The Ends of Tropomyosin Are Major Determinants of Actin Affinity and Myosin Subfragment 1-Induced Binding to F-Actin in the Open State†

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ABSTRACT: Tropomyosin (TM) is thought to exist in equilibrium between two states on F-actin, closed and open [Geeves, M. A., and Lehrer, S. S. (1994) *Biophys. J. 67,* 273−282]. Myosin shifts the equilibrium to the open state in which myosin binds strongly and develops force. Tropomyosin isoforms, that primarily differ in their N- and C-terminal sequences, have different equilibria between the closed and open states. The aim of the research is to understand how the alternate ends of TM affect cooperative actin binding and the relationship between actin affinity and the cooperativity with which myosin S1 promotes binding of TM to actin in the open state. A series of rat α-tropomyosin variants was expressed in *Escherichia coli* that are identical except for the ends, which are encoded by exons 1a or 1b and exons 9a, 9c or 9d. Both the N- and C-terminal sequences, and the particular combination within a TM molecule, determine actin affinity. Compared to tropomyosins with an exon 1a-encoded N-terminus, found in long isoforms, the exon 1b-encoded sequence, expressed in 247-residue nonmuscle tropomyosins, increases actin affinity in tropomyosins expressing 9a or 9d but has little effect with 9c, a brain-specific exon. The relative actin affinities, in decreasing order, are 1b9d > 1b9a > acetylated 1a9a > 1a9d ≫ 1a9a ≥ 1a9c ≅ 1b9c. Myosin S1 greatly increases the affinity of all tropomyosin variants for actin. In this, the actin affinity is the primary factor in the cooperativity with which myosin S1 induces TM binding to actin in the open state; generally, the higher the actin affinity, the lower the occupancy by myosin required to saturate the actin with tropomyosin: 1b9d >1a9d> 1b9a ≥ acetylated 1a9a > 1a9a > 1a9c ≅ 1b9c.

The interaction of myosin with the actin thin filament in muscle and nonmuscle cells has long been recognized to be highly cooperative and allosteric (e.g., refs *1*−*5*; reviewed in ref *6*). The striated muscle thin filament containing actin, tropomyosin (TM),[1] and troponin (Tn) is cooperatively activated by two different ligands: Ca^{2+}, which binds to troponin, and myosin, which binds to and whose ATPase is activated by actin. The cooperativity depends on TM, the two-chained coiled coil that binds along the length of the actin filament (*1*−*9*). The molecular basis for the cooperativity is not known, though it has been generally assumed that end-to-end interactions between neighboring TMs along the thin filament should be responsible (*10*−*12*).

Tropomyosin alone is thought to be in equilibrium between two states on the actin filament, referred to as closed or off and open or on in terms of the McKillop and Geeves three-state model (*6, 13*−*15*). Troponin, in the absence of Ca^{2+}, shifts the equilibrium to a third state, blocked, and myosin binding and Tn ($+Ca^{2+}$) favor the open state in which myosin binds strongly and develops force. The three states in the model reflect different states of actin−myosin interaction and have been proposed to correspond to different positions of TM on the actin filament (*16*−*19*). Tropomyosin isoforms (in the absence of Tn) have different equilibria between the closed and open states, evaluated by myosin binding or inhibition and activation of the actomyosin ATPase (e.g., refs *20*−*24*), or by a fluorescence change of pyrene-labeled TM (*12, 25, 26*).

Tropomyosins are expressed in virtually all eucaryotic cells (reviewed in ref *27*). The diversity among TM isoforms is a consequence of different genes as well as alternative promoter selection and alternative splicing of the RNA transcripts (*28*). The alternatively expressed exons encode the N- and C-terminal regions (exons 1 and 9) and two internal regions of TM (exons 2 and 6). It is well-established through the study of many different TM isoforms, both recombinant and naturally occurring, that the sequences of the N- and C-termini of TM, and modifications to these regions, affect actin affinity and myosin-dependent thin filament cooperativity (e.g., refs *12* and *29*−*44*).

To understand how the sequences of the ends of TM define cooperative TM function, we have constructed and expressed in *Escherichia coli* a series of α-TM variants that are identical except for the ends, which are encoded by exons 1a or 1b and exons 9a, 9c, or 9d (Figure 1). Exon 1a is

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[1] Abbreviations: CD, circular dichroism; myosin S1, myosin subfragment 1; TM, tropomyosin; Tn, troponin.

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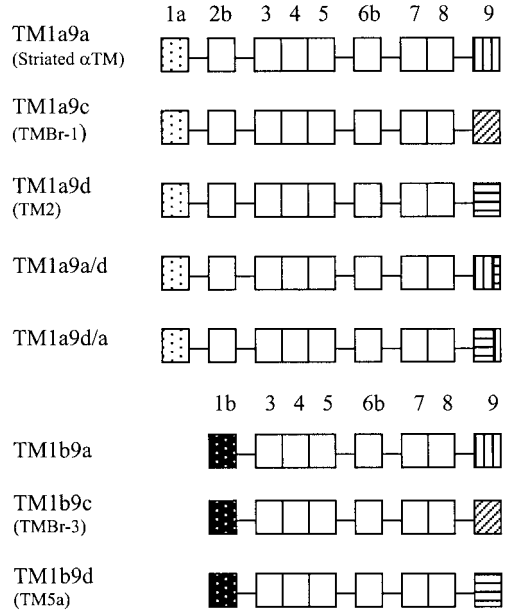

FIGURE 1: Schematic representation of recombinant rat α-tropomyosins used in this study. The sequences of the N-termini were encoded either by exon 1a (white boxes filled with black dots) or by exon 1b (black boxes filled with white dots). C-Terminal sequences were encoded by exon 9a (vertical lines), 9c (diagonal lines), or 9d (horizontal lines). The exon numbering is based on that of Pittenger et al. (*28*).

expressed in 284-residue TMs found in muscle and nonmuscle cells. Exon 1b replaces exons 1a and 2, resulting in TMs 247 amino acids long, found in nonmuscle cells. The C-terminal exon 9a is expressed in striated muscles, and exon 9c in the brain, while exon 9d is expressed in many different nonmuscle cell types and tissues, as well as in smooth muscle (*28*).

The aim of the research is to understand how the alternate ends of TM affect cooperative actin binding and the relationship between actin affinity and the facility with which myosin S1 promotes binding of TM to actin in the open, force-developing state. Our results show that both the N- and C-terminal sequences, and their particular combination within a TM molecule, determine actin affinity. While myosin S1 induces binding of all TM variants to actin, actin affinity is the primary factor in the cooperativity of myosin S1 in inducing TM binding to the actin filament in the open state; the higher the actin affinity, the fewer myosin molecules per TM are required to activate the filament. Portions of this research have been previously reported (*45, 46*). Part of this work was carried out in partial fulfillment for the requirements of the Ph.D. for K. Nicholson-Flynn at Rutgers University and University of Medicine and Dentistry of New Jersey (UMDNJ) (*47*).

