Published in final edited form as:

Prog Mol Biol Transl Sci. 2019 ; 166: 311–340. doi:10.1016/bs.pmbts.2019.03.014.

Role of intrinsic disorder in muscle sarcomeres

Dmitri Tolkatchev*, Garry E. Smith Jr., Alla S. Kostyukova

Voiland School of Chemical Engineering & Bioengineering, Washington State University, Pullman, WA 99164-6515, USA

Abstract

The role and utility of intrinsically disordered regions (IDRs) is reviewed for two groups of sarcomeric proteins, such as members of tropomodulin/leiomodin (Tmod/Lmod) protein homology group and myosin binding protein C (MyBP-C). These two types of sarcomeric proteins represent very different but strongly interdependent functions, being responsible for maintaining structure and operation of the muscle sarcomere. The role of IDRs in the formation of complexes between thin filaments and Tmods/Lmods is discussed within the framework of current understanding of the thin filament length regulation. For MyBP-C, the function of IDRs is discussed in the context of MYBP-C-dependent sarcomere contraction and actomyosin activation.

Keywords

intrinsically disordered region; sarcomere; myosin binding protein C; tropomodulin; leiomodin; tropomyosin; actin; myosin; thin filament; thick filament

Introduction

Sarcomere, which is the basic unit of striated muscles, is a complex multicomponent biological system controllably transforming the chemical energy released upon ATP hydrolysis into mechanical work. Since the change in sarcomere length in a contracting muscle requires the presence of well-coordinated moving parts, many muscle proteins evolved to quickly adapt to the variable molecular environment and geometry.¹ Consequently, protein regions that do not possess a rigid three-dimensional structure are expected to play important roles in the sarcomere functions.

Protein components and architecture of the sarcomere have been extensively reviewed, and the interested reader is directed to a review by Henderson *et al.*² for more comprehensive information on the subject. Here, we will mainly focus on functionalities of intrinsically disordered regions (IDRs) in two key muscle protein groups within the context of the sarcomere structure and operation (Fig. 1). One protein group, comprising skeletal and cardiac isoforms of myosin-binding protein C (MyBP-C), is recognized as having a regulatory role in sarcomere contraction.^{2–4} Another protein group consists of several isoforms of tropomodulin/leiomodin homology family, and it is known to regulate thin filament formation.^{2,5–7} Both groups represent two different yet strongly related aspects of

* dmitri.tolkatchev@wsu.edu.

the sarcomere function, namely its structure and how this structure enables normal (or abnormal, if in disease) muscle performance in a constantly changing environment.

Myosin-binding protein C (MyBP-C)

MyBP-C is a modular multi-domain protein playing a critical part in myofilament function (see e.g. reviews;^{8,9} and more recently published work on cardiac^{10,11} and skeletal MyBP-C isoforms¹²). The cardiac MyBP-C (cMyBP-C) regulates cardiac contraction kinetics and activates actomyosin interactions.^{13–17} Mutations in cardiac MyBP-C are frequently associated with familial hypertrophic cardiomyopathy,^{18–20} a common genetically transmitted cardiovascular disease, which is characterized by hypertrophy of the left ventricle.

MyBP-C exists in three isoforms, slow-skeletal (ssMyBP-C), fast-skeletal (fsMyBP-C), and cardiac (cMyBP-C).^{21,22} Each of the two skeletal MyBP-C isoforms contains ten independently folded β -sandwich domains, specifically seven immunoglobulin-like domains (Ig) and three fibronectin type III-like domains (Fn3). The domains are termed sequentially from C1 to C10. The cMyBP-C includes an additional Ig domain C0, which is located at the protein N-terminus, before C1.⁸ MyBP-C tethers the thin and thick filaments via its N- and C-terminal domains, respectively, and this bridging is believed to modulate filament stability and sliding.²³ On the other hand, the N-terminal region of MyBP-C was shown to also bind to myosin,^{24–26} which suggests the existence of more than one binding mode of MyBP-C. The concurrent existence and repopulation of MyBP-C thin filament and thick filament binding modes could play a role of an additional regulatory mechanism.^{25,26}

Two IDRs in MyBP-C are represented by a myosin-binding motif (M-domain) and a Pro/Ala-rich region, or a PA-linker (PAL).⁸ M-domain connects domains C1 and C2, while PAL precedes the C1 domain. In cMyBP-C, which includes the N-terminal C0 domain (absent in skeletal MyBP-C isoforms), the PAL domain serves as a linker between domains C0 and C1. Since both IDR domains are located in the N-terminal part of MyBP-C, we will focus on the function of the N-terminal regulatory region of MyBP-C, specifically on domains up to and including C2. These will correspond to the N-terminal domains (NTDs) C0-PAL-C1-M-C2 (C0C2) in cMyBP-C and PAL-C1-M-C2 (PALC2) in skeletal MyBP-C isoforms. Both C0C2 (cardiac) and PALC2 (skeletal) regions are known to activate thin filaments at low calcium concentration by pushing tropomyosin from a “blocked” towards a “closed” position and exposing the myosin-binding site, although these MyBP-C isoforms produced different effects.^{12,17}

M-domain—M-domain consists of approximately 100 amino acid residues. Early circular dichroism (CD) and small angle X-ray scattering (SAXS) experiments on cardiac C0C2 and C1-M-C2 (C1C2) fragments led to a proposal that M-domain is a β -strand-rich compact structure with a presumably Ig-like fold.²⁷ However, this conclusion contradicted later structural investigations.^{28,29} Nuclear magnetic resonance (NMR) chemical shift dispersion and heteronuclear ¹H-¹⁵N NOE (nuclear Overhauser effect) experiments for the ¹⁵N-labeled cardiac C1C2 (ref.²⁸) suggested that the M-domain was considerably less rigid than could be expected from the earlier report.²⁷ Mechanical unfolding of the full-length cMyBP-C using

atomic force microscopy (AFM) was consistent with both PAL and M-domain being at least partially unfolded and lacking a well-defined three-dimensional structure.²⁹ In addition, when a smaller fragment of cMyBP-C incorporating the M-domain; i.e., C1C2, was subjected to mechanical stretching, the force extension spectra showed the presence of only two unfolding force peaks, which were assigned to the unfolding of C1 and C2. Since the contour length of the M-domain appeared to be somewhat shorter than the maximum theoretical contour length, the authors concluded that even though the M-domain lacks an overall stable three-dimensional fold, it may contain some secondary structure elements, such as short α -helices.

A better understanding of the structural properties of an isolated M-domain (amino acid residues 255–357 of mouse cMyBP-C) was achieved by using 3D heteronuclear NMR spectroscopy.³⁰ Measured ^1H - ^{15}N NOE values divided the protein into two distinct regions. The N-terminal residues 255–314 appeared to be disordered, while the residues 315–349 showed ^1H - ^{15}N NOE values typical of a protein with stable three-dimensional structure. Long-range ^1H - ^1H NOEs giving proton-proton distances that are typically used for NMR structure calculations were difficult to analyze due to spectral overlap. Therefore, a chemical shift-based structural approach was used to establish that the residues 315–349 form a three-helix bundle (THB) fold often found in actin-binding proteins. The formation of the THB was confirmed independently by solving a three-dimensional NMR structure of the human cMyBP-C fragment that included the THB motif followed by the C2 domain.³¹ Interestingly, upon binding to another protein binding partner of cMyBP-C – calmodulin,³² the THB undergoes large conformational changes.³¹ The three helices were proposed to bind in an extended mode where the helices and a short unfolded linker tethering THB and C2 wrap around calmodulin.³¹ This suggests that the even though THB is a structural domain well defined by NMR, it has some characteristics of an intrinsically disordered region in that its bound conformation to calmodulin has little resemblance to its unbound structural features.

Phosphorylation of the M-domain is an important mechanism of MyBP-C function regulation.^{33–36} Slow skeletal MyBP-C isoform has one known phosphorylation site in the M-domain, the M-domain of cMyBP-C has four known phosphorylation sites, while none were discovered in the M-domain of the fsMyBP-C. M-domain phosphorylation accelerates cross-bridge kinetics in cMyBP-C,³⁷ and it was shown to modulate myofilament length-dependent activation.³⁸ Replacement of three phosphorylatable Ser residues with inert Ala residues led to cardiac hypertrophy.³⁷ These studies are consistent with the finding that upon M-domain phosphorylation, binding of the cMyBP-C N-terminal fragment C1C2 to the myosin-S2 fragment was eliminated.³⁹ Additionally, the phosphorylation was demonstrated to affect binding of the same fragment to F-actin.⁴⁰ Interestingly, the measured dissociation constant (K_d) for the C1C2/F-actin complex was only weakly affected by phosphorylation, however the molar binding ratio between C1C2 and F-actin was reduced approximately twice (from ~1:1 to ~1:2 mol/mol actin) once the fragment was phosphorylated. By testing binding and actin filament cross-linking properties of different cMyBP-C fragments that included or lacked the M-domain the authors proposed that C1 and m-domains bind to independent sites on the surface of actin filaments.

Since the phosphorylation sites are located in the disordered part of the M-domain,^{33,34} this immediately suggests that the phosphorylation status of the M-domain may be related to its folding properties. The impact of M-motif phosphorylation on its structural properties was studied by an AFM characterization of force-extension relations for the cardiac C0-PAL-C1-M-C2-C3 (C0C3) fragment.⁴¹ First, C0C3 control was compared with two mutant inert analogs where either three (S273, S282, S302) or four (S273, S282, S302, and S307) phosphorylation sites were substituted with Ala residues (denoted as C0C3^{AAA} and C0C3^{AAAA}, respectively). The differences in the initial contour length (L_C) values (before the Ig domain unfolding events) of the entire fragment for all three constructs were rather small ($L_C \sim 60$ – 65 nm, $p > 0.05$). Unfolding of Ig domains added a constant value to the contour length ($L_C = 30 \pm 0.29$ nm) regardless of Ser/Ala substitutions. The persistence length L_P was on the order of a single amino acid residue (~ 0.36 nm), consistent with the disordered nature of PAL and the M-domain. An 83% phosphorylated C0C3 fragment⁴² and two its phosphomimetic mutants were used to establish the effect of phosphorylation on its structure.⁴¹

100% phosphorylation of the C0C3 fragment was modeled by substitution of the Ser phosphorylation sites with either three or four negatively charged Asp residues. The major difference observed in the phosphorylated C0C3 and both phosphomimetic mutants denoted as C0C3^{DDD} and C0C3^{DDDD} was a decrease of the initial L_C by 21–29 nm. The decrease in the initial L_C was interpreted as stabilization of the M-domain structure upon phosphorylation, possibly as a consequence of electrostatic interactions between the negatively charged phospho-Ser or Asp residues and several solvent-exposed Arg and Lys residues in the THB. The hypothesis of the electrostatic nature of the putative structure stabilization was in agreement with the effects of ionic strength on the initial L_C of C0C3^{AAA} and C0C3^{DDD}. While L_C of the unphosphorylated C0C3^{AAA} remained at approximately 60 nm for KCl concentration from 25 to 300 mM, the distribution of L_C values for the phosphomimetic C0C3^{DDD} was bimodal. Two populations of L_C values were observed, one shorter (~ 40 nm), corresponding to a more compact protein, and one longer (~ 60 nm) corresponding to the C0C3^{AAA} mutant lacking negative charges in sequence positions 273, 282, and 302.⁴¹ As the ionic strength increased, the relative populations were shifted towards longer L_C values.

