Short Conceptual Overview

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Tropomodulins and tropomyosins – organizers of cellular microcompartments

Abstract: Eukaryotic cells show a remarkable compartmentalization into compartments such as the cell nucleus, the Golgi apparatus, the endoplasmic reticulum, and endosomes. However, organelle structures are not the only means by which specialized compartments are formed. Recent research shows a critical role for diverse actin filament populations in defining functional compartments, here referred to as microcompartments, in a wide range of cells. These microcompartments are involved in regulating fundamental cellular functions including cell motility, plasma membrane organization, and cellular morphogenesis. In this overview, the importance of two multigene families of actin-associated proteins, tropomodulins and tropomyosins, their interactions with each other, and a large number of other proteins will be discussed in the context of generating specialized actin-based microcompartments.

Keywords: actin; cellular compartments; tropomodulin; tropomyosin.

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Introduction

The architecture and dynamic properties of eukaryotic cells are highly dependent on complex arrangements of cytoskeletal elements. With only few exceptions, all eukaryotic cells have a cytoskeleton built from three different filamentous systems: actin filaments, microtubules, and intermediate filaments. These filamentous systems help to build subcellular compartments within the cell and determine the shape of the cell as well as support dynamic processes including cell division, cell migration, intracellular transport, and cell communication. Common to all three filament system is that they are polymers, built from monomeric subunits with a dynamic exchange between the monomeric and the filamentous pool.

Actin is one of the most abundant proteins in eukaryotic cells and constitutes a key component of the cytoskeleton. Precise regulation of the assembly and disassembly of actin filaments is essential to support a wide range of cellular processes and functions. Actin filaments are polymers that are generated through the self-assembly of 42-kDa monomeric (G-actin) subunits, modulated by various actin-associated proteins. Although intermediate filaments exchange subunits between the monomeric and the filamentous pool along the whole length of the filament, the assembly and disassembly of subunits on and off actin filaments and microtubules can only occur from either end of the filaments. In actin filaments, we distinguish between the slow-growing minus (pointed) end and the fast growing (barbed) end. Assembly and disassembly from the barbed ends are regulated by a number of actin-associated proteins, such as capping protein [reviewed by Cooper and Sept (1)] or adducin (2). The most well-characterized pointed-end capping protein is tropomodulin (Tmod), originally identified as a binding partner of the actin-associated protein tropomyosin (Tm) in erythrocytes (3). Although previous comprehensive reviews provided in-depth discussions on the biology of either Tmods or Tms (4–7), the focus of the current overview is to discuss the role of Tmod and the interaction with its major binding partner Tm in generating functionally diverse actin filament populations in suborganellar compartments. These compartments can be very diverse in shape and dimension, such as the cortical actin cytoskeleton underneath the plasma membrane, or different actin filament populations that are found within growth cones of extending neurites in nerve cells. Here we will refer to these compartments collectively as ‘microcompartments’.

Isoform diversity of Tmods

One of the main functions of Tmods in vertebrate cells is the regulation of the length and stability of actin filaments. In addition to its most characterized property as an actin filament pointed-end capping protein, more recent

work has shown that some Tmods also bind actin monomers (8). In vertebrates, four ≈40-kDa isoforms have been identified, Tmod1–4 (9). Furthermore, three ≈65-kDa isoforms have been found in vertebrates, named leiomodin, or Lmod 1–3. This overview focuses on the different Tmod/Lmod transcripts and proteins generated in vertebrates, and structural considerations of each of the isoforms are discussed. The aim of this work is to provide a concise overview of these aspects of the different isoforms. For a more comprehensive discussion of tissue-specific expression of Tms and Tmods, please refer to (6).

Tmod isoforms

Tmod1

The first Tmod isoform that was characterized as a binding partner of Tm in erythrocytes was Tmod1, also referred to as E-Tmod (3). Subsequent studies have found Tmod1 in the ocular fiber cells of the lens, neurons, and striated and some smooth muscle cells (10–17). Two different transcripts of Tmod1 with a size of 1.6 and 2.7 kb are generated by alternative promoter usage and/or alternative splicing, leading to the synthesis of a truncated 26 kDa and a full-length 40-kDa protein, respectively (18).

Tmod2

Tmod2 was originally found during an attempt to identify proteins that bind to TmBr3, a neuron-specific Tm isoform and was subsequently named N-Tmod (19). A single 9.5-kb transcript (9) has been found for Tmod2 with a ≈40-kDa protein expressed. Tmod2 and Tmod1 share 59% amino acid identity, but their expression patterns differ (19).

Tmod3

Due to Tmod3's ubiquitous expression, this isoform was originally referred to as U-Tmod (17). A range of different transcripts (1–9.5 kb) has been found for this isoform with only a single approximately 40-kDa protein found in vertebrate cells so far (9).

Tmod4

Also referred to as skeletal (Sk) Tmod, Tmod4 is expressed in skeletal muscles (20, 21). A 1.3- to 1.5-kb transcript is found in this tissue, resulting in a ≈40-kDa protein (9, 20).