MATERIALS AND METHODS

DNA Constructions, Protein Expression, and Purification. Rat striated and rat smooth muscle α-TM cDNA clones were the gift of Dr. B. Nadal-Ginard (*48*). cDNA clones corresponding to TMBr1 (pOK4), TMBr2 (pOK15), and a partial cDNA clone of TMBr3 (pOk10) were gift of Dr. D. M. Helfman (*49*). Rat striated α-TM (TM1a9a) and TM2 (TM1a9d), and the exon 9 chimera cDNAs were previously constructed and cloned into pET11d (*38, 50*).

TMBr1 (TM1a9c) and TMBr2 (TM1b9b) cDNAs were subcloned from pOK4 and pOK15, respectively, into pET11d at the *Nco*I and *Bam*HI restriction sites (*51*). TM1b9d (TM5a) cDNA was constructed by excising a *Sma*I/*Bam*HI fragment containing exon 9b from TMBr2 and replacing it with the *Sma*I/*Bam*HI fragment containing exon 9d from TM2. Likewise, the chimera TM1b9a was constructed by replacing the same region with the *Sma*I/*Bam*HI fragment containing exon 9a from TM1a9a/pET11d. Each of these constructs was confirmed by extensive restriction digestion analysis to ensure that the appropriate sequences were contained in each subclone. General recombinant DNA methods were as described by Sambrook et al. (*52*) or as recommended by the supplier. The sequences of the inserts were determined by the DNA Synthesis and Sequencing Facility (UMDNJ−Robert Wood Johnson Medical School, Piscataway, NJ).

Recombinant TMs were expressed in *E. coli* BL21(DE3)-pLysS or *E. coli* BL21(DE3) cells (*51*) and purified as described previously (*33, 50*) except the $(NH_4)_2SO_4$ fractionation was 35−70% saturation instead of 35−60%. All TM isoforms expressed at high levels except for TM1b9b (TMBr2), which was expressed at levels too low for purification and analysis.

Actin was isolated from White Leghorn chicken pectoral muscle as previously described (*53*), except that actin was polymerized by addition of KCl and $MgCl_2$ to 20 mM and 0.7 mM, respectively, and incubated at 37 °C for 10 min before polymerization at room temperature. Myosin S1 was prepared by papain digestion of chicken myosin (*54*). Troponin was purified from chicken pectoral muscle (tissue was the gift of Dr. J. Fagan, Rutgers University) according to the method of Potter (*55*), except that alternate Triton X-100 extractions included the following protease inhibitors: 2 μM E64, 1 μg/mL leupeptin, 2 μM amidinophenylmethanesulfonyl fluoride, 0.5 mM benzamidine. The $(NH_4)_2SO_4$ crude Tn pellet was dialyzed against 20 mM Tris-HCl, pH 7.5, and 0.5 mM DTT. This was purified by DE52 ion-exchange chromatography with a gradient of 0−0.6 M NaCl in buffer containing 0.1 mM $CaCl_2$.

The concentrations of actin, myosin S1, and Tn were spectrophotometrically determined by using the extinction coefficients at 280 nm (0.1%) of 1.1, 0.83, and 0.45, respectively. Concentrations of recombinant TM were determined by differential absorption spectra of tyrosine as previously described (*50, 56, 57*).

Molecular Weight Determination. The mass and apparent T_M were determined for TMs we have not previously studied: the TM1b- and TM9c-expressing forms.

The molecular weights were determined by electrospray mass spectrometry at the W. M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT). Most corresponded to the predicted mass: TM1a9c, calculated 32 509 Da, measured 32 499 ± 2 Da; TM1b9a, calculated 28 412 Da, measured 28 412 ± 8 Da; TM1b9d, calculated 28 426 Da, measured 28 430 ± 4 Da. The average mass of TM1b9c was 29 064 ± 6 Da, 149 Da lower than predicted (28 213). The difference does not correspond to the loss of one amino acid from the N- or C-terminus and may result from deamidation. The sample showed some heterogeneity but one major peak had the predicted mass (28 216 Da). The protein appeared to be homogeneous on SDS−PAGE.

Circular Dichroism Analysis. The thermal stability measurements were made by following the ellipticity as a function of temperature: 1.5 μM TM (0.1 mg/mL) in 500 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA, and 1 mM DTT (*26*, *58*). Data were collected on an Aviv Model 62D spectropolarimeter at 222 nm and analyzed by Dr. Norma J. Greenfield (UMDNJ Circular Dichroism Facility, Robert Wood Johnson Medical School, Piscataway, NJ) as previously described (*59*). The apparent T_M is defined here as the temperature at which the ellipticity at 222 nm is at the midpoint between the value found at 0 °C, where all of the TMs studied in this work are fully folded, and 60 °C, where they are fully unfolded. All isoforms were ~100% α-helical at 0 °C on the basis of the mean residue ellipticity.

Actin Binding Assays. TM binding to F-actin was measured by a cosedimentation assay as described previously (*33*) with modifications (*43*). The amounts of bound and free TM in the pellets and supernatants, respectively, were quantified by densitometry of SDS−polyacrylamide gels on a Molecular Dynamics model 300A computing densitometer. The apparent K_a of TM for F-actin and Hill coefficient (α^H) were determined by fitting the experimental data to

$$\upsilon = n[\mathrm{TM}]^{\alpha^H} K_{app}{}^{\alpha^H}/1 + [\mathrm{TM}]^{\alpha^H} K_{app}{}^{\alpha^H} \qquad (1)$$

where υ = fraction maximal TM binding to actin, n = maximal TM bound, and [TM] = $[\mathrm{TM}]_{free}$. The TM:actin ratio was normalized to 1.0 by dividing the TM:actin ratio obtained from densitometry by the TM:actin maximal ratio (n) from each experiment calculated by using eq 1. The TM:actin density ratio at saturation was the same for all TM isoforms, indicating that the same mass of TM binds to actin independent of the length of the TM. We have previously shown that the observed density ratio at saturation reflects stoichiometric binding of TM to actin (*38*). We have normalized the data because the intensity of the staining is somewhat variable from experiment to experiment.

