Tropomyosin Prevents Depolymerization of Actin Filaments from the Pointed End*

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Regulation of the pointed, or slow-growing, end of actin filaments is essential to the regulation of filament length. The purpose of this study is to investigate the role of skeletal muscle tropomyosin (TM) in regulating pointed end assembly and disassembly in vitro. The effects of TM upon assembly and disassembly of actin monomers from the pointed filament end were measured using pyrenyl-actin fluorescence assays in which the barbed ends were capped by villin. Tropomyosin did not affect pointed end elongation; however, filament disassembly from the pointed end stopped in the presence of TM under conditions where control filaments disassembled within minutes. The degree of protection against depolymerization was dependent upon free TM concentration and upon filament length. When filaments were diluted to a subcritical actin concentration in TM, up to 95% of the filamentous actin remained after 24 h and did not depolymerize further. Longer filaments (150 monomers average length) were more effectively protected from depolymerization than short filaments (50 monomers average length). Although filaments stopped depolymerizing in the presence of TM, they were not capped as shown by elongation assays. This study demonstrates that a protein, such as TM, which binds to the side of the actin filament can prevent dissociation of monomers from the end without capping the end to elongation. In skeletal muscle, tropomyosin could prevent thin filament disassembly from the pointed end and constitute a mechanism for regulating filament length.

The mechanisms by which cells determine the lengths of actin filaments are not understood. Actin filament length is tightly regulated in skeletal muscle to achieve the registered thin filament assembly of the sarcomere, where all the actin filaments are uniform lengths of about 1 μm (1). The actin filaments of the muscle sarcomere are also polarized; the fast, or barbed, filament end is associated with the Z disk while the slow, or pointed, ends terminate in the A-band. In this and other cells, control of actin filament assembly requires regulation of elongation and depolymerization at both filament ends. CapZ, a high affinity barbed end capping protein, may function to both cap the barbed end of the filament and anchor it to the Z-disk (2, 3). Although evidence has been provided that the pointed ends may be capped in isolated IZI-

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1 The abbreviations used are: TM, tropomyosin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid.
kinetics of monomer addition and dissociation from the pointed end were measured with pyrenyl-actin filaments capped at the barbed ends by the high affinity capping protein villin (19). This method for assaying pointed end events is quantitative and highly sensitive (for a recent review see Ref. 20). Skeletal muscle TM was found to stop pointed end disassembly completely at actin concentrations far below the critical concentration for elongation. By all measurements, this pointed ends remained uncapped in these assays. This study demonstrates the possible role of TM in the regulation of pointed end disassembly.

Experimental Procedures

Protein Purification—Skeletal muscle TM was purified from rabbit psoas muscle ether powder as described (21). Skeletal muscle actin was purified from chicken breast muscle acetone powder by cycles twice through polymerization and depolymerization (22). Actin was labeled at the penultimate cysteine by reaction with pyrenyl-iodoacetamide as described (23) with the modifications of Bryan and Coluccio (24). Nucleating activity was removed from G-actin stocks by gel filtration. Pyrenyl-actin was purified using anion exchange as described previously (10). Tropomyosin and G-actin were stored at −80 °C and thawed quickly (10). Pyrenyl-G-actin concentration was calculated using an E₂₅₀ = 249 mM⁻¹ cm⁻¹ to compensate for the absorbance of pyrene as described (26). Native G-actin concentration was measured using an E₂₅₀ = 249 mM⁻¹ cm⁻¹ (27). Villin concentration was determined using an E₂₈₀ = 123.5 mM⁻¹ cm⁻¹ (19). Tropomyosin concentrations were determined by the Lowry assay using bovine serum albumin as a standard.

Filament Formation—All assays and incubations were performed at 25 °C. Actin was polymerized in the presence of villin to nucleate filaments and cap the barbed end (19). Filament length was determined by the ratio of villin:actin and was confirmed as described below by measuring filament lengths after negative stain electron microscopy. G-actin, 7.5 mM in G-buffer (0.5 mM ATP, 0.2 mM CaCl₂, 10 mM HEPES, pH 7.5, 0.1 mM dithiothreitol, 0.01% NaN₃) was mixed with villin at the specified ratio. Polymerization was initiated by adding 20 × F-buffer (1 × F-buffer = 0.1 M KCl, 2 mM MgCl₂, 0.5 mM ATP, 0.2 mM CaCl₂, 10 mM HEPES, pH 7.5, 0.1 mM dithiothreitol, and 0.01% NaN₃) to achieve a final concentration of 0.1 mM KCl and 2 mM MgCl₂. Capped filaments were incubated with or without skeletal muscle tropomyosin (7 μM actin and 1.5 μM TM) for 8–14 h prior to depolymerization or elongation assays. Filaments prepared for depolymerization assays contained 100% pyrenyl-actin, whereas elongation assays were performed using 10% pyrenyl actin.

