CELL STRUCTURE AND FUNCTION 22: 191–195 (1997)

Models of Thin Filament Assembly in Cardiac and Skeletal Muscle

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Key words: Tropomodulin/Cardiac Muscle/Skeletal Muscle/Actin Filament/Myofibril Assembly/Capping Proteins

ABSTRACT. **The assembly and functional characteristics of many contractile proteins are different in skeletal and cardiac muscle. Two models for thin filament assembly are consistent with observations from recent studies focused on determining the functional significance of actin filament capping in primary cultures of embryonic chick myogenic cells and cardiac myocytes. Future experiments will test the validity of the proposed models for *in vivo* embryonic development.**

Perfectly aligned myofibrils in striated muscle represent one of the most highly ordered macromolecular structures found in eukaryotic cells. The assembly of contractile proteins into myofibrils is a complex process that requires coordinate synthesis of the constituent proteins, polymerization of actin and myosin (and many associated proteins) into thin and thick filaments, respectively, and association of the two filament systems into highly organized sarcomeres. Newly assembled sarcomeres consist of parallel arrays of approximately 1.0 μm-long thin filaments that interdigitate with laterally aligned 1.6 μm long thick filaments. Rodlike tropomyosin molecules are associated with each other head-to-tail, forming two polymers per thin filament. Each tropomyosin molecule binds one troponin complex (composed of troponins T, I and C); together they mediate the calcium regulation of myosin ATPase (35). Thin filaments are polarized in muscle sarcomeres with the barbed ends crosslinked by α-actinin and anchored at the Z disk, and the pointed ends terminating in the A band and overlapping with the bipolar myosin filaments (11). ("Barbed" and "pointed" ends of actin filaments refer to the orientation of arrowheads generated by myosin subfragment 1 binding to actin filaments and are the fast-growing and the slow-growing ends of the filaments, respectively.)

The regulation of actin filament length and spatial organization is an essential property of all striated muscles: contractile activity is critically dependent on it. Although little is known about the complex mechanisms required for this regulation during myofibrillogenesis, a diverse repertoire of interacting components appears to be involved. For example, there are actin binding proteins responsible for regulating the rate and site of assembly of the filaments (either by binding to influence the filamentous form of the molecule or to sequester soluble subunits), whereas others stabilize and link actin filaments to one another or to other cellular components (for reviews see: 3, 7, 21, 27). In addition, it has been proposed that template molecules exist to specify the characteristic lengths of actin filaments within sarcomeres (e.g., nebulin in skeletal muscle) (for reviews see: 7, 12, 32). Interestingly, thin filament lengths are more precisely determined in the sarcomeres of skeletal muscle compared with cardiac muscle. This suggests that the mechanisms utilized to maintain a stable length distribution of actin filaments in skeletal and cardiac muscle are different. Specifically, thin filaments in cardiac muscle vary in length from 0.8 μm to about 1.3 μm (24), while the distribution of thin filament lengths in skeletal muscle is significantly narrower at 1.11 ± 0.03 μm (30). Although the distribution of filament lengths in cardiac muscle is broader than in skeletal muscle, a regulatory mechanism must be present since the thin filament lengths resemble a Gaussian and not the exponential distribution seen with pure actin filaments *in vitro*.

The length of actin filaments is regulated in part by actin filament capping proteins at both the barbed and pointed ends. Capping proteins inhibit the addition and loss of actin monomers at the ends of actin filaments *in vitro* (for reviews see: 10, 27, 37). In striated muscle, capZ and tropomodulin are the capping proteins for the barbed and the pointed ends of the actin filaments, respectively (5, 9, 36). CapZ is one of a large family of proteins which cap the barbed ends of the actin filaments in many cell types, while tropomodulin is the only cellular protein identified so far to cap the pointed ends of the actin filaments. In contrast to barbed end capping proteins which cap independently of other proteins, tropomodulin requires tropomyosin for its full ac-

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tivity *in vitro* ($K_d \leq 1$ nM). It is likely that "tight" capping by tropomodulin requires binding to both tropomyosin and actin and reflects the sum of the binding energies for both molecules (1, 36). In the absence of tropomyosin, tropomodulin is a "leaky" cap, and only partially blocks elongation and depolymerization at the pointed ends of actin filaments ($K_d = 0.1$–0.4 μm) (36).