Leiomodin isoforms

Lmod1 was originally identified as 64-kDa human autoantigen D1 (22), then named smooth muscle leiomodin (SM-Lmod) due to its abundance in tissues containing smooth muscles and now is referred to as Lmod1 (17). Lmod2, also referred to as C-Lmod, has a more restricted expression profile as compared with Lmod1, being primarily found in the heart and skeletal muscles based on mRNA analysis (23). Lmod3 mRNA has been identified across a number of different vertebrate species, with the highest transcript levels in heart and skeletal muscle tissue (24). This isoform has not yet been characterized.

Tmod structure and functional domains

Tmod structure comprises two major domains as defined by (6): a TM/pointed-end actin capping (TM-Cap) domain at the extended N-terminus (residues 1–182) and a leucine-rich repeat/pointed-end actin capping (LRR-Cap) domain at the C-terminus (residues 183–359) (25–31) (Figure 1).

The TM-Cap domain of Tmod1–4 contains three α-helical sequences. The first and third α-helices (residues 24–35 and 126–135) have been characterized as Tm-binding sites (27, 32, 33), whereas the second α-helix has been shown to interact with actin (34).

The LRR-Cap domain contains five leucine-rich repeat domains (residues 183–321), an α-helical sequence (residues 321–344), and a carboxyl-terminal C-tail (residues 344–359). The actin-binding and actin filament-nucleation domains in the LRR-Cap have been mapped to residues 323–359 and 344–359, respectively (32, 34). Binding of Tmod to the actin filament-stabilizing protein nebulin has been mapped to residues 160–344 within the LRR-Cap.

The LRR-Cap is also the site of a pattern 4 nuclear localization signal (residues 340–343, RKRR), whereas the Tm-Cap domain harbors a nuclear export signal-like motif. Disruption of either of these signals has been shown to impact cycling of some of the Tmod1 pool between the cytosolic and the nuclear compartment (35).

In contrast to Tmod1–4, Lmods1–3 lack the third α-helical sequence (6). However, the largest structural difference between Tmod and Lmods is the carboxyl-terminal extension of Lmods. This extension harbors two predicted polyproline regions, two predicted α-helical sequences, and a WASP homology 2 domain (WH2), which contributes