The authors of the study⁴¹ suggested that the phosphorylation-dependent extensibility of the M-domain is a means to regulate the cMyBP-C affinity for actin and/or myosin, *e.g.* by limiting fly-casting abilities⁴³ of the M-domain or stabilizing a structure incompatible with the binding interface. Generally, changing binding affinities *via* altering flexibility of IDRs connecting binding domains/sites could be a robust regulatory mechanism that would not require fine-tuning the amino acid sequence in the course of evolution. Many interacting biological molecules and macromolecular assemblies are built on the principle of di- or polyvalency, whereby two or more weaker binding constituents are linked to form a much stronger ligand.^{44–46} If the linker is flexible, restraining the flexibility of such linker IDRs through, *e.g.* binding to a third molecule⁴⁷ or incorporation of repulsive charges by phosphorylation⁴⁸ may lead to considerable decreases in binding affinities.

Importantly, the presence of 0.1 M calcium, the primary trigger of muscle contraction,⁴⁹ had little effect on the extensibility of the unphosphorylated C1C2 fragment.⁵⁰ However, when the same amount of calcium was added to the phosphomimetic C1C2^{4D} where all four phosphorylation sites S273, S282, S302, and S307 were replaced with Asp residues, it resulted in an 88% reduction of population of AFM traces typical of molecules with phosphorylation-induced stabilization of the M-domain.⁵⁰ In the same study it was shown by electron microscopy (EM) that MyBP-C has a hinge point in the M-domain, which made the region C0C3 adopt a V-like conformation. When the bacterially expressed C0C3 fragments were examined, three types of V-like conformations with different degrees of bending were observed. One of the three was extended (>14 nm between the ends of the molecule), the other one was more bent (7–14 nm between ends), and the third one was completely folded around the hinge with <7nm between ends (a compact conformation). The wild type C0C3 fragments existed predominantly in extended or bent conformations, while the phosphomimetic C0C3^{4D} molecules - with four phosphorylated Ser replaced with Asp - were mostly compact. Upon addition of calcium, the fraction of C0C3^{4D} in compact conformations reduced by 89% therefore reversing the effects of negative charges provided by the Asp residues which mimic phosphorylated Ser. That observation explained why calcium removed the ability of phosphorylation to regulate the function of MyBP-C in *in vitro* motility experiments performed in this study.⁵⁰ The authors proposed that the interplay between phosphorylation and calcium is dynamic and allows tuning the cMyBP-C modulatory role within each heartbeat. However, one must be careful with conclusions made on Ser-to-Asp phosphomimetic molecules as it has been argued that not all effects of Ser phosphorylation are reproduced by Ser substitution with Asp.⁵¹

In another study, the structural effects of the M-domain phosphorylation were tested in modified mouse cMyBP-C fragment C0C2 with fluorescent and spin labels placed on Cys residues in C1 (Cys248) and C2 (Ser440Cys) domains.⁵² The interprobe distance distribution was quantitated with time-resolved fluorescence resonance energy transfer (TR-FRET) and dipolar electron paramagnetic resonance (DEER). Consistently with the earlier investigation,⁴¹ Protein kinase A phosphorylation of C0C2 led to a narrower distribution of distances between the molecular probes.

To obtain a high-resolution molecular view of processes resulted in the observed changes, 2 μ s-long molecular dynamics simulation (MDS) approaches suitable for the analysis of intrinsically disordered proteins were applied to the M-domain.⁵² In agreement with NMR studies,³⁰ the N-terminal part of the M-domain (His255-Glu315) was disordered. Interestingly, the region remained disordered upon *in silico* phosphorylation, and phosphorylation did not affect the structure of the folded THB motif. Additionally, phosphorylation caused the disordered N-terminal region to rotate away from the THB thus making it seemingly less compact than before phosphorylation. The authors suggested that the increase in C0C2 compactness induced by phosphorylation is a global event, and it is caused by a local decrease in compactness in the M-domain, perhaps by exposing new binding interfaces.

The direct binding of residues from the M-domain region to the thick (myosin) and thin (actin) filaments adds an additional level of complexity into the mechanism of the M-

domain-dependent regulation. The evidence that the M-domain interacts directly with the thick filaments was first provided by experiments on cosedimentation of myosin minifilaments containing 16-18 molecules of myosin with cardiac and slow-skeletal isoforms of C1C2, which showed that C1C2 and myosin bind in solution.²⁴ At the same time cC0C1 and cC2C5 fragments, which did not contain an M-domain, did not cosediment with the minifilaments, suggesting that the M-domain may interact with the filaments directly. Further binding mapping identified the N-terminal 126 residues of the myosin S2 fragment (S2) as the binding site for the C1C2.²⁴

Although the cosedimentation experiments did not show a detectable evidence for the direct interaction between C1 or C2 Ig domains and myosin, a more sensitive approach *via* NMR titrations demonstrated the presence of weak but specific S2 binding sites on C1 (ref.²⁸) and C2.⁵³ Based on the NMR data and mutagenesis, a structural model of the C1C2 complex with S2 was proposed where C1 and C2 occupied adjacent sites on S2. The bound to the S2 fragment M-domain was proposed to form a U-shaped, tuning fork-like conformation with each “prong” being fully extended towards C1/C2 binding sites.²⁸ The authors also suggested that the M-domain was not in a rigid bound conformation but rather undergoing dynamic motions reducing the entropic penalty upon binding. The model provided explanations on the effects of a number of HCM-linked mutations and the effects of phosphorylation on the complex formation.

Quantitative microscale thermophoresis measurements gave K_d for complexes between cMyBP-C and S2 .⁵¹ The K_d for an isolated M-domain was $\sim 40 \mu\text{M}$, for the C1C2 $\sim 20 \mu\text{M}$, and for C1 linked to C2 by a flexible unrelated linker $\sim 480 \mu\text{M}$. This study established that although Ig domains C1 and C2 slightly improve the binding of the M-domain by a factor of ~ 2 , the most important region providing interacting contacts with S2 is the M-domain itself.

Direct *in vitro* interaction of the M-domain with the thin filaments was discovered by testing cosedimentation of F-actin with recombinant murine cMyBP-C fragments.⁴⁰ Recombinant fragments C1-M, M-C2 and a chimeric construct C3-M-C4 bind to F-actin with very similar affinities ($K_d \sim 10 \mu\text{M}$) and it was concluded that M-domain is a ligand for F-actin. In another study, fragments C0C3 and C0C2 were sufficient to reproduce the ability of the full-length cMyBP-C to inhibit actin filament sliding velocity, however, trimming the N-terminal fragment to C0C1 resulted in a very modest inhibition.⁴² Adding to the C1 C-terminus only 17 amino acid residues (HEAIGSGDLRLSAFRR) from the N-terminus of the M-domain restored the inhibition. Replacing three Arg residues in the 17-residue sequence with Ala eliminated the inhibitory effects.

Pro/Ala rich region (PAL)—In cardiac MyBP-C, Pro/Ala-rich region links C0 and C1 domains and, depending on the species, spans from ~ 40 to ~ 60 amino acid residues.⁵⁴ The relative flexibility of the PAL flanked with well-folded C0 and C1 domains was clearly demonstrated by NMR ^{15}N relaxation experiments.⁵⁵ Intuitively, compared to a random polypeptide chain, Pro-rich regions should experience some restrictions in their flexibility, because the Pro ring closure limits ϕ torsional angles to $\sim -50^\circ - -100^\circ$.⁵⁶ TR-FRET and DEER quantitation of interprobe distance distribution was used to test the flexibility of the

PA-linker in modified mouse cMyBP-C fragment C0C2 with fluorescent and spin labels placed on Cys residues in C0 (Ser18Cys) and C1 (Cys248) domains.⁵² The distance distribution was deconvoluted into two major cMyBP-C conformational populations. One is narrowly distributed around a shorter distance corresponding to what was termed a “closed conformation”, and the other had a much wider distribution around a longer distance, corresponding to an “open conformation”. The open conformation represented a disordered state of the PA-linker, while the closed conformation represented a more ordered state where the C0 and C1 domains connected by the linker are likely to form extensive contacts with each other. Interestingly, protein kinase A-catalyzed phosphorylation of an M-domain connecting C1 and C2 domains had an allosteric effect on the population distribution between the two conformational states. In the phosphorylated state the fraction of the closed conformation was appreciably increased.

Even though the M-domain phosphorylation was shown to affect the PA-linker conformation, the role of the PA-linker in the cMyBP-C function and how this role is affected by the phosphorylation is still somewhat unclear. Both C0 and C1 bind thin filaments⁵⁷ and they were found to bind to thin filaments in distinct sites.⁵⁸ The C0-PA-C1 fragment can activate actomyosin interactions in the absence of Ca^{2+} ions in a species-specific manner.⁵⁹ When human C0 and C1 domains were tested separately, they displayed two very different effects on thin filament activation of myosin-subfragment 1 (S1) ATP hydrolysis.⁵⁸ Whereas C0 was inhibitory, C1 displayed an activatory effect with an optimal concentration of $\sim 10 \mu\text{M}$. However, despite the inability of C0 to activate thin filaments, the tandem C0-PA-C1 shows a considerably enhanced activation when compared with C1 alone.¹¹ To understand the structural basis of this cooperativity, the thin filaments decorated with the C0-PA-C1 fragments were studied with cryo-EM. It was shown that C1 links actin and tropomyosin and therefore seems to stabilize the actin/tropomyosin cable complex. Importantly, this interaction moves the tropomyosin cable and traps thin filaments in an activated state under the conditions of low calcium concentrations.

