Periodicities designed in the tropomyosin sequence and structure define its functions

Bipasha Barua

Department of Pathology and Laboratory Medicine; Robert Wood Johnson Medical School; Rutgers University; Piscataway, NJ USA

Keywords: tropomyosin, muscle regulation, actin filament, cytoskeleton, coiled coil

Abbreviations: Tm, tropomyosin; Tn, troponin; myosin S1, myosin subfragment 1; HMW, high molecular weight; LMW, low molecular weight; EM, electron microscopy; a.a., amino acid

Tropomyosin is an actin binding protein that regulates actin filament dynamics and its interactions with actin binding proteins such as myosin, tropomodulin, formin, Arp2/3 and ADF-cofilin in most eukaryotic cells. Tropomyosin is the prototypical two-chained, α -helical coiled coil protein that associates end-to-end and binds to both sides of the actin filament. Each tropomyosin molecule spans four to seven actin monomers in the filament, depending on the size of the tropomyosin. Tropomyosins have a periodic heptad repeat sequence that is characteristic of coiled coil proteins as well as additional periodicities required for its interaction with the actin filament, where each periodic repeat interacts with one actin molecule. This review addresses the role of periodic features of the Tm molecule in carrying out its universal functions of binding to the actin filament and its regulation and the specific features that may determine the isoform specificity of tropomyosins.

Introduction

Tropomyosin (Tm) is an actin binding protein that binds to the actin filament and stabilizes it. Actin filaments carry out diverse cellular functions such as cell migration, cytokinesis, cell morphology, intracellular transport and muscle contraction.¹ Tropomyosin regulates actin filament functions in most eukaryotic muscle and non-muscle cells in organisms ranging from mammals to fungi.^{2,3} Mutations in Tm genes cause skeletal and cardiomyopathies.^{4,5} In striated muscle, Tm and troponin (Tn) regulate actin-myosin crossbridge cycling during muscle contraction in a Ca^{2+} -dependent manner. This regulation is described by a three-state model, where the three kinetic states are suggested to correspond to three positions of Tm on the actin filament.^{6,7} The three states are defined as: (1) blocked or off state, where in the presence of Tn and absence of Ca^{2+} , Tm blocks the interaction of myosin with actin; (2) closed state, where in the presence of Tn and Ca^{2+} , Tm shifts azimuthally on the actin filament to partially expose myosin binding sites and allow weak myosin binding; and (3) open or on state, where in the presence of myosin

heads (myosin S1), Tm azimuthally shifts further on the actin filament, allowing strong myosin binding and force production. Tropomyosins regulate the interaction of the actin filament with actin binding proteins including myosin, tropomodulin, formin, Arp2/3 and ADF-cofilin as well as regulate actin dynamics at the leading edge of cells during migration.^{2,3}

Tropomyosin is the prototypical two-chained, α -helical coiled coil protein that associates end-to-end to form long cables along both sides of the actin filament. Each Tm molecule spans 4 to 7 actin monomers in the actin filament, depending on the length of the Tm. In animals, Tm can be found as ~284-residue high molecular weight (HMW) isoforms spanning 7 actins or ~247-residue low molecular weight (LMW) isoforms spanning 6 actins. In fungi, Tms can be 199- or 161-residues spanning 5 and 4 actins, respectively. The elongated nature of the binding interface of Tm with the actin filament suggests the occurrence of periodic actin binding sites in Tm, where each site binds to one actin monomer. The Tm sequence consists of seven-residue (a to g) repeats of amino acids characteristic of coiled coil proteins known as heptad repeats. Moreover, the Tm gene structure is also believed to be generated through repeated duplications of an ancestral 21-residue sequence. Do the periodicities inherent in the Tm molecule make it specially designed to carry out its universal functions of binding and regulating the actin filament? How are these periodic features related to function? Are specific regions of Tm involved in carrying out specific functions? What determines the isoform specificity of tropomyosins? This review addresses some of these questions in an attempt to enhance our understanding of the specific features of Tm and the mechanisms through which it binds and regulates the actin filament.

Periodicities in the Tropomyosin Sequence

Tropomyosin is a coiled coil protein with two parallel α -helices that can exist as homodimers or heterodimers. The α -helices of Tms from animals (242–284 a.a.) consist of continuous heptad repeats, which are seven-residue (a-g) repeats of amino acids characteristic of coiled coil proteins, where a and d positions are typically occupied by hydrophobic residues, and e and g positions are often occupied by oppositely charged residues that can form inter-helical salt bridges (Fig. 1). The stability of the coiled coil is derived from the packing of the hydrophobic residues at

Correspondence to: Bipasha Barua; Email: baruabi@rwjms.rutgers.edu
Submitted: 06/06/13; Revised: 07/01/13; Accepted: 07/03/13
<http://dx.doi.org/10.4161/bioa.25616>

Figure 1. Helical wheel representation of a coiled coil showing the heptad repeats. Heptad repeats are labeled (a-g) and (a'-g') on the two helices, where (a) and (d) are hydrophobic residues, (e) and (g) are acidic or basic residues and (b), (c), and (f) are surface residues (from Mason et al.).⁴⁷

the coiled coil interface and inter-helical salt-bridge formation. The residues at b, c and f positions are on the surface of the coiled coil and are available for binding other proteins.

