure along virtually the entire length of the protein, and heptad repeat motifs with hydrophobic residues at positions 1 and 4 are found along the entire molecule. These quasirepeat regions appear to match up with actin monomers when tropomyosin binds to F-actin along the filament axis and may serve as weak actin binding regions. Tropomyosins have a basic amino terminus, which is usually N-acetylated, and for most tropomyosins, the acetyl group is believed to be important for preservation of the helical structure at the end of the protein (3). Adjacent tropomyosins form head-to-tail overlapping interactions along the length of the actin filament (4), and these overlaps are believed to be the major site of interaction between F-actin and tropomyosin (5, 6). Recombinant skeletal muscle tropomyosin with either an unacetylated N terminus (7) or with a C-terminal deletion (5) loses the ability to bind to actin. The interaction between actin and the internal quasirepeat regions of tropomyosin is believed to be weaker but still critical for cooperative binding to F-actin (8), and there is a hierarchy of importance among these quasirepeat regions (9).

The role of tropomyosin, along with troponin, as a mediator of calcium regulation of thin filament function in sarcomeric muscle is well established. However, its role in nonmuscle cells, where there is no troponin, is poorly understood. Based on its ability to interact simultaneously with a number of actin monomers along the actin helix, it has been hypothesized that tropomyosin functions as a stabilizer of actin filaments in nonmuscle cells. Even a modest effect of TPM on filament stability would be biologically significant because actin monomer binding proteins in the cell act to amplify any change in stability such as the critical concentration (10). Studies in vitro addressing this question, however, do not yield consistent results. Experiments in vitro using muscle actin and muscle tropomyosins showed that tropomyosin can alter the monomer association and dissociation rates at both ends of the filament, resulting in a small change in critical concentration (11). Brosch et al. (12, 13) demonstrated that nonmuscle and muscle tropomyosins slowed depolymerization at the pointed end of the filament, while another study reported that tropomyosin prevented filament fragmentation but did not alter elongation rate (14). These differing results may derive from differences in muscle versus nonmuscle tropomyosin or from differences in experimental methodology. It is also possible that the low critical concentration and the stability of muscle actin filaments in vitro render the filament-stabilizing properties of tropomyosin difficult to measure reproducibly. This situation would also make it very difficult for one to determine the relative contribution of different parts of the tropomyosin molecule to actin filament stabilization.

A valuable system for the assessment of tropomyosin in nonmuscle cells has been the budding yeast Saccharomyces cerevisiae. This yeast contains two tropomyosins, each encoded by a separate gene. TPM1, which contains five internal quasirepeat regions, constitutes about 85% of the cell’s tropomyosin and is present in sufficient quantities to saturate the F-actin present in the cell (15). TPM2, which contains only four quasirepeat regions, comprises 15% of the cell’s tropomyosin (1). Both bind to actin in a saturable fashion, with the binding of TPM2 less sensitive to higher salt than TPM1. The simultaneous deletion of both TPM genes results in cell death, whereas deletion of the TPM1 gene, alone, results in a mild ts phenotype, and disappearance of actin cables. These phenotypes can be partially rescued by overexpression of TPM2. Deletion of TPM2 alone produces no recognizable phenotype.

We previously generated a mutant yeast actin, GG, with a...
polymerization defect in vitro. Cells expressing this as their only actin are viable but sick (16). In this actin, two hydrophobic residues, Val266 and Leu267, were simultaneously altered to glycines. These residues are at the amino-terminal end of a hydrophobic loop between actin subdomains 3 and 4, which, according to a model advanced by Holmes and colleagues (17), interacts with a hydrophobic surface composed of two neighboring monomers on the opposing strand of the actin filament. Such an interaction would impart cross-strand stabilization to the filament structure. The model predicts that interference with this hydrophobic interaction should destabilize the filament, and we showed that this mutant actin would not polymerize at concentrations as high as 19 μM. Phalloidin, which forms a bridge between the two strands of the filament (18, 19), restores polymerization of GG-actin (20). Beryllium fluoride (BeF\(_2\)) also restores polymerizability to the actin. BeF\(_2\) occupies the position on the actin monomer vacated by the inorganic phosphate that is slowly released following hydrolysis of ATP by actin during polymerization of the protein (21). The binding of BeF\(_2\) converts the filament to a more stable ADP-P\(_i\)-like state by strengthening intermonomer contacts along the filament axis such that the increased stability can compensate for the loss in cross-strand stabilization caused by the GG mutation (20). Hydrophobic interactions are predicted to be cold-sensitive (22). However, while phalloidin rescue is not cold-sensitive, BeF\(_2\) rescue is; the GG-actin depolymerizes as the temperature decreases to 4 °C. Sac6p, the yeast homologue of the actin filament bundling protein fimbrin, binds to the actin filament between neighboring actin monomers along the filament axis, and in agreement with our BeF\(_2\) results, this protein also rescues GG polymerization in a temperature-sensitive fashion (23).