The role of the PA-linker in C0-PA-C1 was demonstrated by comparing the thin filament activation in the presence of the mixture of C0-PA and C1 or the C0-PA-C1 tandem.¹¹ The tandem activated thin filaments to a higher extent than the mixture, suggesting that the PA-linker plays a role in orienting C0 and C1 rather than simply providing a passive avidity effect of a worm-like fully flexible linker.^{60,61} However, since no observable density that could be ascribed to the PA-linker was observed in the study, it is possible that the PA-linker remains somewhat flexible in the complex.

The amino acid sequences of the Pro/Ala region in different species were analyzed by Shaffer & Harris.⁵⁴ They found that the amino acid sequence of the Pro/Ala-rich region in cMyBP-C (PA-linker) is very variable from species to species, while the neighboring domains show a considerably higher degree of conservation. For example, the C0, C1, M and C2 domains share >80% identity between human and mouse proteins, with the C1, M and C2 domains being >90% identical between the species. At the same time, only 46% of PA-linker residues are identical in humans and mice. Importantly, the relative number of Pro and Ala residues differ even more in the mouse and human PA-linker, with 51.0% Pro/Ala content in human, and 28.2% in mouse orthologs. The authors also found that in mammalian

species there exists an inverse correlation between the percentages of the Pro and Ala residues in the PA-linker and the average resting heart rate beat. They proposed that the linker composition and length serve the role of tuning cross-bridge cycling kinetics to optimize the contractile efficiency under the conditions of species-specific hemodynamic loads and contraction frequencies.

Proteins of tropomodulin family

Function and localization

Tropomodulin (Tmod) and leiomodulin (Lmod) are homologous proteins with important roles in muscle sarcomere thin filament assembly and maintenance. Both proteins contain long IDRs that are crucial for their functions. Of all muscle proteins these two display the highest percent of disorder, with ~50 % backbone chain disorder in Tmods and ~70 % in Lmods.

Three of the four known Tmod isoforms, Tmod1, Tmod3, and Tmod4 can be found in muscle. While Tmod1 is found only in heart muscle⁶² and skeletal muscle,⁶³ Tmod3 is expressed in all muscle tissues.⁶⁴ Tmod4 is found exclusively in skeletal muscle.^{64,65} The Tmod isoforms are approximately 60% identical and 70% similar. Although Tmod3 is found in muscle tissue, only Tmod1 and Tmod4 had been shown by immunofluorescence experiments to localize at the pointed end of thin filaments under normal physiological conditions.⁶⁶ In skeletal muscle cells of adult mice where Tmod1 had been knocked out, however, Tmod3, as well as Tmod4, appear at the pointed end of thin filaments.⁶⁶

There are three known isoforms of Lmods, which, like Tmods, are expressed in different muscle tissues. Lmod1 appears primarily in smooth muscle, Lmod2 primarily in cardiac muscle, and Lmod3 primarily in skeletal muscle.^{67–69} Immunofluorescence experiments with both endogenous and overexpressed Lmod2 showed that, like its homolog Tmod1,⁶⁶ Lmod2 localizes near the pointed end of thin filaments.⁷⁰ This observation has been confirmed in many later studies.^{71–75} However, it has been suggested that the localization of Lmod at the pointed end, while similar to Tmod, is somehow distinct; more specifically that Tmod localizes in tighter bands while Lmod localizes in broader bands.⁷¹ Also, endogenous Lmod was shown to localize sparsely along the length of thin filaments,⁷³ and the same was shown for overexpressed Lmod.⁷² When F-actin was cosedimented with Lmod2, the amount of Lmod2 presented is in excess of the amount that would sediment if Lmod2 only bound to the pointed end,^{71,76,77} providing further evidence that Lmod2 binds to sides of actin filaments.

The role of Tmod as a pointed-end capping protein was demonstrated at first in pyrene-actin polymerization assays.⁷⁸ Tmod reduced rates of polymerization and depolymerization in a tropomyosin dependent manner when the barbed ends were capped. It was shown that in myocytes, after thin filaments have matured to the proper length, Tmod assembled at the pointed ends, maintaining the length of the filaments by preventing further polymerization or depolymerization, which provided ultimate evidence for the pointed end capping mechanism.^{79,80} Tropomyosin had to be present at thin filaments for Tmod to be able to bind at the pointed end.⁷⁹

It is generally accepted that Lmod plays a role in thin filament length regulation. Indeed, cellular studies of Lmod2 knockout (KO) and overexpression of Lmod2 in cells show that both have an effect on thin filament length.^{70,72,74,81} The Lmod2 KO cells displayed shorter thin filaments, whereas Lmod2 overexpression led to longer thin filaments. The localization of Lmod along the length of thin filaments suggests that Lmod may play another role through an alternate mode of binding; for example, it was shown in *in vitro* assays that Lmod2 bound along actin filaments decreased the actin-activated Mg^{2+} -ATPase activity of skeletal muscle myosin.⁷⁷

The mechanism by which Lmods regulate thin filament length is still under intensive debate. All Lmod isoforms are strong actin nucleators.^{69,70,82} One proposed mechanism suggests that Lmod nucleates actin to form thin filaments until they are capped by Tmod.⁷³ On the other hand, the localization of Lmod at the pointed end hints that, as with Tmod, the Lmod's role is realized through its binding to the pointed end. Overexpression of Lmod2 was shown to displace Tmod1 from the pointed ends.⁷² Consequently, another mechanism was proposed suggesting that Lmod not only nucleates new thin filaments, but it also competes with Tmod at the pointed end, therefore maintaining the thin filament length via a dynamic Tmod/Lmod exchange process.^{72,75}

The facts that the Lmod2 KO in mice caused shortening of thin filaments⁷⁴ and that the overexpression of Lmod2 induced abnormal actin structures including longer thin filaments and larger bundles of thin filaments,⁷⁰ are both consistent with either of the two mechanisms; i.e., the nucleation mechanism⁷⁰ or the competition mechanism⁷² of thin filament length regulation. However, in chicken cardiomyocytes Lmod2 mRNA transcripts did not appear until after the heart has begun to beat while Tmod1 transcripts appeared earlier.⁷² This suggests that Lmod2 cannot act as a nucleator of thin filaments at the early stages of heart tissue development. Additionally, Lmod2 conditional KO, which decreases expression of Lmod2 only in fully developed adult mice after thin filaments have already been formed, caused both a decrease in the average thin filament length and a loss of uniformity of thin filament lengths.⁸¹ These data support the competition mechanism. The distinction between the two mechanisms can be made on the basis of the three-dimensional structures of Lmod and Tmods and the structure of their complexes with the thin filament. Therefore, proponents of each mechanism refer to structural data to support their preferred models.

The domain organization of Tmod and Lmod

Tmods consist of two structurally distinct halves, the disordered N-terminal half and the ordered C-terminal half represented by a leucine rich repeat (LRR) domain which folds into 5 repeated α/β motifs followed by a C-terminal α -helix.^{83–85} The disordered domain contains three binding sites: a tropomyosin binding site 1 (TMBS1), followed by an actin binding site 1 (ABS1) and finally by a second tropomyosin binding site (TMBS2).^{84,86–92} The C-terminal half contains a second actin binding site (ABS2).^{80,93,94}

In addition to the disordered N-terminal region and the ordered LRR domain as in Tmods, Lmods have a C-terminal extension. Based on homology with Tmod, the Lmods were predicted and later confirmed in tropomyosin binding assays using CD to contain one

tropomyosin binding site (TMBS1) in the disordered region.⁹⁵ Analysis of sequence homology suggested that Lmods also have one actin binding site (ABS1) in the N-terminal region, a second actin binding site (ABS2) in the middle of the protein (the LRR domain), and a third actin binding site (ABS3) in the C-terminal region in the form of a Wiskott-Aldrich syndrome protein homology 2 (WH2) domain.^{67,70} Also, Lmods contain a poly-proline region (polyP) located between the LRR and WH2 domains. Strong nucleating abilities of Lmod2 in comparison with Tmods were first explained by the presence of the WH2 domain.⁷⁰

Structure of the LRR domain

The most homologous domain between Lmod and Tmod is the LRR domain. Since LRR domain operates together with the disordered regions of Lmod and Tmod, to fully understand their function, the structure and function of the LRR domain should be explained. Crystal structures of Tmod and Lmod LRR domains look similar.^{73,85,96} The C-terminal residues of Tmods are disordered and cannot be seen in their crystal structures.^{93,97} Based on the crystal structure of actin in complex with Tmod1 C-terminal fragment fused with the gelsolin fragment, there is no interaction between the C-terminal disordered region of Tmod1 and actin⁹³ (Fig. 2). The truncation of the 15 C-terminal residues (13 of them being disordered) did not affect ability of the truncated Tmod1₁₋₃₄₄ to cap actin filaments in *in vitro* experiments in the presence of tropomyosin⁸⁸ and had no effect on its assembly at the pointed end of thin filaments in myocytes, while removal of the C-terminal helix affected the assembly drastically.⁹⁸ However, the binding of the distant ABS1 is likely to be affected by this truncation because in the absence of tropomyosin, the capping ability of Tmod1₁₋₃₄₄ is decreased.⁸⁸ Even more remarkable results were obtained for Tmod2, a Tmod isoform with the highest of all isoforms ability to nucleate actin since it can bind two G-actin molecules simultaneously. The nucleating ability was lost when homologous truncation was done for Tmod2.⁹⁹ It was also shown by atomic force microscopy (AFM) that despite multiple remaining residues that should still interact with actin in the LRR domain, the binding in ABS2 was lost.¹⁰⁰

C-terminal residues starting from R343 are disordered in the crystal structure of individual Tmod1's LRR domain (pdb 1IO0), however, in the crystal structure of Tmod1's LRR domain fused with the gelsolin fragment in a complex with actin (pdb 4PKI), residues R343-A345 form a last turn of the C-terminal helix. In the C-terminal helix residues R325-L344 were reported to interact with actin.⁹³ Removal of 15 C-terminal residues (A345-V359) affects only formation of the last turn of the C-terminal helix, but there is no effect on the rest of the helix (Fig. 2). Nevertheless, losing helical conformation for R343-A345 results in drastic effect on tropomyosin-independent binding to actin in this site^{88,99} despite multiple interactions with actin along the whole LRR domain (Y170-N179, N201-R206, V232-R235, N258-F263 K286-S291 K314-H318) reported in ref.⁹³ in addition to the remaining part of the C-terminal helix.

