Modulation of Myosin Function by Isoform-specific Properties of Saccharomyces cerevisiae and Muscle Tropomyosins*

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Tropomyosin is an extended coiled-coil protein that influences actin function by binding longitudinally along thin filaments. The present work compares cardiac tropomyosin and the two tropomyosins from Saccharomyces cerevisiae, TPM1 and TPM2, that are much shorter than vertebrate tropomyosins. Unlike cardiac tropomyosin, the phase of the coiled-coil-forming heptad repeat of TPM2 is discontinuous; it is interrupted by a 4-residue deletion. TPM1 has two such deletions, which flank the 38-residue partial gene duplication that causes TPM1 to span five actins instead of the four of TPM2. Each of the three tropomyosin isoforms modulates actin-myosin interactions, with isoform-specific effects on cooperativity and strength of myosin binding. These different properties can be explained by a model that combines opposite effects, steric hindrance between myosin and tropomyosin when the latter is bound to a subset of its sites on actin, and also indirect, favorable interactions between tropomyosin and myosin, mediated by mutually promoted changes in actin. Both of these effects are influenced by which tropomyosin isoform is present. Finally, the tropomyosins have isoform-specific effects on in vitro sliding speed and on the myosin concentration dependence of this movement, suggesting that non-muscle tropomyosin isoforms exist, at least in part, to modulate myosin function.

Tropomyosin is a highly elongated coiled-coil protein that binds to actin filaments in both muscle and non-muscle cells. Tropomyosin, which has many isoforms, stabilizes actin filaments against fragmentation, and by its presence on the thin filament has the potential to influence many aspects of F-actin function. In particular, tropomyosins have complex and incompletely understood effects on actin-myosin interactions. One consistent finding is that vertebrate tropomyosins increase the affinity of myosin subfragment-1 for actin (1–5), despite steric hindrance between the preferred binding sites for tropomyosin and myosin S1 when they bind to actin separately (6–8).

Saccharomyces cerevisiae has two tropomyosin isoforms, TPM1 and TPM2, both of them substantially shorter than vertebrate tropomyosins (9, 10). The 199-residue TPM1 is the predominant isoform by expression level, and it spans five actin monomers. TPM2 is 161 residues and spans four actin monomers, whereas vertebrate tropomyosins span either six or seven actin monomers. Like other tropomyosins, TPM1 and TPM2 have the classical heptad repeat that is responsible for coiled-coil formation, in which hydrophobic residues are found in the first and fourth positions of successive groups of seven amino acids. Below we show that, unlike other tropomyosins, this motif is interrupted once (TPM2) or twice (TPM1) in the amino acid sequence; the phase of the heptad pattern shifts, due to four residue deletions. Both the short length and the interrupted heptad pattern of yeast tropomyosins suggest they could significantly differ from vertebrate tropomyosins functionally. In the present report we describe the solution properties of TPM1 and TPM2, in comparison to each other and to muscle tropomyosin. The results indicate that yeast and vertebrate tropomyosins act in an isoform-specific manner to modulate myosin binding to actin, in vitro motility, cooperative interactions between myosin and tropomyosin, and the distribution of thin filament conformational states.

MATERIALS AND METHODS

Protein Purification—TPM1 and TPM2 were each cloned into the NeoII/BamHI sites of the bacterial expression plasmid pET3d (11). Both plasmids directed high expression levels in DE3 cells, and 20 mg or more were purified from 1 liter of liquid culture. However, actin co-sedimentation experiments (such as described below) showed that neither TPM1 nor TPM2 bound to actin. Similarly, bacterially expressed striated muscle tropomyosin does not polymerize or bind to actin (12), because it lacks N-terminal acetylation and consequently (13) is non-helical at the N terminus. These functional defects can be corrected by encoding an additional Met-Ala-Ser tripeptide at the N terminus of muscle tropomyosin (14). This is then processed to an Ala-Ser dipeptide, which restores polymerization and actin binding of striated muscle tropomyosin and was recently shown to do the same for TPM1 (15). Therefore, the polymerase chain reaction was used to alter the 5′ end of TPM1 and TPM2, adding bases encoding a Met-Ala-Ser tripeptide. These tropomyosins were expressed in DE3 cells and purified to homogeneity by published procedures (14). The resulting proteins bound to F-actin, as shown below. Rabbit skeletal muscle actin (16) and myosin S1 (17) and bovine cardiac tropomyosin (18) were purified as described previously. Rabbit skeletal muscle HMM was prepared by desalting a 5 μM solution of myosin S1 in 1 M guanidine HCl, with the free and bound tropomyosin concentrations determined from the radioactivity of the supernatant and the difference between the unspun and supernatant, respectively (20). The conditions were as follows: 3 μM F-actin, 0 or 3 μM myosin S1, 10 mM Tris–HCl, pH 7.5, 5 mM MgCl2, 1 mM EGTA, 1 mM dithiothreitol, and 100 mM KCl. Data were analyzed using the linear lattice formalism of McGhee and von Hippel (21–23), allowing measurement not only of the overall apparent affinity $K_{app}$.
but also of cooperativity parameter $y$ (note this lowercase y is distinct from Y used below). $K_{mp}$ is increased by is any interactions that occur between adjacent tropomyosins that bind end-to-end, and $y$ equals the fold increase in affinity produced by this effect.