Myosin S1-Induced Tropomyosin Binding to Actin. Actin (3 μM) and TM (1 μM) in 30 mM (or 12 mM where indicated) NaCl, 0.5 mM $MgCl_2$, 1 mM DTT, and 10 mM imidazole, pH 7.0, were mixed with myosin S1 (0−3.6 μM). The mixture was incubated at room temperature for 0.5 h to ensure hydrolysis of residual ATP from F-actin and then centrifuged in a TLA-100 rotor for 25 min at 60 000 rpm, 20 °C, in a Beckman TL-100 ultracentrifuge. The pellets were washed with assay buffer and then solubilized in actin extraction buffer (5 mM imidazole, pH 7.0, 0.5 mM DTT, 0.1 mM $CaCl_2$, and 0.1 mM ATP) by sonication in an ultrasonic cleaner. Pellets were electrophoresed on SDS−12% polyacrylamide gels (*60*). Proteins were visualized with Coomassie Blue. The composition of proteins sedimented in pellets was analyzed by densitometry. The results were plotted as the TM:actin and S1:actin ratio obtained from intensities of protein bands on the gel vs the S1:actin molar ratio in the mixture before centrifugation. Sigma Plot (Jandel Scientific) was used to fit the experimental data to a modified version of eq 1:

$$\upsilon = (n[\mathrm{X}]^{\alpha^H} K^{\alpha^H}/1 + [\mathrm{X}]^{\alpha^H} K^{\alpha^H}) + C \qquad (2)$$

where υ = TM:actin ratio; [X] = S1:actin molar ratio, and C = TM:actin ratio without S1. The S1:actin ratio necessary for half-maximal saturation of actin with TM = 1/K. The maximal TM:actin ratio was the same for all TM isoforms, indicative of full saturation (see above). The myosin S1 line was fitted by a linear regression to the experimental points for S1:actin ratios 0−0.8. The line at saturation was drawn manually.

Table 1: N- and C-Terminal Sequences of Tropomyosin[a]

	N-terminal sequences					
	1	10	20	30	38	
1a	MD**A**IKK**K**MQM**L**KLDKEN**A**LD**RA**EQAEADKKAAEDRSKQ					
1b	MAGSSSLE**A**VRR**K**IRS**L**QEQADA**A**EE**RA**GSLQRELDQERKLRET					
	d a d a d a d a d a d a					
	C-terminal sequences					
	258		284			
9a	DELYAQKLKYKAISEE**L**DHA**L**NDMTSI					
9c	DQLYHQLEQNRRLTNE**L**KLA**L**NED					
9d	EKVAHALEENLSMHQM**L**KQT**L**LELNNM					
	a d a d a d a d					

[a] The rat α-TM exon 1 and exon 9-encoded sequences are taken from ref *92*. The letters a and d correspond to the interface residues in the heptapeptide repeat of the TM coiled coil (*69*). The conserved residues are shown in boldface type. The N-terminal Met encoded by exon 1b is removed following synthesis.

RESULTS

The TMs used in this study are illustrated in Figure 1. The TMs differed only at the N- and C-termini, which are encoded by alternatively expressed exons 1a or 1b and 9a, 9c, or 9d (Table 1). Exons 1a and 1b (which replaces residues 1−80 encoded by exons 1a and 2) are expressed in 284- and 247-residue TMs, respectively. The exon 1a-containing TMs span seven actin monomers in F-actin, whereas the exon 1b-TMs span six actins. Exon 1b has a five amino acid N-terminal extension when the exon 1a and 1b sequences are aligned on the basis of conservation of the pattern of charged and hydrophobic residues in residues 2−9 of exon 1a (Table 1). Exon 9c is three codons shorter than exon 9a and 9d. Here the TMs are named after the exons encoding the N- and C-termini. Most are naturally occurring isoforms of TM (*28*): TM1a9a is striated muscle α-TM; TM1a9c and TM1b9c are brain-specific isoforms (TMBr1 and TMBr3); TM1a9d is equivalent to nonmuscle TM2; TM1b9d is TM5a found in many nonmuscle cells and tissues. TM1b9a is a chimera not yet described in nature. In addition, there are chimeras (TM1a9a/d and TM1a9d/a) in which the N- or C-terminal nine amino acids encoded by exons 9a and 9d were exchanged (*50*). Except for acTM1a9a (*N*-acetylated striated α-TM), which was isolated from chicken pectoral muscle, all TMs were products of the rat α-TM gene expressed in *E. coli*. The N-terminal residue of the recombinant forms is unacetylated.

Conformational Analysis of TM Variants. The conformation and folding of the TM variants were analyzed by circular dichroism spectrophotometry (CD). All exhibited fully reversible folding following thermal denaturation, though they differed in stability (Table 2). The TM9d isoforms were the least stable and the TM9c isoforms were the most stable,

Table 2: Thermal Stability of Tropomyosin Isoforms

tropomyosin variant	T_{Mapp}[a] (°C)
1a9a	42.6
1b9a	41.4
1a9c	44.8
1b9c	43.8
1a9d	40.0
1b9d	37.3
1a9a/d	43.0[b]
1a9d/a	38.7[b]

[a] T_{Mapp} = temperature at which the ellipticity at 222 nm is at the midpoint between the value found at 0 °C, where the TM was fully folded, and 60 °C, where it was fully unfolded. [b] Data from ref *59*.

probably because of a Leu at a d position (the 13th residue encoded by exon 9, Table 1) instead of Ile and Met encoded by exons 9a and 9d. It is not possible to compare the T_{Mapp} of the exon 1a and 1b forms because the 1b-expressing TMs are shorter and do not contain exon 2b, which encodes a stable region (*59*).

Actin Binding of Tropomyosins with Alternative N- and C-Termini. The N- and C-termini of TM are the primary determinants of actin affinity in the absence of accessory proteins (reviewed in ref *61*). We have compared three N-terminal variants (*N*-acetylation, exon 1a, and exon 1b) and three C-terminal variants (exons 9a, 9c, and 9d) here and in previously published work (*38, 43, 50, 62*).

Figure 2A compares the actin affinities of TM1b9a, TM1b9c, and TM1b9d with those of the higher MW counterparts, TM1a9a, TM1a9c, and TM1a9d, in the absence of associated proteins. The chimera TM1b9a bound strongly to actin (Figure 2A, Table 3) with a K_{app} of $(6.5 \pm 0.2) \times 10^6$, an even higher affinity than acetylated striated α-TM isolated from muscle. Recombinant striated α-TM does not bind measurably in 150 mM ionic strength, although in 100 mM salt it binds weakly with K_{app} of about 3×10^5 (Table 3). Thus, exon 1b increased the actin affinity of TM with exon 9a at least 100-fold, similar to other N-terminal extensions of exon 1a (*42, 43, 62*).

As previously reported, TM1a9d binds strongly to actin (*38, 50*). Its exon 1b-containing counterpart, TM5a, bound with even greater affinity, too tightly to measure accurately (Figure 2A, Table 3), consistent with work from other laboratories (*39*).

