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Thin filament architecture in skeletal muscle: a two-segment model

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PREFACE

Correct specification of myofilament lengths is essential for efficient skeletal muscle contraction. Thin filament lengths are best specified by a novel ‘two-segment’ model, wherein the thin filament consists of two concatenated segments, of either constant or variable length, rather than by the classical ‘nebulin ruler’ model, wherein the thin filament is a uniform structure whose length is dictated by nebulin. The two-segment model implicates position-specific ‘differential microregulation’ of actin dynamics as a general principle underlying actin filament lengths and stability.

Keywords

actin; tropomodulin; tropomyosin; nebulin; proximal segment; distal segment

Dynamic actin filament assembly and turnover are hallmarks of diverse biological processes, such as cell motility, adhesion formation, and vesicular trafficking¹. However, in certain differentiated and specialized cell types^{2, 3, 4}, actin filaments form highly organized and stable structures with precisely regulated lengths, providing membrane stability and maintaining cell physiology¹. Some of the most striking natural examples of actin filament organization, stability, and length regulation can be observed in striated muscle cells, where semicrystalline arrays of actin and myosin filaments form densely packed, interdigitating protein lattices (sarcomeres) that are organized into repeating subunits along contractile myofibrils. Contractile force generation in striated muscle arises from crossbridge interactions between thin and thick filaments, with the degree of myofilament overlap determining the amount of active force production (the well-known ‘sliding filament’ model)^{5, 6}. As such, thin and thick filament lengths are among the main determinants of sarcomeric force production^{7, 8}, establishing the operating lengths of the sarcomere and determining muscle-joint biomechanics^{9, 10}.

During myofibril assembly and maintenance, thin and thick filaments each have carefully controlled lengths that are highly uniform within a myofibril¹¹. However, the molecular basis of myofilament length specification in vertebrate muscles is incompletely understood. The lengths of the thick filaments (~1.65 μm) are conserved across vertebrate species and muscle types, suggesting that mechanisms underlying thick filament length specification, involving both myosin and structural M-line proteins^{12–15}, are highly conserved. By contrast, the lengths of the thin filaments (i.e., complexes of F-actin and associated actin-binding proteins in a two-stranded helical ‘pearls-on-a-string’ arrangement^{16, 17}) are

constant within a myofibril, but are highly variable both across species and between different muscles within an organism, with important biomechanical implications (Box 1).

This Opinion article discusses the contributions of thin filament-associated proteins in thin filament length specification in skeletal muscle. Emphasis is placed on the thin filament region that interacts with the thick filament, and not on the Z-line-embedded region, whose characteristics have been detailed previously¹⁸. First, we introduce actin dynamics at thin filament ends, and then we discuss recent data arguing against the classical ‘nebulin ruler’ model and in favor of the ‘two-segment’ model for describing thin filament length specification. The nebulin ruler model depicts the thin filament as a uniform, nebulin-associated structure, whereas the two-segment model depicts the thin filament as a bipartite structure with two concatenated segments: a constant-length, nebulin-coated core region followed by a variable-length nebulin-free extension. The two-segment model can account for the characteristic actin dynamics of the thin filaments, explain variations in thin filament lengths across diverse muscles, and provide insight into the pathogenesis of nemaline myopathy (NM), a hereditary muscle disorder caused by mutations in thin filament structural and regulatory proteins. The two-segment model also implicates position-specific regulation of actin dynamics along a single filament (‘differential microregulation’) as a mechanism underlying the properties of actin filaments in both muscle and nonmuscle cells that have different actin-binding proteins along their lengths.

Thin filament organization

Studies into the molecular organization and actin dynamics of thin filaments have provided clues to the mechanisms of thin filament length regulation and stability.