Fluorescence Measurements—Fluorescence measurements were made at 25 °C using a Perkin-Elmer 650-10B or an SLM SPF-600C fluorescence spectrophotometer. Fluorescence coefficients were determined from concentration curves of pyrenyl-G- and pyrenyl-F-actin. Instrument response was calibrated with a closed standard of anthracene using an excitation wavelength of 365 nm (slit width 4 nm) and an emission wavelength of 408 nm (slit width 5 nm). F- or G-actin concentrations were calculated from the fluorescence measurements as described previously (19, 28). A fluorescence increase of 20–20 fold was typically observed upon polymerization of actin. Samples were illuminated briefly during fluorescence measurements to minimize the effects of photobleaching. The critical G-actin concentration measured for the barbed end was typically 0.12–0.15 μM G-actin and the pointed end was 0.6–0.7 μM G-actin.

Depolymerization of actin filaments from the pointed end was initiated by rapidly diluting an aliquot of capped filaments (7 μM initial actin) to a final concentration of 50 nM total actin in F-buffer. Depolymerization was monitored by fluorescence for 1 h in a thermostated cell; fluorescence measurements were taken at 24 and 48 h after dilution.

Elongation of actin from the pointed ends of capped filaments was performed as described previously (10). Just prior to the assay, the G-actin was converted from Ca²⁺- to Mg²⁺-actin by incubation with 0.2 mM EGTA and 100 μM MgCl₂ in G-buffer (26). Elongation was initiated by the addition of 20 × F-buffer and 0.5 μM actin, 10 nM filament concentration. To assay barbed end elongation, 5 mM EGTA was mixed with the G-actin prior to the addition of capped filaments. As previously shown, dissociation of the villin cap from the barbed end is rapid at subnanomolar calcium concentrations (19), allowing barbed end elongation.

Measurements of steady state critical concentrations in the presence of TM were performed by the same protocol as elongation assays. In each assay actin filaments were diluted to 0.5 μM total F-actin, 10 nM filament concentration, into four different initial G-actin concentrations: 0.4, 0.5, 0.6, and 0.7 μM G-actin. Initial fluorescence and approximate steady state fluorescence were measured for each sample. F-actin and G-actin concentrations at the initial and final points were calculated from the fluorescence measurements as described (10, 26).

Electron Microscopy—Direct measurements of capped filament lengths were made by negative stain electron microscopy. An aliquot of the filament stock solution at the time of the depolymerization assays was rapidly diluted 10-fold, placed on a Formvar- and carbon-coated grid, rinsed with 4 drops of F-buffer, and stained with 1% uranyl acetate. The preparation was then observed rapidly (<30 s) to minimize change in filament length. Grids were viewed with a JEOL 100 CX electron microscope at 60 kV. Lengths of all individual filaments on each micrograph were measured and the mean length (n > 100) was used to confirm filament length.

Results

Tropomyosin Stops Depolymerization from the Pointed End—Depolymerization of villin-capped filaments to subcritical actin concentrations results in the rapid disassembly of the filaments from the pointed end (19). Under ideal conditions as described (29), the rate of elongation and depolymerization of actin filaments from the pointed end obeys Equation 1, where the kₕ and kₐ are the on and off rate constants for the pointed end.

\[ \frac{df}{dt} = \text{filament concentration}(\text{G-actin})k_{\text{on}} - k_{\text{off}} \]