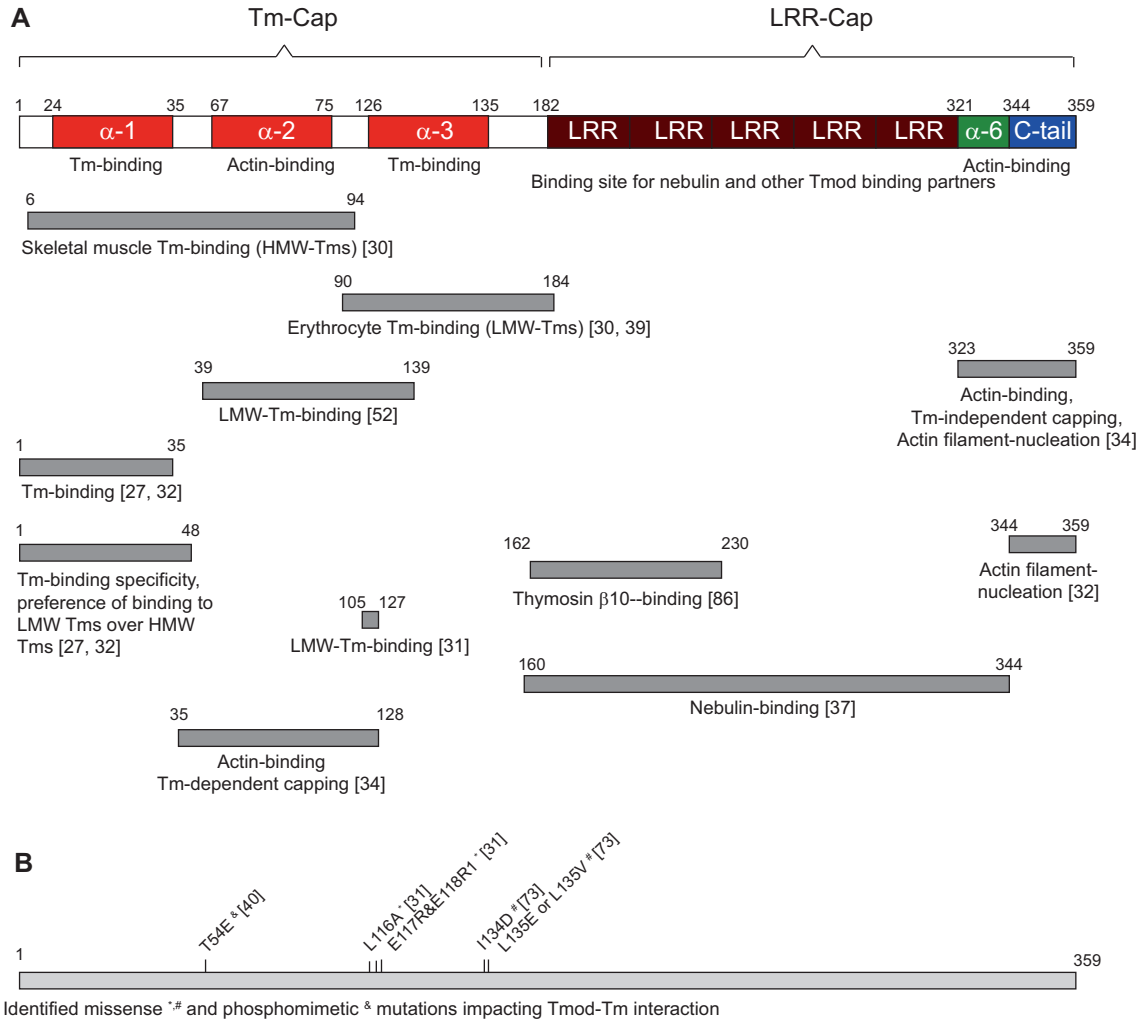

Figure 1 Schematic representation of the functional domain structure of Tmods.
Depicted is the mouse Tmod1 isoform with 359 amino acids as a representative example for Tmod1–4. (A) Tm-, actin-, thymosin-β10-, and nebulin-binding domains of Tmods are shown, and the respective references are shown in brackets. (B) Missense and phosphomimetic mutations that have been shown to impact on Tmod-Tm interactions in *in vitro* experiments are indicated.

to the strong actin nucleation activity of Lmods as shown for Lmod2 (36).

Members of the Tmod protein family are highly conserved in their amino acid sequence both between different isoforms and between different species, e.g., within the same isoforms, the vertebrate Tmods show about 80%–90% homology between different species and Tmod1–4 from the same vertebrate species show about 70%–80% homology (9, 19, 20). As determined for human Tmods, Tmods display the highest amino acid sequence conservation in their LRR-Cap domain (80%–90%), whereas the Tm-Cap domain shows about 70%–80% homology (37, 38). The greater variability in the Tm-Cap domain is likely to account for the functional diversity of Tmod isoforms.

Posttranslational modifications of Tmods

Tmod1 has been shown to be posttranslationally modified. Mass spectrometry analysis of Tmod1 from erythrocytes has shown that the N-terminal methionine is absent and the serine 2 residue is acetylated (39). Potential phosphorylation sites in Tmod1 have been identified at serine 3 and threonine 54, which *in vitro* can be phosphorylated by the kinase transient receptor potential melastatin-like 7 (TRPM7). *In vitro* evidence for a potential regulatory role of this phosphorylation site comes from the use of a phosphomimetic mutant (T54E of $Tmod1_{1–92}$; Figure 1), which reduces the Tm-dependent actin capping activity of Tmod1 (40).

The functional relevance of Tmod phosphorylation by protein kinase C (PKC) in cells comes from a study

that showed that phorbol ester and epidermal growth factor induced PKC-dependent association of Tmod with a 'Triton-insoluble cytoskeletal' fraction in cell extraction experiments (41). However, a specific phosphorylation site in Tmod for PKC has not yet been mapped.

Functional aspects of Tmods in different cellular systems

That Tmods function as an actin filament pointed-end capping protein was first shown for Tmod1 *in vitro* (42). In the absence of Tms, Tmods form a loose cap at the pointed end of actin filaments, which leads to reduced association and dissociation rates of actin monomers onto and away from the pointed end of the filaments. Capping of the actin filament pointed ends by Tmod in the presence of Tms can completely inhibit elongation and prevent depolymerization at the pointed ends. This characteristic is the basis for one of Tmod's major roles in structures containing relatively stable actin filament populations, the sarcomeres of muscle cells, and the membrane skeleton in erythrocytes where it is believed to be involved in precise actin filament length regulation (43–45).

Actin monomer sequestering and nucleation activity of Tmods

Contrary to the classical belief that Tmods exclusively interact with the pointed ends of actin filaments, mammalian Tmods have more recently been found to bind and sequester actin monomers (8, 46) as well as promote actin nucleation (46). Actin sequestering activity has been found for Tmod2 and Tmod3 but not Tmod1, whereas actin-nucleating activity has been established for Tmod1–3 (8, 46). A potential mechanism of actin nucleation has been proposed by Yamashiro et al. (6, 46), which involves both the LRR-Cap and the TM-Cap domains. In a first step, the LRR-Cap domain could bind to spontaneously formed actin dimers, followed by the recruitment of a third actin monomer by the TM-Cap domain.

The different biochemical properties of Tmods, namely the above-discussed actin filament-stabilizing, actin monomer-sequestering, and actin-nucleating activities may provide a basis for diverse cellular functions in different cellular systems and in different subcellular compartments within the same cells. An example for a potential relevance of these differences of isoform properties is the isoform-specific impacts of Tmod1 and Tmod2 on neurite outgrowth in N2a neuroblastoma cells (10). Both Tmod1 and Tmod2 negatively regulate neurite outgrowth but impact on different aspects of neurite formation: shRNA-mediated knockdown of Tmod1 leads to more neurites being formed per cell with a slight attenuation of neurite extension, whereas shRNA-mediated knockdown of Tmod2 has no effect on the number of neurites formed per cells but significantly promotes neurite extension. Crucial for the dynamic behavior of neuronal growth cones and the extension of neurites is the control of the level and distribution of monomeric, globular (G) actin in the growth cone (47). Altering the levels of actin monomers by actin monomer sequestering, for example, by β-thymosin, has previously been shown to impact on neurite extension (48). Inhibition of β-thymosin using dsRNA in cultured PeA neurons from the snail *Lymnaea stagnalis* enhanced neurite extension. In a similar way, more efficient monomer sequestering activity of Tmod2 as compared with Tmod1 provides a potential explanation for the neurite extension-promoting effect of Tmod2 knockdown in these cells. The effect of Tmod1 on initial neurite formation with the observed increase in the number of neurites formed per cell in response to Tmod1 knockdown may be the result of the inhibition of Tmod1-mediated stabilization of the cortical actin cytoskeleton, which is required for neurite initiation (49).