Caps at filament ends

Thin filaments are polarized copolymers of actin subunits complexed with troponin-tropomyosin regulatory units and stabilized at their ends by specialized actin-capping proteins (Figure 1). The fast-growing (barbed) end of the thin filament is capped by the protein CapZ and embedded in the Z-line, an elaborate protein complex that defines the sarcomere periphery and laterally crosslinks thin filaments within sarcomeres^{19–22} (see review²³). By contrast, the slow-growing (pointed) end is at the thin filament free end, localized near the center of the sarcomere and capped by a combination of two sarcomeric tropomodulin (Tmod) isoforms in skeletal muscle, Tmod1 and Tmod4^{24–27} (see review²⁸). In cardiac myocytes, where Tmod1 is the exclusive pointed-end cap, Tmod appears to be more dynamic than CapZ, based on the relative propensities of actin subunits adjacent to these caps to exchange at their respective filament ends²⁹. Tmod and CapZ do not maintain thin filament length and stability via a treadmilling mechanism, in which actin subunits are incorporated at barbed ends and dissociate at pointed ends²⁹. Instead, thin filament length and stability in cardiac myocytes are principally controlled by Tmod capping of pointed ends, whereby Tmod binding to actin at the pointed end reduces actin subunit association and prevents thin filament elongation^{29–32}. Simultaneously, Tmod binding to the terminal tropomyosin at the pointed end reduces both tropomyosin and actin subunit dissociation and prevents filament shortening^{33–35} (see reviews^{11, 28}). With tropomyosin stabilizing thin filaments by binding along filament sides and reducing actin subunit dissociation rates at both Tmod-capped and uncapped pointed ends, tropomyosin and Tmod likely synergize to promote thin filament stability at the pointed end^{34–37} (see reviews^{28, 38, 39}). However, how Tmod-tropomyosin interactions contribute to the muscle-specific thin filament lengths and precisely coordinated alignment of pointed ends in skeletal muscle sarcomeres remains unclear.

The nebulin ruler model

In addition to CapZ, Tmod, and tropomyosin, the giant protein nebulin has historically been considered a key determinant of thin filament lengths in skeletal muscle. Nebulin is a massive (600–900 kDa) and elongated protein that coextends with thin filaments, with its N-terminal domain located in close proximity to the pointed end (and containing a Tmod-binding site) and its C-terminal domain embedded in the Z-line (and containing a CapZ-binding site)^{40–42}. The intervening region of nebulin consists of >100 actin-binding repeat modules and 22 troponin-tropomyosin-binding super-repeat modules⁴⁰. Most notably, the molecular weight of nebulin roughly correlates with thin filament length across various muscles^{43, 44}. This has motivated the long-held textbook depiction of a thin filament, whereby nebulin functions as a ‘molecular ruler’ that specifies end-to-end (i.e., CapZ-to-Tmod) distance in thin filaments, thereby determining thin filament lengths (Figure 1).

The two-segment model

While the nebulin ruler model of thin filament length specification is appealing in its intuitiveness and simplicity, recent studies have provided compelling evidence that this model is incorrect. Thin filaments are more accurately modeled as bipartite structures with the following concatenated segments: (i) a constant-length, nebulin-coated thin filament core region (‘proximal segment’) starting at the Z-line and continuing to a distance of ~0.95 μm away from the Z-line; (ii) a variable-length, nebulin-free, thin filament extension (‘distal segment’) starting at ~0.95 μm away from the Z-line and continuing to the thin filament pointed end, which is capped by Tmod (Figure 2A,B). The constant length of the nebulin-coated proximal segment excludes the possibility of a ruler function for nebulin, since thin filament lengths vary across different muscles^{24, 25, 45–48}, while the variable length of the distal segment implicates Tmod-mediated pointed-end actin dynamics as the chief determiner of skeletal muscle-specific thin filament lengths *in vivo*.

