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Review

Thin filament length regulation in striated muscle sarcomeres: Pointed-end dynamics go beyond a nebulin ruler

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ABSTRACT

The actin (thin) filaments in striated muscle are highly regulated and precisely specified in length to optimally overlap with the myosin (thick) filaments for efficient myofibril contraction. Here, we review and critically discuss recent evidence for how thin filament lengths are controlled in vertebrate skeletal, vertebrate cardiac, and invertebrate (arthropod) sarcomeres. Regulation of actin polymerization dynamics at the slow-growing (pointed) ends by the capping protein tropomodulin provides a unified explanation for how thin filament lengths are physiologically optimized in all three muscle types. Nebulin, a large protein thought to specify thin filament lengths in vertebrate skeletal muscle through a ruler mechanism, may not control pointed-end actin dynamics directly, but instead may stabilize a large core region of the thin filament. We suggest that this stabilizing function for nebulin modifies the lengths primarily specified by pointed-end actin dynamics to generate uniform filament lengths in vertebrate skeletal muscle. We suggest that nebulette, a small homolog of nebulin, may stabilize a correspondingly shorter core region and allow individual thin filament lengths to vary according to working sarcomere lengths in vertebrate cardiac muscle. We present a unified model for thin filament length regulation where these two mechanisms cooperate to tailor thin filament lengths for specific contractile environments in diverse muscles.

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The uniformity of actin (thin) filament lengths in striated muscle has been an intriguing problem since 1954 when striated myofibrils were shown to contract by the relative sliding of thin and myosin (thick) filaments [1–3]. Sliding filament theory is the structure–function relationship for striated myofibrils that relates muscle contraction to the molecular organization of proteins within the sarcomere, the basic contractile unit of striated muscle (Fig. 1A–C). Length is not an intrinsic property of actin filaments, as actin monomers assemble *in vitro* and *in vivo* to many allowed polymer lengths [4,5]. Since striated muscle thin filaments all have the same ~1 μm lengths, their lengths must be regulated by actin-binding proteins. How are their lengths specified? At the advent of sliding filament theory, thin filaments were for all intents and purposes thought to be static. Now, we know that

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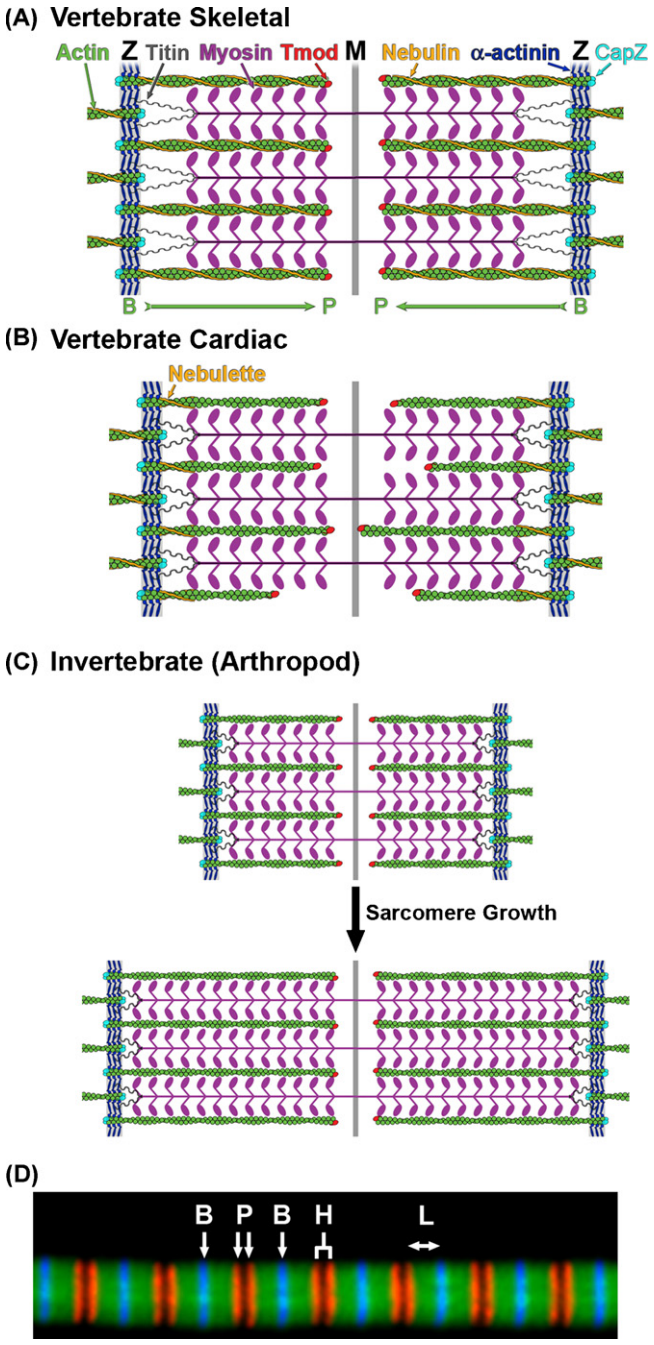