Structure and function of the N-terminal domains in Lmods and Tmods

The N-terminal domains that are represented by regions located before the well-folded LRR domain in Tmods and Lmods (residues 1-182 and 1-198 for Tmod1 and Lmod2,

respectively) are mostly disordered (Fig. 3). The first results demonstrating that Tmods are composed of two distinct domains with different degree of folding came from data obtained by limited proteolysis, CD and fluorescence.⁸³ It was further confirmed by SAXS¹⁰¹ and differential scanning calorimetry (DSC) studies.⁸⁴ Later, using CD and DSC it was shown that N-terminal Tmod fragments of different lengths (residues 1-91, 1-92 and 1-130) had some α -helical content; however, these fragments acquired tertiary structure with the increased α -helical content only upon binding tropomyosin.^{84,89,92}

In attempt to define regions with residual α -helical content and therefore primary candidates for binding sites, secondary structure predictions for the unfolded regions were made. Secondary structure prediction for the N-terminal fragments (residues 1-130 in both Tmod1 and Tmod4) suggested that two α -helical segments with propensity to form a coiled coil may be formed (residues 24-38 and residues 68-81 in Tmod1 and residues 25-39 and 69-82 in Tmod4).⁹² Secondary structure prediction of full length Tmod1 supplemented these with two more predicted helices (residues 126-135 and residues 143-145) in the mainly disordered N-terminal region.⁹¹ Secondary structure prediction in the N-terminal region (up to residue 40) of Lmod1 and (up to residue 42) of Lmod2 suggested that there are two α -helical segments (residues 17-21 and 26-35 in Lmod1, and residues 17-23 and 27-38 in Lmod2).⁹⁵ In addition, residues 134-159 in Lmod2 also had high propensity to form a helix. We ran the Jpred (ref.¹⁰²) secondary structure prediction for human Lmod2 and it gave the following α -helical segments: 18-23, 27-35, 66-79, 124-131, 135-146 (Fig. 3) The Jpred (ref.¹⁰²) secondary structure prediction for human Tmod1 gave the following α -helical segments: 15-20, 24-36, 64-75, 116-120, 126-135, 144-147.

NMR demonstrated that the isolated Tmod1 N-terminal fragment (residues 1-92) contains only one of predicted α -helices formed by residues 24-35 (Fig. 4)⁸⁹. Similar results were shown by CD for the N-terminal fragments of Lmod1 and Lmod2 (~40 residues), they were mostly disordered in solution, acquiring a more helical conformation upon binding to tropomyosin.⁹⁵

Initially, TMBS1 was mapped to residues 1-35 in Tmod1.⁸⁹ Upon addition of the tropomyosin peptide, the ¹⁵N-HSQC crosspeaks corresponding to residues 1-35 in Tmod1₁₋₉₂ broadened and/or shifted, indicating that the tropomyosin binding site is contained within these residues. Since the primary sequence of residues 24-38 in Tmod1 contains the heptapeptide repeat typical of a coiled coil,⁹² the importance of the coiled coil formation for binding with tropomyosin was tested by introducing two different mutations instead of Leu27, the residue in the *a* position of the heptapeptide repeat.⁸⁹ Two mutants were created where L27G was meant to break the helix while L27E was meant to break the hydrophobic interactions necessary for coiled coil formation, keeping the helix intact. Native gel electrophoresis and CD experiments showed that neither mutant formed a stable complex with tropomyosin, indicating that not only the helix is important for binding tropomyosin but also the ability of the helix to form a coiled coil. Later the N-terminal binding site TMBS1 was updated to residues 1-38 (Fig. 3).⁹⁰

Following TMBS1 in Tmod1 is ABS1 (Fig. 3). ABS1 was initially mapped to residues 48-92.^{89,90} This region, containing ~40% charged residues and ~10% Gly and Pro, is highly

flexible as shown by NMR (Fig. 4). Residues 39-47 were not considered to participate in interactions with the thin filaments but to be a link between binding sites TMBS1 and ABS1.

The first evidence for the existence of an actin-binding site in the N-terminal domain was found when the N-terminal fragment of Tmod1 (residues 1-130) showed some weak pointed-end capping activity in the absence of tropomyosin in pyrene-actin polymerization assays.¹⁰³ To localize the actin-binding site, similar experiments were performed with various fragments of Tmod1. The K_d values, calculated by double-reciprocal plots, for full length Tmod1 and truncated Tmod1₃₅₋₃₅₉ were approximately equal. If 94 or more N-terminal residues were excised, the K_d values were greater.¹⁰³ Furthermore, Tmod1 fragment (residues 35-128) capped actin filament pointed ends similarly to Tmod1₁₋₁₃₀.¹⁰³ The ability of a Tmod1 fragment (residues 1-92) to cap actin filaments in the presence of tropomyosin further refined the position of ABS1.⁹⁰ It was assumed that residues 65-75, which predicted to form an amphipathic helix, are important for actin binding, and this idea was tested by introducing the mutation L71D into Tmod1₁₋₉₂ that should destabilize the amphipathic helix. The mutation resulted in a ~50 fold decrease in capping activity shown by pyrene-actin polymerization assay.⁹⁰ Furthermore, introducing a series of mutations (T59A/G60A/P61A) into the disordered part of this region to promote α -helical conformation led to a ~10-fold decrease in capping activity. These indicated that both the formation of an amphipathic helix and the intrinsic disorder of this region are important for Tmod's interaction with actin in this site. Later this was confirmed in studies where direct interaction of Tmod isoforms with actin was investigated for full-length Tmods and with the mutation of L71 or homologous residues to Asp in other Tmod isoforms using gel-shift¹⁰⁴ or AFM.¹⁰⁰

To further understand Tmods and Lmods binding and function at the pointed end, there was an urgent need in detailed structure of binding interfaces between disordered Tmod's N-terminal region and actin or tropomyosin. While several attempts to crystallize N-terminal fragments of Tmod and Lmod with tropomyosin did not succeed, likely due to the disordered nature of the fragments,^{105,106} the crystal structure of Tmod1's ABS1 with actin was finally obtained.⁹³ For crystallization, Tmod1's N-terminal fragment (residues 50-101) was fused at the N-terminus with gelsolin segment 1 via a 9-aa flexible linker to ensure binding to actin. The authors chose the fragment slightly different from what was suggested in other publications.⁹⁰ Their choice was based on the data obtained by isothermal calorimetry (ITC) where this fragment was shown to interact with actin with $K_d=7.5 \mu\text{M}$.⁹³ Only residues P58-K99 were seen in the crystal structure (Fig. 5) and K63-E77 formed an amphipathic helix that interacted with actin via a hydrophobic surface predicted in earlier studies. The authors concluded that residues before P58 do not interact with actin.

Based on this result, homologous fragments were chosen for Lmod2 (residues 52-103) and Lmod1 (residues 51-102).⁷³ The affinity of Tmod1 fragment to actin measured by ITC in this paper gave $K_d=15 \mu\text{M}$ for of Tmod1, twice higher than in the previous study.⁹³ Surprisingly, in spite of high homology of Lmod fragments with the corresponding site in Tmods, no binding to actin was shown by ITC.⁷³ This result led authors to a conclusion that although Lmod2 has a region homologous to ABS1 in Tmod1, there is no ABS1 in Lmods and therefore the roles of Lmod and Tmod should be completely different in cells. This is

consistent with the notion that Lmod acts only as a nucleating factor. However, as it was shown in later studies, residues 45-41 are crucial for Lmod binding to actin.^{75,100} Of these seven residues, five residues are conserved in both Tmod and Lmod isoforms.

Using 1D proton NMR, several overlapping Lmod2 fragments were titrated with monomeric actin and it was showed that there is, in fact, interaction between the two for the fragment containing residues 43-90.⁷⁵ Furthermore, AFM unbinding force experiments, which has the resolution to detect individual forces involved in protein-protein interaction, showed that full length Lmod2 has a trimodal unbinding force distribution with actin. A Lmod2 fragment without the WH2 domain (residues 1-514) has a bimodal unbinding force distribution with actin, and a Lmod2 fragment without both the WH2 and LRR domains (residues 1-201) has a unimodal force distribution.¹⁰⁰ The trimodal unbinding force distribution of Lmod2 suggests Lmod2 and actin contact each other at three distinct locations, while the bimodal and unimodal unbinding force distribution of the truncated Lmods suggest that those contact actin at two and one distinct locations, respectively. Since the fragment that consists of residues 1-201 contains neither the LRR nor the WH2, a detectable unbinding force between this fragment and actin provided the ultimate evidence for the existence of ABS1 in Lmod2 that was localized to residues 43-90.⁷⁵

Removing residues 43-49 from the fragment used for crystallization and moreover attaching gelsolin fragment via the N-terminus of the Tmod1 fragment 50-101 most likely did not allow residues before P58 to bind properly to actin. The distance between C α atoms of the C-terminus of the gelsolin fragment and the P58 of Tmod1 on actin is ~ 47 Å, which corresponds to the distance spanned by ~ 13 residues in a fully extended peptide chain.¹⁰⁷ A polypeptide chain of seventeen residues used between them (9 residues of the linker and 8 residues of Tmod1 (residues 50-57)) is only slightly longer than a fully extended conformation. Even if residues 50-57 bind to actin in a full-length protein, they may be unable to reach their binding location in this chimeric peptide, or the entropic penalty paid to maintain the nearly extended conformation could be too high to allow the binding to happen. Taking in account all described data, the updated ABS1 should be mapped to residues 43-99 in Tmod1 and 43-90 in Lmod2 (Fig. 3).