Evidence for the two-segment model

Recently, high-resolution immunofluorescence localization of Tmod and the nebulin N-terminus and computational measurements of their distances from the Z-line (Box 2) have shown that they do not colocalize *in vivo*^{24, 25, 45}, despite the fact that they can interact *in vitro*⁴¹. Indeed, investigations of chicken, mouse, rabbit, and human skeletal muscles have revealed that the nebulin N-terminus is a relatively constant distance (~0.95 μm) from the Z-line in all skeletal muscles, while both Tmod and the actin filament pointed ends are located at diverse distances from the Z-line (ranging from ~1.00 μm to ~1.40 μm , but always further than the nebulin N-terminus)^{24, 25, 45–47}. Moreover, the variation in nebulin isoform sizes across muscles appears to be due to variable-length insertions in the nebulin C-terminus region within the Z-line^{45, 49, 50}.

Additional evidence arguing against the nebulin ruler hypothesis has come from phenotypic analysis of neonatal-lethal nebulin-knockout mice. One-day-old nebulin-null skeletal muscles have shorter thin filaments as compared to age-matched wild-type muscles, but, even more intriguingly, muscle-specific thin filament length heterogeneity also disappears, with all nebulin-null muscles reverting to a ‘generic’ thin filament length approaching ~0.95 μm ⁵¹. This length of ~0.95 μm is also nearly identical to the average thin filament length in cardiac myocytes, which normally lack nebulin and, instead, express nebullette, a much shorter nebulin isoform that barely extends out from the Z-line^{50, 52, 53} (see review⁴⁰). In these nebulin-free systems, the proximal segment of the thin filament is absent, the entirety of the thin filament is composed of the distal segment, and thin filament lengths are more variable (Figure 2C). Notably, ~0.95 μm is also the length of the proximal segment of a wild-type skeletal muscle thin filament^{24, 25, 45}, suggesting that ~0.95 μm may define the maximum length that a thin filament can establish in the absence of nebulin. To establish a

longer functional thin filament length, nebulin may be required to stabilize the ~0.95- μ m-long proximal segment, facilitating actin polymerization and extension of the Tmod-capped distal segment.

Several experiments have provided support for nebulin functioning as a 'proximal segment stabilizer' instead of a molecular ruler. When nebulin is depleted from chick skeletal myotubes via siRNA knockdown, the lengths of the nebulin-free thin filaments are indistinguishable from the lengths of the original nebulin-containing filaments, as determined by unchanged Tmod localization with respect to the Z-line⁵⁴. Furthermore, if endogenous nebulin is replaced by an exogenous ~250-kDa mini-nebulin construct containing the N- and C-termini of human nebulin but lacking 18 (out of 22) central super-repeat modules, the relative lengths of the proximal and distal segments are shortened and lengthened, respectively, so that the N-terminal end of mini-nebulin is a mere ~0.22 μ m from the Z-line, while the distance of Tmod from the Z-line remains normal⁵⁴ (Figure 2D). Thus, the thin filaments do not adjust their overall lengths to match the shorter length of mini-nebulin – a finding in direct opposition to a ruler mechanism⁵⁴. However, mini-nebulin confers protection against latrunculin A-induced actin depolymerization in a manner similar to full-length endogenous nebulin⁵⁴, indicating that nebulin provides stabilization to the entire thin filament independent of the proportion of the thin filament length occupied by nebulin in the proximal segment. Such a model is corroborated by the observation that the dynamics of GFP-actin, GFP-Tmod, and GFP-tropomyosin are increased in the absence of nebulin, as determined by fluorescence recovery after photobleaching (FRAP), and these dynamics are reduced (i.e., rescued) by introducing mini-nebulin into the nebulin-depleted myotubes⁵⁴. Moreover, electron microscopy observations have shown that nebulin-null skeletal muscles rapidly degenerate after being subjected to postnatal contraction and use^{51, 55, 56}. Thus, by association with the proximal segment of the thin filament, nebulin confers a long-range stabilizing effect on the actin filament that protects both the proximal and distal segments from biochemical and biomechanical challenges. Nebulin may control distal segment dynamics and stability by regulating the structural polymorphism of F-actin⁵⁷, but this remains unproven.