Surprisingly, TMs with the brain-specific exon 9c (TM1a9c and TMb9c) did not bind to actin at physiological ionic strength, with either N-terminal exon (Figure 2A, Table 3). The exon 9c-expressing TMs are three residues shorter than their exon 9a and 9d counterparts, and the region forms a more stable coiled coil, features that may reduce actin affinity. Four of the six C-terminal amino acids encoded by exon 9c are identical or homologous to the exon 9a sequence, the region known to be responsible for the lower actin affinity of TM1a9a relative to TM1a9d (*50*).

Tropomyosin binding proteins, such as caldesmon and troponin, can promote TM binding to F-actin (*63*; reviewed in ref *27*). Troponin ($+Ca^{2+}$) requires exon 9a to increase the affinity of TM1a9a for actin, having little effect on the actin affinity of TM1a9d (Figure 2B, Table 3; *38, 50*). Troponin did increase the affinity of TM1a9c and TM1b9c, but binding was still 10-fold weaker than TM1a9a or TM1a9d (Figure 2B, Table 3). Nevertheless, this result shows

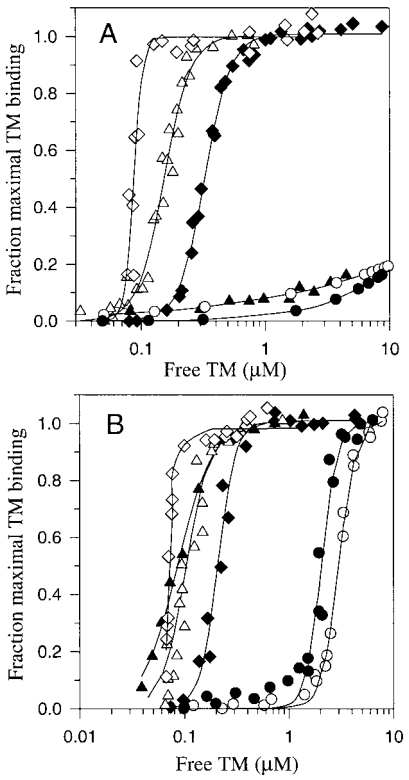

FIGURE 2: Binding of N- and C-terminal α-tropomyosin variants to actin alone (A) and in the presence of troponin and Ca^{2+} (B). Tropomyosin (0−10 μM) was cosedimented with 5 μM actin in 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 0.5 mM DTT, and 0.2 mM $CaCl_2$. Troponin concentration was at 1.2-fold molar excess over TM. Symbols: TM1a9a (▲), TM1b9a (△), TM1a9c (●), TM1b9c (○), TM1a9d (◆), and TM1b9d (◇). The data shown are from 2−3 experiments for each tropomyosin analyzed. The curves were fit to the data by use of the Hill equation.

that exon 9c-containing proteins have the ability to bind specifically to actin and that Tn can promote binding. Although only two residues are conserved in all three exon 9-encoded sequences, the homology between 9c and 9a is greater than between the other two pairs, particularly the last nine residues known to be important for Tn binding (*50*). The 9c-encoded sequence is in ways a hybrid of 9a and 9d.

For Tn to enhance specifically actin affinity, however, the combination of N- and C-termini is important; exon 9a must be combined with exon 1a, as in striated α-TM, for Tn to increase actin affinity by more than 100-fold (Figure 2B, Table 3). In addition to the exon 9a-encoded C-terminus, the exon 1a-encoded N-terminus appears to be specialized for Tn-induced binding to actin. Troponin ($+Ca^{2+}$) increased the actin affinity of TM1b9a for actin, but the effect was less than 2-fold. There was almost no effect of Tn ($+Ca^{2+}$) on the actin affinity of TM1a9d or TM1b9d (Figure 2B; also refs *38* and *50*), although the F-actin affinity of TM1b9d was difficult to quantitate in this tight-binding range. When Ca^{2+} was removed, the affinity of all six TM variants increased; TM1a9a, TM1b9a, TM1a9d, and TM1b9d all bound too tightly to be measured in our assay (data not shown; also refs *38* and *50*).

The major effect of the N- and C-terminal sequences is on the affinity of a TM molecule for an isolated single site on F-actin, rather than on the cooperativity of binding. This is reflected in the similar slopes and Hill coefficients, 4−5, for TMs that bind with apparent affinities $<10^7$ M^{-1} (Figure

Table 3: Tropomyosin Binding to Actin Alone and in the Presence of Troponin or Myosin S1

	TM-actin binding constant K_{app} (M^{-1}) (TM alone)	TM-actin binding constant K_{app} (M^{-1}) (Tn + Ca^{2+})	S1/actin molar ratio[b]	number of S1 per 7 actin subunits[b]
ac-TM1a9a	$(4.7 \pm 0.8) \times 10^6$ [c]	$>10^8$ [c]	0.22 ± 0.02	1.5 ± 0.1
TM1a9a	$\ll 10^5$ [d]	$(1.2 \pm 0.4) \times 10^7$	0.36 ± 0.01	2.5 ± 0.2
TM1a9c	$\ll 10^5$	$(4.9 \pm 0.1) \times 10^5$	0.45 ± 0.02	3.1 ± 0.3
TM1a9d	$(3.1 \pm 0.1) \times 10^6$	$(4.8 \pm 0.1) \times 10^6$	0.19 ± 0.02 0.29 low salt[e]	1.3 ± 0.3 2.0 low salt[e]
TM1a9a/d	$3.2 \pm 0.2 \times 10^6$ [f]	$(1.3 \pm 0.1) \times 10^7$ [f]	0.25 ± 0.01	1.7 ± 0.2
TM1a9d/a	$\ll 10^5$ [f]	$(4.6 \pm 0.6) \times 10^5$ [f]	0.36 ± 0.02	2.5 ± 0.3
	K_{app} (M^{-1}) (TM alone)	K_{app} (M^{-1}) (Tn + Ca^{2+})	S1/actin molar ratio	number of S1 per 6 actin subunits
TM1b9a	$(6.5 \pm 0.2) \times 10^6$	$(9.2 \pm 0.5) \times 10^6$	0.21 ± 0.03	1.3 ± 0.2
TM1b9c	$\ll 10^5$	$(3.3 \pm 0.1) \times 10^5$	0.41	2.9
TM1b9a	$(1.1 \pm 0.2) \times 10^7$	$(1.4 \pm 0.1) \times 10^7$	0.20 low salt [e]	1.2 low salt [e]

[a] Values are shown with standard errors. [b] Myosin S1/actin ratio for half-maximal saturation of F-actin with TM. [c] Data from ref *43*. [d] In 100 mM NaCl, TM1a9a binds to actin with $K_a \approx 3 \times 10^5$ (*47*). [e] In low salt conditions, NaCl was reduced from 30 to12 mM. [f] Data from ref *50*. The K_{app} values published in ref *50* were high by a factor of 2 because of a calculation error.