Most α -helical coiled coils such as those in myosin, kinesin, intermediate filaments, lamin and fibrinogen have breaks or disruptions in the heptad repeat, but Tm is different from other coiled coils of similar length in having uninterrupted heptad repeats.⁸ An exception is fungal Tms, which are shorter (161–199 a.a.) and have one or two 4-residue deletions that disrupt the heptad repeats, designated as “stammers.”^{8,9} Also, unlike typical coiled coil proteins, the interface *a* and *d* positions in animal Tms are often occupied by small apolar residues such as Ala and Ser, designated as Ala clusters or by charged residues such as D137 and E218, which are highly conserved in Tms. The presence of these residues at the interface allows the formation of bends and staggers and introduces local flexibility in the Tm coiled coil. Again, this is not true of fungal Tms, which do not have Ala clusters but it is believed that the shorter coiled coil length and the stammer(s) in the heptad repeat provide the instability and flexibility similar to the Ala clusters in animal Tms.

The 284-residue rabbit skeletal muscle α -tropomyosin sequence was the first to be extensively analyzed in order to determine if there were significant long-range patterns that could explain how Tm binds to seven actin monomers in the actin filament. These initial studies to relate Tm sequence to function were all done with the only protein sequence information that was available at the time, the rabbit skeletal muscle α -tropomyosin sequence. There was no DNA sequence information available for this protein and hence no information about the gene structure of Tms. Based on Fourier analysis of the amino acid sequence, in addition to the heptad repeat pattern, ~ 20 - and ~ 40 -residue periodicities were observed at non-interface residues.^{10–12} The 20- and 40-residue periodicities were postulated to correspond to 14 and 7 quasi-equivalent actin binding sites along the length of one Tm molecule that can bind to seven consecutive actins on one side of the filament. McLachlan and Stewart proposed that the 14 bands

of ~ 20 -residues each, consist of alternative sets of sites designated as α and β that are actin binding sites in the off and on states of the thin filament, respectively.¹⁰ They also noted a periodic distribution of small nonpolar amino acids (such as Ala) at the interface that they suggested would allow flexibility and bending of the Tm supercoil on the actin filament helix. Taking into account the azimuthal positions of residues, Phillips identified a 7-fold quasi-equivalent periodic repeat, designated as periods, where each period included a seven-residue repeat of charged and nonpolar residues at surface positions that were proposed as actin binding sites.¹³ The observation of long-range periodicity in the Tm supercoil led to the conclusion that the periodic repeats must be related to its interaction with the actin filament, since long-range periodicity is not required to maintain the Tm coiled coil structure.

Tropomyosin Gene Structure and Evolution

On the basis of the periodic patterns present in the Tm protein sequence, it was suggested that the periodicities are a consequence of gene duplications of a 42-residue segment.¹⁰ However, with the discovery of the Tm gene structure and exon organization, it was suggested that the Tm genes originated through repeated duplication of an ancestral 21 a.a. exon with the loss of introns between duplicated exons to form longer exons.¹⁴ The exon sizes in Tm genes range from 21 a.a. to 45 a.a. in vertebrates and correspond roughly with the putative actin binding periodic repeats of ~ 20 - or ~ 40 -residues thus strengthening the notion that the functional periodicities in the Tm molecule arise from the exon duplications. However, fungi do not have this exon structure and some invertebrates have shorter exons.^{15,16}

Tropomyosins are a highly-conserved family of proteins. In addition to the conserved structural motifs to ensure the adoption of the coiled coil, the extent of primary sequence conservation is quite remarkable, and suggests that the entire surface of Tms (especially in vertebrates) is under evolutionary selective pressure.^{15,17} Tropomyosin diversity arises from the presence of various isoforms that are produced by the use of alternate promoters (exons 1a/1b) and alternative splicing of three exons: 2a/2b, 6a/6b, and 9a/9b/9c/9d, that are expressed in a developmental- and tissue-specific manner from 4–6 genes (in vertebrates) (Fig. 2).² Invertebrate Tms either have no alternate exons or have alternative splicing of exons that are constitutive in the vertebrates (Fig. 2). The difference in length of HMW and LMW forms arises from the use of alternate promoters for exons 1a and 2 or 1b. The same Tm gene can express both muscle and non-muscle isoforms of the protein. For example, the rat *TPM1* gene gives rise to one striated muscle, one smooth muscle and at least seven non-muscle isoforms of Tm including three brain-specific isoforms.² Tropomyosin isoform diversity increases with increasing complexity of organisms- fungi and lower invertebrates such as cnidarians have one or two Tm genes that produce single isoforms with no alternative exons, but mammals have four Tm genes that undergo alternative splicing of exons to generate multiple Tm isoforms from each gene.^{15,16} In contrast to invertebrates where the diversity of gene structure