Since the initial G-actin concentration is very low when filaments are diluted to a total concentration of 50 nM actin in F-buffer, the initial net depolymerization rate of filaments is the product of the off rate constant, kₕ, and the filament concentration. The effect of skeletal muscle TM upon depolymerization from the pointed end was tested with filaments of two different lengths, averaging either 50 or 150 monomers long. The net rate of depolymerization should be lower in the long filament preparation since the filament concentration was one third that of the shorter filament preparation. When villin-capped filaments (50-mers) were diluted to 50 nM total actin, they rapidly depolymerized from the pointed end as shown in Fig. 1A, and reach complete depolymerization within 60 min. Longer control filaments (150-mers) depolymerized at a slower net rate, as shown in Fig. 1B. Preincubation of filaments with skeletal muscle TM slowed but did not stop depolymerization, as shown in Fig. 1, A and B. When actin filaments of both lengths were preincubated with skeletal muscle TM and then diluted into F-buffer without free TM, they depolymerized at a slower rate than control filaments, but completely depolymerized within 1–2 h of dilution. Preincubation of filaments with TM resulted in an inhibition of the net depolymerization rate compared to control filaments, regardless of filament length, even though the total TM concentration was only 11 nM. This result suggests that the rate-limiting step in the disassembly of a TM filament is the dissociation of TM from the filament.

The presence of free skeletal TM stopped disassembly of actin filaments even though the actin concentration was far below the critical for the pointed filament end. Fluorescence measurements of actin filaments diluted to subcritical actin concentrations in the presence of 0.5–2 μM free TM indicated that a population of stable actin filaments remained even 24 or 48 h later. As shown in Fig. 1, C and D, the amount of F-actin which did not depolymerize was dependent upon the free TM concentration and was maximal when filaments were preincubated with TM. When short control filaments (50-mers) were diluted into TM, their initial depolymerization rate was similar to the control rate, but slowed rapidly, in a
TM concentration-dependent manner. Short filaments which were preincubated with TM depolymerized slowly when diluted into 0.5–2 μM free TM at subcritical actin concentrations (Fig. 1C), and contained more F-actin 24 h after dilution than control filaments diluted into equivalent free TM concentrations. Filaments which averaged 150 monomers in length were protected from disassembly by TM to a greater extent than the shorter filaments, as shown in Fig. 1D. Preincubation of longer filaments with TM did not greatly enhance their stability, unlike the result with shorter filaments. Both control and TM-preincubated filaments depolymerized only slightly when diluted into a 2 μM skeletal TM (Fig. 1D). The effects of TM upon filament stability did not depend upon filament concentration. When short filaments (50-mers) were diluted to 16 nM total actin (resulting in a filament end concentration one-third that used in other assays), the absolute depolymerization rate was unchanged and the same percentage of the F-actin remained after 24 h as in samples with 50 nM actin (data not shown).

The percentage of the initial F-actin remaining polymerized 24 h after dilution to subcritical actin concentrations is summarized in Fig. 2. The point at which filament disassembly stopped was dependent upon the free TM concentration. When short filaments were tested, filament stability was maximized by preincubation with TM prior to dilution. A greater percentage of F-actin remained when longer filaments were diluted than when short filaments were diluted into the same TM concentrations. Filaments were not disassembling slowly, since further depolymerization did not occur upon incubation for 48 h, as shown in Table I. After 24–48 h at subcritical actin concentrations a population of stable actin
filaments remained in the presence of TM.

As predicted by the fluorescence measurements at 24 and 48 h after dilution to 50 nM actin, actin filaments were observed by negative stain electron microscopy only in the presence of TM (Fig. 3). Grids of control filaments were virtually free of filaments (Fig. 3A); rarely one filament might be found on an entire grid, while filaments were easily found on the grids of TM-treated actin (Fig. 3B). Filament lengths were not quantitated due to their low concentration; however, filament length was varied and no short filaments (<75 monomers) were observed.

Tropomyosin Did Not Inhibit Assembly from the Pointed End—Elongation assays were performed using short actin filaments capped at the barbed end by villin as nuclei for elongation. The slower depolymerization rate observed in the presence of TM was not due to a dramatic change in filament number during the preincubation period as shown by elongation assays in Fig. 4. Elongation of filaments from the pointed end in 1 \( \mu \text{M} \) G-actin did not differ when control or TM-preincubated filaments were used as nuclei. When villin was removed from the barbed end by EGTA, the net elongation rate increased and the critical concentration decreased as predicted (19) when either control or TM filaments were used as nuclei. TM filaments elongated more slowly than control filaments from the barbed end; as shown previously (11, 13, 14), skeletal TM inhibits barbed end elongation in a concentration-dependent manner. Filament number must be very similar in the TM-preincubated and control filament stocks since they elongated from the pointed end at the same initial rate when added to 1 \( \mu \text{M} \) G-actin. These data also suggest that filaments remained capped by villin in the presence of TM.