The viability of GG-actin-expressing cells, despite the polymerization defect exhibited by the actin in vitro, implies that another protein within the yeast can rescue GG-actin function. A candidate for such a protein is tropomyosin due to its mode of binding to the actin filament and to the amount of TPM1 relative to the amount of actin in the cell. Although the binding of tropomyosin to actin depends on the integrity of the ends of TPM, the contribution of the internal repeats to formation of a stable tropomyosin-actin complex is still under debate. The side-binding nature of TPM suggests that it should rescue GG-actin polymerizability in much the same way that Sac6p does, and the availability of tropomyosins of different lengths allows us to also address the relative contribution of the ends of TPM versus the internal quasirepeat regions in actin filament stabilization. In this paper, we assess the ability of N-acetylated and non-N-acetylated yeast TPM1 and TPM2 and bovine cardiac tropomyosin (CTPM) to rescue GG-actin polymerization at different temperatures.

**EXPERIMENTAL PROCEDURES**

**Materials**—Affi-Gel 10 activated resin was purchased from Bio-Rad, and DNase I (grade D) was purchased from Worthington. DNase I affinity columns were made as described previously (24). ATP was purchased from Sigma. Yeast cakes were obtained from a local food store. All other chemicals used were reagent grade quality. Recombinant yeast TPM1 and TPM2, isolated from Escherichia coli and therefore possessing an unblocked NH\(_2\) terminus, were the generous gift of Dr. Larry Tobacman (University of Iowa). Recombinant chicken striated muscle \(\alpha\)-tropomyosin produced from E. coli was a generous gift of Sarah Hitchcock-DeGregori (University of Medicine and Dentistry of New Jersey).

**Actin Purification**—Yeast wild type and mutant GG-actins were purified in the Ca\(^{2+}\) form by a DNase I affinity chromatography protocol as described (20, 24) and stored in Ca\(^{2+}\)-G buffer (10 mM Tris-HCl, pH 7.5, containing 0.2 mM ATP, 0.2 mM CaCl\(_2\), and 0.1 mM dithiothreitol). Actins were converted to their Mg\(^{2+}\) form at 4 °C according to Strella-Golaszewksa et al. (25) and used within 12 h.

**Tropomyosin Purification**—Native bovine cardiac tropomyosin was prepared from an acetone powder of heart tissue according to the procedure of Butters et al. (5) based on heat fractionation, salt fractionation, and DEAE-cellulose chromatography. To produce yeast TPM1, we first constructed a htpm1 haploid yeast strain (yP\(_R\)-5) and transformed it with a TPM1 overexpression plasmid in which expression of TPM1 was under control of the GAL1 promoter. For TPM2 production, a haploid htpm1 strain (yP\(_R\)-6) was constructed and transformed with a TPM2 overexpression plasmid, also under galactose regulation. The TPM1 and TPM2 plasmids were kindly provided by A. Bretscher (Cornell University). TPM1 and TPM2 were purified according to a modified protocol (1). Following induction of the plasmids with galactose for 12 h, cell lysates were made from 4-liter cultures and subjected to heat and salt fractionation followed by ion exchange chromatography on a Pharmacia MonoQ column. These two tropomyosins were subjected to an additional gel filtration step on Sephracyl 200 in 10 mM Tris-HCl, pH 7.5, containing 150 mM KCl and 1 mM dithiothreitol. The resulting protein was then dialyzed against the same buffer without the KCl. In each case, single bands were obtained following SDS gel electrophoresis. Tropomyosin concentrations (dimers) were determined by UV absorbance at 280 nm using the following extinction coefficients: \(\Delta A_{280} = 0.01356\) cm\(^{-1}\)M\(^{-1}\), \(\Delta A_{285} = 0.02788\) cm\(^{-1}\)M\(^{-1}\), and \(\Delta A_{280} = 0.03068\) cm\(^{-1}\)M\(^{-1}\). Yields of the tropomyosins were 8.1 mg of TPM1 and 4 mg of TPM2 from 10 g of each cell type.