Figure 2. Tropomyosin gene structure in animals. Exon organization and alignment of the vertebrate and invertebrate TPM genes. Alternatively spliced exons are labeled a–d in vertebrates and A–E in invertebrates (from Barua et al.).¹⁵

is quite variable, there is a remarkable conservation of both gene structure and alternative splicing among vertebrates.

Phylogenetic trees of Tm genes show that the four Tm genes of the vertebrates arose through gene duplications specific to the lineage leading to vertebrates, while Tm gene duplications in the invertebrate lineages arose independently.^{15,16,18} Similarly, exon duplications leading to alternatively spliced exons within Tm genes took place independently and on multiple occasions in the vertebrate and invertebrate lineages. For example, the human *TPM1* gene has alternative splicing of exons 1, 2, 6 and 9 whereas in the *C. elegans TPM* gene, exons 3, 4 and 9 are alternatively spliced. The number of exons is not strictly conserved between invertebrate and vertebrate genes.^{15,16} Some exons are split and others are fused relative to the vertebrate exon structure, however, the exon-intron boundaries are mostly conserved. Therefore, it appears that the repetitive gene structure of Tm has been evolutionarily conserved in eukaryotes and the Tm genes evolved under selective pressure to maintain the periodicity to be able to bind to the actin filament and regulate its functions.

Actin Binding and Actomyosin Regulation by Tropomyosin

Experimental evidence based on mutagenesis studies of Tms has confirmed the importance of the periodic features of the Tm molecule that were predicted based on the analysis of the protein sequence alone. These include an uninterrupted heptad repeat pattern, regions of local instability at the coiled coil interface (Ala clusters), and the sequence pattern on the surface of the coiled coil that was proposed as actin binding sites.¹⁹ Previous studies have also shown that individual periodic repeats of Tm are quasi-equivalent and contribute in different ways to actin binding and regulatory function.^{20–24} Crystal structures of Tm fragments have shown that the coiled coil structure is not uniform along the length of the molecule and there are variations in the interhelical distance and pitch of the coiled coil as well as bends and staggers in the molecule.^{25–27} A solution structure of the striated muscle Tm overlap complex gave some insight into how the proposed periodic actin binding sites on Tm relate to the actin monomers in the actin filament.²⁸ Based on the 7-fold periodicity in the sequence, it may be expected that there will be seven half-turns of the supercoil per Tm molecule to correspond to the seven actins along the length of one HMW Tm molecule. However, based on an analysis of the 7 Å crystal structure of Tm and the overlap complex structure, Greenfield et al. showed that there

Figure 3. Tropomyosin mutations at evolutionarily-conserved surface residues. The rat striated α Tm sequence showing conserved b, c, and f residues that were mutated to Ala. Each mutant had 1–4 mutations within the first-half (magenta) of periods 1–7 or the second-half (cyan) of periods 2–6. The pattern of basic and acidic residues in the first-half of periods at positions f, b, and f are indicated by the blue (position f, basic residues) and red (positions b and f, acidic residues) boxes.^{15,30,31}

are only 5.75 half-turns of the supercoil.^{28,29} Modeling of Tm molecules joined by the overlap complex on the actin filament showed that the actin binding sites proposed by Phillips present a similar face to the actin monomers from one Tm molecule to the next, even though the periodic repeats do not correspond to supercoil repeats.

In an attempt to determine the functionally important sites of Tm independent of previous models based on sequence analysis, an evolutionary analysis of Tm was performed to identify its conserved residues.¹⁵ Since actin is a highly conserved protein it was postulated that the periodic actin binding sites of Tm would have been conserved through evolution to retain its actin-binding and other conserved functions. Evolutionarily-conserved surface b, c, and f residues in periodic repeats (periods) 1–7 were mutated to Ala, where each mutant had 1–4 sites mutated within the first-half or the second-half of each period (Fig. 3). The mutations in the first-half of periods 1–7 included a pattern of basic and acidic surface residues that are conserved at positions f, b and f of the heptad repeat as shown in Figure 3.³⁰ Mutations in the first-half of periods resulted in ≥ 4 -fold reduction in actin affinity, indicating these mutations include residues in actin binding sites. This was in contrast to mutations in the second-half of the periods, which had

