Cell. Mol. Life Sci. 65 (2008) 563 – 569
1420-682X/08/040563-7
DOI 10.1007/s00018-007-7347-9

Cellular and Molecular Life Sciences

Review

Tropomodulins and tropomodulin/tropomyosin interactions

A. S. Kostyukova

Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854 (USA), Fax: +1 732 235 4029, e-mail: kostyuas@umdnj.edu

Received 30 July 2007; received after revision 2 October 2007; accepted 10 October 2007
Online First 29 October 2007

Abstract. The tropomodulins are a family of proteins that cap the slow-growing (pointed) end of actin filaments and require tropomyosin for optimal function. Tropomodulin is an elongated molecule with a molecular mass of about 40 kDa. The C-terminal half of tropomodulin contains one compact cooperatively melting domain, whereas the N-terminal half has no cooperatively melting structure. The N-terminal half of tropomodulin contains two tropomysin-binding sites and a tropomyosin-dependent actin-binding site, the tropomyosin-independent actin-binding site being located at the C terminus. One tropomodulin molecule binds two tropomyosin molecules, and thus one molecule of tropomodulin is necessary and sufficient for capping at the pointed end. Tropomyosin/tropomodulin interactions are isoform specific. Differences in tropomyosin affinity for the two binding sites in tropomodulin may regulate its correct positioning at the pointed end as well as effectiveness of capping the actin filament.

Keywords. Tropomodulin, tropomyosin, actin filament, pointed end, capping.

Introduction

Actin filaments play an important role in many biological functions, including muscle contraction, cytoskeletal organization, cell migration, and organelle transport [1–4]. Actin filaments form vastly different structures in different cell types and at different locations within the cell. While actin filaments in skeletal muscle sarcomeres are 1.1 ± 0.025 μm, actin filaments from the spectrin network in erythrocytes have lengths of 33 ± 5 nm [5, 6]. Of central importance in the assembly of these various structures is regulation of the dynamics at the actin filament ends. Actin filaments are polar, have fast-growing (barbed) and slow-growing (pointed) ends that differ in structure and dynamic properties. The exclusive properties of these ends are exploited by a wide range of actin-binding proteins. There are over 160 distinct actin-binding proteins, including those that cap, sever, or cross-link filaments [7]. The barbed end is directed toward the Z-line in sarcomeres of striated muscles and toward the membrane in microvilli [8]. Several capping proteins are known for the barbed end, such as gelsolin, CapZ, and adducin [7–9]. However, the length of muscle thin filaments is controlled by regulation of actin assembly at the pointed not the barbed end [10]. The only known capping protein for the pointed end is tropomodulin (Tmod) [11–13].

Tmod was originally found in erythrocyte membranes as a tropomyosin (TM)-binding protein with a molecular mass of about 40 kDa [14]. When Tmod was first cloned and sequenced, no sequence homologies were found with any other known protein [15]. Later, Tmod was shown to bind specifically to the pointed end of the actin filament, inhibiting polymerization and depolymerization of actin monomers [11, 16]. The affinity of Tmod for the pointed end is low in the absence of TM (K_d ~ 0.3–0.4 μM), whereas it increases substantially in the presence of TM (K_d ~ 50 pM)

[3]. In *in vitro* experiments, actin capping is tight; however, in living myocytes, capping is transient [17]. Actin capping is a dynamic process: *in vivo* actin and Tmod molecules bound to the pointed end may exchange with free molecules. The mechanism by which Tmod capping may be downregulated in muscle is not known. There may be factors directly influencing Tmod function by binding or modifying it. Isoform-specific Tmod/TM interactions may be one of the regulatory mechanisms.

TM is an elongated two-chained α-helical coiled coil [for review see ref. 18]. TM molecules lie along both sides of the helical actin filament; the N terminus of each molecule interacts with the C terminus of the following one. The N terminus of TM is directed toward the pointed end of the actin filament. TM isoforms are encoded by four genes, α, β, γ, and δ, with additional diversity resulting from alternative exon expression. There are long (284 residues) and short (246 residues) TM isoforms. The distribution of TM isoforms varies in different cells and tissues and changes during their development. For example, in striated muscle, long α- and β-TMs interact with thin filaments of a contractile apparatus in sarcomeres, whereas a short γ-TM, TM5NM1, is situated in the cytoskeleton adjacent to the Z-line [19].

TM isoforms, their distribution and significance

At present four Tmod isoforms are known [20–22]. Tmod1, previously E(erythrocyte)-Tmod, is found mainly in erythrocytes, in heart and slow skeletal muscles, although it is detected in many other tissues. Tmod4, Sk(skeletal)-Tmod, prevails in fast skeletal muscles and replaces Tmod1 during development. Tmod2, N(neuron)-Tmod, is found in brain. Tmod3, U(ubiquitous)-Tmod, is widely expressed. These isoforms are 60% identical and 70% similar in amino acid sequence.