**Actin Polymerization**—Actin polymerization was followed by monitoring the increase in light scattering as a function of time. Experiments were performed in a 120-μl cuvette in a thermostatted cell chamber of a SpeX Fluorolog 3 fluorescence spectrometer with excitation and emission wavelengths set at 360 nm. For experiments at 25 °C, tropomyosin of the desired type and concentration was mixed with Mg\(^{2+}\)-GG-actin in Mg\(^{2+}\)-G buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM MgCl\(_2\), and 0.1 mM dithiothreitol). Polymerization was initiated by the addition of MgCl\(_2\) and KCl to final concentrations of 2 and 50 mM, respectively. The steep deflection observed at the beginning of the kinetic trace is due to the opening and closing of the sample chamber during the addition of the salt. Actin polymerization data are normalized against the net increase in light scattering caused by the polymerization of 19 μM WT actin in the presence of saturating amounts of CTPM. Temperature was regulated with a thermostatted water bath attached to the cuvette chamber. In those cases where the temperature was lowered from 25 °C, the rate of decrease was about 2 °C per 3 min. In cases where we increased the temperature from 4 °C, the temperature was raised 5 °C over a 2-min period, after which the sample was allowed to remain at the new temperature for 10 min.

**Electron Microscopy**—Samples containing 19 μM actin were deposited on carbon-coated Formvar grids, negatively stained with 1.5% uranyl acetate, and observed on a Hitachi 7000 transmission electron microscope in the University of Iowa Central Electron Microscopy Facility.

**RESULTS**

**Tropomyosin Restores Polymerization of GG-actin**—We first assessed the ability of different tropomyosins to restore the polymerizability of the Mg\(^{2+}\) form of GG-actin at 25 °C (Fig. 1). As reported previously, the addition of salt in the absence of tropomyosin causes an initial small increase in light scattering followed by a slow decrease toward base-line values. This change occurs without the formation of stable filaments as judged by electron microscopy (Fig. 3) and may involve an abortive attempt at filament formation following salt-induced nucleation. At saturating levels of tropomyosin, the three tropomyosins tested, CTPM, TPM1, and TPM2 (Fig. 1, A–C) all induced the same increase in light scattering in the presence of 19 μM GG-actin, although the rate of increase for the two yeast tropomyosins was faster than that observed for the cardiac protein. In each of these cases, electron microscopy showed that this stable increase in light scattering was accompanied by F-actin formation and did not merely result from salt-induced aggregation. Filaments were also observed (data not shown) at sub saturating tropomyosin concentrations in which the final extent of increased light scattering was reduced by about 60%.

We wished to determine whether the ability of tropomyosin to restore GG-actin polymerizability depended on the nature of the divalent cation bound in the high affinity site at the base of
of its enhanced stability. Previous work has shown that there are distinct differences in the dynamic properties of these two forms of actin. We thus determined the ability of TPM1 to induce polymerization of the Ca$^{2+}$ form of GG-actin (Fig. 2). With Ca$^{2+}$ actin, the initial rise in light scattering seen in the absence of tropomyosin does not occur, in contrast to the situation with the Mg$^{2+}$ form of actin, in agreement with previous results (20). The addition of saturating TPM1 induced the same degree of light scattering increase observed with the Mg$^{2+}$ form, and EM demonstrated again that this increased light scattering resulted from formation of F-actin (Fig. 3). However, the rate of increase for the Ca$^{2+}$ form of actin is slower than that observed with the Mg$^{2+}$ form and may simply reflect the faster rate of polymerization of the Mg$^{2+}$ form of WT actin alone in comparison with its Ca$^{2+}$ form.