Fig. 1. Thin filament organization in striated sarcomeres. Diagrams of vertebrate skeletal (A), vertebrate cardiac (B) and invertebrate (C) striated muscle sarcomeres showing the location of actin-binding proteins and the organization of thin and thick filaments. Elastic titin filaments (gray) extend an entire half-sarcomere from Z-line (Z) to M-line (M) to form a stable, yet flexible myofibril scaffold. Actin (thin) filaments (green) are precisely aligned with their fast-growing (barbed, B) ends at the Z-line where they are capped by CapZ (cyan) and crosslinked by α-actinin (blue) to thin filaments in adjacent sarcomeres. The non-crosslinked (free) portions of the thin filaments extend ~1 μm away from the Z-line, overlap with the 1.6 μm bipolar myosin (thick) filaments (purple), and their slow-growing (pointed, P) ends are capped by tropomodulin (Tmod, red). Tropomyosin/troponin (TM/Tn) complexes copolymerize along the entire free portions of the thin filaments where they regulate myosin motor binding and bind Tmod at the pointed filament end (not shown). Nebulin (gold, A) is anchored at the Z-line and extends ~1 μm along skeletal muscle thin filaments. Nebulette (gold, B), a nebulin homolog, extends ~150 nm along cardiac muscle thin filaments. Invertebrate (arthropod) muscles (C, shown at a reduced scale) have titin-like proteins that connect thick filaments to Z-lines (gray) and do not have nebulin homologs. Thin and thick filaments elongate to maintain overlap during sarcomere growth. (D) Thin filaments (green, phalloidin) are readily observed by light microscopy. Barbed (B) and pointed (P) ends are visualized using α-actinin (blue) and Tmod (red) antibodies, respectively. A gap in phalloidin staining (H zone, H) indicates that thin filaments are regulated in length. Narrow Tmod striations indicate that thin filament lengths are uniform. Narrow α-actinin striations indicate that thin filament barbed ends are precisely aligned at Z-lines. The distance between Tmod and associated Z-lines (L) is equal to thin filament length [81].

actin subunits continuously associate and/or dissociate from the polymer ends despite the uniformity of filament lengths in these and other actin structures [6–8]. Thus, mechanisms to control thin filament length must ultimately rely upon a length-dependent control of actin association and dissociation rates at the polymer ends.

Striated muscle is probably one of the best-studied systems of actin filament length regulation. This is due to the repetitive and hierarchical organization of actin filaments into sarcomeres, myofibrils, and muscle fibers. Alternating stripes of thin filament barbed and pointed ends in sarcomeres are located along the myofibrils (Fig. 1D). The uniform lengths of thin filaments are evidenced by a gap in the middle of the sarcomere (H zone). The locations of barbed ends at the Z-lines, tropomodulin (Tmod) at the pointed ends, and the distance between them (filament length) can be resolved easily by light microscopy, making precise length measurements along with analyses of actin dynamics at filament ends uniquely accessible to experimentation. Nevertheless, challenges arise because the sarcomere is a complex nano-machine [9,10]. Nearly every protein in the sarcomere binds to actin and could in theory affect actin monomer–polymer dynamics and thin filament lengths. Many sarcomeric proteins also regulate one another's interactions, and are regulated by extracellular and cellular signals and mechanics. Obligatory changes in sarcomere length that accompany contraction can interfere with the interpretation of experiments, if not taken into account. Besides being possible candidates for regulating thin filament lengths, most other sarcomere proteins have established roles in contraction or myofibril assembly. Consequently, mutations or knockout approaches often block earlier events during myofibril assembly, such as thin filament alignment, precluding measurements of thin filament lengths.

Since our previous review on thin filament length regulation [10], there has been significant and exciting experimental progress in the field, especially on the roles of pointed-end capping by Tmods and the proposed molecular ruler protein, nebulin. We have organized this review into five sections: First, we discuss evidence that regulation of actin filament pointed end dynamics by Tmod and other actin regulatory proteins controls thin filament lengths, since this provides the underpinnings of any mechanistic explanation for filament length regulation. Second, we discuss how indirect effects due to perturbation of the actin monomer economy can confound analysis of thin filament length regulation during thin filament assembly and muscle development. Third, we review the nebulin ruler model and possible mechanisms for how it may specify uniform thin filament lengths within a sarcomere, and particular thin filament lengths for different muscles. Fourth, we review recent evidence suggesting that a nebulin-independent mechanism may specify thin filaments in vertebrate muscle. Finally, we discuss the relationship between filament length regulation, sarcomere length and muscle contraction; it is the thesis of this review that mechanisms controlling thin filament lengths are tailored to sarcomere physiology. In this context we suggest that mechanisms specifying thin filament lengths emerge from mechanisms that regulate myofilament overlap.