Functional role of Tmods in different tissues

Tmods in muscle tissue

Tmods show a distinct expression profile in different muscle types. Although Tmod4 is the predominant isoform in fast-twitch and Tmod1 in slow-twitch skeletal muscle in chicken, these isoforms are coexpressed in mammalian skeletal muscles (50). Lmod2 is an antagonist of Tmod1 at the pointed end of the thin filaments in mammalian cardiac muscle (28). In muscle, Tmods play a major role in myofibril assembly, contractile function and the regulation of thin filament length (7). In skeletal muscle, Tmod3 is found in a sarcoplasmic reticulum (SR) microdomain where it forms an M-line complex that is distinct from Tmod1- and Tmod4-containing SR microdomains flanking the Z-line (51).

Tmods in erythrocytes, polarized epithelial cells, and motile cells

The major Tmod in human erythrocytes is Tmod1 (3), which is a critical constituent of the spectrin-based

membrane skeleton (44, 52, 53). Tmod3 is usually absent from mature erythrocytes but shows expression in erythroblasts at early stages of erythropoiesis (54). In erythrocytes deficient for Tmod1, Tmod3 upregulation is likely to provide partial functional compensation (45). In chicken erythrocytes, the major Tmod found in the membrane skeleton is Tmod4 (55). Tmod1 is also the major Tmod isoform found associated with the spectrin-based membrane skeleton of mammalian lens fiber cells, whereas in chicken, Tmod4 takes the place of Tmod1 (11, 56, 57). In polarized epithelial cells, including intestinal, bronchiole, and kidney epithelial cells, Tmod3 can be found in the membrane skeleton (58).

Tmod1 has been shown to be involved in the regulation of the adhesion properties of human ligament fibroblasts, suggesting that Tmods influence cell dynamics (59). A more recent study has shown a direct impact of Tmod3 depletion on the motile behavior of human microvascular endothelial cells, confirming the role of Tmods in regulating cell migration (60).

Tmods in the nervous system

Tmod1–3 isoforms are expressed in the nervous system, with Tmod2 being exclusively expressed in neurons (19), whereas Tmod4 and Lmod1–3 are largely absent from neuronal tissue (9, 19, 23, 61). Expression of Tmod1 has been detected in the olfactory bulb, hippocampus, cerebral cortex, basal ganglia, brain stem, cerebellum, and in retinal horizontal cells (13, 62–64). Tmod1 and Tmod2 show differential expression and localization patterns in developing neurons in culture (10). Furthermore, results from a study where Tmod1 and Tmod2 were overexpressed in differentiating N2a neuroblastoma cells suggest an isoform-dependent role of Tmod in neuronal morphogenesis (10), as discussed above. Tmods likely play an important role also at later stages of development. Mice deficient for Tmod2 expression have strain-dependent hyperactivity, reduced sensorimotor gating, and impaired learning and memory (61), and electrophysiological recordings have shown enhanced long-term potentiation (61). However, whether this effect is due to the lack of Tmod2 or an observed eightfold upregulation of Tmod1 has yet to be determined.

Further evidence for a potential role of Tmods in signal transmission in the nervous system comes from studies analyzing altered Tmod1 and Tmod2 expression in the context of seizure activity (63, 65). *In situ* hybridization analysis of rat brains after subcutaneous injection of kainic acid showed a strong increase in Tmod1 mRNA levels in hippocampal formation. However, the upregulation of mRNA levels was not shown to lead to changes at the protein level (63). A more recent proteomic study on hippocampal tissue from patients with mesial temporal lobe epilepsy has shown a downregulation of Tmod2 protein as compared with control tissue (65). Data for changes in protein levels for Tmod1 in this study were not reported. Hence, it will be important to test in future studies for differential regulation of different Tmod isoforms under seizure-inducing conditions. This will also help to better understand a potential involvement of Tmods in signal transmission in the brain under other pathological conditions, e.g., altered Tmod2 expression has been reported from patients with Down syndrome (66). The role of the third isoform present in neuronal tissue, Tmod3, has not yet been studied in the context of neuronal function. However, considering the role of Tmod3 in cell adhesion and migration, expression of Tmod3 in neuronal cells has likely important implications during early neuronal development when intense migratory behavior is required for correct tissue organization.

Interactions among Tmods, Tms, and other proteins

The association of actin monomers to the filament ends is regulated by proteins that cap the filaments at either the pointed or barbed end and by the available pool of monomeric actin. Tmods regulate the on and off rate of actin monomers by binding to the pointed ends of the filaments. The affinity of Tmods for the pointed end of the actin filament increases more than a thousand-fold in the presence of Tm (K_d=0.3–0.4 μM to 50 pM) (32, 42, 67).

The dependency of the actin filament pointed end-capping activity on Tm has been shown to be restricted to the actin-binding region in the Tm-Cap domain. This dependency is also reflected by structural changes in the α-helicity of the Tm-Cap domain upon binding of Tm to Tmod (26, 27, 68). Actin filament pointed-end capping mediated by the Tmod actin-binding region located in the LRR domain has been shown to be independent of Tm (34, 42, 69). Different models have been proposed for the stoichiometry of Tmod binding to actin pointed-end filament ends [see (6) for a comprehensive discussion of these models]. The two main models suggest the binding

of either one (68, 69) or two (14, 70) Tmod molecules to one actin filament pointed end.