Figure 4. Summary of contributions of conserved residues of individual periodic repeats to tropomyosin function. Tropomyosin residues important for actin binding (first-half of periods, magenta) and actomyosin regulation (second-half of periods, cyan) shown in the 7 Å striated muscle α Tm structure (1C1G).²⁹

a minimal (≤ 2 -fold) effect on affinity indicating these residues may be involved in other conserved functions such as actomyosin regulation. These studies also showed that actin binding sites on Tm follow a pattern of basic and basic-acidic residues in alternating periods (Fig. 3) in the first-half of each period. Hydrophobic interactions were found to be relatively less important for actin binding consistent with the primarily electrostatic nature of actin-Tm binding.³⁰ The effect of Tm mutations at conserved surface positions in the second-half of periods 2–6 on actomyosin regulation was determined using in vitro motility assays.³¹ Tropomyosin with mutations in the second-half of periods 3–6 showed a large inhibition of actin-Tm filament velocities compared with WT Tm, indicating that the conserved residues in the second halves of the periods are involved in actomyosin regulation. The inhibition of myosin regulation suggests that the mutated residues of Tm are required to shift the equilibrium of actin-Tm filaments to the open state and may be binding sites for actin and/or myosin in the open or activated state. These studies suggest that each period of Tm includes a site in the first-half for actin binding in the closed state and a site in the second-half for cooperative activation by myosin to the open state (Fig. 4). The extent of contribution to these two functions varies from period to period.

The mutations at evolutionarily-conserved residues in the first and second halves of periods correspond approximately to the α - and β -bands of Tm that were proposed by McLachlan and Stewart to be actin binding sites in the closed and open states, respectively.¹⁰ Phillips identified a pattern of charged and hydrophobic residues, six at b, c, f and one at an e position in the first half of each period that approximately correspond to the “ α -sites” of McLachlan and Stewart. Of the 49 residues identified in the Phillips pattern, 22 were found to be evolutionarily-conserved and mutations at 21 of the b, c, f positions, singly or in combination, reduced actin affinity ≥ 4 -fold. Unlike the conserved residues in the first-half of periods that correspond to the “ α -sites” of McLachlan and Stewart, the conserved residues in the second-half of periods that correspond to the “ β -sites” do not show a definite periodic pattern (Fig. 3). Therefore, the evolutionary analysis identified periodic patterns in Tm that closely resemble those predicted from sequence analysis alone and provides an independent basis for establishing models for how Tm binds to the actin filament in the different regulatory states and the residues involved in switching it from one state to another.

The average azimuthal positions of Tm on the actin filament in the absence and presence of $\text{Tn} \pm \text{Ca}^{2+}$ and myosin S1

are known from EM reconstructions. A computational model of the actin-Tm filament combined with EM reconstructions in the absence of Tn and myosin (closed state) was determined by optimization of electrostatic interactions between Tm and actin, while a 8-Å resolution structure of the rigor actin-Tm-myosin S1 complex (open state) has been determined by cryo-electron microscopy.^{32,33} An important caveat of EM reconstructions is that the molecular ends of Tm as well as the axial and rotational position of Tm with respect to actin are not defined due to the necessity of applying helical averaging to achieve maximum resolution. Structural models for the actin-Tm interface were also constructed on the basis of crystal structures of Tm fragments and mutagenesis studies that are in agreement with the computational and EM models.^{15,26,31} The models indicate periodic interactions of Tm with actin monomers in the filament in the closed state and with both actin and myosin in the open state. To test the current models of the actin-Tm interface in the closed state, mutations were made at actin residues predicted to make electrostatic interactions with Tm residues in the repeating motif.³⁰ Tropomyosin failed to bind the mutant actin filaments, thus supporting the models. In the absence of high resolution structures for the actin-Tm and actin-Tm-myosin complexes, we have to rely on experimental validation of the current EM and computational models in order to move forward our understanding of the actin-Tm and actin-Tm-myosin interface and the manner in which the periodic quasi-repeats of Tm interact with actin and myosin.

Isoform Specificity of Tropomyosins

There are more than 40 isoforms of Tm that are expressed in mammals with the majority being non-muscle isoforms. The same *TPM* gene can express both muscle or non-muscle isoforms through the use of alternate promoters (1a/1b) and alternatively spliced exons (2a/2b, 6a/6b and 9a/9b/9c/9d). Non-muscle Tms can be HMW or LMW depending on the use of alternate promoters for exons 1a or 1b, respectively, but muscle Tms are always HMW. It has been shown that the Tm isoforms are not redundant and carry out specific functions through developmental, tissue-specific and spatial regulation. The periodic features of Tm that define its structure and function such as the heptad repeat pattern, Ala clusters, actin binding and regulatory sites are conserved among Tm isoforms, so the question that arises is what determines the isoform specificity of Tms? Isoform specific roles of Tm are most likely due to the selective use of alternate exons that could alter

actin affinity, actin filament dynamics or interaction of actin with other actin binding proteins. The combination of alternate promoters 1a or 1b at the N-terminus with alternate exons 9a, 9c, or 9d at the C-terminus determine the actin affinity of Tms due to altered end-to-end interactions between Tm molecules. The use of alternate exons may also have long-range effects by altering the structure of the Tm molecule in regions far away from the exon or by altering the position of Tm on the actin surface.