Incubation of Skeletal Muscle TM with Short Capped Actin Filaments Results in a Change in Length Distribution—The length distribution of short filaments (50 monomers average length) just prior to dilution assays indicated that preincubation with TM promoted formation of longer filaments (Fig. 5). The average length of filaments incubated with TM typically was twice that of control filaments. Control filaments exhibited a Poisson length distribution; filaments incubated with TM did not have a Poisson length distribution and

### Table I

F-actin remaining 24 and 48 h after dilution into F-buffer

| Pre-incubated filaments: | [TM] | % F-actin remaining |
|--------------------------|------|---------------------|
|                         | \( \mu \text{M} \) | 24 h (n) | 48 h (n) |
| Control                  | 0    | 0 (5) | 1.5 (2) |
| Tropomyosin              | 0    | 0 (5) | 0 (2)  |
| Control                  | 0.5  | 9 (3) | 11 (1) |
| Control                  | 2    | 25 (3) | 34 (1) |
| Tropomyosin              | 0.5  | 37 (5) | 37 (2) |
| Tropomyosin              | 1    | 50 (5) | 51 (2) |
| Tropomyosin              | 2    | 54 (5) | 50 (2) |

**Fig. 3.** Electron microscopy of actin filaments present 48 h after dilution into F-buffer. A, 24 h after the start of the depolymerization assay (Fig. 1), an aliquot of control filaments which had been diluted to 50 nM actin in F-buffer contained no visible filaments. B, a typical micrograph of filaments 48 h after TM filaments were diluted to 50 nM actin in 0.5 \( \mu \text{M} \) TM. Aliquots were placed on Formvar- and carbon-coated grids. Following a 3-min period, the grids were rinsed with F-buffer, stained, and viewed as described in Fig. 5. Bar = 0.5 \( \mu \text{m} \).

**Fig. 4.** Elongation of actin from filaments incubated plus/minus skeletal TM. Filaments (actin:villin, 50:1) prepared with 5% pyrenyl-G-actin were polymerized, preincubated plus and minus TM overnight, and diluted to 0.5 \( \mu \text{M} \) actin (10 nM filament concentration) into 1 \( \mu \text{M} \) Mg\(^{2+}\)-G-actin in F-buffer (26). Open symbols, without EGTA; solid symbols, 5 mM EGTA. Elongation of TM filaments in 0.5 \( \mu \text{M} \) TM (A); control filaments (C); 5 mM EGTA, control filaments (○); 5 mM EGTA, TM filaments and 0.63 \( \mu \text{M} \) tropomyosin (▲); 5 mM EGTA, TM filaments and 0.13 \( \mu \text{M} \) TM (▲).

**Fig. 5.** Length distribution of preincubated filaments prior to dilution assays. The length distribution of stock filaments was quantitated by measuring the length of all filaments on negatives taken at either 29,000 or 36,000 magnification. This distribution is typical of other experiments. An aliquot of stock filaments was rapidly diluted 10-fold in F-buffer, pipetted onto a Formvar- and carbon-coated grid, rinsed with F-buffer, and stained with 1% uranyl acetate. Grids were viewed at 60 kV with a JEOL CX100 transmission electron microscope. Each filament preparation was measured at the end of the depolymerization or elongation assay period. Filament lengths were recorded and the monomer number calculated using a value of 360 monomers/\( \mu \text{m} \). Control filament average length was 49 monomers (n = 246). TM filament average length was 115 monomers (n = 338).
Pointed End Regulation by Muscle Tropomyosin

Each value is the mean free G-actin concentration measured after 20–24 h of incubation following the dilution of nucleot into four different concentrations of G-actin (0.4, 0.5, 0.6, and 0.7 μM). S.D. is calculated for each experimental mean.

| Expt. | Mean free G-actin concentration μM ± S.D. | Mean μM |
|-------|-----------------------------------------|---------|
| Control | 0.60 ± 0.05 | 0.69 ± 0.01 | 0.63 ± 0.02 | 0.64 |
| 0.5 μM TM | 0.39 ± 0.01 | 0.52 ± 0.02 | 0.42 ± 0.02 | 0.44 |
| 1 μM TM | 0.33 ± 0.01 | 0.48 ± 0.01 | 0.41 ± 0.01 | 0.41 |
| 2 μM TM | 0.26 ± 0.01 | 0.43 ± 0.02 | 0.36 ± 0.01 | 0.35 |