The region between ABS1 and the LRR domain in Tmods contains the second tropomyosin-binding site TMBS2. Early studies suggested that Tmod1 contains two tropomyosin binding sites, each site being specific for short or long tropomyosin isoforms.⁸⁶ Using dot-blotting experiments with long and short I¹²⁵-labeled tropomyosin isoforms and three fragments of Tmod1 (residues 6-94, 6-184, 90-359), it was shown that the first fragment binds only to long muscle tropomyosin, the second binds to both, and the third binds only to short non-muscle tropomyosin. The authors concluded not only that Tmod1 contains two distinct binding sites, but also that the binding site contained in residues 6-94 is specific for long muscle tropomyosins while the binding site contained in residues 90-359 is specific for short non-muscle tropomyosins. However, a later study demonstrated through pyrene-actin polymerization assay that the pointed end capping activity of a Tmod1 fragment (residues 95-359) is increased in the presence of long muscle tropomyosin,¹⁰³ indicating that the second binding site should bind skeletal muscle tropomyosin, contrary to the previous finding.

Later, a different approach was used in studying localization of TMBS2. Since secondary structure prediction suggested that residues 126-135 form an amphipathic helix, and since hydrophobic interactions had been shown to be important for binding in TMBS1⁸⁹ it was thought that the hydrophobic side of this helix could have interactions with tropomyosin.⁹¹ The combination of mutations L27G and L71D in a Tmod1 fragment (residues 1-159), which destroy the binding capabilities of helices at residues 24-35 and residues 65-75, failed to abolish the peptide's binding to tropomyosin in gel-shift experiments and its pointed end capping function in pyrene-actin polymerization assays.⁹¹ Addition of a third mutation, I131E, which would disrupt formation of the hydrophobic side of the helix at residues 126-135, abolished tropomyosin binding and caused a 30-fold decrease in pointed end capping activity.⁹¹ Eventually, the second tropomyosin-binding site was mapped to residues 109-144 in Tmod1. CD experiments were performed to study interactions between Tmod peptides and tropomyosin peptides containing the Tmod binding site.^{89-91,108} K_d values for complex formation between peptides from all Tmod and tropomyosin isoforms were calculated and compared, and it was demonstrated that Tmod/tropomyosin interactions were isoform dependent and K_d values varied from 1 nM to 1 μ M.

Immunoprecipitation of Tmod and actin from myofibrils showed that there are between 1.2 and 1.6 Tmod molecules per thin filament.⁶³ With a stoichiometric ratio between one and two, it was not clear whether one or two Tmod molecules bind to the pointed end. However, it was shown by introducing several disabling point mutations of the established binding sites and by performing native gel electrophoresis that Tmod binds two separate tropomyosin molecules, one for each of its binding sites, implying that only one Tmod molecule is present at the pointed end.⁹¹

No second tropomyosin-binding site was found in Lmods. While it was shown that interaction between Lmod fragments containing TMBS1 and peptides containing N-terminal residues from different tropomyosin isoforms is also isoform-dependent, no interaction with tropomyosin was detected with a Lmod fragment containing residues between ABS1 and the LRR domain.⁹⁵ That low-complexity disordered region in Lmods (residues 97 to 154, Fig. 3) contains ~50% negatively charged residues. In later studies the absence of TMBS2 in that region of Lmod2 was confirmed; however, NMR data indicated that some residues in that region might interact with actin.⁷⁵

The N-terminal domain of Lmod, through its binding to tropomyosin, is thought to control Lmod localization at the pointed end, thereby modulating actin nucleation and the pointed end dynamics in sarcomeres. In several studies, pyrene-actin polymerization assays on different fragments of Lmod2 containing all or part of the N-terminal domain were used to investigate these roles. Tropomyosin enhanced nucleation activity of Lmod and its fragments, but only when TMBS1 was present in the fragments.⁷⁰ Lmod2 fragments, either containing just the N-terminal TMBS1 and ABS1 (residues 1-94) or containing the whole N-terminal domain (residues 1-201) inhibited actin polymerization in the presence of tropomyosin.⁷⁵ A mutation disabling TMBS1 caused a 5-fold decrease in the ability of the Lmod2 fragments to inhibit actin polymerization in the presence of tropomyosin, indicating that, like Tmod1, Lmod2 binding to the pointed end is dependent on tropomyosin.⁷⁵ Similarly, a mutation disabling ABS1 caused a 3-fold decrease in the ability of the Lmod2

fragments to inhibit actin polymerization.⁷⁵ Further, addition of Tmod1 to seeds capped by Lmod2 caused more G-actin to be nucleated, indicating that Tmod1 frees Lmod2 from the pointed-end.⁷²

The C-terminal extension in Lmod isoforms

Different from Tmods, Lmods contain an additional ~110-180 residue C-terminal extension bearing a disordered region, which includes the polyP domain of different lengths in different isoforms⁶⁷ followed by the WH2 domain, or ABS3.^{67,70} The crystal structure of Lmod2 without its N-terminal domain in a complex with monomeric actin⁹⁶ updated in ref.⁸² is missing the electron density of the region between ABS2 and ABS3, confirming that the region is disordered.

Pyrene-actin polymerization assays have been used to investigate the role of the WH2 domain. Before it was shown that the LRR domain in Lmods has its own strong nucleation effect, it was thought that the C-terminal extension in Lmods is responsible for nucleation activity. However, a hybrid of Tmod1 with the C-terminal extension of Lmod2 showed only ~20-30% nucleation activity compared to Lmod2 and Lmod1.⁷³ A mutation thought to incapacitate the WH2 domain in Lmod2 (⁵³⁷LKRV⁵⁴⁰ to AAAA) only slightly reduced nucleation activity.⁸² The fragment that contained residues 180-367 of Lmod2 (the LRR domain) had weak nucleating activity,⁸² this being in contradiction with previous reports from this laboratory where they stated that the LRR domain by itself has strong nucleating abilities.⁷³ Comparison of data from different laboratories showed that the truncated Lmod2 without the WH2 domain (residues 1-514) had weaker nucleation⁷² than Lmod2 with the mutated WH2 domain, but stronger than the Lmod2 fragment missing both the N-terminal region and the C-terminal extension (residues 180-367).⁸² This indicates that the mutation introduced in the WH2 domain might not completely abolish the function of the C-terminal extension in actin nucleation. The truncated Lmod2₁₋₅₁₄ inhibits both polymerization and depolymerization at the pointed end, however, weaker than Tmod; the inhibition is enhanced by the presence of tropomyosin.⁷² Furthermore, expression of several truncated Lmod2 fragments (residues 1-394, 1-443 and 1-514) in cardiomyocytes had the same effect on thin filaments as Lmod2 KO by making them shorter.⁷² It appears that the WH2 domain is important not only for nucleation activity, but also for thin filament elongation at the pointed end.

It should be noted that in the first papers describing Lmod2 structure and localization the Lmod2 constructs contained deletions in low complexity regions. Either a total of 52 residues were missing: S99-E130, which is part of the disordered region located between ABS1 and ABS2, and P421-P440, which is part of the polyP domain⁷⁰ or a total of 12 residues were missing, i.e. P433-P444.⁷² This may explain some discrepancies in the results obtained for Lmod fragments in earlier and later publications.

Importance of unfolded binding sites

Since more than one Tmod isoform are expressed in each type of various tissues, and they bind to tissue-specific tropomyosin isoforms with variable affinity, it was suggested that tropomyosins regulate Tmod capping in this way.^{86,108} Pyrene-actin polymerization assays

showed that the ability of Tmod1 to inhibit polymerization and depolymerization at the pointed end of actin filaments varies depending on a tropomyosin isoform used in the assay.^{88,109} The ability of Tmod isoforms to perform different functions in different tissues is a result of the ability of tropomyosin-binding sites in the intrinsically disordered N-terminal region to bind to various tropomyosin isoforms with different affinity. It is very possible that Tmods assume slightly different conformations dependent on a tropomyosin isoform present at the pointed end of actin filaments. Moreover, for Tmods to be able to bind and nucleate actin monomers and to be able to cap the pointed end of thin filaments, different conformations are required, and the ABS1 in the flexible N-terminal region allows Tmod to adopt those.

To investigate differential binding of Tmods to tropomyosin isoforms, residues Ala21 and Glu33 in Tmod1 fragment (residues 1-38) containing TMBS1 were mutated to Lys and Val respectively, since these residues are present in Tmod2 and Tmod3 in homologous positions.¹⁰⁸ The combination of the two mutations resulted in the Tmod1 fragment that had similar affinity for short tropomyosins as homologous fragments of the Tmod2 and Tmod3 isoforms. The differential binding was investigated further by studying the competition between Tmod isoforms 1-3 for binding to the pointed end in the presence of Tpm3.1, a short tropomyosin isoform found in many non-muscle cells.⁹⁹ Cosedimentation of Tpm3.1 decorated actin filaments in the presence of all Tmod isoforms 1-3 showed that Tmod3 preferentially binds to the pointed end. Performing the same experiment with one of two mutations in Tmod3, L29E and L134D, which were meant to disrupt TMBS1 or TMBS2, respectively, revealed that the TMBS2 in Tmod3 confers its preferential binding.⁹⁹ These findings demonstrate that Tmods compete for binding to the pointed end and that their competition is a function of their affinity for the available tropomyosin.

Concluding remarks

To summarize, in muscle, the flexible adaptive nature of IDRs can provide multiple benefits to efficient governing of processes responsible for the sarcomere structure and function. The roles of the IDRs in sarcomeres can vary in scope, and they can range from ensuring effective binding of multiple partners to the control of binding kinetics and thermodynamics by processes altering the backbone dynamic properties of the IDRs.

We reviewed two types of proteins containing IDRs, the members of the Tmod/Lmod homology group, responsible for the thin filament assembly, and MyBP-C, a regulator of sarcomere contraction. Both of the groups are expressed as a set of isoforms playing different roles in different tissues and at different times of the organism development. The IDRs are capable of adaptation to changing environment. As a result, only a limited number of isoforms are required to perform a large number of tasks related to normal muscle operation.