Tmod regulation of distal segment lengths

While nebulin clearly specifies the constant length of the proximal segment of the thin filament, most likely via an F-actin stabilization mechanism, it remains less intuitive how Tmod-regulated pointed-end actin turnover might establish and fine-tune the variable and muscle-specific lengths of the distal segments. Tmod is a dynamic cap that inhibits actin monomer association and dissociation from actin filament pointed ends and regulates actin filament lengths²⁸. Studies in cultured cardiac myocytes, mouse cardiac muscle, and *Drosophila* indirect flight muscle – all of which lack nebulin – have shown that thin filament lengths are inversely proportional to the extent of Tmod-mediated capping, with reduced Tmod levels and capping associated with enhanced actin monomer addition (longer lengths) and increased Tmod levels associated with actin monomer dissociation (shorter lengths)^{29, 30, 32, 58} (see review²⁸). Such a mechanism predicts that elimination of either Tmod1 or Tmod4 from skeletal muscle (i.e., less total Tmod) would result in longer thin filaments, with longer distal segments. However, Tmod1-knockout mice display unexpectedly normal thin filament lengths (with normal proximal and distal segment lengths) in skeletal muscle, due to structural compensation by a sarcoplasmic reticulum-associated Tmod isoform, Tmod3, that does not normally cap the pointed ends of the sarcomeric thin filaments^{25, 59} (see reviews^{28, 60}). Compensation by Tmod3 suggests that preservation of normal thin filament lengths is highly prioritized in skeletal muscle, necessitating additional transgenic systems, such as single-, double-, and triple-knockouts of Tmods 1, 3, and 4, to probe the precise roles of Tmods in distal segment length specification

in skeletal muscle. Moreover, striated muscle expresses a larger Tmod family variant, leiomodlin-2 (Lmod2), that can antagonize Tmod1's pointed-end capping activity under certain conditions ⁶¹. Namely, overexpression of Lmod2 in cultured cardiac myocytes leads to loss of Tmod1 association with thin filament pointed ends and concomitant increases in thin filament lengths ⁶¹. However, other experiments have suggested that Lmod2 functions during myofibril assembly to nucleate assembly of actin filaments that elongate from their barbed ends ⁶². Understanding the timing and mechanisms of Lmod2 function is essential for future refinement of the two-segment model.

Length control by the thick filament

Correlative data have identified the thick filament as an unexpected player in specifying the length of the thin filament distal segment. In fish, mouse, rabbit, and human muscles, muscles with a fast-twitch fiber type composition have shorter thin filament and distal segment lengths, while muscles with a slow-twitch fiber type composition have longer thin filament and distal segment lengths ^{7, 24, 25, 45, 46}. (Note that the lengths of the nebulin-coated proximal segments appear to be independent of fiber type composition ^{24, 25, 45}.) Moreover, in human skeletal muscle fiber bundles, distal segment lengths are negatively correlated with type-2 (fast) myosin heavy chain (MHC) expression, and type-2 MHC-expressing fibers have shorter thin filaments than type-1 (slow) MHC-expressing fibers ²⁴ (Figure 2B). Communication between thin and thick filament components to specify myofilament lengths is also suggested by the correlation between distal segment length and the molecular weight of the giant thick filament scaffold, titin ⁴⁵, which may be involved in setting resting sarcomere tension such that the thin filament pointed ends coincide with the thick filament bare zone at neutral sarcomere lengths (see reviews ^{18, 63}).

It is appealing to speculate that a bare zone-associated protein acts as a targeting signal for Tmod and positions the thin filament pointed end at the bare zone. This targeting signal would function to halt thin filament elongation once the pointed end has cleared the myosin head-containing region of the thick filament and entered the head-free bare zone. The existence of such a targeting signal is supported by observations in *Drosophila* indirect flight muscle, wherein deletion of obscurin leads to thick filament asymmetry, loss of bare zone positioning, and adjustment of pointed-end targeting and thin filament lengths to match the aberrant bare zone positioning ⁶⁴. However, obscurin-knockout mice exhibit normal myofilament dimensions and sarcomere symmetry in skeletal muscle ⁶⁵. Instead, the activity of myosin heads themselves may control Tmod capping and actin association/dissociation rates at pointed ends via actomyosin crossbridge interactions, as proposed previously ¹⁸. Intriguing experiments have suggested that actomyosin contractility influences myofibril assembly and actin dynamics ^{66–69}, but these experiments did not measure thin filament lengths when myosin activity was perturbed. Additional studies are required to identify a specific pointed-end targeting signal or myosin-based F-actin modulation mechanism for the distal segments of mammalian sarcomeres.