TPM1 has no cysteines for labeling, so its binding to actin was assessed by competition with [3H]TPM2. Increasing concentrations of TPM1 were added to [3H]TPM2-saturated actin filaments in the absence or presence of myosin S1. This resulted in increasing concentrations of displaced, supernatant [3H]TPM2. This pattern depends upon $K_{mp}$ defined as the ratio of the apparent binding constants ($K_{mp}$) of TPM1 and [3H]TPM2 for actin. Since $K_{mp}$ for [3H]TPM2 is determined independently (preceding paragraph), $K_{mp}$ for TPM1 can be calculated from $K_{mp}$. Conditions are as follows: 3 mM F-actin, 0 or 3 mM myosin S1, 1.5--2 mM [3H]TPM2 (constant in any experiment), 10 mM Tris-HCl, pH 7.5, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM dithiothreitol, and 100 mM KCl. Based upon conservation of mass, the different number of TPM1 versus TPM2 molecules that can pack onto the filament (a 4 to 5 ratio), and the assumption that the filament remains saturated, the following quadratic expression was derived (Equation 1),

$$0 = x^2(1 - K_a) + x(5/4 C + H - S) + H K_a + H K_b (S - 5/4 C - H)$$

where $C$ is the concentration of competitor TPM1 (the independent variable); $x$ is the measured free concentration of [3H]TPM2 (the dependent variable); $K_a$ is the unit-less ratio of TPM1 affinity for actin relative to that of [3H]TPM2; $H$ is the total concentration of free plus bound [3H]TPM2, and $S$ is the (saturating) concentration of actin-bound [3H]TPM2 in the absence of competitor. Competitive binding data were fit to this equation by a nonlinear least squares algorithm (Scientist 2.0 by MicroMath).

**Myosin S1-ADP Binding to Thin Filaments**—As described previously (24), actin was labeled with N-(1-pyrenyl)iodoacetamide on Cys$^{774}$, and strong myosin binding to actin was monitored by the decrease in fluorescence intensity as aliquots of myosin S1 were sequentially added (25). The conditions are as follows: 1.0 mM modified actin, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM dithiothreitol, 100 mM KCl, 0.2 mM ADP, 25 units of hexokinase, 10 mM ATP, 1 mM $\gamma$-glucose, 0.2 mg/ml bovine serum albumin, and either 6 mM TPM1, 6 mM TPM2, 3 mM cardiac tropomyosin, or no tropomyosin.