Larger proteins with molecular masses of about 64 kDa, leiomodins, were cloned based on homology with Tmod [22, 23]. There are three leiomodin isoforms: Lmod1 found in many tissues but mainly in smooth muscles, Lmod2 found in heart and skeletal muscle, and Lmod3, whose distribution is not known. Lmod function is not yet well studied. They bind TM [23, 24]. There is evidence that Lmod2 may act as an actin filament nucleating factor [25].

In muscles, Tmod was immunolocalized not only at the pointed (free) ends of thin filaments [26], but also in the Z-disc region [27]. This indicates the presence of Tmod in the Z-line-associated actin filament network [28]. Tmod overexpression in mice myocardium causes myofibril degeneration, which leads to dilated cardiomyopathy [29]. Reduced Tmod expression results in the formation of abnormally long actin filaments [30]. Overexpression of GFP-Tmod in cardiac myocytes results in shorter thin filaments [17]. In a Tmod1 knockout mouse, heart defects, including aborted development of the myocardium and inability to pump, lead to embryonic lethality [31, 32]. Cardiomyocyte differentiation was studied in Tmod1 null embryonic stem cells and it was shown that Tmod1 function is critical for late stages of myofibrillogenesis [33].

Unlike Tmod1 and Tmod4 that bind only to F-actin, Tmod3 is able to sequester actin monomers and Tmod2 presumably has a similar ability [34]. At low concentrations, Tmod3 increases actin polymerization by nucleating actin filaments; at high concentration, it decreases actin filament polymerization, not only by capping the pointed end, but also by binding actin monomers [10]. Overexpression of Tmod3 leads to decreased endothelial cell motility [35]. Tmod2 expression was found to be altered in pathological conditions and human diseases, such as epilepsy or celebral ischemia [36–38].

Of all the isoforms, Tmod1 is the best understood; its structure, function and interactions with other proteins have been studied in detail. Tmod1 is an elongated molecule; its C-terminal half consists of one compact, cooperatively melting domain [39–41]. The crystal structure of Tmod1 C-terminal domain is represented by a right-handed superhelix composed of alternate α helices and β strands [42]. This structure is characteristic for leucine-rich repeat (LRR) motif typically involved in protein-protein interactions [43–46]. In contrast, the N-terminal half has no cooperatively melting structure; it is flexible and disordered [39–41, 47]. The disordered nature of Tmod1 N-terminal domain was confirmed by solving the solution structure of an N-terminal 92-residue fragment using nuclear magnetic resonance (NMR) [48]. Residues 24–35 are helical but the rest of the peptide has no regular secondary structure.

Tmod-binding partners: binding site localization

Despite the fact that the Tmod N-terminal domain has no cooperatively melting structure, adding a Tmod1 N-terminal fragment (residues 1–91) to long-muscle TM drastically changed the melting curves as indicated by circular dichroism (CD) and differential scanning calorimetry (DSC) [41]. The heat denaturation of TM is a multi-step process [49], and after forming the Tmod-TM complex, major changes occur in the high-temperature transition, which corresponds to the TM N terminus. As a result of binding, the

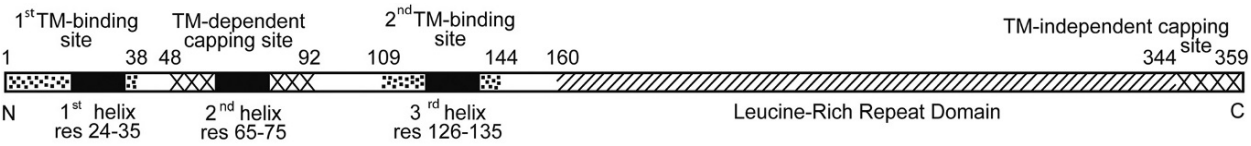

Figure 1. Schematic model of the TM-binding and actin-capping sites on the Tmod1 molecule.

temperature of the transition and the excess heat of denaturation increased, as did the α-helical content. Similar data were obtained when longer N-terminal fragments (aa 1–130) of Tmod1 and Tmod4 were mixed with TM peptides [50]. Complex formation between Tmod and TM fragments results in increased α-helical content and stability. N-terminal acetylation that stabilizes the coiled coil of the N terminus of long tropomyosin was found to be essential for its interaction with Tmod [50].

In an earlier study, the binding domain for erythrocyte TMs (homodimers or heterodimers of α and γ short TM [51, 52]) was mapped to residues 39–138 [15]. Soon after it was suggested that the site of interaction of Tmod1 depends on the type of target TM: residues 6–94 of Tmod1 interact with skeletal muscle TM, whereas residues 90–184 interact with short non-muscle erythrocyte TM [53]. However, the finding that Tmod1 residues 95–359 exhibited a 160-fold increase in capping activity in the presence of skeletal muscle TM indicates the presence of a second binding site for long TM in this region [54]. Moreover, the Tmod1 N-terminal fragment, residues 1–92, was able to bind not only long skeletal α-TM but also short non-muscle α-TM5a [55]. In recent studies, Vera et al. [56] mapped a binding site for TM5 (a short TM encoded by the γ-TM gene) to residues 105–127, and Kong and Kedes [57] found that residues L134 and L135 are crucial for TM5 binding. Neither study found binding of γ-TM to the first TM-binding site or of long muscle TM to the second TM-binding site. The number of TM-binding sites in a Tmod molecule and their specificity remained unresolved.