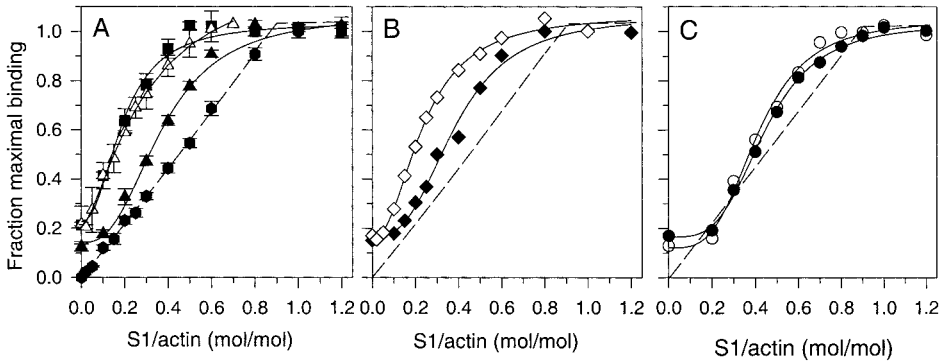

FIGURE 3: Myosin S1-induced binding of α-tropomyosins to actin. Binding of TM and myosin S1 are shown. (A) ac-TM1a9a, acetylated TM isolated from chicken pectoral muscle (■), TM1a9a (▲), and TM1b9a (△); (B) TM1a9d (◆) and TM1b9d (◇); (C) TM1a9c (●), and TM1b9c (○). Binding of TM (1 μM) and S1 to actin (3 μM) was measured as a function of S1 concentration (0−3.6 μM) in 30 mM NaCl (A, C) or 12 mM NaCl (B), 0.5 mM $MgCl_2$, 1 mM DTT, and 10 mM imidazole, pH 7.0, as described in Materials and Methods. Myosin S1 binding to actin (dashed line, ⬢) is an average from five independent experiments; in B and C the averaged points were not included for simplicity. Tropomyosin binding data in panel A are averages from 3−6 experiments; data in panels B and C are from a single representative experiment. The TM binding curves were fit to the data by use of the Hill equation.

2). The Hill coefficients for the TMs with the highest affinity are ≫10, but like the K_{app} the Hill coefficients are inaccurate because it is impossible to measure $[TM]_{free}$ in this range using our assay.

Myosin S1-Induced Binding of TM Variants to Actin. Myosin S1 increases the affinity of TM for actin (*20, 64*). In terms of the Geeves and Lehrer model, the binding of myosin heads (myosin S1 or myosin S1-ADP) to actin shifts the equilibrium of the actin-TM from the closed state to the open state in which TM and myosin bind to actin with higher affinity (*15;* reviewed in refs *6* and *65*).

To understand the structural requirements of TM for this fundamental function, we have investigated the effect of alternatively expressed N- and C-terminal exons on thin filament cooperativity. For each TM variant we determined the number of myosin heads bound per actin (or per TM molecule) required to switch the actin filament from the weak TM binding state (the closed state) to the tight TM binding open state. Since some TM variants do not bind well to actin alone, we could not use a fluorescence change in TM to report the transition from the closed to the open state (*25*). Instead, we monitored the closed-to-open state transition using a direct cosedimentation binding assay (*20*). We have set the experimental conditions so that TM binds weakly to actin in the absence of myosin S1 (10−20% saturation, depending on the TM affinity, predominantly the closed state) but binds well when myosin S1 binding to actin allows strong, cooperative binding of TM in the open state. Myosin S1 induced all TM forms to bind to actin, but the amount of S1 required for half-maximal TM binding depended on the N- and C-terminal sequences.

The effect of the N-terminal sequence of TM on myosin S1-induced TM binding was found to be similar to that on actin affinity. *N*-Acetylation of TM1a9a increased the cooperativity of myosin S1-induced TM binding to actin, requiring 1.5 heads/7 actins for half-maximal TM binding, compared to 2.5 heads/7 actins for unacetylated TM1a9a (Figure 3A, Table 3). The effect of lack of *N*-acetylation of TM1a9a on actin binding and myosin S1-induced binding was compensated by replacement of exons 1a and 2b with exon 1b (1.3 heads/6 actins; Figure 3A, Table 3). Myosin S1 binding to actin was strong and stoichiometric in the conditions of these experiments (Figure 3), as observed when myosin S1 binding to actin-TM is followed by light scattering (e.g., ref *25*).

The myosin S1-induced binding of exon 1b-containing TMs depended on the C-terminal exon. With exon 9d (TM1b9d), the actin affinity was so high in 30 mM NaCl

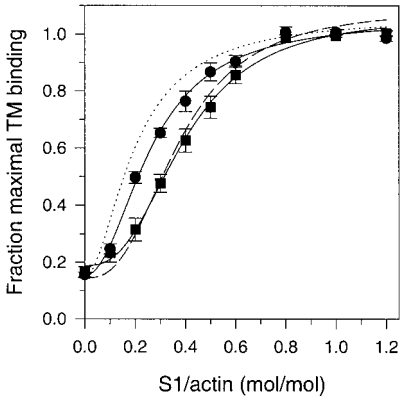

FIGURE 4: Myosin S1-induced binding of chimeric α-TMs to actin. Chimeras TM1a9a/d (●) and TM1a9d/a (■) are compared to TM1a9a (dashed line) from Figure 3A and to TM1a9d (dotted line) from Figure 3B. Conditions as in Materials and Methods and Figure 3. The curves were fit to the data by use of the Hill equation.

that the actin filament was 80% saturated even without S1 (results not shown). At a lower salt concentration (12 mM NaCl), TM1b9d saturated the actin filament at a lower myosin S1 occupancy than TM1a9d (1.2 heads/6 actins vs 2.0 heads/7 actins; Figure 3B, Table 3), a similar effect to that seen with the exon 9a-expressing TMs. However, exon 1b did not increase the myosin S1-induced actin binding of TMs expressing the brain-specific exon 9c (Figure 3C); both TM1a9c and TM1b9c required high S1 occupancy for half-maximal binding (3.1 heads/7 actins, 2.9 heads/6 actins, Table 3), a result that is consistent with the low actin affinity of the brain TM isoforms.

Even though weak-binding TMs (TM1a9a, TM1a9c, and TM1b9c) required nearly full occupancy of the actin with myosin S1 for maximal binding (Figure 3A,C, Table 3), saturating amounts of myosin S1 increased the actin affinity of these TMs more than 500−1000-fold. The binding was too tight to measure by our assay ($\gg 10^7$ M^{-1}) (results not shown; *47*).