**In Vitro Motility**—In vitro motility was determined by labeling actin filaments with rhodamine phalloidin and monitoring their movement over a rabbit skeletal heavy meromyosin-coated surface using epifluorescence microscopy and quantitative analysis of motion (26). Prior to motility measurements, the motility surface was incubated with concentration of 0.1 mg/ml rhodamine-phalloidin between 50 and 200 mg/ml. These values were converted to the density of bound HMM molecules per $\mu$m, using parameters determined previously (27). Rhodamine-phalloidin-labeled F-actin (designated RPH-F-actin) was prepared as described previously (28) and used within 2 weeks. Nitrocellulose-coated glass coverslips and flow cells were prepared as described previously (28). An HMM solution (50--300 $\mu$g/ml) in assay buffer (25 mM MOPS, 20 mM KCl, 2 mM MgCl$_2$, 2 mM $\alpha$-K$_2$EGTA (pCa 9), 1 mM dithiothreitol, pH 7.4) was injected into the 20-$\mu$l flow cell and replaced 2 min later by 35 $\mu$l of 0.5 mg/ml bovine serum albumin in assay buffer. One minute later 70 $\mu$l of assay buffer was used to rinse the chamber prior to introduction of the RPH-labeled thin filament. For controls, 35 $\mu$l of a 20 mM solution of RPH-labeled F-actin plus 20 mM RPH was introduced into the chamber and incubated for 1 min. For experimental solutions, 35 $\mu$l F-actin was incubated with 2 mM BVC Tn, TPM1, or TPM2 for at least 12 h at 4 °C. These solutions were then 20 mM F-actin with 2 mM BVC Tn, TPM1, or TPM2, and 35 $\mu$l was put into the flow chamber and allowed to incubate and bind to the HMM-coated surface for 2 min. Next 35 $\mu$l of a motility buffer or a buffer containing 2 $\mu$l TPM1, TPM2, or cardiac tropomyosin solution in motility buffer without ATP was perfused through the chamber and allowed to incubate for 2 min. This was replaced by 35 $\mu$l of a motility buffer or one containing 2 $\mu$l TPM1, TPM2, or cardiac tropomyosin solution in motility buffer which contained 2 mM ATP (pH 7.4), 20 mM KCl, 2 mM MgCl$_2$, 2 mM $\alpha$-K$_2$EGTA (pCa 9), 10 mM dithiothreitol, and 1 mM MgATP. All solutions also contained 14 mM glucose, 240 units of glucose oxidase ml$^{-1}$ (Sigma), and 9 $\times$ 10$^5$ units of catalase ml$^{-1}$ (Sigma) to slow photo-bleaching (19). The flow cell was then placed on a temperature-controlled (25 °C) stage in an epifluorescence microscope, and movement of the labeled thin filaments was recorded, and sliding speed of the thin filaments was analyzed for thin filament sliding speed as described previously (28).

**RESULTS**

The Coiled Coils of S. cerevisiae Tropomyosins Are Not Continuous—Coiled-coil homodimers such as tropomyosins result from a characteristic, repeating heptad motif, in which the first and fourth amino acids are apolar within each successive group of seven residues. This pattern is seen in TPM1 and TPM2, but heptad alignment (Fig. 1) shows the motif is not continuous within either isoform. In each case the first 10 heptads are out of phase with the final 12 heptads. For example, the N-terminal and C-terminal sections of TPM2 can both adopt the expected coiled-coil structure only if the phase of the heptad repeat shifts ahead by four residues somewhere between these two regions. This kind of transition is termed a “stammer” and has been predicted to cause a locally over-wound but not necessarily interrupted coiled-coil (29). Stammers have not been described for other tropomyosins, so this is an unexpected feature of the yeast isoform, suggesting a site of increased flexibility in its structure (29). TPM1 and TPM2 share high homology (64%). They differ primarily by a partial duplication in TPM1; 38 additional residues are inserted in TPM1 following amino acid 69, with this insertion 86% identical to residues 32--69 (9). This repeated segment is approximately the length calculated to span one additional actin monomer on the filament, 39 1/3 residues (30). Notably, TPM1 contains four residue gaps in the heptad repeat at two sites in the sequence, producing two stammers in the coiled-coil. Interestingly, these stammers flank or nearly flank the 38-residue insertion that distinguishes TPM1 from TPM2, i.e. after amino acid 69 in both isoforms, and also after residue 107 in TPM1.

**Fig. 1.** Breaks in the coiled-coil heptad repeats of S. cerevisiae tropomyosins. The figure shows alignments of the TPM1 and TPM2 amino acid sequences with the heptad motif that is characteristic of coiled coils: $a$--$g$, with apolar residues at positions $a$- and $d$- successful alignments require four residue deletions, in which the phase shifts ahead by four residues, but the precise locations of these deletions are uncertain. Four residue deletions are termed stammers (29) and are proposed to be sites of increased flexibility. A coiled-coil prediction algorithm (50) suggests the gaps occur approximately after residues 69 and 111 in TPM1 and after residue 72 in TPM2. The figure illustrates a similar proposal that they occur at the sites of the 38-amino acid insertion that distinguishes TPM1 from TPM2, i.e. after amino acid 69 in both isoforms, and also after residue 107 in TPM1.
length plus coiled-coil stammers may substitute for alanine-induced bends, combining to provide the flexibility in the tropomyosin strand that is required for wrapping around the actin filament and moving on its surface.

Cooperative Binding of [3H]TPM2 to Actin—Drees et al. (9) reported that TPM2 binds to actin cooperatively. Presumably, end-to-end interactions between adjacent, actin-bound tropomyosins are primarily responsible for this pattern. However, the end-to-end overlap of adjacent TPM2 molecules is much shorter than the overlap for muscle tropomyosin, only four amino acids instead of nine. This difference arises because muscle tropomyosin is 123 amino acids longer than TPM2, several residues more than the distance needed to span three additional actins. Thus, TPM2 could be hypothesized to have weak end-to-end interactions.