In mammals, exon 9a is mainly expressed in striated muscle Tms while exon 2a is expressed only in smooth muscle α Tms. It was shown through in vitro studies that exon 9a is required for Tn interaction, which is only present in striated muscle.³⁴ Exon 9a is also expressed from the *TPM3* gene in LMW non-muscle Tms in mammals, and it was observed that when exon 9c is knocked out in mouse brain the levels of exon 9a-containing Tms are upregulated to compensate.^{17,35,36} However, the specific biological functions of these isoforms are not yet known. Tropomyosins have been shown to have isoform-specific effects on actomyosin ATPase activity in in vitro studies, depending on the Tm and myosin isoforms.³⁷⁻³⁹ Smooth muscle α Tm activates skeletal muscle actomyosin ATPase activity under conditions where skeletal muscle α Tm inhibits.³⁸ A HMW non-muscle Tm isoform was shown to inhibit the actin-activated ATPase of skeletal muscle myosin but a LMW non-muscle isoform activated it.³⁷ However, both the HMW and LMW isoforms as well as skeletal muscle α Tm inhibited the ATPase activity of myosin I. Fission yeast Tm (cdc8p) promoted the ATPase activity of fission yeast myosin V (Myo51p and Myo52p) and myosin II (Myo2p), but inhibited myosin-I (Myo1p) activity, whereas budding yeast Tm (Tpm1p) was essential for the processive motion of the budding yeast myosin V (Myo2p) on actin.⁴⁰⁻⁴²

Tropomyosins have also been shown to have isoform-specific localization and functions in vivo. Tm5NM2 is sorted specifically to the Golgi complex, whereas Tm5NM1, which differs by a single alternatively spliced internal exon (6b in Tm5NM2 vs. 6a in Tm5NM1), is incorporated into stress fibers showing that alternative splice choice can determine the sorting of a Tm isoform.⁴³ There is extensive compartmentalization of Tms in neurons that is developmentally regulated.² Tm5NM1/2 is localized to the axon in embryonic neurons but is displaced by TmBr3, a brain-specific Tm, in the axon and relocates to the somatodendritic compartment in mature neurons. Elevated expression of Tm5NM1 in neuroepithelial cells promoted formation of stress fibers, and decreased lamellipodia and cell migration, whereas TmBr3 induced lamellipodial formation, increased cell migration and loss of stress fibers.⁴⁴ There is differential sorting of Tm

isoforms within stress fibers: Tm1 and Tm5NM1/2 regulate the stability of actin filaments in focal adhesions, while Tm2 and Tm3 promote stability along the length of dorsal stress fibers.⁴⁵ In contrast, Tm4 promotes stress fiber assembly by recruiting myosin II to stress fiber precursors. Introduction of skeletal muscle α Tm into migrating epithelial cells inhibited the formation of the leading edge lamellipodium by displacing Arp2/3 and ADF/cofilin that are normally concentrated in the lamellipodium and produce rapid actin treadmilling at the leading edge of cells.⁴⁶

Concluding Remarks

Tropomyosins play a pivotal role in regulating striated muscle contraction with troponin in a Ca^{2+} -dependent manner. There is increasing evidence for the role of Tms in cellular functions such as cell migration, cell shape, cell division and intracellular transport. The ability to bind actin and regulate actomyosin MgATPase activity constitute universal functions of Tm that are dependent on the periodicities embedded in the Tm sequence and structure. The Tm sequence, structure and functions are highly conserved among eukaryotes ranging from mammals to fungi. However, the multitude of Tm isoforms found in vertebrates points to isoform-specific functional roles of Tm in the cytoskeleton. How does a simple coiled-coil protein carry out isoform-specific functions while maintaining the conserved periodic features required for structure and function? How does Tm regulate the interaction of the actin filament with actin binding proteins including myosin, tropomodulin, formin, Arp2/3 and ADF-cofilin in an isoform-specific manner? The complexity of the cellular interactions increases further due to the presence of multiple isoforms of actin, myosin and other actin binding proteins and their differential interactions with Tm isoforms. High-resolution structural studies combined with in vivo and in vitro functional studies of Tm isoforms and their interactions with various isoforms of actin and actin binding proteins can provide insights into the specific features of Tms that lead to specificity of Tm functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The author would like to thank Dr Sarah Hitchcock-DeGregori for her helpful comments, discussion and critical reading of this manuscript. This work was supported by NIH grant GM093065 (to Sarah Hitchcock-DeGregori).