Myosin S1-Induced Binding of C-Terminal TM Chimeras to Actin. In the six TM isoforms discussed this far, the myosin occupancy required for cooperative binding of TM in the open state correlates with TM affinity for actin. Tropomyosins with higher actin affinity require lower occupancy of actin by myosin for TM to bind. Further support for this relationship comes from analysis of TM9a/9d chimeras in which the last nine residues were exchanged (TM1a9a/d and TM1a9d/a; Figure 1). Hammell and Hitchcock-DeGregori (*50*) showed that the C-terminal nine amino acids allow for the ∼100-fold difference in actin affinity between TM1a9a and TM1a9d. Similarly, the myosin S1-induced actin binding of TM1a9d/a, which has only a third of the striated-specific exon 9a, was comparable to that of the TM with the entire exon 9a-encoded sequence (Figure 4, Table 3). The last nine residues encoded by exon 9d increased S1-induced binding of chimera TM1a9a/d; however, it required slightly more S1 for half-maximal saturation of actin filament than TM1a9d (Figure 4, Table 3).

DISCUSSION

Systematic analysis of TMs that differ in the N- and C-terminal sequences has given insight into the roles of these regions in cooperative binding of TM to actin and cooperativity of myosin S1 in inducing binding of TM to actin in the open state. The results show that the ends of TM affect these two functions in parallel: the TMs that bind to actin with the highest affinity require the lowest occupancy by myosin S1 to bind to actin in the open state.

It is well-established that the N- and C-termini of TM are major functional determinants of actin affinity (e.g., refs *12* and *29*−*44*). Here we have shown that 247-residue TMs with a N-terminus encoded by exon 1b and either exon 9a or 9d encoding the C-terminus bind with higher affinity than their 284-residue counterparts expressing exon 1a. One explanation for the higher affinity with exon 1b may be that the N-terminal five amino acid extension (Table 1) functions the way *N*-acetylation or N-terminal extensions of exon 1a do on striated α-TM (TM1a9d) to increase actin affinity over unacetylated TM (*33*, *42*−*44*, *62*). Similarly, TMs with an exon 1b-encoded N-terminus increase the affinity of TMs with C-termini encoded by exon 9d or related exons (this work; refs *39*−*41*).

The effect of exon 1b does not extend to TMs with an exon 9c-encoded C-terminus, which do not bind well with either N-terminal sequence. The N- and C-terminal sequences may be specialized for interaction with accessory proteins. For example, exon 9a encodes a Tn-specific binding site (*38*, *50*, *66*), and the results reported herein suggest that exons 1a and 9a both must be present for Tn to promote optimal binding to actin. Evidence for the association between TM1a9c and TM1b9c and F-actin in the brain suggests there may be brain proteins that promote actin binding (unpublished results).

The most significant outcome of the present work is the correlation between the myosin S1-induced binding of TM to actin and actin affinity. It is known that TM isoforms differ in cooperative activation of the acto-myosin S1 ATPase, and in the myosin S1-induced switch of actin-TM to the open state (*12*, *21*−*24*). The TMs compared in earlier studies differed in their N- and C-terminal sequences, as well as in other regions. The TMs compared here were identical except at the ends. We show here that the ends, by defining the actin affinity, are the primary determinants of the myosin S1 dependence of induced TM binding to actin, though certainly other regions of TM contribute to thin filament activation (unpublished results; ref *67*). Comparison of TM1a and TM1b-encoding isoforms shows that the myosin S1 dependence of TM binding in the open conformation is not related to TM length, in support of Lehrer et al. (*12*).

Both the N- and C-termini of TM are crucial functional determinants, but the structural basis for effect of the ends of TM on myosin-dependent thin filament activation is unknown. We cannot yet conclude that end-to-end interactions have a major role (*12*). Tropomyosin is aligned head-to-tail along the actin filament, and it has been generally assumed that head-to-tail association is responsible for cooperative binding as well as cooperative activation (*10*, *68*); more recent models have also incorporated this idea (*11*, *14*). While it has been proposed that the N- and C-termini of striated TM overlap by about nine amino acids (*69*), the ends are unresolved in the crystal structure (*70*, *71*). The only known structure is of a model peptide containing the N-terminus of exon 1a, which forms a coiled coil (*72*). Preliminary structural analysis of a TM1b-containing model peptide indicates that the N-terminal extension is not

α-helical (*73*). There is no structural information on the C-terminus, or the complex between the N- and C-termini, of TM alone or on actin.

Evidence for strong head-to-tail association of TMs is primarily based on viscosity measurements at low ionic strength, which may reflect other intermolecular interactions. While TMs have vastly different viscosities, and enzymatic removal of the C-terminus of striated or smooth muscle TM results in loss of viscosity (*29, 30, 74*), there is poor correlation between viscosity and actin affinity and the cooperativity of actin binding or activation (*12, 33, 38, 40, 43;* reviewed in ref *61*). We have suggested that cooperative binding to actin and activation are intrinsic to TM, do not require strong TM−TM interaction, and may be communicated through the actin filament without strong direct intermolecular contact between TM ends (*38, 62*), an idea that has received support from others (*11, 75, 76*).

The binding of myosin S1 to actin must change the conformation of the actin filament, or the actin monomer within the filament, to allow binding of TM in the open, high-affinity state. The TM isoforms with the highest affinity require about 1−2 myosins to bind to actin per length of one TM molecule for half-maximal TM binding. The TM binding exceeds the threshold level when the actin filament has less than one myosin S1/TM molecule; the effect of binding of one S1 (or one TM) is carried beyond one TM binding site, as emphasized by Geeves and Lehrer (*12, 14*). For the TMs with the lowest affinity (TM1a9c and TM1b9c), approximately two myosin S1 per seven actins must bind for TM binding to exceed the basal level, and the actin filament is nearly half-saturated with myosin S1 for half-saturation with TM. In terms of the Geeves and Lehrer model (*14*), this means that about three myosin heads must cooperate to induce TM binding to actin in the open conformation; in functional terms, the thin filament is more difficult to switch into the active conformation.

We suggest that the effect of the TM ends on myosin-dependent cooperativity of the switch primarily depends on the intrinsic actin affinity of the TM; the higher the affinity, the less myosin is required to activate the filament (*37*). Proteins that increase the cooperativity of activation, such as the N-terminal half of TnT (*11, 75, 77−79*), may have their effect primarily through increasing the affinity of TM for actin (but not the cooperativity of TM binding). Another possible consideration, that cannot be evaluated in the context of the present experiments, is TM may bind directly to myosin (*80, 81*). It may seem counterintuitive that the TMs with the highest actin affinity require the lowest occupancy by myosin S1 for binding in the open state, since TM alone binds to actin in the closed state, where it is proposed to occupy partially the myosin binding site on actin. The binding of TM and myosin S1 and the mechanism by which these events facilitate activation of the thin filament involve multiple sources of cooperativity that remain to be described in molecular and structural terms.