To analyze end-to-end interactions quantitatively, the effect of this process on tropomyosin binding to actin was determined. If tropomyosin-actin binding were, hypothetically, non-cooperative, then this would result in binding data that appeared negatively cooperative (21). This is because of the statistical impediment to parking each long tropomyosin molecule along the actin filament without gaps. Instead, binding appears positively cooperative, as shown by the S-shaped curve in Fig. 2A. Qualitatively, the stronger the end-to-end interactions, the more S-shaped the binding isotherm. Quantitatively, the strength of the interactions must be dissected out from the opposing effect of the parking problem, which in turn varies directly with the number of actins spanned by the tropomyosin. Curve-fitting analysis of Fig. 2A indicates that each end-to-end interaction strengthens TPM2 binding to actin 50-fold (i.e. \( y = 49 \pm 9 \)). Equivalently, it is 50-fold preferred for TPM2 to bind adjacent to another bound molecule, rather than to an isolated site on the filament. These results are similar to those found for striated muscle \( \alpha \)-tropomyosin (\( y = 42 \)) using similar methods (20, 31). Despite very different lengths for the end-to-end overlap, tropomyosin-tropomyosin interactions are the same strength for TPM2 and for muscle tropomyosin. The most likely explanation is that few tropomyosin-tropomyosin contacts are needed to produce the small free energy required (\( \Delta G = -RT \ln 49 = -2.3 \) kcal/mol). Alternatively, the cooperativity may involve tropomyosin-induced changes in actin (32).

Effect of Myosin S1 on TPM2 Binding to Actin—Muscle tropomyosin binds much more tightly to myosin-S1-decorated actin than to actin alone (33–36). Under conditions where muscle tropomyosin binds only weakly to bare actin, tropomyosin binding to actin-S1 is, in contrast, too tight to measure by co-sedimentation assay. The effect of myosin S1 is at least 100-fold from these earlier data and is likely to be 4 orders of magnitude based upon the equilibrium linkage implications that follow from the effect of tropomyosin on actin-myosin affinity (5). In contrast, myosin S1 barely alters [3H]TPM2 affinity for actin, increasing it from 3.45 \( \pm \) 0.08 to 5.08 \( \pm \) 0.44 \( \times \) 10^6 M\(^{-1}\) (Fig. 2, A versus B). This absence of a major effect of myosin S1 was unexpected, because it qualitatively differs from the findings with muscle tropomyosin.

Comparison of TPM1 and TPM2 Binding to Actin—TPM1 and TPM2 cooperatively interact to promote each other’s binding to actin, indicating that they are able to co-polymerize along the thin filament (9). We have shown that tropomyosin binding to the thin filament can be assessed by competition, in which an unlabeled tropomyosin competes with a labeled reference tropomyosin with known affinity for actin (36). By using this method, increasing concentrations of TPM1 progressively displaced [3H]TPM2 from actin, as shown by the representative experiment in Fig. 3A. Pooled results from this and replicate experiments (not shown) indicate that the affinity of TPM1 for actin was 1.42 \( \pm \) 0.26 \( \times \) 10^6 M\(^{-1}\) (similar to \( K_{app} = 1.6 \times 10^6 \) M\(^{-1}\) for \( \alpha \)-striated tropomyosin (31)), which is 41% the affinity seen for [3H]TPM2 in the absence of myosin (Fig. 2A). However, control competition experiments using unlabeled TPM2 (Fig. 3B) showed that it also bound more weakly than the labeled form, with affinity 1.04 \( \pm \) 0.18 \( \times \) 10^6 M\(^{-1}\). (This effect of TPM2 labeling has some precedent, since it alters the surface charge of tropomyosin and comparable changes on the actin surface can have similar effects (37, 38).) Therefore, unlabeled TPM1 and TPM2 have nearly the same affinity for actin, the same within experimental error. This contrasts with the findings of Drees et al. (9) who reported that TPM2 binds more tightly to
actin than does TPM1. However, the difference had not been assessed quantitatively, and the previous ionic conditions differ from the present work. Finally, the effect of myosin S1 on TPM1 binding to actin was evaluated (Fig. 3C) and found to increase the binding constant 1.4-fold, to $2.0 \pm 0.5 \times 10^6$ M$^{-1}$.

Myosin S1 has similar small effects on the actin binding of both tropomyosin isoforms from *S. cerevisiae*.