A series of mutagenic studies and analyses using model peptides has resolved two TM-binding sites in Tmod. In addition to helix 24–35 which was determined by NMR, there are several predicted helical regions in the N-terminal half of Tmod1 [47]. While the α helix formed by residues 24–35 has a high probability to form a coiled coil, the two other putative helices, residues 65–75 and residues 126–135 are amphipatic helices. Correct folding of these helices is crucial for the formation of the binding sites. The L27E mutation destroys potential α helix coiled coil formation of the helical region, residues 24–35, located in the first TM-binding site [48]. This mutation causes a loss of TM-binding ability in this site. Mutation L71D inhibits formation of the hydrophobic surface in the amphipathic helix, residues 65–75, which is responsible for TM-dependent capping activity [58]. The third mutation, I131D, postulated to destroy the hydrophobic surface in a putative helix, residues 126–135, caused a loss of TM binding in the second binding site [47]. Collectively, these three mutations cause a 30-fold decrease in capping ability in a full-length Tmod1.

Figure 1 shows the positions of TM-binding and actin-capping sites on the Tmod1 molecule that were determined using the pyrene-actin polymerization assay, native gelelectrophoresis, and CD [47, 48, 54, 55, 58]. Two binding sites were localized for both short and long TM isoforms on Tmod1 to residues 1–38 and 109–144 and a TM-independent actin-capping site was localized to residues 48–92. The TM-independent actin-capping site is located at the C terminus of the Tmod1 molecule. Tmod1 without 15 C-terminal residues has drastically lower ability to cap actin filaments in the absence of TM [55]. Due to high homology of these regions, other Tmod isoforms are likely to have the same binding sites for TM and actin. In Tmod3, two regions, residues 31–40 and 149–169, were suggested to be involved in actin monomer binding, an ability that is absent in Tmod1 and Tmod4 [10].

Besides actin and TM, Tmod1 interacts with another protein, nebulin, at the pointed end of actin filaments in striated muscle. Nebulin, originally found in vertebrate skeletal muscles, is a giant protein with a molecular mass of 800 kDa that extends along the actin filament [for a review see ref. 59]. Later nebulin was found in cardiac muscle [60] together with nebulette, a nebulin-like protein of smaller molecular mass (~100 kDa) [61]. In nebulin-deficient mice, thin filaments have reduced lengths and approximately 15% of their Z-disks are abnormally wide [62]. Phenotypically, this model recapitulates human nemaline myopathy. The nebulin N terminus is directed towards the pointed end. Its molecules mainly consist of short repeats that are likely to form an α helix [63]. It has been suggested that nebulin acts as a molecular ruler defining the length of actin filaments in striated muscle by specifying pointed- and barbed-end thin filament capping [33, 62] and it may therefore also regulate binding of Tmod at the pointed end. A

Table 1. Binding of Tmod1, Lmod1, and Lmod2 fragments containing TM-binding sites to tropomyosin peptides.

Peptide (source)	TM sequence	Tmod1 aa 1–38	Tmod1 aa 109–144	Lmod2 aa 5–42	Lmod1 aa 3–40
αTM1aZip (αstTM)	MDAIKKKMQMLKLD	1.1 ± 0.4	1.3 ± 0.3	0.8 ± 0.2	1.98
αTM1bZip (αTM5a)	AGSSSLEAVRRKIRSLQEQ	0.22 ± 0.10	0.003 ± 0.001	0.011 ± 0.008	0.016 ± 0.009
γTM1bZip (γTM5NM1)	AGSTTIEAVKRKIQVLQQQ	NC	0.04 ± 0.03	0.6 ± 0.1	0.61 ± 0.07
δTM1bZip (δTM4)	AGLNSLEAVKRKIQALQQQ	NC	0.09 ± 0.02	0.24 ± 0.07	0.43 ± 0.08

K_d values (μM) were estimated from the thermodynamics of unfolding of the complexes compared with the Tmod/Lmod fragments and TMZips alone. TM sequences are aligned according to their homology. NC, binding cannot be calculated in these conditions.

nebulin fragment containing three N-terminal modules of nebulin M1-M2-M3 can bind to Tmod [64]. The binding site to nebulin is not yet localized, but it is within the C-terminal domain of Tmod1 (residues 160–344) [42].

Isoform specificity of Tmod-TM interactions

Specificity of TM binding to Tmod1 was first shown by Sussman and Fowler [65] with TM isoforms from erythrocyte, brain, platelet, and skeletal muscle tissue. Tmod1 forms complexes with all of these isoforms, but binds preferentially to erythrocyte TM. At that time, it was known that Tmod binds to the end of TM [66], and it was therefore assumed that binding ability reflected the heterogeneity in the N- or C-terminal sequences characteristic of the different TM isoforms. For the first time, it was suggested that isoform-specific interactions of Tmod with TM may represent a novel mechanism for selective regulation of TM-actin interactions. Each of the short TMs, γ-TM5 and α-TM5b, identified as the major TM isoforms in erythrocytes, demonstrates a higher affinity toward Tmod1 than do long TM isoforms [51, 52].