The processes of binding and dissociating of the MyBP-C NTBs involve dynamic transitions between multiple conformational states of the M-domain and PAL. These processes can be regulated by phosphorylation. Since a large conformational space is accessible to IDRs, the regulation mechanisms are difficult to study and properly characterize. However, these

regulation mechanisms may have evolutionary advantages in that fine-tuning can be achieved without the constraints that would be imposed if the domains were to be kept well folded.

Tropomodulins/leiomodins contain large IDRs that simultaneously perform the function of binding sites and flexible linkers connecting the sites and well folded domains. The Tmod/Lmod binding is realized through multivalent interactions with the thin filaments. Since regulation of the actin filament assembly also requires fast exchange between Tmods/Lmods, multivalency could provide an indispensable mechanism accelerating the exchange between high affinity complexes. Indeed, multiple binding domains connected by linkers can achieve an extremely high binding affinity.⁴⁵ However, dissociation of the tightly bound multivalent complexes is accelerated in the presence of competitors through repopulation of partially bound intermediates. This aspect of utilization of IDRs makes them extremely valuable in the condition of dynamic exchange between protein complexes responsible for sarcomere structure.

Acknowledgements

This work was supported by the National Institutes of Health grant GM120137 to ASK.

Bibliography

1. van der Velden J, Stienen GJM. Cardiac Disorders and Pathophysiology of Sarcomeric Proteins. *Physiological reviews*. 1 1 2019;99(1):381–426. [PubMed: 30379622]
2. Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC. Overview of the Muscle Cytoskeleton. *Comprehensive Physiology*. 6 18 2017;7(3):891–944. [PubMed: 28640448]
3. Sequeira V, Witjas-Paalberends ER, Kuster DW, van der Velden J. Cardiac myosin-binding protein C: hypertrophic cardiomyopathy mutations and structure-function relationships. *Pflugers Archiv : European journal of physiology*. 2 2014;466(2):201–206. [PubMed: 24240729]
4. McNamara JW, Sadayappan S. Skeletal myosin binding protein-C: An increasingly important regulator of striated muscle physiology. *Arch Biochem Biophys*. 12 15 2018;660:121–128. [PubMed: 30339776]
5. Gokhin DS, Fowler VM. Tropomodulin capping of actin filaments in striated muscle development and physiology. *Journal of biomedicine & biotechnology*. 2011;2011:103069. [PubMed: 22013379]
6. Colpan M, Moroz NA, Kostyukova AS. Tropomodulins and tropomyosins: working as a team. *Journal of muscle research and cell motility*. 8 2013;34(3–4):247–260. [PubMed: 23828180]
7. Fowler VM, Dominguez R. Tropomodulins and Leiomodins: Actin Pointed End Caps and Nucleators in Muscles. *Biophysical journal*. 5 9 2017;112(9):1742–1760. [PubMed: 28494946]
8. Flashman E, Redwood C, Moolman-Smook J, Watkins H. Cardiac myosin binding protein C: its role in physiology and disease. *Circulation research*. 5 28 2004;94(10):1279–1289. [PubMed: 15166115]
9. Ackermann MA, Kontogianni-Konstantopoulos A. Myosin binding protein-C: a regulator of actomyosin interaction in striated muscle. *Journal of biomedicine & biotechnology*. 2011;2011:636403. [PubMed: 22028592]
10. Previs MJ, Beck Previs S, Gulick J, Robbins J, Warshaw DM. Molecular mechanics of cardiac myosin-binding protein C in native thick filaments. *Science*. 9 7 2012;337(6099):1215–1218. [PubMed: 22923435]
11. Risi C, Belknap B, Forgacs-Lonart E, et al. N-Terminal Domains of Cardiac Myosin Binding Protein C Cooperatively Activate the Thin Filament. *Structure*. 12 4 2018;26(12):1604–1611 e1604. [PubMed: 30270174]

12. Lin BL, Li A, Mun JY, et al. Skeletal myosin binding protein-C isoforms regulate thin filament activity in a Ca(2+)-dependent manner. *Scientific reports*. 2 8 2018;8(1):2604. [PubMed: 29422607]
13. Stelzer JE, Dunning SB, Moss RL. Ablation of cardiac myosin-binding protein-C accelerates stretch activation in murine skinned myocardium. *Circulation research*. 5 12 2006;98(9):1212–1218. [PubMed: 16574907]
14. Korte FS, McDonald KS, Harris SP, Moss RL. Loaded shortening, power output, and rate of force redevelopment are increased with knockout of cardiac myosin binding protein-C. *Circulation research*. 10 17 2003;93(8):752–758. [PubMed: 14500336]
15. Palmer BM, Georgakopoulos D, Janssen PM, et al. Role of cardiac myosin binding protein C in sustaining left ventricular systolic stiffening. *Circulation research*. 5 14 2004;94(9):1249–1255. [PubMed: 15059932]
16. Razumova MV, Shaffer JF, Tu AY, Flint GV, Regnier M, Harris SP. Effects of the N-terminal domains of myosin binding protein-C in an in vitro motility assay: Evidence for long-lived cross-bridges. *The Journal of biological chemistry*. 11 24 2006;281(47):35846–35854. [PubMed: 17012744]
17. Mun JY, Previs MJ, Yu HY, et al. Myosin-binding protein C displaces tropomyosin to activate cardiac thin filaments and governs their speed by an independent mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 2 11 2014;111(6):2170–2175. [PubMed: 24477690]
18. Richard P, Charron P, Carrier L, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*. 5 6 2003;107(17):2227–2232. [PubMed: 12707239]
19. Bonne G, Carrier L, Bercovici J, et al. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nature genetics*. 12 1995;11(4):438–440. [PubMed: 7493026]
20. Watkins H, Conner D, Thierfelder L, et al. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nature genetics*. 12 1995;11(4):434–437. [PubMed: 7493025]
21. Weber FE, Vaughan KT, Reinach FC, Fischman DA. Complete sequence of human fast-type and slow-type muscle myosin-binding-protein C (MyBP-C). Differential expression, conserved domain structure and chromosome assignment. *European journal of biochemistry*. 9 1 1993;216(2):661–669. [PubMed: 8375400]
22. Carrier L, Bonne G, Bahrend E, et al. Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. *Circulation research*. 3 1997;80(3):427–434. [PubMed: 9048664]
23. Luther PK, Winkler H, Taylor K, et al. Direct visualization of myosin-binding protein C bridging myosin and actin filaments in intact muscle. *Proceedings of the National Academy of Sciences of the United States of America*. 7 12 2011;108(28):11423–11428. [PubMed: 21705660]
24. Gruen M, Gautel M. Mutations in beta-myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin-binding protein-C. *Journal of molecular biology*. 2 26 1999;286(3):933–949. [PubMed: 10024460]
25. Kampourakis T, Yan Z, Gautel M, Sun YB, Irving M. Myosin binding protein-C activates thin filaments and inhibits thick filaments in heart muscle cells. *Proceedings of the National Academy of Sciences of the United States of America*. 12 30 2014;111(52):18763–18768. [PubMed: 25512492]
26. Nag S, Trivedi DV, Sarkar SS, et al. The myosin mesa and the basis of hypercontractility caused by hypertrophic cardiomyopathy mutations. *Nature structural & molecular biology*. 6 2017;24(6):525–533.
27. Jeffries CM, Whitten AE, Harris SP, Trewhella J. Small-angle X-ray scattering reveals the N-terminal domain organization of cardiac myosin binding protein C. *Journal of molecular biology*. 4 4 2008;377(4):1186–1199. [PubMed: 18313073]

28. Ababou A, Rostkova E, Mistry S, Le Masurier C, Gautel M, Pfuhl M. Myosin binding protein C positioned to play a key role in regulation of muscle contraction: structure and interactions of domain C1. *Journal of molecular biology*. 12 19 2008;384(3):615–630. [PubMed: 18926831]
29. Karsai A, Kellermayer MS, Harris SP. Mechanical unfolding of cardiac myosin binding protein-C by atomic force microscopy. *Biophysical journal*. 10 19 2011;101(8):1968–1977. [PubMed: 22004751]
30. Howarth JW, Ramisetty S, Nolan K, Sadayappan S, Rosevear PR. Structural insight into unique cardiac myosin-binding protein-C motif: a partially folded domain. *The Journal of biological chemistry*. 3 9 2012;287(11):8254–8262. [PubMed: 22235120]
31. Michie KA, Kwan AH, Tung CS, Guss JM, Trewella J. A Highly Conserved Yet Flexible Linker Is Part of a Polymorphic Protein-Binding Domain in Myosin-Binding Protein C. *Structure*. 11 1 2016;24(11):2000–2007. [PubMed: 27720588]
32. Lu Y, Kwan AH, Jeffries CM, Guss JM, Trewella J. The motif of human cardiac myosin-binding protein C is required for its Ca²⁺-dependent interaction with calmodulin. *The Journal of biological chemistry*. 9 7 2012;287(37):31596–31607. [PubMed: 22801425]
33. Barefield D, Sadayappan S. Phosphorylation and function of cardiac myosin binding protein-C in health and disease. *Journal of molecular and cellular cardiology*. 5 2010;48(5):866–875. [PubMed: 19962384]
34. Ackermann MA, Kontogianni-Konstantopoulos A. Myosin binding protein-C slow is a novel substrate for protein kinase A (PKA) and C (PKC) in skeletal muscle. *Journal of proteome research*. 10 7 2011;10(10):4547–4555. [PubMed: 21888435]
35. Ackermann MA, Ward CW, Gurnett C, Kontogianni-Konstantopoulos A. Myosin Binding Protein-C Slow Phosphorylation is Altered in Duchenne Dystrophy and Arthrogryposis Myopathy in Fast-Twitch Skeletal Muscles. *Scientific reports*. 8 19 2015;5:13235. [PubMed: 26287277]
36. Ackermann MA, Kerr JP, King B, C WW, Kontogianni-Konstantopoulos A. The Phosphorylation Profile of Myosin Binding Protein-C Slow is Dynamically Regulated in Slow-Twitch Muscles in Health and Disease. *Scientific reports*. 8 19 2015;5:12637. [PubMed: 26285797]
37. Tong CW, Stelzer JE, Greaser ML, Powers PA, Moss RL. Acceleration of crossbridge kinetics by protein kinase A phosphorylation of cardiac myosin binding protein C modulates cardiac function. *Circulation research*. 10 24 2008;103(9):974–982. [PubMed: 18802026]
38. Mamidi R, Gresham KS, Verma S, Stelzer JE. Cardiac Myosin Binding Protein-C Phosphorylation Modulates Myofilament Length-Dependent Activation. *Frontiers in physiology*. 2016;7:38. [PubMed: 26913007]
39. Gruen M, Prinz H, Gautel M. cAPK-phosphorylation controls the interaction of the regulatory domain of cardiac myosin binding protein C with myosin-S2 in an on-off fashion. *FEBS letters*. 6 25 1999;453(3):254–259. [PubMed: 10405155]
40. Shaffer JF, Kensler RW, Harris SP. The myosin-binding protein C motif binds to F-actin in a phosphorylation-sensitive manner. *The Journal of biological chemistry*. 5 1 2009;284(18):12318–12327. [PubMed: 19269976]
41. Michalek AJ, Howarth JW, Gulick J, et al. Phosphorylation modulates the mechanical stability of the cardiac myosin-binding protein C motif. *Biophysical journal*. 1 22 2013;104(2):442–452. [PubMed: 23442866]
42. Weith A, Sadayappan S, Gulick J, et al. Unique single molecule binding of cardiac myosin binding protein-C to actin and phosphorylation-dependent inhibition of actomyosin motility requires 17 amino acids of the motif domain. *Journal of molecular and cellular cardiology*. 1 2012;52(1):219–227. [PubMed: 21978630]
43. Shoemaker BA, Portman JJ, Wolynes PG. Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 8 1 2000;97(16):8868–8873. [PubMed: 10908673]
44. Mammen M, Choi SK, Whitesides GM. Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors. *Angew Chem Int Ed Engl*. 11 2 1998;37(20):2754–2794. [PubMed: 29711117]