**Effects of Tropomyosin Isoforms on in Vitro Motility**—Thin filament sliding over a HMM-covered surface represents unloaded movement, and sliding speed in many respects parallels the unloaded shortening of muscle fibers (26). The effects of the tropomyosin isoforms on this process are shown in Table I and in Fig. 4 as a function of the concentration of HMM that was attached to the sliding surface. To ensure saturation of the actin filaments and to obtain consistent results, 2 μM tropomyosin was maintained in the motility chamber. Bare actin filaments had nearly maximal sliding speed at the lowest concentration of HMM applied to the motility surface; speed at 50 μg/ml HMM was fully 89% of the speed in the presence of 300 μg/ml HMM (3.79 versus 4.25 μm/s). This was not true for actin-tropomyosin filaments. Thin filaments containing cardiac tropomyosin, TPM1, or TPM2, when observed after 50 μg/ml HMM was applied to the motility surface, had only 60, 49, and 72%, respectively, of their maximum observed speeds. Also, these maximum speeds were altered by the presence of tropomyosin. Compared with actin-only filaments, both of the yeast isoforms increased the speed determined with 300 μg/ml HMM, a 18% increase for TPM1 filaments and a 30% increase for TPM2 filaments. In contrast, cardiac tropomyosin decreased the speed by 4%. The difference between TPM1-containing and TPM2-containing filaments was particularly large at 50 μg/ml HMM; filaments with TPM2 moved 62% faster than those with TPM1 (4.00 versus 2.47 μm/s). These several comparisons among mean speeds are statistically significant, since each speed is based upon more than 100 filaments, and the standard errors are 1.5% or less (standard deviations shown in Table I). The motility results indicate that tropomyosin isoforms differentially affect the speed of myosin-propelled movement.

**Fig. 3. TPM1 binding to actin.** Representative experiments show the association of TPM1 with actin (A) and with actin-myosin S1 (C), as measured by competitive displacement of saturating amounts of [3H]TPM2. B shows similar competition between unlabeled TPM2 and [3H]TPM2. The thin filament affinity of the unlabeled competing tropomyosin of interest, relative to that of [3H]TPM2, was 0.33 (A), 0.30 (B), and 0.44 (C).
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The sliding speeds of thin filaments containing different tropomyosins (Tm) were measured as a function of the concentration of heavy meromyosin applied to the motility surface. Mean speeds are also shown in Fig. 4. Conditions are as stated under “Materials and Methods.” The term filaments sliding refers to thin filaments that were moving continuously, and the listed speeds are for these filaments.

| Filament type     | HMM (μg/ml) | Speed (μm/s ± S.D.) | No. of filaments | No. of filaments sliding | % filaments sliding |
|-------------------|-------------|---------------------|------------------|--------------------------|---------------------|
| Actin             | 300         | 4.25 ± 0.57         | 493              | 407                      | 82.5                |
| Actin             | 150         | 3.97 ± 0.61         | 286              | 214                      | 74.7                |
| Actin             | 50          | 3.79 ± 0.58         | 450              | 214                      | 47.5                |
| Actin-cardiac Tm  | 300         | 4.08 ± 0.76         | 470              | 392                      | 83.4                |
| Actin-cardiac Tm  | 150         | 3.34 ± 0.67         | 253              | 178                      | 70.4                |
| Actin-cardiac Tm  | 50          | 2.47 ± 0.58         | 537              | 210                      | 39.1                |
| Actin-TPM1        | 300         | 5.00 ± 0.63         | 514              | 354                      | 68.9                |
| Actin-TPM1        | 150         | 4.35 ± 0.67         | 269              | 136                      | 50.6                |
| Actin-TPM1        | 50          | 2.47 ± 0.58         | 600              | 219                      | 36.5                |
| Actin-TPM2        | 300         | 5.51 ± 0.62         | 572              | 461                      | 80.6                |
| Actin-TPM2        | 150         | 4.37 ± 0.62         | 279              | 214                      | 76.7                |
| Actin-TPM2        | 50          | 4.00 ± 0.68         | 552              | 223                      | 40.4                |