Later it was shown that Tmod binds to the N-terminus of TM [51]. Without the first 19 residues, short non-muscle γ-TM, TM5, did not bind to Tmod1 and the binding site was mapped to residues 7–14 of TM5 [67]. The first 14 residues of long TMs are homologous to residues 6–19 of short TM (Table 1) and contain a Tmod-binding site [50]. To measure the affinities of TM isoforms to Tmod1, model peptides were used. These peptides were originally designed to study the structure of TM N termini and TM overlap complexes [68, 69]. They contained the 19 N-terminal residues of short or the 14 N-terminal residues of long TMs. In addition to the TM N-terminal region, these peptides contain the 18 C-terminal residues of the GCN4 leucine zipper domain, which help to stabilize the coiled coil structure [68]. The validity of peptide models is well established. The structures of two of these peptides, αTM1aZip and αTM1bZip, were solved: the peptides bind C-terminal TM fragments and form a ternary complex with troponin [68, 69]. Because the TM N terminus contains a Tmod-binding site [67], the peptides were later used to study TM/Tmod interactions [47, 48, 50, 54, 55, 58, 70].

Affinities to the individual TM-binding sites were studied in detail using Tmod1 fragments, residues 1–38 and 109–144, and four TM peptides representing different isoforms: long muscle TM, αTM1aZip, and the short non-muscle TMs: αTM1bZip, γTM1bZip, and δTM1bZip. Dissociation constants calculated from the CD unfolding curves are presented in Table 1 [24, 47, 70]. The N-terminal sequence of long-muscle β-TM encoded by exon 1a is identical to the α-TM sequence and there is only one conservative replacement of Asp2 to Glu in long γ-TM. No long TM coded by the δ gene has been found. Therefore, dissociation constants determined for αTM1aZip-Tmod complexes should be the same or very similar in the case of other long TMs. The N-terminal sequences of short TMs are similar and different from long TM. Despite the similarity, the difference in binding abilities was striking. While αTM1bZip and αTM1aZip both bind well to both sites in Tmod1 (though αTM1bZip does so with higher affinity), the peptides γTM1bZip and δTM1bZip bind only to the second binding site. In contrast, the N-terminal fragments of Lmod1, residues 3–40, and Lmod2, residues 5–42, which is highly homologous to the first TM-binding site of Tmod, bind with much higher affinity to γTM1bZip and δTM1bZip (Table 1). The binding can be easily detected either by native gelelectrophoresis or CD [24].

Even though the γTM1bZip peptide only binds the second Tmod1 binding site, full-length γTM, γTM5NM1, inhibits pointed-end elongation in a pyrene-actin fluorescence assay with Tmod1 N-terminal fragment, residues 1–92, containing only the first TM-binding site and the TM-independent actin-capping site, although with less effectiveness than with full-length Tmod1 [70]. To do this, there should be interaction of γTM5NM1 with the first binding site. It was shown using cross-linking that there is an inter-

action of the first TM-binding site with both α- and γTM1bZip. Increasing concentration of fragments in CD experiments also showed weak interaction with γ- and δTM1bZip [70].

The residues responsible for the isoform specificity of TM binding were determined [70]. Changing Ser4 of αTM1bZip to Thr as in γTM1bZip decreased the binding ability fourfold, and changing Arg14 to Gln resulted in the loss of binding. These residues are not involved in coiled coil formation, which is important for TM-Tmod interaction [48].

The analysis resolves a long-standing debate in the literature concerning the location of TM-binding sites on Tmod. All TM isoforms bind to both TM-binding sites on Tmod1, but short γ-TM and δ-TM bind to the first site with much lower affinity than α-TMs. Subtle sequence differences among TM isoforms can have major effects on the affinity for the first Tmod1 binding site and thereby modulate the dynamics of the actin filament pointed end.

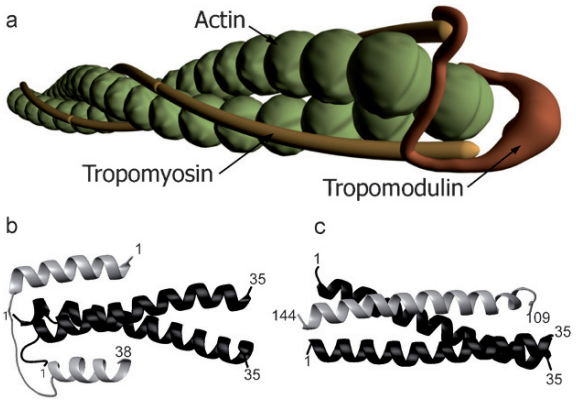

Figure 2. A cartoon representation of the pointed end of the actin filament. The N-terminal half of Tmod1 binds to both TMs and interacts with actin (*a*). Schematic of possible binding modes of αTM1bZip to two binding sites on Tmod1, residues 1–38 (*b*) and 109–144 (*c*).