Implications for NM

Several exciting recent studies point to both the proximal and distal segments of the thin filaments as structures whose destabilization contributes to the pathogenesis of NM. NM is characterized by nonprogressive muscle weakness and formation of pathological aggregates of thin filament and Z-line protein components ('nemaline bodies') in muscle tissue ⁷⁰. Specific NM-linked nebulin mutations have been shown to reduce thin filament lengths ^{71, 72}, most likely by destabilizing both the proximal and distal segments of the thin filament via mechanisms similar to those observed in nebulin-null mice and nebulin-depleted myotubes ^{51, 54, 55}. Destabilization mechanisms can also account for the phenotype

of a zebrafish NM model, *neb*, which also exhibits shorter overall thin filament lengths, nemaline body formation, and muscle weakness secondary to nebulin deficiency⁷³. On the other hand, a recent study has shown that a specific tropomyosin mutation (R167H in *TPM3*) also reduces thin filament lengths by shortening only the distal segment, with no effects on the length of the nebulin-containing proximal segment⁴⁷ (Figure 2E). The *TPM3*-R167H mutation does not involve the Tmod-binding site on tropomyosin³⁹, suggesting that, instead, the molecular defect induced by this mutation may be reduced affinity of the mutant tropomyosin for actin, leading to thin filament instability, although the effect of the *TPM3*-R167H mutation on the actin-binding activity of tropomyosin has not been examined.

Together, these observations motivate a novel mechanism that contributes to the complex cascade of events leading to the pathogenesis of NM^{70, 74}. Namely, actin depolymerization from the pointed ends leads to net thin filament shortening; simultaneously, the actin that is lost from the thin filaments repolymerizes in the cytosol, where it is cross-linked by α -actinin to form nemaline bodies. The notion that misregulated actin dynamics may be a pathogenic mechanism for NM is consistent with findings that mutations in actin depolymerizing factor (ADF)/cofilin also cause NM, with ADF/cofilin present in nemaline bodies^{75, 76}. Interestingly, thin filament shortening is associated with aberrant troponin-tropomyosin-mediated thin filament activation in both nebulin- and *TPM3*-R167H-linked NM^{47, 71, 72, 77} as well as in nebulin-null mice^{51, 55, 71, 78–80}, suggesting a relationship between thin filament length regulation and tropomyosin-troponin regulation of actomyosin crossbridge cycling. Dissecting the origins of the diverse thin filament abnormalities in muscles from NM patients is a key unresolved ‘chicken-and-egg’ problem at the interface of muscle physiology and cell biology.

Conclusion/perspective

The studies discussed here provide extensive evidence that, in contrast to their classical depictions, the thin filaments in skeletal muscle sarcomeres are not static and uniform structures whose lengths are dictated by a simple nebulin ruler mechanism. Indeed, a single ~1.00-to-1.30- μ m-long thin filament may be host to a fine-tuned gradient of actin dynamics conditions, ranging from remarkable stability in the Z-line-embedded region to rapid subunit exchange at the Tmod-capped pointed end, with two heterogeneous segments in between – the relatively stable, nebulin-containing proximal segment and the comparatively dynamic, nebulin-free distal segment. Sophisticated differential microregulation of actin stability and dynamics along the length of a single filament may be particularly relevant to other long, stable actin-containing structures, such as intestinal epithelial cell microvilli and auditory hair-cell stereocilia. The long actin filaments in these organelles contain distinct inventories of actin-binding proteins associated with the filament rootlet and protrusion regions, which may differentially alter actin dynamics and stability in these regions^{3, 4, 81–83}. Differential microregulation along regionally specialized actin filaments may provide a universal strategy for orchestrating diverse cytoskeletal architectures in functionally specialized metazoan cells.