The Importance of Tropomyosin N-terminal Acetylation in the Rescue of Mg$^{2+}$-GG-actin Polymerizability—It had been shown previously that removal of the N-terminal acetyl group from sarcomeric tropomyosins prevented their binding to F-actin. We therefore assessed the effect of this post-translational modification on the ability of muscle and both yeast tropomyosins to rescue the polymerization of GG-actin. The unacetylated proteins were obtained from E. coli that had been transformed with plasmids carrying the tropomyosin coding sequences. We utilized chick skeletal muscle α-tropomyosin instead of the CTPM because of its availability as a recombinant protein. None of these nonacetylated tropomyosins were able to restore GG-actin polymerization at concentrations where the rescue by the acetylated proteins was maximal (Fig. 4), and EM confirmed the absence of actin filaments in these preparations (Fig. 3, F–H). It had previously been demonstrated that extension of the N terminus of a nonacetylated sarcomeric tropomyosin by addition of an Ala-Ser restored the ability of the tropomyosin to bind to F-actin and to restore thin filament regulation (26). This tropomyosin also restored GG-actin polymerization although to a lesser extent than WT acetylated tropomyosin (data not shown). These experiments underscore the importance of the tropomyosin N terminus in the rescue of GG-actin polymerization and suggest that tropomyosin end-end overlap plays an important role in the establishment of GG-actin filament stability.

GG-actin Polymerization as a Function of Tropomyosin Concentration—To further establish the relative importance of the
troponymosin ends versus internal quasirepeat regions in the rescue of GG-actin polymerization, we assessed the relative ability of different concentrations of CTPM (seven quasirepeating regions), TPM1 (five quasirepeating regions), and TPM2 (four quasirepeating regions) to polymerize 19 μM Mg²⁺-GG-actin. Per length of actin filament, at saturating conditions, there should be more ends with the shorter troponymosins and hence a tighter interaction between actin and troponymosin if the hypothesis is correct. The results are displayed in Fig. 5 and are presented on a weight basis with respect to the amount of troponymosin used. The use of weight rather than molarity allows one to account for differences in the molecular weights of the three different troponymosins used. Because of this molecular weight difference, approximately the same weight of each of the troponymosins, not the same number of molecules, should be bound per length of actin filament at saturating concentrations. The results show that the efficiency of rescue of GG-actin polymerization varies inversely with the length of the troponymosin used. Saturation was approximately achieved with 360 μg/ml of CTPM, 225 μg/ml of TPM1, and 171 μg/ml of TPM2. A plot of these concentrations versus the number of quasirepeats in the troponymosin yields a straight line (Fig. 6), suggesting that the number of ends per length of actin is a central factor in rescue efficiency.

**Apparent Critical Concentration of GG-actin in the Presence of Tropomyosin**—The incomplete polymerization of GG-actin produced in the presence of saturating tropomyosin concentrations (Figs. 1 and 8) indicates that the critical concentration of GG-actin in the presence of tropomyosin is much higher than the value for wild type actin, 0.4 μM (27). To determine an approximate value for the critical concentration of GG-actin, we incubated the G form of Mg²⁺-GG-actin at concentrations between 1 and 19 μM with 7.5 μM CTPM, TPM1, or TPM2 in F-buffer at 25 °C and recorded the final increase in light scattering. This concentration of tropomyosin is well above that needed for saturation for each of the troponymosins based on the results of Fig. 7. The final net increase in light scattering was then plotted as a function of actin concentration. Fig. 7 shows that for each of the three troponymosins, the critical concentration for Mg²⁺-GG-actin was approximately 5.5 μM. Thus, although each of the troponymosins tested can lower the critical concentration of actin from >20 to about 5 μM, the final value is still 10–15 times that observed for the critical concentration of WT actin in the absence of troponymosin.

**Temperature Dependence of the Rescue of GG-actin Polymerization by Tropomyosin**—The side-binding nature of the tropomyosin-F-actin interaction predicts that tropomyosin’s ability to rescue GG-actin polymerization should display a cold sensitivity similar to that seen with BeFx or Sac6p-induced rescue. To test this hypothesis, 19 μM GG-actin was polymerized in the presence of 500 μg/ml CTPM, TPM1, or TPM2 at 25 °C (Fig. 8A). This concentration was well above saturating levels for each of the troponymosins in question. The results show a TPM-dependent acceleration of polymerization with TPM2 > TPM1 > CTPM. The temperature was then lowered to 4 °C over about a 30-min period with continuous monitoring of the light scattering of the sample. With each of the three troponymosins, we observed a decrease in light scattering, which reached a plateau at between 4 and 8% above background (Fig. 8B). Electron microscopic examination of these samples revealed mostly small aggregates with occasional filament-like structures observed (Fig. 9). The relative rates of depolymerization were much more similar for these three samples than were the relative polymerization rates. Additionally, the fila-

![Fig. 3. Electron microscopic images of mixtures of GG-actin with various tropomyosins at the completion of polymerization.](image)

At the end of each experiment in Figs. 1 and 2, an aliquot was examined under the electron microscope following negative staining of the sample with uranyl acetate as described under “Experimental Procedures.” A, WT actin plus CTPM; B, GG-actin plus CTPM; C, GG-actin plus TPM1; D, GG-actin plus TPM2; E, GG-actin alone; F, GG-actin plus unacetylated CTPM; G, GG-actin plus unacetylated TPM1; H, GG-actin plus unacetylated TPM2.