45. Rao J, Lahiri J, Isaacs L, Weis RM, Whitesides GM. A trivalent system from vancomycin-D-ala-D-Ala with higher affinity than avidin-biotin. *Science*. 5 1 1998;280(5364):708–711. [PubMed: 9563940]
46. Song J, Ni F. NMR for the design of functional mimetics of protein-protein interactions: one key is in the building of bridges. *Biochemistry and cell biology = Biochimie et biologie cellulaire*. 1998;76(2–3):177–188. [PubMed: 9923687]
47. Tolkachev D, Vinogradova A, Ni F. Transforming bivalent ligands into retractable enzyme inhibitors through polypeptide-protein interactions. *Bioorganic & medicinal chemistry letters*. 12 1 2005;15(23):5120–5123. [PubMed: 16202582]
48. Ni F, Tolkachev Dmitri, Su, Zhengding, Inventor; National Research Council of Canada (Ottawa, CA), assignee. Bivalent thrombin binding molecules comprising linkers. US patent 8,063,018 11 22, 2011.
49. Szent-Gyorgyi AG. Calcium regulation of muscle contraction. *Biophysical journal*. 7 1975;15(7):707–723. [PubMed: 806311]
50. Previs MJ, Mun JY, Michalek AJ, et al. Phosphorylation and calcium antagonistically tune myosin-binding protein C's structure and function. *Proceedings of the National Academy of Sciences of the United States of America*. 3 22 2016;113(12):3239–3244. [PubMed: 26908872]
51. Kampourakis T, Ponnamm S, Sun YB, Sevrieva I, Irving M. Structural and functional effects of myosin-binding protein-C phosphorylation in heart muscle are not mimicked by serine-to-aspartate substitutions. *The Journal of biological chemistry*. 9 14 2018;293(37):14270–14275. [PubMed: 30082313]
52. Colson BA, Thompson AR, Espinoza-Fonseca LM, Thomas DD. Site-directed spectroscopy of cardiac myosin-binding protein C reveals effects of phosphorylation on protein structural dynamics. *Proceedings of the National Academy of Sciences of the United States of America*. 3 22 2016;113(12):3233–3238. [PubMed: 26908877]
53. Ababou A, Gautel M, Pfuhl M. Dissecting the N-terminal myosin binding site of human cardiac myosin-binding protein C. Structure and myosin binding of domain C2. *The Journal of biological chemistry*. 3 23 2007;282(12):9204–9215. [PubMed: 17192269]
54. Shaffer JF, Harris SP. Species-specific differences in the Pro-Ala rich region of cardiac myosin binding protein-C. *Journal of muscle research and cell motility*. 12 2009;30(7-8):303–306. [PubMed: 20217194]
55. Jeffries CM, Lu Y, Hynson RM, et al. Human cardiac myosin binding protein C: structural flexibility within an extended modular architecture. *Journal of molecular biology*. 12 16 2011;414(5):735–748. [PubMed: 22041450]
56. Lovell SC, Davis IW, Arendall WB 3rd, et al. Structure validation by C α geometry: ϕ , ψ and C β deviation. *Proteins*. 2 15 2003;50(3):437–450. [PubMed: 12557186]
57. Orlova A, Galkin VE, Jeffries CM, Egelman EH, Trewheella J. The N-terminal domains of myosin binding protein C can bind polymorphically to F-actin. *Journal of molecular biology*. 9 23 2011;412(3):379–386. [PubMed: 21821050]
58. Harris SP, Belknap B, Van Sciver RE, White HD, Galkin VE. C0 and C1 N-terminal Ig domains of myosin binding protein C exert different effects on thin filament activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2 9 2016;113(6):1558–1563. [PubMed: 26831109]
59. Shaffer JF, Wong P, Bezold KL, Harris SP. Functional differences between the N-terminal domains of mouse and human myosin binding protein-C. *Journal of biomedicine & biotechnology*. 2010;2010:789798. [PubMed: 20379391]
60. Zhou HX. Single-chain versus dimeric protein folding: thermodynamic and kinetic consequences of covalent linkage. *Journal of the American Chemical Society*. 7 11 2001;123(27):6730–6731. [PubMed: 11439075]
61. Zhou HX. The affinity-enhancing roles of flexible linkers in two-domain DNA-binding proteins. *Biochemistry*. 12 18 2001;40(50):15069–15073. [PubMed: 11735389]
62. Sussman MA, Sakhi S, Barrientos P, Ito M, Kedes L. Tropomodulin in Rat Cardiac-Muscle - Localization of Protein Is Independent of Messenger-Rna Distribution during Myofibrillar Development. *Circulation Research*. 8 1994;75(2):221–232. [PubMed: 8033336]

63. Fowler VM, Sussmann MA, Miller PG, Flucher BE, Daniels MP. Tropomodulin Is Associated with the Free (Pointed) Ends of the Thin-Filaments in Rat Skeletal-Muscle. *J Cell Biol.* 1 1993;120(2):411–420. [PubMed: 8421055]
64. Cox PR, Zoghbi HY. Sequencing, expression analysis, and mapping of three unique human tropomodulin genes and their mouse orthologs. *Genomics.* 1 1 2000;63(1):97–107. [PubMed: 10662549]
65. Almenar-Queralt A, Lee A, Conley CA, de Pouplana LR, Fowler VM. Identification of a novel tropomodulin isoform, skeletal tropomodulin, that caps actin filament pointed ends in fast skeletal muscle. *Journal of Biological Chemistry.* 10 1 1999;274(40):28466–28475. [PubMed: 10497209]
66. Gokhin DS, Lewis RA, McKeown CR, et al. Tropomodulin isoforms regulate thin filament pointed-end capping and skeletal muscle physiology. *J Cell Biol.* 4 5 2010;189(1):95–109. [PubMed: 20368620]
67. Conley CA, Fritz-Six KL, Almenar-Queralt A, Fowler VM. Leiomodins: larger members of the tropomodulin (Tmod) gene family. *Genomics.* 4 15 2001;73(2):127–139. [PubMed: 11318603]
68. Conley CA. Leiomodins and tropomodulin in smooth muscle. *American journal of physiology. Cell physiology.* 6 2001;280(6):C1645–1656. [PubMed: 11350761]
69. Yuen M, Sandaradura SA, Dowling JJ, et al. Leiomodins-3 dysfunction results in thin filament disorganization and nemaline myopathy. *J Clin Invest.* 11 2014;124(11):4693–4708. [PubMed: 25250574]
70. Chereau D, Boczkowska M, Skwarek-Maruszewska A, et al. Leiomodins are actin filament nucleators in muscle cells. *Science.* 4 11 2008;320(5873):239–243. [PubMed: 18403713]
71. Skwarek-Maruszewska A, Boczkowska M, Zajac AL, et al. Different localizations and cellular behaviors of leiomodins and tropomodulin in mature cardiomyocyte sarcomeres. *Mol Biol Cell.* 10 1 2010;21(19):3352–3361. [PubMed: 20685966]
72. Tsukada T, Pappas CT, Moroz N, Antin PB, Kostyukova AS, Gregorio CC. Leiomodins-2 are an antagonist of tropomodulin-1 at the pointed end of the thin filaments in cardiac muscle. *J Cell Sci.* 9 15 2010;123(Pt 18):3136–3145. [PubMed: 20736303]
73. Boczkowska M, Rebowski G, Kremneva E, Lappalainen P, Dominguez R. How Leiomodins and Tropomodulin use a common fold for different actin assembly functions. *Nat Commun.* 9 15 2015;6(1):8314. [PubMed: 26370058]
74. Pappas CT, Mayfield RM, Henderson C, et al. Knockout of Lmod2 results in shorter thin filaments followed by dilated cardiomyopathy and juvenile lethality. *Proceedings of the National Academy of Sciences of the United States of America.* 11 3 2015;112(44):13573–13578. [PubMed: 26487682]
75. Ly T, Moroz N, Pappas CT, et al. The N-terminal tropomyosin- and actin-binding sites are important for leiomodins' function. *Mol Biol Cell.* 8 15 2016;27(16):2565–2575. [PubMed: 27307584]
76. Ly T, Pappas CT, Johnson D, et al. Effects of cardiomyopathy-linked mutations K15N and R21H in tropomyosin on thin-filament regulation and pointed-end dynamics. *Mol Biol Cell.* 1 15 2019;30(2):268–281. [PubMed: 30462572]
77. Szatmari D, Bugyi B, Ujfalusi Z, Grama L, Dudas R, Nyitrai M. Cardiac leiomodins bind to the sides of actin filaments and regulate the ATPase activity of myosin. *PLOS ONE.* 2017/10/12/ 2017;12(10):e0186288. [PubMed: 29023566]
78. Weber A, Pennise CR, Babcock GG, Fowler VM. Tropomodulin caps the pointed ends of actin filaments. *J Cell Biol.* 12 1994;127(6 Pt 1):1627–1635. [PubMed: 7798317]
79. Gregorio CC, Fowler VM. Mechanisms of thin filament assembly in embryonic chick cardiac myocytes: tropomodulin requires tropomyosin for assembly. *J Cell Biol.* 5 1995;129(3):683–695. [PubMed: 7730404]
80. Gregorio CC, Weber A, Bondad M, Pennise CR, Fowler VM. Requirement of pointed-end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes. *Nature.* 1995.
81. Pappas CT, Farman GP, Mayfield RM, Konhilas JP, Gregorio CC. Cardiac-specific knockout of Lmod2 results in a severe reduction in myofilament force production and rapid cardiac failure. *J Mol Cell Cardiol.* 9 2018;122:88–97. [PubMed: 30102883]