A model of the pointed end: one Tmod binds two TMs

The question now is how many TM molecules does one Tmod molecule bind? By titration of Tmod1 with αTM1bZip, it was shown that one Tmod1 molecule binds two TM molecules in a cooperative manner [47]. The model was proposed for actin capping where one Tmod molecule binds two TM molecules at the pointed end (Fig. 2a). This changed the previous concept of pointed-end organization, whereby one molecule of Tmod binds to one molecule of TM [7] and resolves a long-standing controversy in the field. The NMR studies using αTM1bZip and Tmod1 fragments show that the TM structures are different in the two complexes, therefore different in the two sites [70]. While the structures of Tmod1-TM complexes remain to be solved, possible models of binding at Tmod-TM binding sites have been suggested [70]. These models are based on circular dichroism spectra of the complexes, the effects of complex formation on the ^{1}H-^{15}N HSQC spectra of αTM1bZip, and mutagenesis studies [47, 48, 56, 57, 70].

For the first TM-binding site, residues 1–13 of Tmod1 bind antiparallel to the αTM1bZip coiled coil on one side of the TM interface, residues 15–26 loop around the N terminus of TM, and residues 27–38 bind parallel to the other side of the TM coiled coil interface (Fig. 2b). Possible hydrophobic interactions are consistent with such a model. For the second TM-binding site, it was proposed that Tmod1 residues 109–144 bind antiparallel to αTm1bZip to form a three-helix bundle (Fig. 2c). These figures illustrate hypothetical models; the arrangement of the helices and the significance of specific residues will be learned only by solving the structures of the complexes.

The position of a single Tmod molecule at the pointed end of the actin filament that forms fundamentally different complexes with the TM molecules on the two sides of the filament helix increases the asymmetry of the end. According to Table 1, long striated muscle α-TM binds to both sites in Tmod1 with the same affinity, while short non-muscle TMs have higher affinity to the second site, residues 109–144. Differences in TM affinity for the two binding sites in Tmod may regulate its correct positioning at the pointed end in the absence of another Tmod-binding protein, nebulin, which is found only in striated muscle. Isoform-specific differences in affinity for the two sites contribute to the efficiency in capping the pointed end of the actin filament. Since small sequence variations in the N terminus of TM can have major effects on Tmod1 binding and the ability to cap the pointed end, the end becomes a significant regulatory site. TMs are recognized to be a major regulator of the actin filaments in cells, having the ability to protect filaments against severing and branching [71–73], to recruit specific myosins [74], alter cell shape, and now to regulate the pointed end. Regulation of Tmod binding as well as the effectiveness of capping by specific TMs may have significant consequences for local cytoskeletal formation and filament dynamics in cells.

Acknowledgements. I thank Dr. S. Hitchcock-DeGregori for discussing this manuscript. Supported by the American Heart Association Grant 0535328N and UMDNJ Foundation Grant.