FIG. 4. Effects of tropomyosin isoforms on in vitro motility. The speed of thin filament sliding was examined for sliding surfaces that had been exposed to three different concentration of HMM. Each data point shows the average speed of 136–461 continuously moving filaments, with details in Table I. The solid line connecting the filled circles is a hyperbolic fit to the data for naked actin filaments (maximum speed = 4.52 ± 0.09 μm/s, an apparent $K_m$ of 178 ± 30 molecules/μm$^2$, $r^2 = 0.999$); the solid line connecting the open circles is a hyperbolic fit to the data for bovine cardiac tropomyosin containing filaments (maximum speed = 6.34 ± 0.29 μm/s, an apparent $K_m$ of 1344 ± 138 molecules/μm$^2$, $r^2 = 0.999$); the dashed line connecting the open triangles is a hyperbolic fit to the data for TPM1-containing actin filaments (maximum speed = 5.66 ± 3.489 μm/s, an apparent $K_m$ of 2105 ± 1392 molecules/μm$^2$, $r^2 = 0.975$); the dotted line connecting the open squares is a hyperbolic fit to the data for TPM2 containing actin filaments (maximum speed = 6.95 ± 0.75 μm/s, an apparent $K_m$ of 714 ± 240 molecules/μm$^2$, $r^2 = 0.993$). The HMM density on the surface was computed from the measured relationship density (molecules/μm$^2$) = 3850 × c/ (c + 168 μg/ml), where c is the HMM concentration applied to the motility surface (25). The tropomyosins had isoform-specific effects on sliding speed, either increasing or decreasing speed compared with bare actin. Also, bare actin filaments approached their maximal speed at lower HMM concentrations than did any of the actin-tropomyosin filaments, as indicated by the much lower $K_m$.

Isomform-specific Effects of Tropomyosin on Cooperative Binding of Myosin S1-ADP to Thin Filaments—Vertebrate tropomyosins, including those from striated muscle, smooth muscle, and fibroblast, increase the affinity of myosin S1 for actin, an effect that is between 3- and 7-fold in magnitude (1, 2, 5, 39). Data presented in Fig. 5 confirmed that muscle tropomyosin strengthens S1 binding substantially, in this case a 6-fold effect from 8.0 × 10$^{-6}$ to 4.7 × 10$^{-6}$ M$^{-1}$. (This comparison is upon the simple hyperbolic portions of the two dashed binding curves after the initial cooperative portions and are model-independent.) Interestingly, the effects of the yeast tropomyosins on the upper portions of the curves were much less than the effect of muscle tropomyosin, indicating less “potentiation” of binding. The affinity of myosin S1 for actin was increased only 1.6-fold by yeast tropomyosins, indicating less cooperativity in the absence of calcium (40, 41), thereby preventing contraction (reviewed in Refs. 42 and 43). Fig. 5
shows that this cooperativity also occurs with yeast tropomyosins and is isoform-specific; the results are qualitatively more sigmoidal in the presence of TPM1 (filled circles) than the C-state or if they oscillate between these two states in the absence of myosin.

The diverse results for the different tropomyosins (Fig. 5) are consistent with this model and suggest several functional distinctions among the three isoforms. The three actin-tropomyosin curves in Fig. 5 were fitted to Equation 3 from Ref. 5, and the actin-only data were fitted to a non-cooperative isotherm with \( K_{app} = K_s^0 K_{S1}^0 (1 + K_s^0) \). (In Ref. 5 an erroneous expression for this \( K_{app}^0 \) was used, resulting in a miscalculation of \( K_s^0 \) as 0.15 instead of 0.17. \( K_s^0 = 0.17 \) was used in the present study.) The greatest curve-fitting success was for the TPM1 data, from which both \( L \) and the product \( K_s^0 L \) could be determined, as well as cooperativity parameter \( Y \) (Table II). In the model (and as implied by experimental data (8)), tropomyosin oscillation between positions on actin characteristically occurs over single long regions of the tropomyosin strand, in preference to an equivalent shift involving several shorter regions. \( Y \) is defined as the statistical preference for one back and forth shift, rather than two shifts. The only isoform for which \( Y \) could be determined from Fig. 5 was TPM1. \( Y = 19 \pm 6 \) for TPM1, which for comparison, is less than the result for muscle tropomyosin in the presence of troponin-\( \text{Ca}^{2+} \); \( Y = 97 \pm 20 \) (5). It is unclear whether this greater flexibility for TPM1 position is due to the absence of troponin or is attributable to structural features of TPM1 (Fig. 1). Curve fitting of the Fig. 5 data for cardiac tropomyosin without troponin gave large errors for \( Y \), precluding resolution of these two possibilities.