82. Boczkowska M, Yurtsever Z, Rebowksi G, Eck MJ, Dominguez R. Crystal Structure of Leiomodlin 2 in Complex with Actin: A Structural and Functional Reexamination. *Biophys J*. 8 22 2017;113(4):889–899. [PubMed: 28834725]
83. Kostyukova A, Maeda K, Yamauchi E, Krieger I, Maeda Y. Domain structure of tropomodulin: distinct properties of the N-terminal and C-terminal halves. *Eur J Biochem*. 11 2000;267(21):6470–6475. [PubMed: 11029591]
84. Kostyukova AS, Tiktopulo EI, Maeda Y. Folding properties of functional domains of tropomodulin. *Biophys J*. 7 2001;81(1):345–351. [PubMed: 11423419]
85. Krieger I, Kostyukova A, Yamashita A, Nitanaï Y, Maeda Y. Crystal structure of the C-terminal half of tropomodulin and structural basis of actin filament pointed-end capping. *Biophys J*. 11 2002;83(5):2716–2725. [PubMed: 12414704]
86. Babcock GG, Fowler VM. Isoform-specific Interaction of Tropomodulin with Skeletal Muscle and Erythrocyte Tropomyosins". *Journal of Biological Chemistry*. 1994/11/04/ 1994;259(44):9.
87. Vera C, Sood A, Gao KM, Yee LJ, Lin JJ, Sung LA. Tropomodulin-binding site mapped to residues 7-14 at the N-terminal heptad repeats of tropomyosin isoform 5. *Arch Biochem Biophys*. 6 1 2000;378(1):16–24. [PubMed: 10871039]
88. Kostyukova AS, Hitchcock-DeGregori SE. Effect of the structure of the N terminus of tropomyosin on tropomodulin function. *The Journal of biological chemistry*. 2 13 2004;279(7):5066–5071. [PubMed: 14660556]
89. Greenfield NJ, Kostyukova AS, Hitchcock-DeGregori SE. Structure and tropomyosin binding properties of the N-terminal capping domain of tropomodulin 1. *Biophys J*. 1 2005;88(1):372–383. [PubMed: 15475586]
90. Kostyukova AS, Rapp BA, Choy A, Greenfield NJ, Hitchcock-DeGregori SE. Structural requirements of tropomodulin for tropomyosin binding and actin filament capping. *Biochemistry*. 3 29 2005;44(12):4905–4910. [PubMed: 15779917]
91. Kostyukova AS, Choy A, Rapp BA. Tropomodulin binds two tropomyosins: a novel model for actin filament capping. *Biochemistry*. 10 3 2006;45(39):12068–12075. [PubMed: 17002306]
92. Greenfield NJ, Fowler VM. Tropomyosin requires an intact N-terminal coiled coil to interact with tropomodulin. *Biophys J*. 5 2002;82(5):2580–2591. [PubMed: 11964245]
93. Rao JN, Madasu Y, Dominguez R. Mechanism of actin filament pointed-end capping by tropomodulin. *Science*. 7 25 2014;345(6195):463–467. [PubMed: 25061212]
94. Fowler VM, Greenfield NJ, Moyer J. Tropomodulin Contains Two Actin Filament Pointed End-capping Domains. *Journal of Biological Chemistry*. 2003/10/10/ 2003;278(41):40000–40009. [PubMed: 12860976]
95. Kostyukova AS. Leiomodlin/tropomyosin interactions are isoform specific. *Arch Biochem Biophys*. 9 1 2007;465(1):227–230. [PubMed: 17572376]
96. Chen X, Ni F, Kondrashkina E, Ma J, Wang Q. Mechanisms of leiomodlin 2-mediated regulation of actin filament in muscle cells. *Proceedings of the National Academy of Sciences of the United States of America*. 10 13 2015;112(41):12687–12692. [PubMed: 26417072]
97. Lu S, Symersky J, Li S, et al. Structural genomics of *Caenorhabditis elegans*: crystal structure of the tropomodulin C-terminal domain. *Proteins*. 8 1 2004;56(2):384–386. [PubMed: 15211521]
98. Tsukada T, Kotlyanskaya L, Huynh R, et al. Identification of residues within tropomodulin-1 responsible for its localization at the pointed ends of the actin filaments in cardiac myocytes. *The Journal of biological chemistry*. 1 21 2011;286(3):2194–2204. [PubMed: 21078668]
99. Colpan M, Moroz NA, Gray KT, Cooper DA, Diaz CA, Kostyukova AS. Tropomyosin-binding properties modulate competition between tropomodulin isoforms. *Arch Biochem Biophys*. 6 15 2016;600:23–32. [PubMed: 27091317]
100. Arslan B, Colpan M, Gray KT, Abu-Lail NI, Kostyukova AS. Characterizing interaction forces between actin and proteins of the tropomodulin family reveals the presence of the N-terminal actin-binding site in leiomodlin. *Arch Biochem Biophys*. 1 15 2018;638:18–26. [PubMed: 29223925]
101. Fujisawa T, Kostyukova A, Maeda Y. The shapes and sizes of two domains of tropomodulin, the P-end-capping protein of actin-tropomyosin. *FEBS Lett*. 6 1 2001;498(1):67–71. [PubMed: 11389900]

102. Drozdetskiy A, Cole C, Procter J, Barton GJ. JPred4: a protein secondary structure prediction server. *Nucleic Acids Research*. 2015/07/01/ 2015;43(W1):W389–W394. [PubMed: 25883141]
103. Fowler VM, Greenfield NJ, Moyer J. Tropomodulin contains two actin filament pointed end-capping domains. *The Journal of biological chemistry*. 10 10 2003;278(41):40000–40009. [PubMed: 12860976]
104. Yamashiro S, Speicher KD, Speicher DW, Fowler VM. Mammalian tropomodulins nucleate actin polymerization via their actin monomer binding and filament pointed end-capping activities. *The Journal of biological chemistry*. 10 22 2010;285(43):33265–33280. [PubMed: 20650902]
105. Meshcheryakov VA, Krieger I, Kostyukova AS, Samatey FA. Structure of a tropomyosin N-terminal fragment at 0.98 Å resolution. *Acta crystallographica. Section D, Biological crystallography*. 9 2011;67(Pt 9):822–825. [PubMed: 21904035]
106. Ly T, Krieger I, Tolkachev D, et al. Structural destabilization of tropomyosin induced by the cardiomyopathy-linked mutation R21H. *Protein science : a publication of the Protein Society*. 2 2018;27(2):498–508. [PubMed: 29105867]
107. Pauling L, Corey RB. The pleated sheet, a new layer configuration of polypeptide chains. *Proceedings of the National Academy of Sciences of the United States of America*. 1951;37(5):251–256. [PubMed: 14834147]
108. Uversky VN, Shah SP, Gritsyna Y, Hitchcock-DeGregori SE, Kostyukova AS. Systematic analysis of tropomodulin/tropomyosin interactions uncovers fine-tuned binding specificity of intrinsically disordered proteins. *Journal of molecular recognition : JMR*. Jul-Aug 2011;24(4):647–655. [PubMed: 21584876]
109. Kostyukova AS, Hitchcock-DeGregori SE, Greenfield NJ. Molecular basis of tropomyosin binding to tropomodulin, an actin-capping protein. *J Mol Biol*. 9 21 2007;372(3):608–618. [PubMed: 17706248]
110. Chojnacki S, Cowley A, Lee J, Foix A, Lopez R. Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Research*. 2017/07// 2017;45(W1):W550–W553. [PubMed: 28431173]

Fig. 1.

A schematic view of a sarcomere. Shown in the figure are schematic locations of tropomodulin, leiomodlin and myosin-binding protein C (MyBP-C). The inset shows two putative binding modes of MyBP-C.

Fig. 2.
Alignment of structures of Tmod1's LRR domain solved with (pdb 4PKI, tan) and without actin (pdb 1IO0, teal). Actin is shown in grey. Side chains of R343 and L344 are shown.

Fig. 3.

Amino acid sequences of the N-terminal disordered regions of Tmod1 from *Homo sapiens* (Accession No. AAH02660.1) and Lmod2 from *Homo sapiens* (Accession no. Q6P5Q4). Pairwise sequence alignment was made using EMBOSS stretcher¹¹⁰ at https://www.ebi.ac.uk/Tools/psa/emboss_stretcher/. Identical amino acid residues are highlighted in light gray. Similar amino acids are highlighted in dark gray. Secondary structure prediction was made using Jpred¹⁰² at <http://www.compbio.dundee.ac.uk/jpred/index.html>. Amino acid residues predicted to be in α -helical conformation are italicized. Amino acid residues observed to be in α -helical conformation are bolded. Binding sites are boxed.

Fig. 4. Alignment of the ten best NMR structures of the N-terminal Tmod1 fragment (residues 1-92). In the structure only residues E24-E35 that should be within TMBS1 formed a helix, all other were disordered⁸⁹.

Fig. 5. Crystal structure of the complex of actin (grey) with Tmod1's N-terminal fragment (tan), fused by its N-terminus to the gelsolin fragment (orange) via a 9 amino acid flexible linker (pdb 4PKG). Of Tmod1's residues 50-101, only residues P58-K99 can be seen in the structure.