Since all of the barbed ends of the thin filaments are precisely aligned in mature Z-disks, the variability in length seen in cardiac muscle must result from differential regulation at the pointed ends of the filaments (in the center region of the sarcomere) (24). Consistent with the strict alignment of the barbed ends of the filaments in both cardiac and skeletal muscle, capZ assembles early during myofibril assembly in both primary cultures of chick embryonic skeletal myogenic cells and cardiac myocytes [that is, before the appearance of actin filament striations (25, 26)]. This observation, together with the characteristics of capZ deduced from *in vitro* studies (2, 4), suggests that capZ functions to nucleate actin filament polymerization at the Z-line, and to organize (e.g., establish polarity) and maintain thin filament length at the barbed end from the earliest stages of myofibril assembly in both of these striated muscle cell types. Thus, while similar mechanisms of length regulation appear to be utilized in skeletal and cardiac muscle at the barbed ends of the thin filaments, it seems likely that the mechanism for length regulation at the pointed ends are considerably different.

Increasing evidence supports the notion that thin filament length regulation at the pointed ends differs in the two muscle types. For example, in contrast to capZ, the assembly properties of the same isoform of the pointed end capping protein tropomodulin are significantly different in skeletal and heart muscle. In skeletal myogenic cells, tropomodulin is assembled early during myofibrillogenesis (A. Almenar-Queralt, C.C. Gregorio and V.M. Fowler, Mol. Biol. Cell, 546a. 1996). It is detected in a periodic pattern in premyofibrils, preceding the detection of actin striations. This suggests that thin filament pointed ends become capped early in skeletal myofibril assembly before the separation of actin filaments into two half sarcomeres and the appearance of H zones (gaps in the center of the sarcomere which give actin filaments their mature striated appearance). In contrast, in cardiac myocytes, tropomodulin is incorporated into sarcomeres late during myofibril assembly (8). It is not detected in the assemblies of contractile proteins present during early stages of myofibril assembly (for example, nonstriated premyofibrils) and is incorporated into myofibrils only after other myofibrillar proteins including titin, the thick filament proteins myosin and C-protein, and the thin filament proteins α-actinin, actin and tropomyosin are assembled in their characteristic mature striated patterns. The delayed appearance of tropomodulin with respect to the other contractile proteins indicates that the pointed ends are uncapped in initial stages of myofibril assembly in cardiac muscle. It should be pointed out that the existence of a new, currently undetectable, isoform of tropomodulin or a more distantly related pointed end capping protein might be associated with the pointed ends of thin filaments in immature cardiac myofibrils. Interestingly, however, once tropomodulin is assembled in cardiac myocytes it functions to maintain the length of actin filaments and is essential for cell beating (9). Based on the assembly and functional characteristics of tropomodulin, it is likely that this protein is directly involved in conferring the different molecular mechanisms of length regulation that exist in skeletal and cardiac muscle.

Other contractile proteins are also known to have different properties in skeletal and cardiac muscle. For example skeletal muscle, but not cardiac muscle, contains the giant protein nebulin ($\sim$776 kDa: Labeit and Kolmerer, 1995). A single molecule of nebulin inserts in the Z-disk, associates with the thin filament along its length and terminates in close proximity to the pointed ends of the thin filaments. Nebulin has been proposed to be a template for actin filament length specification based on its repeating domain structure, close association with thin filaments, inextensible nature during contraction and stretching of myofibrils, and the observation that the molecular mass of nebulin correlates with the length of thin filaments found in different skeletal muscles (14, 15, 34). To date, no putative template molecule has been identified in cardiac muscle, although a small 107 kDa nebulin-related molecule (nebulette) is present in this tissue (20). A role for nebulette in length regulation is improbable since it is predicted to interact with the Z-disk and maximally extend only about 25% of the length of the thin filaments.

Two thick filament-associated proteins also have different assembly properties in skeletal and cardiac muscle. Zuegmatin, a protein which is now known to be part of the Z-disk region of the giant protein titin, associates with nascent myofibrils of skeletal muscle prior to the addition of α-actinin (17, 33). On the other hand, in cardiac myocytes, it is not associated with the myofibrils until after the addition of α-actinin (23). Additionally, myomesin is only found associated with striated myofibrils in chicken cardiac myocytes, whereas in skeletal myogenic cells, it is found associated with both nonstriated premyofibrils and mature striated myofibrils (28).

Although other models of thin filament assembly might be plausible, a "Fixed Length" model of a thin filament assembly in skeletal muscle and a "Variable Length" model of thin filament assembly in cardiac muscle are consistent with the known properties of actin fila-

ment capping proteins and other sarcomeric components.

Fixed Length Model for Skeletal Thin Filament Assembly.