The effects of skeletal TM upon pointed end events differ significantly from those observed at the barbed end. As shown by several laboratories, TM inhibits both elongation and depolymerization of monomers at the barbed end but does not stop filament disassembly (11, 13, 15, 16). There is agreement that TM does not change the barbed end critical concentration, whereas we have shown that both nonmuscle and skeletal TMs lower the pointed end critical concentration (Ref. 10 and this paper). The effects of skeletal TM upon pointed end depolymerization described in this study would not have been detected by the methods employed in earlier reports. Neither the skeletal nor nonmuscle TM isoforms appear to inhibit elongation from the pointed end (10, 11). A very recent report indicates that troponin-tropomyosin slows pointed end events, in partial agreement with the data presented here (33). They conclude that troponin-TM moderately inhibits pointed end elongation at 0.6–1.0 μM G-actin. I did not find that skeletal TM inhibited elongation of nuclei in 1.0 μM G-actin. This difference may be due to the presence of troponin-TM. However, the effect of skeletal TM, like that of nonmuscle TM, appears to be predominantly upon the off rate constant, k₀, with little effect upon the on rate constant, k₊.

This is the first description of a mechanism for stopping actin filament disassembly by the lateral binding of an actin binding protein to the actin filament. The effect of TM upon pointed end disassembly is similar to that of the mushroom toxin phalloidin. Phalloidin lowers the apparent off rate constant to 0 and also lowers the critical actin concentration for polymerization to 0 (34). In the presence of phalloidin, actin polymerization is promoted so that virtually all of the actin monomers are incorporated into filaments. Like phalloidin, the effect of skeletal TM upon depolymerization from the pointed end is to effectively lower the off rate constant to 0. Although the pointed end critical concentration was lowered in the presence of TM, TM did not promote the complete polymerization of actin as might be predicted. While TM and phalloidin may share a similar mechanism for preventing monomer dissociation, the differences in their effects on filament assembly may be due to the difference in the number of actin monomers which each molecule binds. Tropomyosin spans a length of 7 actin monomers, which actually in the filament requires the addition of 14 actin monomers before one complete TM binding site is added to the filament. Since phalloidin binds an equimolar ratio of actin, a much smaller addition to the filament end would create an additional phalloidin site. The difference in the effects of phalloidin and TM could result if TM did not bind to the filament end prior to the complete extension of an additional 14 monomers. An important conclusion, also noted recently (33), is that in the contained a population of longer filaments (200–700 monomers in length) which were not present in the control filaments. The number of very short filaments (<10 monomers) was underestimated by this technique, which did not visualize filaments less than 8–10 monomers in length. A 2-fold increase in average length of TM filaments cannot account for the magnitude by which the initial net depolymerization rate was inhibited or the subsequent halt of depolymerization in the presence of TM.

Skeletal Muscle TM Lowers the Critical G-actin Concentration at the Pointed End—Since skeletal muscle TM stopped pointed end depolymerization, the effect of TM upon the pointed end critical actin concentration was measured by determining the amount of G-actin remaining at approximate steady state. Shown in Table II, the critical actin concentration was lowered by the presence of skeletal muscle TM, from 0.6 to 0.25–0.3 μM G-actin. The degree to which pointed end polymerization was promoted was dependent upon the TM concentration. At higher TM concentrations, less G-actin remained at apparent steady state. TM did not in any case cause the complete polymerization of all actin. It is important to note that regardless of the initial G-actin concentration (0.4–0.7 μM), the end points were very similar, indicating that this assay measured the extent to which polymerization would occur in the presence of TM.

DISCUSSION

The results described here demonstrate that skeletal muscle TM stops actin filament disassembly from the pointed filament end without inhibiting filament elongation. Since filament disassembly stops in the presence of TM, but the pointed filament end is not capped, disassembly of a TM-saturated filament must require that TM dissociate from the filament end before actin monomers can then dissociate. The percentage of filamentous actin which remained stable at subcritical actin concentrations was dependent upon free TM concentration and actin filament length but was independent of filament concentration. Nonmuscle TM also did not inhibit elongation from the pointed end but was not capable of stopping filament disassembly (10). If skeletal muscle TM slowed but did not stop disassembly, several other possible events could explain these observations. First, TM has been shown to inhibit spontaneous or mechanical fragmentation of filaments (11, 30, 31). Both of these events result in the creation of a new barbed and pointed end. Free barbed ends generated during these assays would have been detected in both the depolymerization and elongation assays since the rate constants for the barbed end are much higher than the pointed end (19). Free barbed ends were not detected in either the control or the TM-containing samples. Second, skeletal muscle TM did not appear to promote filament annealing in these assays, as has been reported when TM and caldesmon are incubated with gelsolin-capped filaments (32). Longer filaments were formed during preincubation in the presence of TM (Fig. 5). However, no evidence was produced in these experiments that TM promoted the dissociation of villin from the barbed end as was suggested for gelsolin (32). This observation could also result if monomers dissociate from short filaments and polymerize onto longer filaments, which would be more stable than short filaments in the presence of TM. As shown by the elongation assays, the filament concentration remained the same in the presence or absence of TM, and the barbed ends remained capped by villin unless the free calcium concentration was reduced to sub-nanomolar levels. Therefore, the observation that TM stops filament disassembly is most likely due to its influence on the dissociation of monomers from the pointed filament end.