![Fig. 4. Unacetylated yeast or cardiac tropomyosins do not restore GG-actin polymerizability.](image)

The experiment in Fig. 1 was repeated except that unacetylated recombinant troponymosins were used instead of their acetylated counterparts. Curve 1, GG-actin alone; curve 2, CTPM; curve 3, TPM1; curve 4, TPM2.
ment stability order was reproducibly different as well from what we observed for polymerization efficiency: CTPM > TPM2 > TPM1. We then determined whether this cold-induced depolymerization was reversible and, if so, what the temperature dependence of repolymerization was. No light scattering increase was observed when any of the samples were raised to 10 or 15 °C (Fig. 8C). However, elevation of the sample to 20 °C resulted in an increased light scattering (Fig. 8D), which EM showed was due to repolymerization of the actin (Fig. 9). These last results are remarkable for two reasons. First, the plateau level reached is significantly lower than that achieved initially at 25 °C, demonstrating the extreme temperature sensitivity of GG F-actin stability in the presence of tropomyosin. Second, the order of repolymerization efficiency with respect to tropomyosin is identical to the polymerization efficiency order seen originally at 25 °C. However, the relative differences between
FIG. 8. Temperature sensitivity of tropomyosin-dependent rescue of Mg$^{2+}$-GG-actin polymerizability. For all experiments, 19 μM GG-actin was combined with 500 μg/ml of CTPM (curve 1), TPM1 (curve 2), or TPM2 (curve 3), and polymerization was allowed to go to completion at 25°C (A). The thermostat was then lowered to 4°C, and the decrease in light scattering for each sample was followed over time (B). When the decrease in light scattering reached a plateau, the temperature was first raised to 10°C and then to 15°C, and each temperature was maintained for 10 min (C). The temperature of the sample was then raised to 20°C, and the increase in light scattering was monitored over time (D). Polymerization was normalized to the amount of light scattering achieved with an equivalent amount of WT actin in the presence of saturating CTPM at 25°C.

FIG. 9. Electron microscopic images of samples obtained during the temperature sensitivity study described in the legend to Fig. 8. At the desired point, samples were removed and examined, following negative staining, under the electron microscope. A–C, 4°C; D–F, 20°C repolymerization; A and D, CTPM; B and E, TPM1; C and F, TPM2.

The ability of these three tropomyosins to induce polymerization are much greater at lower temperatures, suggesting that as the temperature is lowered, the tropomyosin end interactions become more crucial in the rescue process. If the sample is jumped to 25°C (data not shown), the light scattering reaches the same plateau that we observed in Fig. 8A, demonstrating the total reversibility of this process.

**DISCUSSION**

The focus of this work was to determine whether or not tropomyosin, because of its ability to bind F-actin along the filament axis, could restore polymerizability to GG-actin, a polymerization-defective mutant yeast actin. If so, then tropomyosin would become a prime candidate for a protein that is required for GG-actin to function in vivo in a manner compatible with cell viability. Our results clearly demonstrate the ability of not only both yeast tropomyosins but also a sarcomeric tropomyosin to restore GG-actin polymerizability. All three tropomyosins do this in a saturable manner by a mechanism that apparently requires the ability of the ends of tropomyosin to interact with one another, based on our results with the nonacetylated proteins. Furthermore, all three tropomyosins confer the same apparent critical concentration on the actin, about 5.5 μM. In the absence of TPM, the critical concentration is so high that stable polymers will not form at 25°C at concentrations as high as 19 μM GG-actin. Therefore, the interaction of the TPM with the actin monomers at or near the end of the filament must change their conformations via strengthened intermonomer contacts such that the addition of a new monomer to the filament end becomes favorable. We believe this is, if not the first, then certainly one of the clearest demonstrations of tropomyosin’s ability to act as an F-actin stabilizer by significantly lowering the critical concentration of the actin as opposed to protecting the filament against severing.