1 Fowler, V. M. (1996) Regulation of actin filament length in erythrocytes and striated muscle. Curr. Opin. Cell Biol. 8, 86–96.
2 Fowler, V. M. (1997) Capping actin filament growth: tropomodulin in muscle and nonmuscle cells. Soc. Gen. Physiol. Ser. 52, 79–89.
3 Weber, A., Pennise, C. R. and Fowler, V. M. (1999) Tropomodulin increases the critical concentration of barbed end-capped actin filaments by converting ADP.P(i)-actin to ADP-actin at all pointed filament ends. J. Biol. Chem. 274, 34637–34645.
4 Pollard, T. D. and Borisy, G. G. (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112, 453–465.
5 Shen, B. W., Josephs, R. and Steck, T. L. (1986) Ultrastructure of the intact skeleton of the human erythrocyte membrane. J. Cell Biol. 102, 997–1006.
6 Sosa, H., Popp, D., Ouyang, G. and Huxley, H. E. (1994) Ultrastructure of skeletal muscle fibers studied by a plunge quick freezing method: myofilament lengths. Biophys. J. 67, 283–292.
7 dos Remedios, C. G., Chhabra, D., Kekic, M., Dedova, I. V., Tsubakihara, M., Berry, D. A. and Nosworthy, N. J. (2003) Actin binding proteins: regulation of cytoskeletal microfilaments. Physiol. Rev. 83, 433–473.
8 Cooper, J. A. and Schafer, D. A. (2000) Control of actin assembly and disassembly at filament ends. Curr. Opin. Cell Biol. 12, 97–103.
9 Kuhlman, P. A., Hughes, C. A., Bennett, V. and Fowler, V. M. (1996) A new function for adducin: calcium/calmodulin-regulated capping of the barbed ends of actin filaments. J. Biol. Chem. 271, 7986–7991.
10 Fischer, R. S., Yarmola, E. G., Weber, K. L., Speicher, K. D., Speicher, D. W., Bubb, M. R. and Fowler, V. M. (2006) Tropomodulin 3 binds to actin monomers. J. Biol. Chem. 281, 36454–36465.
11 Weber, A., Pennise, C. R., Babcock, G. G. and Fowler, V. M. (1994) Tropomodulin caps the pointed ends of actin filaments. J. Cell Biol. 127, 1627–1635.
12 Schafer, D. A. and Cooper, J. A. (1995) Control of actin assembly at filament ends. Annu. Rev. Cell Dev. Biol. 11, 497–518.
13 Weber, A. (1999) Actin binding proteins that change extent and rate of actin monomer-polymer distribution by different mechanisms. Mol. Cell. Biochem. 190, 67–74.
14 Fowler, V. M. (1987) Identification and purification of a novel Mr 43,000 tropomyosin- binding protein from human erythrocyte membranes. J. Biol. Chem. 262, 12792–12800.
15 Sung, L. A., Fowler, V. M., Lambert, K., Sussman, M. A., Karr, D. and Chien, S. (1992) Molecular cloning and characterization of human fetal liver tropomodulin: a tropomyosin-binding protein. J Biol Chem. 267, 2616–2621.
16 Gregorio, C. C. and Fowler, V. M. (1995) Mechanisms of thin filament assembly in embryonic chick cardiac myocytes: tropomodulin requires tropomyosin for assembly. J. Cell Biol. 129, 683–695.
17 Littlefield, R., Almenar-Queralt, A. and Fowler, V. M. (2001) Actin dynamics at pointed ends regulates thin filament length in striated muscle. Nat. Cell Biol. 3, 544–551.
18 Perry, S. V. (2001) Vertebrate tropomyosin: distribution, properties and function. J. Muscle Res. Cell Motil. 22, 5–49.
19 Gunning, P. W., Schevzov, G., Kee, A. J. and Hardeman, E. C. (2005) Tropomyosin isoforms: divining rods for actin cytoskeleton function. Trends Cell Biol. 15, 333–341.
20 Watakabe, A., Kobayashi, R. and Helfman, D. M. (1996) N-tropomodulin: a novel isoform of tropomodulin identified as the major binding protein to brain tropomyosin. J. Cell Sci. 109, 2299–2310.
21 Almenar-Queralt, A., Lee, A., Conley, C. A., de Pouplana, L. R. and Fowler, V. M. (1999) Identification of a novel tropomodulin isoform, skeletal tropomodulin, that caps actin filament pointed ends in fast skeletal muscle. J. Biol. Chem. 274, 28466–28475.