References

- Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. *Science* 2009; 326:1208-12; PMID:19965462; <http://dx.doi.org/10.1126/science.1175862>
- Gunning P, O'Neill G, Hardeman E. Tropomyosin-based regulation of the actin cytoskeleton in time and space. *Physiol Rev* 2008; 88:1-35; PMID:18195081; <http://dx.doi.org/10.1152/physrev.00001.2007>
- Wang CL, Coluccio LM. New insights into the regulation of the actin cytoskeleton by tropomyosin. *Int Rev Cell Mol Biol* 2010; 281:91-128; PMID:20460184; [http://dx.doi.org/10.1016/S1937-6448\(10\)81003-2](http://dx.doi.org/10.1016/S1937-6448(10)81003-2)
- Kee AJ, Hardeman EC. Tropomyosins in skeletal muscle diseases. *Adv Exp Med Biol* 2008; 644:143-57; PMID:19209820; http://dx.doi.org/10.1007/978-0-387-85766-4_12
- Wieczorek DE, Jagatheesan G, Rajan S. The role of tropomyosin in heart disease. *Adv Exp Med Biol* 2008; 644:132-42; PMID:19209819; http://dx.doi.org/10.1007/978-0-387-85766-4_11
- Lehman W, Craig R. Tropomyosin and the steric mechanism of muscle regulation. *Adv Exp Med Biol* 2008; 644:95-109; PMID:19209816; http://dx.doi.org/10.1007/978-0-387-85766-4_8
- McKillop DF, Geeves MA. Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. *Biophys J* 1993; 65:693-701; PMID:8218897; [http://dx.doi.org/10.1016/S0006-3495\(93\)81110-X](http://dx.doi.org/10.1016/S0006-3495(93)81110-X)
- Brown JH, Cohen C, Parry DA. Heptad breaks in α -helical coiled coils: stutters and stammers. *Proteins* 1996; 26:134-45; PMID:8916221; [http://dx.doi.org/10.1002/\(SICI\)1097-0134\(199610\)26:2<134::AID-PROT3>3.0.CO;2-G](http://dx.doi.org/10.1002/(SICI)1097-0134(199610)26:2<134::AID-PROT3>3.0.CO;2-G)