In the "Fixed Length" model of skeletal muscle thin filament assembly (Fig. 1, right side), during early stages actin polymerizes with tropomyosin, the troponins, capZ and tropomodulin into 1 μm long thin filaments. At this stage, capZ is responsible for nucleating actin filament assembly and specifying the polarity of the actin filaments within the sarcomere. Additionally, both capZ and tropomodulin cap the ends of the thin filaments, preventing net elongation or depolymerization of the filaments. The presence of both the barbed and pointed end capping proteins in premyofibrils suggests that in skeletal muscle the length of the thin filaments may be determined and maintained from the earliest stages of myofibril assembly. The filaments are crosslinked together by α-actinin forming "I-Z-I"-like complexes (see 6 and references therein). In most premyofibrils, the complexes appear to be linearly arranged with an average spacing of 1.5 μm between the nascent Z-disks (A. Almenar-Queralt, C.C. Gregorio and V.M. Fowler, Mol. Biol. Cell, 546a. 1996, 31). This distance is sufficient to accommodate the 1 μm long filaments, although the thin filaments would have to overlap extensively at their pointed ends in the short sarcomeres. The overlap of the filaments results in a nonstriated staining pattern for the actin filaments. This staining pattern might be consistent with electron micrographs of nascent myofibrils which show that some actin filaments are continuous through the developing Z-disk and the A-band of newly formed sarcomeres in young myo-

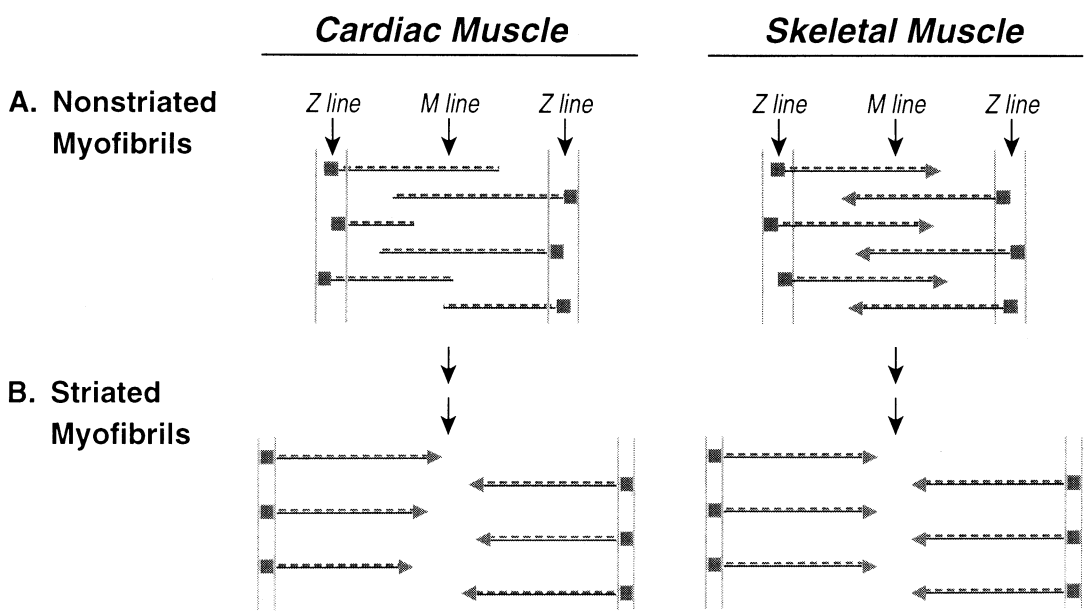

Fig. 1. Models for Thin Filament Assembly in Striated Muscle.
"Variable Length" model for Cardiac Muscle Thin Filament Assembly. During early stages of myofibril assembly (Nonstriated Myofibrils), nascent Z-disks containing capZ are assembled with actin, tropomyosin and the troponins to variable lengths and the filaments are crosslinked by α-actinin into nascent I-Z-I-like structures which are spaced ~1.5 μM apart. While the pointed ends in the nonstriated premyofibrils are uncapped, the thin filament barbed ends are capped and maintained by capZ. The overlap of the variable length actin filaments can account for the nonstriated appearance of actin filament staining at this stage. During subsequent stages of assembly, by as yet unknown mechanisms, the barbed ends of the filaments become precisely aligned into narrow striations and elongation and/or shortening of filaments occurs to generate the 1 μM long mature thin filaments (Striated Myofibrils). Later, thin filaments become mature in the presence of a putative "third factor" and their length is subsequently maintained (capped at their pointed ends) by tropomodulin.
"Fixed Length" model for Skeletal Muscle Thin Filament Assembly. During early stages of myofibril assembly (Nonstriated Myofibrils), actin polymerizes with tropomyosin, the troponins, capZ and tropomodulin into 1 μm long thin filaments. The filaments are cross-linked together by α-actinin into nascent I-Z-I-like structures which are spaced ~1.5 μM apart. The thin filament barbed and pointed ends are capped and maintained by capZ and tropomodulin, respectively. The filaments overlap extensively at their pointed ends in the short sarcomeres; thus, the staining for the actin filaments appears nonstriated. Later in myofibril assembly (Striated Myofibrils) the pointed and barbed ends of the filaments become precisely aligned into narrow striations and the thin filaments in each half sarcomere separate to form gaps (H-zones).
For simplicity, the myosin-containing thick filaments, as well as the titin and nebulin "third filament" systems, have been omitted from these models of the sarcomere. *Vertical lines*, Z-line components such as α-actinin; *boxes*, capZ; *arrowheads*, tropomodulin; *dashed lines*, tropomyosin and the troponins; and *solid horizontal lines*, actin filaments.