To understand the central role of Tm-dependent functions of Tmods, a brief overview will be provided on the Tm gene family and studies that have tested the nature of Tmod-Tm interactions. Tms are actin-associated proteins that associate along the major groove of actin filaments. Like Tmods, Tms are a multigene family. In mammalian cells, more than 40 different Tm isoforms are expressed, which are generated from four different genes (*TPM1–4*) by alternative splicing and alternative promoter usage (71). The expression of these isoforms is tissue and cell specific and developmentally regulated [for detailed reviews on Tms, see (4, 5)].

Tmod interaction with Tm

The interaction between Tmods and Tms has been shown to be isoform specific. *In vitro* studies, using small peptide sequences of the first (within residues 1–38) and second (within residues 109–144) Tm-binding domains of Tmod1 have revealed different Tm-binding affinities for these domains (Figure 1). The first binding domain showed a high binding affinity with *TPM1* gene products (90 nM) as compared with *TPM3* and *TPM4* gene products (nondetectable at 10 μM). The second binding domain, however, showed high binding affinities to all Tm gene products tested (*TPM1*, 2.5 nM; *TPM3* and *TPM4*, 40–90 nM) (72). However, point mutations of residue 135 in the second binding domain has revealed a Tm isoform-dependent impact on Tmod-Tm interaction (73). The missense mutations L135E and L135V strongly reduce binding affinity to the low-molecular-weight Tm, Tm5NM1, but do not impact the interaction with high-molecular-weight Tm isoforms (Figure 1).

Isoform specificity of Tmod-Tm interaction has also been shown for the neuron-specific isoform Tmod2 and the neuronally expressed *TPM1* gene products TmBr1 and TmBr3 (19). Blot overlay in combination with peptide experiments have shown Tmod2 binding with TmBr3 but a lack of interaction with TmBr1 (19).

Although the interaction of Tmods and Tms mutually influence the function of these actin cytoskeleton-regulatory proteins, a large number of studies over the past two decades have investigated the interactions of Tmod and Tm gene family products with structural/scaffolding, signaling, and actin-associated proteins including actin filament-severing proteins and actin motors. In the following section, we will briefly examine the most prominent of these interactions and their functional consequences where known.

Interactions of Tmods-Tms with scaffolding proteins

Filensin and CP49

One of the most characterized interactions between Tmods and cytoskeletal proteins of nonactin filament systems is the binding to the intermediate filament (IF) proteins filensin and CP49. Filensin and CP49 are lens-specific intermediate filament proteins that together form an important structural component, namely, the beaded filament of lens fiber cell architecture (74, 75). Chicken Tmod4 binds to the head, tail, or both ends of filensin alone and also in association with CP49 but not to CP49 alone (76). Tmod-filensin binding does not interfere with Tmod4 capping of the pointed ends of Tm-free actin filaments (76), but its effect on Tmod-Tm binding is yet to be studied. It is speculated that Tmod may be the determinant of the unique characteristics of the lens cell membrane that maintains the elongated cell shape of these cells, but there is no evidence for Tmod-IF interaction in other cell types.

Nebulin

Nebulin is a thin filament-associated actin-binding and stabilizing protein in vertebrate striated muscle sarcomeres [reviewed by (77)] and human brain (78), whose N-terminus M1M2M3 module interacts with the C-terminus of Tmod1 and Tmod4 (37, 79). Binding of Tmod to the N-terminus M1M2M3 module of nebulin is stronger for Tmod4 than for Tmod1 (79). In addition to its binding to actin and Tmods, nebulin has been shown to interact with other binding partners including Tms, troponin, and calmodulin (80), thereby providing a potential scaffolding link among actin, Tmods, and Tms. Although these interactions have been characterized *in vitro*, immunohistochemical analysis of human, rabbit, and chicken skeletal muscle does not show colocalization of Tmods with the M1M2M3 module of nebulin (50, 81, 82). Whether nebulin, Tmods, and Tms are forming complex scaffolding structures in the nervous system has not yet been studied.

sAnk1.5

sAnk1.5 is a splice variant of ankyrin-R (Ank1), which contains a 73-amino-acid-long N-terminal hydrophobic membrane-binding domain in place of the N-terminal tandem ankyrin repeats (83). Originally, sAnk1.5 was identified as a direct interaction partner of Tmod3 in glutathione

S-transferase pull-down experiments (7) and forms larger complexes with low-molecular-weight Tm isoforms (Tm4 and Tm5NM1), Tmod3, and cytoplasmic γ-actin. These complexes have been shown to be associated with the SR at the M-line of skeletal muscle sarcomeres (51). The function of this interaction has been postulated to be of structural importance in mechanically stabilizing the SR membrane (7, 50).