9. Strand J, Nili M, Homsher E, Tobacman LS. Modulation of myosin function by isoform-specific properties of *Saccharomyces cerevisiae* and muscle tropomyosins. *J Biol Chem* 2001; 276:34832-9; PMID:11457840; <http://dx.doi.org/10.1074/jbc.M104750200>
10. McLachlan AD, Stewart M. The 14-fold periodicity in alpha-tropomyosin and the interaction with actin. *J Mol Biol* 1976; 103:271-98; PMID:950663; [http://dx.doi.org/10.1016/0022-2836\(76\)90313-2](http://dx.doi.org/10.1016/0022-2836(76)90313-2)
11. McLachlan AD, Stewart M, Smillie LB. Sequence repeats in alpha-tropomyosin. *J Mol Biol* 1975; 98:281-91; PMID:1195388; [http://dx.doi.org/10.1016/S0022-2836\(75\)80118-5](http://dx.doi.org/10.1016/S0022-2836(75)80118-5)
12. Parry DA. Analysis of the primary sequence of alpha-tropomyosin from rabbit skeletal muscle. *J Mol Biol* 1975; 98:519-35; PMID:1195399; [http://dx.doi.org/10.1016/S0022-2836\(75\)80084-2](http://dx.doi.org/10.1016/S0022-2836(75)80084-2)
13. Phillips GN Jr. Construction of an atomic model for tropomyosin and implications for interactions with actin. *J Mol Biol* 1986; 192:128-31; PMID:3820300; [http://dx.doi.org/10.1016/0022-2836\(86\)90469-9](http://dx.doi.org/10.1016/0022-2836(86)90469-9)
14. Ruiz-Opazo N, Nadal-Ginard B. Alpha-tropomyosin gene organization. Alternative splicing of duplicated isotype-specific exons accounts for the production of smooth and striated muscle isoforms. *J Biol Chem* 1987; 262:4755-65; PMID:3558368
15. Barua B, Pamula MC, Hitchcock-DeGregori SE. Evolutionarily conserved surface residues constitute actin binding sites of tropomyosin. *Proc Natl Acad Sci U S A* 2011; 108:10150-5; PMID:21642532; <http://dx.doi.org/10.1073/pnas.1101221108>
16. Vrhovski B, Thézé N, Thiébaud P. Structure and evolution of tropomyosin genes. *Adv Exp Med Biol* 2008; 644:6-26; PMID:19209810; http://dx.doi.org/10.1007/978-0-387-85766-4_2
17. Schevzov G, Whittaker SP, Fath T, Lin JJ, Gunning PW. Tropomyosin isoforms and reagents. *Bioarchitecture* 2011; 1:135-64; PMID:22069507; <http://dx.doi.org/10.4161/bioa.1.4.17897>
18. Irimia M, Maeso I, Gunning PW, Garcia-Fernández J, Roy SW. Internal and external paralogy in the evolution of tropomyosin genes in metazoans. *Mol Biol Evol* 2010; 27:1504-17; PMID:20147436; <http://dx.doi.org/10.1093/molbev/msq018>
19. Hitchcock-DeGregori SE. Tropomyosin: function follows structure. *Adv Exp Med Biol* 2008; 644:60-72; PMID:19209813; http://dx.doi.org/10.1007/978-0-387-85766-4_5
20. Hitchcock-DeGregori SE, Song Y, Greenfield NJ. Functions of tropomyosin's periodic repeats. *Biochemistry* 2002; 41:15036-44; PMID:12475253; <http://dx.doi.org/10.1021/bi026519k>
21. Kawai M, Lu X, Hitchcock-DeGregori SE, Stanton KJ, Wandling MW. Tropomyosin period 3 is essential for enhancement of isometric tension in thin filament-reconstituted bovine myocardium. *J Biophys* 2009; 2009:380967; PMID:20130792; <http://dx.doi.org/10.1155/2009/380967>
22. Landis C, Back N, Homsher E, Tobacman LS. Effects of tropomyosin internal deletions on thin filament function. *J Biol Chem* 1999; 274:31279-85; PMID:10531325; <http://dx.doi.org/10.1074/jbc.274.44.31279>
23. Oguchi Y, Ishizuka J, Hitchcock-DeGregori SE, Ishiwata S, Kawai M. The role of tropomyosin domains in cooperative activation of the actin-myosin interaction. *J Mol Biol* 2011; 414:667-80; PMID:22041451; <http://dx.doi.org/10.1016/j.jmb.2011.10.026>
24. Singh A, Hitchcock-DeGregori SE. A peek into tropomyosin binding and unfolding on the actin filament. *PLoS One* 2009; 4:e6336; PMID:19629180; <http://dx.doi.org/10.1371/journal.pone.0006336>
25. Brown JH, Kim KH, Jun G, Greenfield NJ, Dominguez R, Volkmann N, et al. Deciphering the design of the tropomyosin molecule. *Proc Natl Acad Sci U S A* 2001; 98:8496-501; PMID:11438684; <http://dx.doi.org/10.1073/pnas.131219198>
26. Brown JH, Zhou Z, Reshetnikova L, Robinson H, Yammani RD, Tobacman LS, et al. Structure of the mid-region of tropomyosin: bending and binding sites for actin. *Proc Natl Acad Sci U S A* 2005; 102:18878-83; PMID:16365313; <http://dx.doi.org/10.1073/pnas.0509269102>
27. Nitanai Y, Minakata S, Maeda K, Oda N, Maeda Y. Crystal structures of tropomyosin: flexible coiled-coil. *Adv Exp Med Biol* 2007; 592:137-51; PMID:17278362; http://dx.doi.org/10.1007/978-4-431-38453-3_13
28. Greenfield NJ, Huang YJ, Swapna GV, Bhattacharya A, Rapp B, Singh A, et al. Solution NMR structure of the junction between tropomyosin molecules: implications for actin binding and regulation. *J Mol Biol* 2006; 364:80-96; PMID:16999976; <http://dx.doi.org/10.1016/j.jmb.2006.08.033>