Our analysis, as does that of Lehrer and colleagues (e.g., ref *12*), assumes myosin S1 binds randomly to F-actin. At equilibrium, binding of substoichiometric amounts of myosin S1 to actin is noncooperative (*82*), though S1 binding may be nonuniform in certain conditions (*83−85*). Extensive work has shown that myosin binding to actin results in a cooperative structural change in F-actin, in the absence of TM, and that the change is reflected beyond the site of myosin binding on the actin (e.g., ref *86*; reviewed in refs *87−90* and references therein). It is very possible that myosin binding may change the structure of the subdomain 3/4 region of the actin subunit in the filament where TM is believed to bind in the open conformation (*16, 17, 19, 91*). There is little knowledge about changes in actin that result from TM binding. Until there are atomic-resolution structures of F-actin with myosin and/or TM bound, it is premature to propose detailed molecular models for the effect of myosin S1 on TM binding to actin.

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REFERENCES

1. Ebashi, S., Endo, M., and Otsuki, I. (1969) *Q. Rev. Biophys. 2*, 351−84.
2. Brandt, P. W., Diamond, M. S., and Schachat, F. H. (1984) *J. Mol. Biol. 180*, 379−384.
3. Bremel, R. D., Murray, J. M., and Weber, A. (1973) *Cold Spring Harbor Symp. Quant. Biol. 37*, 267−275.
4. Greene, L. E., and Eisenberg, E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2616−20.
5. Trybus, K. M., and Taylor, E. W. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7209−13.
6. Geeves, M. A., and Lehrer, S. S. (2000) in *Molecular Interactions of Actin* (Dos Remedios, C., and Thomas, D., Ed.) Springer-Verlag (in press).
7. O'Brien, E. J., Bennett, P. M., and Hanson, J. (1971) *Philos. Trans. R. Soc. London B 261*, 201−208.
8. Lehrer, S. S., and Morris, E. P. (1982) *J. Biol. Chem. 257*, 8073−80.
9. Milligan, R. A., Wittaker, M., and Safer, D. (1990) *Nature 348*, 217−221.
10. Hill, T. L., Eisenberg, E., and Greene, L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3186−3190.
11. Hill, L. E., Mahegan, J. P., Butters, C. A., and Tobacman, L. (1992) *J. Biol. Chem. 267*, 16106−16113.
12. Lehrer, S. S., Golitsina, N. L., and Geeves, M. A. (1997) *Biochemistry 36*, 13449−54.
13. McKillop, D. F., and Geeves, M. A. (1993) *Biophys. J. 65*, 693−701.
14. Geeves, M. A., and Lehrer, S. S. (1994) *Biophys. J. 67*, 273−82.
15. Lehrer, S. S., and Geeves, M. A. (1998) *J. Mol. Biol. 277*, 1081−9.
16. Al-Khayat, H. A., Yagi, N., and Squire, J. M. (1995) *J. Mol. Biol. 252*, 611−32.
17. Lehman, W., Vibert, P., Uman, P., and Craig, R. (1995) *J. Mol. Biol. 251*, 191−6.
18. Vibert, P., Craig, R., and Lehman, W. (1997) *J. Mol. Biol. 266*, 8−14.
19. Xu, C., Craig, R., Tobacman, L., Horowitz, R., and Lehman, W. (1999) *Biophys. J. 77*, 985−992.
20. Eaton, B. L. (1976) *Science 192*, 1337−9.
21. Sobieszek, A., and Small, J. V. (1981) *Eur. J. Biochem. 118*, 533−9.
22. Lehrer, S. S., and Morris, E. P. (1984) *J. Biol. Chem. 259*, 2070−2.
23. Williams, D. L., Jr., Greene, L. E., and Eisenberg, E. (1984) *Biochemistry 23*, 4150−5.
24. Yamaguchi, M., Ver, A., Carlos, A., and Seidel, J. C. (1984) *Biochemistry 23*, 774−9.
25. Ishii, Y., and Lehrer, S. S. (1985) *Biochemistry 24*, 6631−8.
26. Ishii, Y., and Lehrer, S. S. (1990) *Biochemistry 29*, 1160−6.