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Biographies

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Velia M. Fowler is a professor in the Department of Cell Biology at The Scripps Research Institute. Her laboratory studies actin cytoskeleton-mediated regulation of cellular architecture and physiology, with an emphasis on the tropomodulin and tropomyosin families of actin-regulatory proteins and their functions in erythrocytes, the ocular lens, and striated muscle.

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BOX 1

Dependence of the sarcomere length-tension relationship on thin filament lengths

The sarcomere length-tension relationship, which quantifies a muscle's force output as a function of myofilament overlap, was first described in classic physiological experiments performed on frog muscle^{9, 10}, leading to the prediction that alterations in thin filament length may lead to a change in the shape of the length-tension relationship⁸. This prediction was first validated in perch muscles⁷ and, subsequently, in muscles from nebulin-knockout mice, nebulin-deficient NM patients^{56, 71}, and, most recently, a NM patient with a *TPM3*-R167H mutation⁴⁷. In (A), theoretical sarcomere length-tension curves for frog sartorius and human pectoralis major muscles were constructed based on known myofilament dimensions^{7–10}. The frog sartorius and human pectoralis major have thin filament lengths of ~ 0.94 and ~ 1.37 μm , respectively, but identical thick filament lengths of 1.65 μm ^{24, 84}. This wide range of thin filament lengths is functionally relevant, as the optimum sarcomere length for force production commences at twice the thin filament length, while the maximum sarcomere length for force production is the sum of twice the thin filament length plus the thick filament length^{7–10}. These functionally significant sarcomere lengths are ~ 1.88 μm and ~ 3.53 μm in the frog sartorius, and ~ 2.74 μm and ~ 4.39 μm in the human pectoralis major. *a–d* indicate sarcomere lengths corresponding to critical points on the length-tension curves. In (B), schematics of myofilament configurations corresponding to the critical sarcomere lengths *a–d* labeled in (A) are shown for both the frog sartorius and human pectoralis major. Note that, while the resting sarcomere length homogeneity and near-perfect myofibril alignment in frog and fish muscles allow the critical points of experimental sarcomere length-tension relationships to be clearly discerned (as depicted here), actual experimental length-tension curves collected from mammalian muscles are 'smoothed' due to sarcomere length heterogeneity, non-negligible myofibril misalignment, and contributions from non-contractile material (e.g., connective tissue).

FIGURE BOX 1

BOX 2**Imaging thin filament architecture: methodological considerations**

Mapping skeletal muscle-specific thin filament architecture requires high-precision measurements of: (i) total thin filament length; (ii) the position of the Z-line (e.g., α -actinin or CapZ); (iii) distance of the nebulin N-terminus from the Z-line; (iv) distance of Tmod from the Z-line. Classically, myofilament lengths were measured using transmission electron microscopy⁸⁴, and, recently, electron microscopy has been refined via electron tomography of tilt series, enabling three-dimensional visualization of thin filament trajectories within sarcomeres⁸⁵. While electron microscopy methods are impressively precise, affording nanometer-scale resolution, these methods are also constrained by labor-intensive and time-consuming tissue processing procedures, fixation/shrinkage artifacts, and an inability to identify specific protein targets (most notably the nebulin N-terminus, which does not localize to a discernable structural landmark along a thin filament viewed by electron microscopy).