As impressive as this rescue is, it is much less efficient than the ability of yeast fimbrin to restore GG-actin polymerizability. At 25°C, saturating concentrations of the yeast fimbrin, Sac6p, at any of the actin concentrations we examined, drove all of the GG-actin into filament bundles, implying that the critical concentration for actin in this case was equal to or less than that exhibited by WT actin. We believe this difference in rescue efficiency reflects the very different modes by which Sac6p and tropomyosin bind to the actin filament. For tropomyosin, it has been hypothesized that the strongest interactions with actin monomers involve the interacting ends of adjacent TPMs with weaker interactions involving the internal quasirepeat regions. Thus, with the tropomyosins we employed, there would be a strong contact between every four and seven actin monomers depending on the tropomyosin. On the other hand, the actin binding domain of Sac6p binds between adjacent monomers, forming a very tight bridge between them, and we have shown that one Sac6p contact per two actin monomers was needed for stable filament formation. Furthermore, at 25°C with Sac6p in vitro, one does not observe single filaments but ordered bundles due to the cross-linking nature of the protein. This added stabilization may well play a significant role in the enhanced ability of the Sac6p to restore GG-actin polymerization. At very low temperatures, isolated single actin filaments were observed in the presence of Sac6p, presumably due to the effect of the lowered temperature on the ability of the protein to cross-link filaments.

Tropomyosin’s longitudinal stabilization of monomers within the F-actin helix predicts that tropomyosin-dependent rescue should exhibit the same cold sensitivity observed with BeFx- and Sac6p-dependent rescue of GG-actin polymerization. In terms of the Holmes model of the actin filament, the hydrophobic plug, which was mutated to form GG-actin, is involved in a cross-strand stabilization of F-actin by virtue of its ability to contact a hydrophobic surface made up by the interface of two monomers on the opposing strand (17, 19). Because hydrophobic interactions are cold-sensitive, elimination of two-thirds of the hydrophobicity in this plug reduces this interaction to such an extent that filament stability can no longer be maintained. Enough residual stabilization remains at 25°C such that
strenthening longitudinal monomer-monomer contacts can at least partially overcome this plug deficit. However, lowering the temperature reduces this residual cross-strand stabilization to the point where it can no longer be compensated for by increased longitudinal contacts, and the filament disassembles.

To a first approximation, our data support this cold sensitivity prediction. However, there are significant differences between the sensitivity of the GG-actin to depolymerization in the presence of Sac6p versus tropomyosin. Possibly because of the enhanced longitudinal stabilization or the additional stabilization due to filament bundling, lowering the temperature to 4 °C in the presence of Sac6p did not result in complete depolymerization based on light scattering and what looked like the appearance of small aggregates under EM. The residual cross-strand stabilization, however, is at least partially overcome this plug deficit. However, lowering the temperature to 20 °C when recovery was complete. EM showed that this increased light scattering represented F-actin formation. Sac6p when recovery was complete. EM showed that this in-

appearance of small aggregates under EM. Subsequent raising this to 15 °C in the presence of Sac6p did not result in complete depolymerization of the filament (15 °C). With tropomyosin, depolymerization appeared complete at 4 °C, and repolymerization, based on light scattering, did not occur until ≥20 °C. This temperature dependence again reflects the relative lack of efficiency of tropomyosin in allowing GG-actin repolymerization, and it also implies that tropomyosin-induced repolymerization requires de novo polymer nucleation.

Two of our observations provide further insight into what might be the mechanism by which tropomyosin facilitates the polymerization of GG-actin: the requirement for N-terminal acetylation of tropomyosin and the inverse relationship between the number of tropomyosin internal quasirepeats and the kinetic efficiency with which the tropomyosin affects rescue of polymerization. Rescue clearly depends heavily on the length of the tropomyosin and the ability of neighboring tropomyosins to interact with one another through their ends. The binding of a single tropomyosin molecule to a stable F-actin filament, let alone three or four actin monomers, is very weak, and if the actin filament itself is inherently unstable, this is not likely to occur to any significant extent. We suggest that the addition of salt induces nucleation and subsequent elongation, leading to the generation of unstable GG F-actin oligomers of different lengths. Since stable filaments do not form with GG-actin alone, the distribution of these oligomers will probably be skewed toward those with shorter rather than longer lengths. The extended structure of tropomyosin together with its quasirepeat structure makes it somewhat like a template, suggesting that a stable interaction between the two proteins requires a long enough oligomer to span the length of the tropomyosin. The relatively greater efficiency of rescue exhibited by the shorter tropomyosin could then be attributed to a greater number of oligomers available to interact with the shorter tropomyosin molecules during the polymerization process than the longer ones. Subsequent filament formation could then result from elongation and stabilization by tropomyosin or by annealing of these tropomyosin-stabilized oligomers. This oligomer capture model is supported by other experiments in our laboratory.2 This work shows that exposure of GG-actin to F-buffer, in the absence of a stabilizing factor, induces an F-actin-like ATPase activity that reflects a need for oligomerization despite the inability of the protein to form stable actin filaments.