tubes (13, 22, 29). The incomplete alignment of the ends of the filaments at this stage is reflected by the broad width (>1.5-fold wider than observed in mature myofibrils) of the Z-disks and the tropomodulin stained regions in nonstriated premyofibrils (13, 18, 19, 22, and A. Almenar-Queralt, C.C. Gregorio and V.M. Fowler, Mol. Biol. Cell, 546a. 1996). Later in myofibril assembly, by unknown mechanisms the pointed and barbed ends of the filaments become precisely aligned into narrow striations and the thin filaments separate to form well defined H-zones in the middle of the sarcomere giving the actin filaments their mature striated appearance.

Variable Length Model of Thin Filament Assembly in Cardiac Muscle.

In the "Variable Length" model of cardiac muscle thin filament assembly (Fig. 1, left side), nascent Z-disks containing capZ and α-actinin are assembled with the thin filament proteins actin, tropomyosin and troponins to form I-Z-I-like complexes. At this stage, like in the "Fixed Length" model, capZ is required for nucleating assembly and specifying the polarity of the actin filaments within the sarcomere. In addition, the lengths of actin filaments at their barbed ends are maintained by capZ during these early stages of sarcomere assembly. However, in the absence of tropomodulin, the pointed ends are predicted to be uncapped and the absolute length of the individual polarized thin filaments in nonstriated premyofibrils is expected to be less well defined (i.e., both shorter *and* longer than their mature 1 μm lengths) than the length of thin filaments found in mature sarcomeres. As in the "Fixed Length" model, the overlap of the actin filaments at their pointed ends accounts for the nonstriated appearance of actin filament staining in premyofibrils. During subsequent stages of assembly, by as yet unknown mechanisms, the barbed ends of the filaments become precisely aligned into narrow striations and elongation and/or shortening of filaments occurs to generate the 1 μM long mature thin filaments: these processes give the actin filaments their mature striated appearance. In this model, the acquisition of mature thin filament length may be contingent on the absence of tropomodulin and thus uncapped actin filament pointed ends in nonstriated myofibrils. It should be noted that it is unclear how actin and tropomyosin can assume their striated appearance in the absence of tropomodulin, especially based on tropomodulin's critical role as an actin filament capping protein in mature myofibrils in this cell type. Lastly, thin filaments become mature in the presence of a putative "third" factor and the actin and tropomyosin polymers are subsequently stabilized (capped) at their pointed ends by tropomodulin. In this capacity, the assembly of tropomodulin into sarcomeres is required to maintain the final length of thin filaments, as well as perhaps being the rate-limiting step required to orchestrate muscle contraction.

Interestingly, although tropomodulin is present in the soluble pool during *all* stages of myofibril assembly in cardiac myocytes (as in skeletal myogenic cells) it only assembles onto actin filaments after the actin filaments have attained their mature lengths (8, A. Almenar-Queralt, C.C. Gregorio and V.M. Fowler, Mol. Biol. Cell, 546a. 1996). Therefore, it is hypothesized that an additional sarcomeric component(s), besides for actin or tropomyosin is responsible for targeting the early assembly of tropomodulin to the pointed ends of the thin filaments in skeletal muscle nonstriated premyofibrils and to the pointed ends of the thin filaments in mature cardiac muscle striated myofibrils.

Summary

Based on the known role of tropomodulin in capping and maintaining actin filament length, it is hypothesized that the different assembly properties of tropomodulin contribute to the different mechanisms of thin filament assembly and length regulation which lead to a 30% variation in actin filament lengths in cardiac muscle, and a 3% variation in length in skeletal muscle. Future experiments are required to verify the proposed models during embryonic striated muscle differentiation.

Acknowledgment. I would like to thank Dr. Velia Fowler (The Scripps Research Institute, La Jolla, CA) for her insightful comments and support during my tenure as a postdoctoral fellow in her laboratory.

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