Interactions of Tmods-Tms with other actin-associated proteins

Both Tmods and Tms have been shown to influence the activity of proteins that lead to the disassembly of actin filaments. The best characterized families in this category are the thymosins [reviewed by (84)] and ADF/cofilin [reviewed by (85)]. Thymosin β10 and Tmod1 have been identified as interacting proteins in a yeast two-hybrid screen, and the binding region on Tmod1 has been mapped to residues 162–230 (86). Tmod capping of the pointed end of actin filaments inhibited the actin depolymerization activity of ADF/cofilin in *in vitro* experiments (87). Tms have also been shown to control the access of ADF/cofilin to actin filaments in an isoform-specific manner (88). For example, whereas the *TPM1* gene product TmBr3 allows the access of ADF/cofilin to the filaments, the association of the *TPM3* gene product Tm5NM1 prevents the severing of actin filaments by ADF/cofilin, allowing for more stable filaments.

Myosin II and V

Actin-associated myosin motor proteins are involved in a large range of cellular functions. The primary roles are to drive intracellular transport, support cell motility, and determine the mechanochemical properties of cells. The interaction of myosin II and V with actin filaments is influenced by the association of Tms with filaments in an isoform-specific manner (88). A recent study of the formation of complexes among actin, myosin, and Tm using cryo-electron microscopy (EM) analysis suggested a direct interaction between myosin and Tm (89). Since these experiments have been carried out with recombinant muscle Tm to investigate the actin-myosin-Tm interaction in the context of sarcomere function, potential isoform-dependent interactions of cytoskeletal Tms with nonmuscle myosins remain to be addressed. A recent study confirmed Tm isoform-dependent association of myosins with actin filaments by testing Tm-dependent processivity of the class V myosin homologue Myo2p in yeast along actin cables (90). In these assays, the Tm isoform *Tpm1p* showed higher run frequency and longer run length compared with the isoform *Tpm2p*.

Interactions of Tmods-Tms with signaling molecules

The actin cytoskeleton is the subject of continuous remodeling in response to both intracellular events and extracellular signaling cues. Many of these signaling cues involve mediators of the small GTPase family of proteins (91). The most prominent and well-studied small GTPases include RhoA, Rac, and cdc42. Links between the functions of Tmod and Tms and small GTPases have largely been indirect.

RhoA is a small GTPase protein known to be involved in the formation of stress fibers and regulating the actin cytoskeleton. *TPM1* gene silencing caused loss of stress fibers *via* ubiquitination of RhoA, whereas transgenic expression of synaptopodin – which promotes RhoA signaling by blocking the Smurf1-mediated ubiquitination of RhoA – resulted in the restoration of stress fibers in cancer cells, fibroblasts, and *Drosophila* (92). The study by Wong and colleagues identified RhoA as a ™-binding protein and provides evidence for a direct functional interaction between small GTPases and Tms (92).

Distinct actin filament populations as an organizing platform of functionally different microcompartments

Through their interactions with each other as well as with structural/scaffolding and signaling molecules, Tmods and Tms are able to form complex organizing platforms for cellular microcompartments. These can be relatively stable such as building blocks of the spectrin membrane skeleton in erythrocytes, polarized epithelial cells and lens fiber cells, or dynamic such as actin filament populations with high turnover in migratory endothelial cells.

Organization of the membrane skeleton

Actin supports an astonishing plethora of diverse cellular function considering the highly conserved structure

of actin and the presence of only two cytosolic isoforms, β- and γ-actin (93). The diversity of actin filament populations is therefore not a result of the filaments' monomeric building blocks and most likely lies in specific assortments of actin-associated proteins with the filaments. For example, the organization of the membrane skeleton appears to follow some common pattern in a number of different cell types. One of the preferred Tmod-Tm interacting partners in these filament populations in human erythrocytes and lens fiber cells are Tmod1 (or Tmod4 in chicken) and Tm5a/b or Tm5NM1. However, the composition of this actin filament population can differ between different cell types. Evidence suggests that the choice of a specific Tmod isoform as a component of the membrane skeleton is functionally important. Although Tmod3 is present in the membrane skeleton of polarized epithelial cells, the upregulation of Tmod3 in erythrocytes derived from Tmod1$^{-/-}$ mice does not provide full functional compensation for the lack of Tmod1 (45, 51).

Although Tmod1 is believed to provide tight length control of cortical actin filaments, the Tm isoform Tm5NM1 has been show to recruit the actin motor protein myosin II to the filaments (88), thereby directly influencing the mechanochemical properties of the cell membrane.

Organization of sarcomere-associated microcompartments in muscle cells

Arguably, the most prominent formation of microcompartments defined by the localization of specific Tmod and Tm isoforms can be found in muscle cells. Here, Tmod3 defines an M-line-associated SR complex with Tm4 and Tm5NM1, whereas Tmod1 and Tmod4 are segregated to a microdomain adjacent to the Z-line (51), which is likely to be t-tubule-associated, as previous studies have shown that Tm5NM1 associates with t-tubules (94). The depletion of Tmod1 from sarcomeres in a Tmod1$^{-/-}$ mouse model leads to a compensatory relocation of Tmod3 (and Tmod4) to the now uncapped pointed ends of actin filaments within the sarcomeres, reducing the Tmod3 pool at the M-line. This also resulted in a slight redistribution of Tm4 and Tm5NM1 from the M-line to the Z-line, confirming a functional interaction between Tmod3 and Tm5NM1/Tm4 in skeletal muscle cells.

There is growing evidence that suggests that the specificity of Tm-Tmod interactions are important contributing factors in determining the functional properties of distinct, locally restricted actin filament populations not only at the plasma membrane but also in various other cellular microcompartments.