The curve fitting also offers an explanation for the cooperativity in myosin S1 binding to filaments containing TPM1, contrasting with non-cooperative binding to those with TPM2. The equilibrium away from the actin inner domain, \( L \), was found to be \( 2.5 \pm 0.2 \) for TPM1 and \( 1.1 \pm 0.2 \) for TPM2. Tropomyosin must shift in the other direction, away from the C-state, for myosin to bind to actin, and in the absence of myosin S1 more of the actins are in the C-state for TPM1 filaments than for TPM2 filaments. We suggest that the sigmoidal shape of the curve can be explained by cooperative shifting of tropomyosin away from the C-state as myosin binds, and more of this shift occurs (primarily because \( L \) is greater) for thin filaments with TPM1 than for those with TPM2. Inserting the Table II values into Equation 6 from Ref. 5, TPM1-containing filaments are 84% in the C-state in the absence of myosin, so most of the tropomyosin strand must shift out of this state for the actins to become saturated with myosin. In contrast, TPM2-containing filaments are only 30% in the C-state in the absence of myosin.

**DISCUSSION**

Genetic experiments in *S. cerevisiae* imply different functions for TPM1 and TPM2. Deletion of the predominant isoform, TPM1, impairs growth rate and vesicular traffic to the cell surface, and these defects are not corrected by overexpression of TPM2. Also, overexpression of TPM2 but not TPM1 changes budding morphology (9). We now report biochemical observations that potentially explain these in vivo observations, i.e., isoform-specific effects of yeast tropomyosin on binding of myosin to actin. Under cellular conditions where myosin number is limiting and few cross-bridges are bound, Figs. 4 and 5 suggest that TPM1-containing filaments would be more resistant to movement and to cross-bridge attachment than TPM2-containing filaments. More generally, both experiments show that myosin-thin filament interactions depend quantita-
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Table II

| L | Equilibrium toward M-state actin | Y | S1K_{app} \times 10^6 |
|---|----------------------------------|---|---------------------|
| Actin | 0.17 ± 0.02 (K_T \times L)^a | 0.005 ± 0.001 | |
| Actin-TPM1 | 2.5 ± 0.2 | 19 ± 6 | 1.84 ± 0.03 |
| Actin-TPM2 | 1.1 ± 0.2 | ND | 1.32 ± 0.03 |
| Actin-cardiac Tm | >1 | ND | 4.7 ± 0.2 |
| Actin-cardiac Tm-Tn-Ca^{2+} | 3.4 ± 0.5 | 2.5 ± 0.6 (K_T \times L)^a | 97 ± 20 | (1.0)(\mu = 0.32) |

| a | See Ref. 5. | b | K_T for actin alone. K_T \times L, when tropomyosin present. See Fig. 6. |

Equilibrium constants summarized in Table II make it possible to comment on how myosin binding to the thin filament is related to the interaction of yeast tropomyosins with the specific difference between TPM1 and TPM2. First, the extra stammer in TPM1 is expected to produce more flexibility than for TPM2, and this in principle might have decreased the cooperativity of myosin-thin filament binding. Instead, myosin binding was more cooperative to filaments containing TPM1 than to those with TPM2. Furthermore, back and forth shifts in the actin position of the TPM1 strand were unfavorable (Y > 1), despite the two stammers in each TPM1. These results suggest that the intrinsic stiffness of tropomyosin is not the only property governing the cooperativity of the shifts in its position on the actin surface. As an additional mechanism, we suggest that cooperativity of tropomyosin binding (and therefore cooperativity of myosin-thin filament binding) is due to localized energy minima for tropomyosin position on actin (the M-, C-, and B-state positions) (6, 8), with an energetic penalty (equaling RT lnY 1/2) for each site along the actin filament where the tropomyosin strand crosses from one minimum to another.

The 100- to 10,000-fold effect of myosin on vertebrate tropomyosin-actin binding implies either direct contacts between the two proteins or else an indirect interaction mediated by a mutated or uninserted protein. Experiments using tropomyosin mutants in which this process is greatly suppressed suggest (5) that the interaction is in fact indirect, because the mutations did not simply weaken myosin-thin filament binding as would have been expected if direct interactions were eliminated. Instead, myosin-thin filament binding exhibited exaggerated cooperativity, as is characteristic of a switch in quaternary structure. Therefore, to explain how tropomyosin and myosin promote each other’s binding to actin, it was proposed that the changes in actin that accompany myosin binding (47–49) result in a strengthening of the association of tropomyosin with the actin inner domain (5). Correspondingly, when tropomyosin is bound to the actin inner domain, it was proposed to promote some of the same changes in actin, thereby increasing the fraction of actin monomers in the M-state (schematically indicated by stripes in Fig. 6).