22 Conley, C. A., Fritz-Six, K. L., Almenar-Queralt, A. and Fowler, V. M. (2001) Leiomodins: larger members of the tropomodulin (tmod) gene family. Genomics 73, 127–139.
23 Conley, C. A. (2001) Leiomodin and tropomodulin in smooth muscle. Am. J. Physiol. Cell Physiol. 280, C1645–C1656.
24 Kostyukova, A. (2007) Leiomodin/tropomyosin interactions are isoform specific. Arch. Biochem. Biophys. 465, 227–230.
25 Chereau, D., Boczkowska, M., Fujiwara, I., Rebowski, G., Hayes, D. B., Erhardt, P., Pollard, T. D. and Dominguez, R. Leiomodin: a novel actin filament nucleating factor. 46th ASCB Annual Meeting, 2006, San Diego.
26 Fowler, V. M., Sussmann, M. A., Miller, P. G., Flucher, B. E. and Daniels, M. P. (1993) Tropomodulin is associated with the free (pointed) ends of the thin filaments in rat skeletal muscle. J. Cell Biol. 120, 411–420.
27 Sussman, M. A., Ito, M., Daniels, M. P., Flucher, B., Buranen, S. and Kedes, L. (1996) Chicken skeletal muscle tropomodulin: novel localization and characterization. Cell Tissue Res. 285, 287–296.
28 Kee, A. J., Schevzov, G., Nair-Shalliker, V., Robinson, C. S., Vrhovski, B., Ghoddusi, M., Qiu, M. R., Lin, J. J., Weinberger, R., Gunning, P. W. and Hardeman, E. C. (2004) Sorting of a nonmuscle tropomyosin to a novel cytoskeletal compartment in skeletal muscle results in muscular dystrophy. J. Cell Biol. 166, 685–696.
29 Sussman, M. A., Welch, S., Cambon, N., Klevitsky, R., Hewett, T. E., Price, R., Witt, S. A. and Kimball, T. R. (1998) Myofibril degeneration caused by tropomodulin overexpression leads to dilated cardiomyopathy in juvenile mice. J. Clin. Invest. 101, 51–61.
30 Sussman, M. A., Baque, S., Uhm, C. S., Daniels, M. P., Price, R. L., Simpson, D., Terracio, L. and Kedes, L. (1998) Altered expression of tropomodulin in cardiomyocytes disrupts the sarcomeric structure of myofibrils. Circ. Res. 82, 94–105.
31 Chu, X., Chen, J., Reedy, M. C., Vera, C., Sung, K. L. and Sung, L. A. (2003) E-Tmod capping of actin filaments at the slow-growing end is required to establish mouse embryonic circulation. Am. J. Physiol. Heart Circ. Physiol. 284, H1827–1838.
32 Fritz-Six, K. L., Cox, P. R., Fischer, R. S., Xu, B., Gregorio, C. C., Zoghbi, H. Y. and Fowler, V. M. (2003) Aberrant myofibril assembly in tropomodulin1 null mice leads to aborted heart development and embryonic lethality. J. Cell. Biol. 163, 1033–1044.
33 McElhinny, A. S., Schwach, C., Valichnac, M., Mount-Patrick, S. and Gregorio, C. C. (2005) Nebulin regulates the assembly and lengths of the thin filaments in striated muscle. J. Cell Biol. 170, 947–957.
34 Fischer, R. S., Sept, D., Weber, K. L., Speicher, D. W. and Fowler, V. M. (2004) Tmod3 binds actin monomer in vitro and in vivo. Mol. Biol. Cell 15, 147a.
35 Fischer, R. S., Fritz-Six, K. L. and Fowler, V. M. (2003) Pointed-end capping by tropomodulin3 negatively regulates endothelial cell motility. J. Cell Biol. 161, 371–380.
36 Yang, J. W., Czech, T., Felizardo, M., Baumgartner, C. and Lubec, G. (2006) Aberrant expression of cytoskeleton proteins in hippocampus from patients with mesial temporal lobe epilepsy. Amino Acids 30, 477–493.
37 Iwazaki, T., McGregor, I. S. and Matsumoto, I. (2006) Protein expression profile in the striatum of acute methamphetamine-treated rats. Brain Res. 1097, 19–25.
38 Chen, A., Liao, W. P., Lu, Q., Wong, W. S. and Wong, P. T. (2007) Upregulation of dihydropyrimidinase-related protein 2, spectrin alpha II chain, heat shock cognate protein 70 pseudogene 1 and tropomodulin 2 after focal cerebral ischemia in rats – A proteomics approach. Neurochem. Int. 50, 1078–1086.
39 Kostyukova, A., Maeda, K., Yamauchi, E., Krieger, I. and Maeda, Y. (2000) Domain structure of tropomodulin: distinct properties of the N-terminal and C-terminal halves. Eur. J Biochem. 267, 6470–6475.