Actin function in vivo in yeast requires the involvement of individual actin filaments, not bundles like those formed by Sac6p. Our work clearly demonstrates that tropomyosin can allow GG-actin to form individual filaments by significantly affecting the critical concentration of the actin. The question remains as to whether or not tropomyosin is required to function in this manner in GG-actin-expressing cells and to what extent tropomyosin functions this way in general in nonmuscle cells as has so often been hypothesized. We are currently addressing this question in yeasts expressing GG-actin as their sole actin.

REFERENCES

1. Drees, E., Brown, C., Barrell, B. G., and Bretscher, A. (1995) J. Cell Biol. 128, 383–392
2. Huxley, H. E. (1973) Nature 243, 445–449
3. Greenfield, N. J., Stafford, W. F., and Hitchcock-DeGregori, S. E. (1994) Protein Sci. 3, 402–410
4. McLachlan, A. D., and Stewart, M. (1976) J. Mol. Biol. 103, 271–298
5. Butters, C. A., Willadsen, K. A., and Tobaacman, L. S. (1993) J. Biol. Chem. 268, 15565–15570
6. Hill, L. E., Mehegan, J. P., Butters, C. A., and Tobaacman, L. S. (1992) J. Biol. Chem. 267, 16106–16113
7. Matsumura, F., and Yamashiro-Matsumura, S. (1985) J. Biol. Chem. 260, 13851–13859
8. Hitchcock-DeGregori, S. E., and An, Y. (1996) J. Biol. Chem. 271, 3600–3603
9. Landis, C., Back, N., Homsher, E., and Tobaacman, L. S. (1999) J. Biol. Chem. 274, 31279–31285
10. Tobaacman, L. S., and Korn, E. D. (1982) J. Biol. Chem. 257, 4166–4170
11. Lal, A. A., and Korn, E. D. (1986) Biochemistry 25, 1154–1158
12. Broschat, K. O. (1990) J. Biol. Chem. 265, 21323–21329
13. Broschat, K. O., Weber, A., and Burgess, D. R. (1989) Biochemistry 28, 8501–8506
14. Hitchcock-DeGregori, S. E., Sampath, P., and Pollard, T. D. (1988) Biochemistry 27, 9182–9185
15. Liu, H. P., and Bretscher, A. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 90–93
16. Kuang, B., and Rubenstein, P. A. (1997) J. Biol. Chem. 272, 4412–4418
17. Kabesch, W., Mannherz, H. G., Suck, D., Pui, E. F., and Holmes, K. C. (1990) Nature 347, 37–44
18. Druhin, D. G., Jones, H. D., and Wiertman, K. F. (1993) Mol. Biol. Cell 4, 1277–1294
19. Landza, M., Popp, D., and Holmes, K. C. (1993) J. Mol. Biol. 234, 826–836
20. Kuang, B., and Rubenstein, P. A. (1997) J. Biol. Chem. 272, 1237–1247
21. Orlova, A., and Egelman, E. H. (1992) J. Mol. Biol. 227, 1043–1053
22. Privalov, P. L., and Gill, S. J. (1988) Adv. Protein Chem. 39, 191–234
23. Cheng, D., Marner, J., and Rubenstein, P. A. (1999) J. Biol. Chem. 274, 35873–35880
24. Cook, R. K., Blake, W. T., and Rubenstein, P. A. (1992) J. Biol. Chem. 267, 9430–9436
25. Strylecka-Golaszewska, H., Moraczewska, J., Khaitilina, S. Y., and Mossa-kowska, M. (1993) Eur. J. Biochem. 211, 731–742
26. Monteiro, P. B., Latoro, R. C., Ferro, J. A., and Reinach, F. (1994) J. Biol. Chem. 269, 10461–10466
27. Chen, X., Cook, R. K., and Rubenstein, P. A. (1993) J. Cell Biol. 123, 1185–1195

2X. Yao and P. A. Rubenstein, unpublished data.
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