27. Lin, J. J.-C., Warren, K. S., Wamboldt, D. D., Wang, T., and Lin, J. L.-C. (1997) *Int. Rev. Cytol. 170*, 1−38.
28. Pittenger, M. F., Kazzaz, J. A., and Helfman, D. M. (1994) *Curr. Opin. Cell Biol. 6*, 96−104.
29. Ueno, H., Tawada, Y., and Ooi, T. (1976) *Biochemistry 80*, 283−290.
30. Johnson, P., and Smillie, L. B. (1977) *Biochemistry 16*, 2264−9.
31. Mak, A. S., and Smillie, L. B. (1981) *J. Mol. Biol. 149*, 547−550.
32. Dabrowska, R., Nowak, E., and Drabikowski, W. (1983) *J. Muscle Res. Cell Motil. 4*, 143−61.
33. Hitchcock-DeGregori, S. E., and Heald, R. W. (1987) *J. Biol. Chem. 262*, 9730−5.
34. Heeley, D. H., Smillie, L. B., and Lohmeier-Vogel, E. M. (1989) *Biochem. J. 258*, 831−6.
35. Heeley, D. H., Watson, M. H., Mak, A. S., Dubord, P., and Smillie, L. B. (1989) *J. Biol. Chem. 264*, 2424−30.
36. Pan, B. S., Gordon, A. M., and Luo, Z. X. (1989) *J. Biol. Chem. 264*, 8495−8.
37. Cho, Y. J., Liu, J., and Hitchcock-DeGregori, S. E. (1990) *J. Biol. Chem.* 256, 538−45.
38. Cho, Y. J., and Hitchcock-DeGregori, S. E. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10153−7.
39. Pittenger, M. F., and Helfman, D. M. (1992) *J. Cell Biol. 118*, 841−58.
40. Novy, R. E., Lin, J. L., Lin, C. S., and Lin, J. J. (1993) *Cell Motil. Cytoskeleton 25*, 267−81.
41. Fanning, A. S., Wolenski, J. S., Mooseker, M. S., and Izant, J. G. (1994) *Cell Motil. Cytoskeleton 29*, 29−45.
42. Montiero, P. B., Lataro, R. C., Ferro, J. A., and de Castro Reinach, F. (1994) *J. Biol. Chem. 269*, 10461−10466.
43. Urbancikova, M., and Hitchcock-DeGregori, S. E. (1994) *J. Biol. Chem. 269*, 24310−5.
44. Kluwe, L., Maeda, K., Miegel, A., Fujita-Becker, S., Maeda, Y., Talbo, G., Houthaeve, T., and Kellner, R. (1995) *J. Muscle Res. Cell Motil. 16*, 103−10.
45. Nicholson-Flynn, K., Levitt, P., and Hitchcock-DeGregori, S. E. (1997) *Mol. Biol. Cell 8*, 154a.
46. Moraczewska, J., and Hitchcock-DeGregori, S. E. (1998) *Biophys. J. 74*, A53.
47. Nicholson-Flynn, K. (1998) Ph.D. Thesis, Department of Neuroscience and Cell Biology, Rutgers, The State University of New Jersey and University of Medicine and Dentistry of New Jersey, Piscataway, NJ.
48. Ruiz-Opazo, N., and Nadal-Ginard, B. (1987) *J. Biol. Chem. 262*, 4755−65.
49. Lees-Miller, J. P., Goodwin, L. O., and Helfman, D. M. (1990) *Mol. Cell. Biol. 10*, 1729−42.
50. Hammell, R. L., and Hitchcock-DeGregori, S. E. (1996) *J. Biol. Chem. 271*, 4236−42.
51. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol. 185*, 60−89.
52. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
53. Hitchcock-DeGregori, S. E., Mandala, S., and Sachs, G. A. (1982) *J. Biol. Chem. 257*, 12573−12580.
54. Margossian, S. S., and Lowey, S. (1982) *Methods Enzymol. 85*, 55−71.
55. Potter, J. D. (1982) *Methods Enzymol. 85*, 241−63.
56. Edelhoch, H. (1967) *Biochemistry 6*, 1948−54.
57. Lehrer, S. S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3377−81.
58. Ishii, Y., Hitchcock-DeGregori, S., Mabuchi, K., and Lehrer, S. S. (1992) *Protein Sci. 1*, 1319−25.
59. Greenfield, N. J., and Hitchcock-DeGregori, S. E. (1995) *Biochemistry 34*, 16797−805.
60. Laemmli, U. K. (1970) *Nature 227*, 680−5.
61. Hitchcock-DeGregori, S. E. (1994) *Adv. Exp. Med. Biol. 358*, 85−96.
62. Heald, R. W., and Hitchcock-DeGregori, S. E. (1988) *J. Biol. Chem. 263*, 5254−9.
63. Wegner, A., and Walsh, T. P. (1981) *Biochemistry 20*, 5633−42.
64. Cassell, M., and Tobacman, L. S. (1996) *J. Biol. Chem. 271*, 12867−72.
65. Lehrer, S. S. (1994) *J. Muscle Res. Cell Motil. 15*, 232−236.
66. Hammell, R. L., and Hitchcock-DeGregori, S. E. (1997) *J. Biol. Chem. 272*, 22409−16.
67. Landis, C. A., Bobkova, A., Homsher, E., and Tobacman, L. S. (1997) *J. Biol. Chem. 272*, 14051−6.
68. Wegner, A. (1979) *J. Mol. Biol. 131*, 839−53.
69. McLachlan, A. D., and Stewart, M. (1975) *J. Mol. Biol. 98*, 293−304.
70. Phillips, G. N., Jr., Fillers, J. P., and Cohen, C. (1986) *J. Mol. Biol. 192*, 111−31.
71. Whitby, F. G., Kent, H., Stewart, F., Stewart, M., Xie, X., Hatch, V., Cohen, C., and Phillips, G. N., Jr. (1992) *J. Mol. Biol. 227*, 441−52.
72. Greenfield, N. J., Montelione, G. T., Farid, R. S., and Hitchcock-DeGregori, S. E. (1998) *Biochemistry 37*, 7834−43.
73. Greenfield, N. J., and Hitchcock-DeGregori, S. E. (1999) *Biophys. J. 76*, A156.
74. Nowak, E., and Dabrowska, R. (1985) *Biochim. Biophys. Acta 829*, 335−41.
75. Willadsen, K. A., Butters, C. A., Hill, L. E., and Tobacman, L. S. (1992) *J. Biol. Chem. 267*, 23746−52.
76. Butters, C. A., Willadsen, K. A., and Tobacman, L. S. (1993) *J. Biol. Chem. 268*, 15565−70.
77. Pan, B. S., Gordon, A. M., and Potter, J. D. (1991) *J. Biol. Chem. 266*, 12432−12438.
78. Schaertl, S., Lehrer, S. S., and Geeves, M. A. (1995) *Biochemistry 34*, 15890−4.
79. Hinkle, A., Goranson, A., Butters, C. A., and Tobacman, L. S. (1999) *J. Biol. Chem. 274*, 7157−64.
80. Tao, T., and Lamkin, M. (1984) *FEBS Lett. 168*, 169−73.
81. Merkel, L., Ikebe, M., and Hartshorne, D. J. (1989) *Biochemistry 28*, 2215−20.
82. Orlova, A., and Egelman, E. H. (1997) *J. Mol. Biol. 265*, 469−74.
83. Craig, R., Greene, L. E., and Eisenberg, E. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3247−51.
84. Pollard, T. D., Bhandari, D., Maupin, P., Wachsstock, D., Weeds, A. G., and Zot, H. G. (1993) *Biophys. J. 64*, 454−71.
85. Walker, M., Zhang, X. Z., Jiang, W., Trinick, J., and White, H. D. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 465−70.
86. Miki, M., Wahl, P., and Auchet, J. C. (1982) *Biochemistry 21*, 3661−5.
87. Oosawa, F. (1983) *Macromolecular assembly of actin* in *Muscle and Non-muscle Motility* (Stracher, A., Ed.) pp 151−216, Academic Press, New York.
88. Egelman, E. H., and Orlova, A. (1995) *Curr. Opin. Struct. Biol. 5*, 172−80.
89. Feng, L., Kim, E., Lee, W. L., Miller, C. J., Kuang, B., Reisler, E., and Rubenstein, P. A. (1997) *J. Biol. Chem. 272*, 16829−37.
90. Moens, P. D., and dos Remedios, C. G. (1997) *Biochemistry 36*, 7353−60.
91. Lorenz, M., Poole, K. J., Popp, D., Rosenbaum, G., and Holmes, K. C. (1995) *J. Mol. Biol. 246*, 108−19.
92. Lees-Miller, J. P., and Helfman, D. M. (1991) *BioEssays 13*, 429−37.

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