A more technically straightforward approach is confocal laser scanning microscopy-based localization of fluorescently labeled thin filament components, viewed in either isolated myofibril preparations or muscle tissue cryosections. However, point-and-click measurements of Z-line, nebulin N-terminus, and Tmod localizations are constrained by user error as well as the intrinsic impossibility of locating the center of a light source's point-spread function without the aid of computational tools, likely leading to substantial length measurement errors of ~0.2–0.3 μm , as limited by the resolution of optical microscopy. A series of studies from our laboratory has combined the ease of immunofluorescence microscopy with dramatically improved resolution by using Distributed Deconvolution (DDecon), a super-resolution computational averaging technique that computes thin filament and proximal/distal segment lengths with a precision of 10–20 nm by directly determining the peak positions of fluorescently labeled thin filament proteins with respect to the Z-line using line-scans of fluorescence intensities along myofibrils⁴⁶ (as depicted here for an immunostained cryosection of human pectoralis major muscle). Using the line-scan data, the DDecon algorithm generates the best-fit of a model intensity distribution function for a given thin filament component to an experimental one-dimensional myofibril fluorescence intensity profile.⁴⁶ DDecon enables rapid collection of large volumes of high-precision thin filament length data along with proximal and distal segment length data, providing a tractable and efficient assay for analyzing thin filament architecture in experimental muscles of interest^{24, 25, 45, 47}.

FIGURE BOX 2

Figure 1. The nebulin ruler model for thin filament length regulation in skeletal muscle

In this now-obsolete model, thin filaments are uniform structures whose lengths (i.e., CapZ-to-Tmod distances) are specified by nebulin functioning as a molecular ruler (see text), with increasing nebulin molecular weight (MW) associated with increasing thin filament length^{44, 45}. For simplicity, thin filaments are depicted with only one actin filament strand, one tropomyosin polymer, and one nebulin molecule. B, thin filament barbed end; P, thin filament pointed end; Z, Z-line; M, M-line.

Figure 2. A novel two-segment model for thin filament architecture in skeletal muscle

In vertebrate skeletal muscle, thin filaments consist of two concatenated segments (see text). The proximal segment originates at the Z-line, where CapZ caps the barbed end of the thin filament. The remainder of the proximal segment is coated with nebulin and has a constant length ($\sim 0.95 \mu\text{m}$ in most vertebrate muscles) ^{24, 25, 45}. The proximal segment is followed by the distal segment, which is nebulin-free, dynamically capped by Tmod at the pointed end, and has variable length, depending on the muscle type and species ^{24, 25, 45–47}. Z, Z-line; M, M-line. (A) At resting sarcomere lengths, the thin filament pointed ends coincide with the edge of the thick filament bare zone at the center of the sarcomere ¹⁸. (B) In vertebrate skeletal muscle, distal segments are longer in slow-twitch fibers than in fast-twitch fibers ^{24, 25, 45}. (C) Cardiac muscle and nebulin-null skeletal muscles lack nebulin and, hence, display thin filaments that lack proximal segments and consist exclusively of distal segments ^{50–54}. Nebulin-free thin filaments have an average length of $\sim 0.95 \mu\text{m}$ and may exhibit more rapid actin turnover at their pointed ends, resulting in more variable lengths as compared to nebulin-containing thin filaments ^{51, 54}. (D) Skeletal myocytes expressing mini-nebulin ($\sim 250 \text{ kDa}$) have a much shorter proximal segment length of $\sim 0.22 \mu\text{m}$. Reduced proximal segment length is not accompanied by changes in the distance of Tmod from the Z-line, thereby resulting in a dramatically longer distal segment ⁵⁴. Mini-nebulin also reduces (i.e., rescues) actin and Tmod dynamics in nebulin-depleted myotubes ⁵⁴. (E) Human skeletal muscle from a patient with an NM-causing *TPM3*-R167H mutation shows a dramatically attenuated distal segment, such that the entire thin filament consists of the proximal segment ⁴⁷. The *TPM3*-R167H mutation also reduces Ca^{2+} sensitivity during troponin-tropomyosin-mediated thin filament activation ⁴⁷. For simplicity, thin filaments are depicted with only one actin filament strand, one tropomyosin polymer, and one nebulin molecule. The relative lengths of the proximal and distal segments of the thin filaments are approximately to scale.