40 Fujisawa, T., Kostyukova, A. and Maeda, Y. (2001) The shapes and sizes of two domains of tropomodulin, the P-end-capping protein of actin-tropomyosin. FEBS Lett. 498, 67–71.
41 Kostyukova, A. S., Tiktopulo, E. I. and Maeda, Y. (2001) Folding properties of functional domains of tropomodulin. Biophys. J. 81, 345–351.
42 Krieger, I., Kostyukova, A., Yamashita, A., Nitanai, Y. and Maeda, Y. (2002) Crystal structure of tropomodulin C-terminal half and structural basis of actin filament pointed-end capping. Biophys. J. 83, 2716–2725.
43 Kobe, B. and Deisenhofer, J. (1995) A structural basis of the interactions between leucine-rich repeats and protein ligands. Mol. Cell Neurosci. 6, 97–105.
44 Papageorgiou, A. C., Shapiro, R. and Acharya, K. R. (1997) Molecular recognition of human angiogenin by placental ribonuclease inhibitor – an X-ray crystallographic study at 2.0 A resolution. EMBO J. 16, 5162–5177.
45 Price, S. R., Evans, P. R. and Nagai, K. (1998) Crystal structure of the spliceosomal U2B"-U2A' protein complex bound to a fragment of U2 small nuclear RNA. Nature 394, 645–650.
46 Kobe, B. and Kajava, A. V. (2001) The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. 11, 725–732.
47 Kostyukova, A. S., Choy, A. and Rapp, B. A. (2006) Tropomodulin binds two tropomyosins: a novel model for actin filament capping. Biochemistry 45, 12068–12075.
48 Greenfield, N. J., Kostyukova, A. S. and Hitchcock-Degregori, S. E. (2005) Structure and tropomyosin binding properties of the N-terminal capping domain of tropomodulin 1. Biophys. J. 88, 372–383.
49 Potekhin, S. A. and Privalov, P. L. (1982) Co-operative blocks in tropomyosin. J. Mol. Biol. 159, 519–535.
50 Greenfield, N. J. and Fowler, V. M. (2002) Tropomyosin requires an intact N-terminal coiled coil to interact with tropomodulin. Biophys. J. 82, 2580–2591.
51 Sung, L. A. and Lin, J. J. (1994) Erythrocyte tropomodulin binds to the N-terminus of hTM5, a tropomyosin isoform encoded by the gamma-tropomyosin gene. Biochem. Biophys. Res. Commun. 201, 627–634.
52 Sung, L. A., Gao, K. M., Yee, L. J., Temm-Grove, C. J., Helfman, D. M., Lin, J. J. and Mehrpouryan, M. (2000) Tropomyosin isoform 5b is expressed in human erythrocytes: implications of tropomodulin-TM5 or tropomodulin-TM5b complexes in the protofilament and hexagonal organization of membrane skeletons. Blood 95, 1473–1480.
53 Babcock, G. G. and Fowler, V. M. (1994) Isoform-specific interaction of tropomodulin with skeletal muscle and erythrocyte tropomyosins. J. Biol. Chem. 269, 27510–27518.
54 Fowler, V. M., Greenfield, N. J. and Moyer, J. (2003) Tropomodulin contains two actin filament pointed end-capping domains. J. Biol. Chem. 278, 40000–40009.
55 Kostyukova, A. S. and Hitchcock-DeGregori, S. E. (2004) Effect of the structure of the N terminus of tropomyosin on tropomodulin function. J. Biol. Chem. 279, 5066–5071.
56 Vera, C., Lao, J., Hamelberg, D. and Sung, L. A. (2005) Mapping the tropomyosin isoform 5 binding site on human erythrocyte tropomodulin: further insights into E-Tmod/TM5 interaction. Arch. Biochem. Biophys. 444, 130–138.
57 Kong, K. Y. and Kedes, L. (2006) Leucine-135 of tropomodulin-1 regulates its association with tropomyosin, its cellular localization and the integrity of sarcomeres. J. Biol. Chem. 281, 9589–9599.
58 Kostyukova, A., Rapp, B., Choy, A., Greenfield, N. J. and Hitchcock-DeGregori, S. E. (2005) Structural requirements of tropomodulin for tropomyosin binding and actin filament capping. Biochemistry 44, 4905–4910.
59 Wang, K. (1996) Titin/connectin and nebulin: giant protein rulers of muscle structure and function. Adv. Biophys. 33, 123–134.
60 Fock, U. and Hinssen, H. (2002) Nebulin is a thin filament protein of the cardiac muscle of the agnathans. J. Muscle Res. Cell Motil. 23, 205–213.
61 Moncman, C. L. and Wang, K. (1995) Nebulette: a 107 kD nebulin-like protein in cardiac muscle. Cell Motil. Cytoskel. 32, 205–225.
62 Witt, C. C., Burkart, C., Labeit, D., McNabb, M., Wu, Y., Granzier, H. and Labeit, S. (2006) Nebulin regulates thin filament length, contractility, and Z-disk structure in vivo. EMBO J. 25, 3843–3855.
63 Pfuhl, M., Winder, S. J., Castiglione Morelli, M. A., Labeit, S. and Pastore, A. (1996) Correlation between conformational and binding properties of nebulin repeats. J. Mol. Biol. 257, 367–384.
64 McElhinny, A. S., Kolmerer, B., Fowler, V. M., Labeit, S. and Gregorio, C. C. (2001) The N-terminal end of nebulin interacts with tropomodulin at the pointed ends of the thin filaments. J. Biol. Chem. 276, 583–592.
65 Sussman, M. A. and Fowler, V. M. (1992) Tropomodulin binding to tropomyosins: isoform-specific differences in affinity and stoichiometry. Eur. J. Biochem. 205, 355–362.
66 Fowler, V. M. (1990) Tropomodulin: a cytoskeletal protein that binds to the end of erythrocyte tropomyosin and inhibits tropomyosin binding to actin. J. Cell Biol. 111, 471–481.
67 Vera, C., Sood, A., Gao, K. M., Yee, L. J., Lin, J. J. and Sung, L. A. (2000) Tropomodulin-binding site mapped to residues 7–14 at the N-terminal heptad repeats of tropomyosin isoform 5. Arch. Biochem. Biophys. 378, 16–24.
68 Greenfield, N. J., Montelione, G. T., Farid, R. S. and Hitchcock-DeGregori, S. E. (1998) The structure of the N-terminus of striated muscle alpha-tropomyosin in a chimeric peptide: nuclear magnetic resonance structure and circular dichroism studies. Biochemistry 37, 7834–7843.
69 Greenfield, N. J., Huang, Y. J., Palm, T., Swapna, G. V., Monleon, D., Montelione, G. T. and Hitchcock-DeGregori, S. E. (2001) Solution NMR structure and folding dynamics of the N terminus of a rat non-muscle alpha-tropomyosin in an engineered chimeric protein. J. Mol. Biol. 312, 833–847.
70 Kostyukova, A., Hitchcock-DeGregori, S. E. and Greenfield, N. J. (2007) Molecular basis of tropomyosin binding to tropomodulin, an actin capping protein. J. Mol. Biol. 372, 608–618.
71 Bernstein, B. W. and Bamburg, J. R. (1982) Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). Cell Motil. 2, 1–8.
72 DesMarais, V., Ichetovkin, I., Condeelis, J. and Hitchcock-DeGregori, S. E. (2002) Spatial regulation of actin dynamics: a tropomyosin-free, actin-rich compartment at the leading edge. J. Cell Sci. 115, 4649–4660.
73 Blanchoin, L., Pollard, T. D. and Hitchcock-DeGregori, S. E. (2001) Inhibition of the Arp2/3 complex-nucleated actin polymerization and branch formation by tropomyosin. Curr. Biol. 11, 1300–1304.
74 Gupton, S. L., Anderson, K. L., Kole, T. P., Fischer, R. S., Ponti, A., Hitchcock-DeGregori, S. E., Danuser, G., Fowler, V. M., Wirtz, D., Hanein, D. and Waterman-Storer, C. M. (2005) Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. J. Cell Biol. 168, 619–631.