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the resultant affinities of TM for binding to filaments of differing lengths are illustrated using the estimates of binding affinities calculated by Wegner (35). The affinity of TM for filament lengths greater than 42 monomers would increase greatly due to the availability of high affinity binding sites which involve interaction between two adjacent TM molecules.

FIG. 6. Model of the estimated affinities of skeletal muscle tropomyosin for different sites on the actin filament. The affinity of TM for binding to different sites on an actin filament, and the resultant affinities of TM for binding to filaments of differing lengths are illustrated using the estimates of binding affinities calculated by Wegner (35). The affinity of TM for filament lengths greater than 42 monomers would increase greatly due to the availability of high affinity binding sites which involve interaction between two adjacent TM molecules.

presence of TM the elongation and depolymerization of actin filaments from the pointed end may not obey the equations for reversible polymerization of a linear polymer.

Major functional differences exist between TM isoforms, including actin binding affinity, ability to promote filament annealing, and polymerization by head to tail interaction (17, 18, 32). The ability of skeletal muscle TM to prevent actin filament disassembly represents another significant functional difference between TM isoforms. We previously demonstrated that enterocyte TM is capable of lowering the apparent affinity of skeletal muscle TM increased to lo6 for binding to the filament and one adjacent TM (35). The affinity of TM for the very end of the actin filament. These results suggest that the effectiveness of TM isoforms in their ability to stop pointed end disassembly reflects their affinities for the end site on an actin filament and differs significantly between isoforms. It will be important to determine whether the high and low molecular weight classes of TM found in nonmuscle cells differ in their ability to stop pointed end disassembly.

Why would TM better protect longer filaments from depolymerization than short filaments? Tropomyosin binds to actin filaments with high cooperativity (17). Wegner estimated that the apparent $K_a$ of skeletal muscle TM increased from $10^3$ for a single TM molecule binding to an actin filament to $10^6$ for binding to the filament and one adjacent TM (35). If the increase in affinity is equivalent at both ends of TM, the highest affinity site where a TM is binding to two adjacent TMs could increase the $K_a(app)$ to $10^6$ (35). The affinity of TM for that site is not measured in bulk binding assays, since the end sites determine the free TM concentration. However, TM's high cooperativity would greatly affect the net affinity of TM for short actin filaments. As illustrated in Fig. 6, the estimated affinity of TM for a filament that is 14 or 28 monomers in length would be much less than the affinity of TM for a longer filament, where TM would predominantly be binding to the highest affinity sites. In a population of filaments which average 50 monomers in length, roughly one-half of the filaments were less than 48 monomers in length, having a lower affinity for binding to TM than longer filaments. Short filaments could also have a much higher probability of completely losing TM than longer filaments, since the dissociation of one TM would leave the adjacent TM in a very low affinity site. The enhanced stability of short filaments preincubated with TM may be due to the formation of a population of longer filaments during the preincubation period.

Tropomyosin may function in the skeletal muscle sarcomere to both regulate myosin activity and also prevent actin monomers from dissociating from the pointed end of the thin filament. This regulatory mechanism would allow the muscle cell to assemble stable actin filaments and lower the monomeric actin concentration to levels that prevent spontaneous nucleation. Since tropomodulin further increases the affinity of TM for the filament in skeletal muscle (36), the assembled thin filament should be fully saturated with TM. In muscle, regulation of TM addition could therefore determine actin filament length. Since skeletal muscle TM spans 7 actin monomers, and the filament is a 2-start helix, the thin filament could then be assembled in multiples of 14 monomers. The presence of TM at the filament end would be required for filament stabilization. Regulation of the binding of TM at the pointed filament end may determine the stability of the pointed filament end and, therefore, filament length.

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