Organization of actin filament-based microcompartments in dynamic structures of neuronal cells

The presence of different structurally and functionally distinct actin filament populations in small subcellular compartments has attracted particular attention in two well-characterized structures of nerve cells: (1) growth cones at the tips of growing dendrites and axons and (2) dendritic spines, which are small protrusions on the dendritic shafts of neurons, which form the postsynaptic receptive regions of excitatory neurons in the central nervous system. In both of these compartments, multiple actin filament populations have been described [reviewed by Schevzov (5)]. *In vitro* studies using rat hippocampal neurons have shown that Tmod1 and Tmod2 define distinct expression patterns and spatial segregation in microcompartments at the cell edge and within the growth cones at different stages of neurite outgrowth (10). Before the initial formation of neurites, Tmod2 is absent from lamellipodia, which forms the outer edge of the cells, whereas Tmod1 is detected at the proximal segments of actin filament bundles in the lamellipodia. Shortly after the formation of neurites (1 day after plating of cells), Tmod2 is detected in growth cones where it fully decorates actin-rich structures, whereas Tmod1 maintains a distribution limited to the proximal ends of actin filament bundles. At later stages of neurite outgrowth (4 days after plating), Tmod1 and Tmod2 display a similar subcellular segregation within the growth cone. The segregation of Tmod1 and Tmod2 to different microcompartments within the growth cones suggests that they support different actin filament populations during early neurite formation and extension. Isoform-specific effects of altered Tmod and Tm protein levels on neurite formation have been demonstrated in a number of recent studies in differentiating neuroblastoma cells (10, 95, 96). Reduced expression of Tmod2 resulted in increased neurite extension, whereas reducing Tmod1 by siRNA knockdown resulted in a slight but significant decrease in neurite length and an increase in the number of neurites formed per cell

(10). Although the precise mechanism for Tmod-regulated control of neurite outgrowth remains unknown, one may speculate that the spatial segregation, the different functional properties of Tmod1 and Tmod2, or a combination of both are responsible for the different phenotypes that have been observed. Alternative to the hypothesis that the higher actin monomer-sequestering efficiency of Tmod2 as compared with Tmod1 (as discussed above), the increase in the number of neurites formed in response to Tmod1 but not Tmod2 knockdown may be explained by the absence of Tmod2 from the outer edge before the initial formation of neurites. Increased neurite outgrowth has been shown to be associated with a Tm5NM1-mediated increase in the pool of filamentous actin in the growth cone (97). Increased Tmod2-dependent neurite extension may therefore be mediated by increased filamentous actin in the growth cone. However, no data exist about specific Tmod-Tm interactions in the growth cones. Both Tm5NM1 and Tm4 have been shown to segregate to the growth cone compartment (95, 98), but a detailed study of neurite outgrowth stage-specific distribution of different Tms within the growth has not been carried out.

Although the presence of different actin filaments in dendritic spines has been well established (99–101), the contribution of Tmods and Tms to spine function is still poorly understood. Tmod is highly concentrated at the postsynaptic domain of human and rat neuromuscular junctions (102), but so far, no studies have addressed the expression profile of Tmods at the postsynapse of the central nervous system synapse. Immuno-gold EM and immunohistochemical studies have identified the nonmuscle Tm isoforms TmBr3, Tm5NM1, and Tm4 at rat cerebellar and mouse hippocampal synapses, with an isoform-specific distribution (98, 103). Tm5NM1 and Tm4 were enriched at the postsynaptic site, whereas TmBr3 segregated to the presynaptic compartment. Studies of the potential segregation of Tm4 and Tm5NM1 to different regions within the postsynapse would provide greater insight into the functional involvement of these isoforms in synaptic function. To date, such studies are yet to be done.

Summary and outlook

This overview summarizes some of the current knowledge and more recent key findings in the field of Tmod and Tm biology with a focus on highlighting Tmod-Tm interaction-based cellular processes. The simultaneous binding of different partners of Tmods, as shown for Tmod3 in skeletal muscle (51), allows Tmods to serve as scaffolds for anchoring other proteins and subcellular structures, including components of other filament systems and signaling molecules. By forming these scaffolds, the actin filament network could serve as a relay system for spatially restricted biochemical reactions in the cells. The composition of distinct actin filament populations could be instrumental in determining the recruitment of signaling molecules and enzymes that are required for these biochemical reactions.

Careful studies of the cell type-specific Tmod-Tm-dependent formation of microcompartments, which are defined by specific functionally distinct actin filament populations, and the analysis of local activity of signaling molecules, such as small GTPases, in these microcompartments will be important to better understand some of the most fundamental processes in eukaryotic cells.

Recent advances in the development of new imaging tools to study dynamic cellular processes at super-resolution will be instrumental in obtaining new insights into the organization of Tmod-Tm-based microcompartments. For example, super-resolution imaging could reveal the macromolecular compositions of scaffolds built by the scaffolding protein nebulin in neuronal cells and will likely provide novel insights in the architecture of the discussed microcompartments in these cells. As discussed for actin filament-driven processes in the growth cones of neurons, these microcompartments can be very dynamic in cells that undergo morphological changes. Analysis of fixed samples at the level of super-resolution will be insufficient to reveal the role of Tmods and Tms in these actin filament populations, which emphasizes the need to carry out these experiments in living cells. Furthermore, to study the mechanisms by which Tmods, Tms, and their binding partners assemble in filamentous actin-rich structures, imaging approaches will require both high temporal and spatial resolution.