The current results suggest that a major difference between muscle tropomyosin and the yeast isoforms is in the equilibrium constant for actin to convert to the M-state once the tropomyosin is located on the actin inner domain. Schematically, this is the process in Fig. 6 that has an equilibrium value indicated by the product K_T \times L. (See also Table II, column Equilibrium toward M-state actin.) The equilibrium for conversion to the M-state is biased toward formation for muscle tropomyosin, because K_T \times L = 6 ± 3, i.e. > 1, but is less than 1 and biased against M-state formation for TPM1 and TPM2 (K_T \times L = 0.63 ± 0.06 and 0.32 ± 0.15, respectively), and even further against M-state formation for actin alone: K_T \times L = 0.17 (from Ref. 5). Describing these measurements another way, myosin alters actin when it binds, and vertebrate tropomyosins interact with the actin inner domain so as to assist this process. Yeast tropomyosins interact more weakly with the actin inner domain and so provide less assistance to myosin binding.

Since the N- and C-terminal regions of TPM1 are homologous to those of TPM2, the functional differences between the two proteins are most likely due to the extra duplicated region present within TPM1. From Fig. 5 and Table II, inclusion of this region significantly alters the equilibria among the various thin filament states. The effects of the duplicated region on L (increased), K_T (unchanged), and K_T \times L (increased) can be explained if the extra region either selectively destabilizes tropomyosin binding to the inner domain of non-M-state actin (i.e. destabilizes tropomyosin binding to the intermediate state in Fig. 6) or else selectively strengthens formation of both the C-state and the M-state. The former explanation is more likely, because it is simpler and also because the latter explanation conflicts with the absence of an effect of myosin S1 on the TPM1 versus TPM2 competition data in Fig. 2. In either case, the result would be a tendency for TPM1 to move away from the inner domain position, producing cooperative myosin binding to the thin filament.

Further experiments will be required to determine whether the effects of tropomyosin depend not only on tropomyosin isoform but also on actin and/or myosin isoform. However, the strong myosin-tropomyosin interaction observed with muscle tropomyosin does not seem to depend upon the use of muscle actin, since myosin-decorated yeast actin binds to muscle tropomyosin with very high affinity (37, 38). Also preliminary experiments (not shown) suggest little difference in TPM2 binding to muscle versus yeast actin. Finally, the myosin isoform may not make much difference either, since even weak myosin binding to actin strengthens tropomyosin association to the actin inner domain (25).

The Fig. 6 model characterizes the effects of tropomyosins on thin filament sliding. Nevertheless, the model
is qualitatively consistent with the in vitro motility data. The myosin concentration requirement for filament sliding was increased by TPM1 than by TPM2, and was particularly increased by cardiac tropomyosin. Each of these observations is consistent with the steric blocking effect that is part of the model, an effect that may be greater for cardiac tropomyosin because it is located on the actin outer domain (the B-state (6)). Steric blocking is greater for TPM1 than for TPM2 for a different reason, because its movement to the M-state position is more unfavorable (L is greater). On the other hand, it is difficult to relate the model to the effects of tropomyosin on maximal sliding speed, in part because determining the maximum speeds would require higher myosin concentrations than were tested. More significantly, conclusions are difficult because even at maximal speed the densities of myosin attachment are unknown, as are the myosin concentration requirement for filament sliding was increased by TPM1 than by TPM2, and was particularly increased by cardiac tropomyosin. Each of these observations is consistent with the steric blocking effect that is part of the model, an effect that may be greater for cardiac tropomyosin because it is located on the actin outer domain (the B-state (6)). Steric blocking is greater for TPM1 than for TPM2 for a different reason, because its movement to the M-state position is more unfavorable (L is greater). On the other hand, it is difficult to relate the model to the effects of tropomyosin on maximal sliding speed, in part because determining the maximum speeds would require higher myosin concentrations than were tested. More significantly, conclusions are difficult because even at maximal speed the densities of myosin attachment are unknown, as are the densities of myosin attachment are unknown, as are the steric blocking effect that is part of the model, an effect that may be greater for cardiac tropomyosin because it is located on the actin outer domain (the B-state (6)). Steric blocking is greater for TPM1 than for TPM2 for a different reason, because its movement to the M-state position is more unfavorable (L is greater). On the other hand, it is difficult to relate the model to the effects of tropomyosin on maximal sliding speed, in part because determining the maximum speeds would require higher myosin concentrations than were tested. More significantly, conclusions are difficult because even at maximal speed the densities of myosin attachment are unknown, as are the densities of myosin attachment are unknown, as are the densities of myosin attachment are unknown, as are the densities of myosin attachment are unknown, as are the...
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