29. Whitby FG, Phillips GN Jr. Crystal structure of tropomyosin at 7 Ångströms resolution. *Proteins* 2000; 38:49-59; PMID:10651038; [http://dx.doi.org/10.1002/\(SICI\)1097-0134\(20000101\)38:1<49::AID-PROT6>3.0.CO;2-B](http://dx.doi.org/10.1002/(SICI)1097-0134(20000101)38:1<49::AID-PROT6>3.0.CO;2-B)
30. Barua B, Fagnant PM, Winkelmann DA, Trybus KM, Hitchcock-DeGregori SE. A periodic pattern of evolutionarily conserved basic and acidic residues constitutes the binding interface of actin-tropomyosin. *J Biol Chem* 2013; 288:9602-9; PMID:23420843; <http://dx.doi.org/10.1074/jbc.M113.451161>
31. Barua B, Winkelmann DA, White HD, Hitchcock-DeGregori SE. Regulation of actin-myosin interaction by conserved periodic sites of tropomyosin. *Proc Natl Acad Sci U S A* 2012; 109:18425-30; PMID:23091026; <http://dx.doi.org/10.1073/pnas.1212754109>
32. Behrmann E, Müller M, Penczek PA, Mannherz HG, Manstein DJ, Raunser S. Structure of the rigor actin-tropomyosin-myosin complex. *Cell* 2012; 150:327-38; PMID:22817895; <http://dx.doi.org/10.1016/j.cell.2012.05.037>
33. Li XE, Tobacman LS, Mun JY, Craig R, Fischer S, Lehman W. Tropomyosin position on F-actin revealed by EM reconstruction and computational chemistry. *Biophys J* 2011; 100:1005-13; PMID:21320445; <http://dx.doi.org/10.1016/j.bpj.2010.12.3697>
34. Hammell RL, Hitchcock-DeGregori SE. Mapping the functional domains within the carboxyl terminus of alpha-tropomyosin encoded by the alternatively spliced ninth exon. *J Biol Chem* 1996; 271:4236-42; PMID:8626768; <http://dx.doi.org/10.1074/jbc.271.8.4236>
35. Dufour C, Weinberger RP, Schevzov G, Jeffrey PL, Gunning P. Splicing of two internal and four carboxyl-terminal alternative exons in nonmuscle tropomyosin 5 pre-mRNA is independently regulated during development. *J Biol Chem* 1998; 273:18547-55; PMID:9660825; <http://dx.doi.org/10.1074/jbc.273.29.18547>
36. Vrhovski B, Lemckert F, Gunning P. Modification of the tropomyosin isoform composition of actin filaments in the brain by deletion of an alternatively spliced exon. *Neuropharmacology* 2004; 47:684-93; PMID:15458840; <http://dx.doi.org/10.1016/j.neuropharm.2004.07.011>
37. Fanning AS, Wolenski JS, Mooseker MS, Izant JG. Differential regulation of skeletal muscle myosin-II and brush border myosin-I enzymology and mechanochemistry by bacterially produced tropomyosin isoforms. *Cell Motil Cytoskeleton* 1994; 29:29-45; PMID:7820856; <http://dx.doi.org/10.1002/cm.970290104>
38. Lehrer SS, Morris EP. Comparison of the effects of smooth and skeletal tropomyosin on skeletal actomyosin subfragment 1 ATPase. *J Biol Chem* 1984; 259:2070-2; PMID:6230348
39. Ostap EM. Tropomyosins as discriminators of myosin function. *Adv Exp Med Biol* 2008; 644:273-82; PMID:19209828; http://dx.doi.org/10.1007/978-0-387-85766-4_20
40. Clayton JE, Sammons MR, Stark BC, Hodges AR, Lord M. Differential regulation of unconventional fission yeast myosins via the actin track. *Curr Biol* 2010; 20:1423-31; PMID:20705471; <http://dx.doi.org/10.1016/j.cub.2010.07.026>
41. Stark BC, Sladewski TE, Pollard LW, Lord M. Tropomyosin and myosin-II cellular levels promote actomyosin ring assembly in fission yeast. *Mol Biol Cell* 2010; 21:989-1000; PMID:20110347; <http://dx.doi.org/10.1091/mbc.E09-10-0852>
42. Hodges AR, Krementsova EB, Bookwalter CS, Fagnant PM, Sladewski TE, Trybus KM. Tropomyosin is essential for processive movement of a class V myosin from budding yeast. *Curr Biol* 2012; 22:1410-6; PMID:22704989; <http://dx.doi.org/10.1016/j.cub.2012.05.035>
43. Percival JM, Hughes JA, Brown DL, Schevzov G, Heimann K, Vrhovski B, et al. Targeting of a tropomyosin isoform to short microfilaments associated with the Golgi complex. *Mol Biol Cell* 2004; 15:268-80; PMID:14528022; <http://dx.doi.org/10.1091/mbc.E03-03-0176>
44. Bryce NS, Schevzov G, Ferguson V, Percival JM, Lin JJ, Matsumura F, et al. Specification of actin filament function and molecular composition by tropomyosin isoforms. *Mol Biol Cell* 2003; 14:1002-16; PMID:12631719; <http://dx.doi.org/10.1091/mbc.E02-04-0244>
45. Tojkander S, Gateva G, Schevzov G, Hotulainen P, Naumanen P, Martin C, et al. A molecular pathway for myosin II recruitment to stress fibers. *Curr Biol* 2011; 21:539-50; PMID:21458264; <http://dx.doi.org/10.1016/j.cub.2011.03.007>
46. Gupton SL, Anderson KL, Kole TP, Fischer RS, Ponti A, Hitchcock-DeGregori SE, et al. Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. *J Cell Biol* 2005; 168:619-31; PMID:15716379; <http://dx.doi.org/10.1083/jcb.200406063>
47. Mason JM, Müller KM, Arndt KM. Considerations in the design and optimization of coiled coil structures. *Methods Mol Biol* 2007; 352:35-70; PMID:17041258