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References

1. Cooper JA, Sept D. New insights into mechanism and regulation of actin capping protein. Int Rev Cell Mol Biol 2008; 267: 183–206.
2. Kuhlman PA, Hughes CA, Bennett V, Fowler VM. A new function for adducin. Calcium calmodulin-regulated capping of the barbed ends of actin filaments. J Biol Chem 1996; 271: 7986–91.
3. Fowler VM. Identification and purification of a novel Mr 43,000 tropomyosin-binding protein from human erythrocyte membranes. J Biol Chem 1987; 262: 12792–800.
4. Gunning P, O'Neill G, Hardeman E. Tropomyosin-based regulation of the actin cytoskeleton in time and space. Physiol Rev 2008; 88: 1–35.
5. Schevzov G, Curthoys NM, Gunning PW, Fath T. Functional diversity of actin cytoskeleton in neurons and its regulation by tropomyosin. Int Rev Cell Mol Biol 2012; 298: 33–94.
6. Yamashiro S, Gokhin DS, Kimura S, Nowak RB, Fowler VM. Tropomodulins: pointed-end capping proteins that regulate actin filament architecture in diverse cell types. Cytoskeleton (Hoboken) 2012; 69: 337–70.
7. Gokhin DS, Fowler VM. Tropomodulin capping of actin filaments in striated muscle development and physiology. J Biomed Biotechnol 2011; 2011: 103069.
8. Fischer RS, Yarmola EG, Weber KL, Speicher KD, Speicher DW, Bubb MR, Fowler VM. Tropomodulin 3 binds to actin monomers. J Biol Chem 2006; 281: 36454–65.
9. Cox PR, Zoghbi HY. Sequencing, expression analysis, and mapping of three unique human tropomodulin genes and their mouse orthologs. Genomics 2000; 63: 97–107.
10. Fath T, Fischer RS, Dehmelt L, Halpain S, Fowler VM. Tropomodulins are negative regulators of neurite outgrowth. Eur J Cell Biol 2011; 90: 291–300.
11. Fischer RS, Lee A, Fowler VM. Tropomodulin and tropomyosin mediate lens cell actin cytoskeleton reorganization in vitro. Invest Ophthalmol Vis Sci 2000; 41: 166–74.
12. Sussman MA, McAvoy JW, Rudisill M, Swanson B, Lyons GE, Kedes L, Blanks J. Lens tropomodulin: developmental expression during differentiation. Exp Eye Res 1996; 63: 223–32.
13. Yao W, Sung LA. Specific expression of E-Tmod (Tmod1) in horizontal cells: implications in neuronal cell mechanics and glaucomatous retina. Mol Cell Biomech 2009; 6: 71–82.
14. 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 1993; 120: 411–20.
15. 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 nyocytes. Nature 1995; 377: 83–6.
16. Sussman Ma, Ito M, Daniels MP, Flucher B, Buranen S, Kedes L. Chicken skeletal muscle tropomodulin: novel localization and characterization. Cell Tissue Res 1996; 285: 287–96.
17. Conley CA. Leiomodin and tropomodulin in smooth muscle. Am J Physiol Cell Physiol 2001; 280: C1645–56.
18. Yao W, Sung LA. Erythrocyte tropomodulin isoforms with and without the N-terminal actin-binding domain. J Biol Chem 2010; 285: 31408–17.
19. Watakabe A, Kobayashi R, Helfman DM. N-tropomodulin: a novel isoform of tropomodulin identified as the major binding protein to brain tropomyosin. J Cell Sci 1996; 109: 2299–310.
20. 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. J Biol Chem 1999; 274: 28466–75.
21. Fischer RS, Fowler VM. Tropomodulins: life at the slow end. Trends Cell Biol 2003; 13: 593–601.
22. Dong Q, Ludgate M, Vassart G. Cloning and sequencing of a novel 64-kDa autoantigen recognized by patients with autoimmune thyroid disease. J Clin Endocrinol Metabol 1991; 72: 1375–81.
23. Conley CA, Fritz-Six KL, Almenar-Queralt A, Fowler VM. Leiomodins: larger members of the tropomodulin (Tmod) gene family. Genomics 2001; 73: 127–39.
24. Nanda V, Miano JM. Leiomodin 1, a new serum response factor-dependent target gene expressed preferentially in differentiated smooth muscle cells. J Biol Chem 2012; 287: 2459–67.
25. 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 2001; 498: 67–71.
26. Greenfield NJ, Fowler VM. Tropomyosin requires an intact N-terminal coiled coil to interact with tropomodulin. Biophys J 2002; 82: 2580–91.
27. Greenfield NJ, Kostyukova AS, Hitchcock-DeGregori SE. Structure and tropomyosin binding properties of the N-terminal capping domain of tropomodulin 1. Biophys J 2005; 88: 372–83.
28. 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 2000; 267: 6470–5.
29. Kostyukova AS, Tiktopulo EI, Maeda Y. Folding properties of functional domains of tropomodulin. Biophys J 2001; 81: 345–51.
30. Babcock GG, Fowler VM. Isoform-specific interaction of tropomodulin with skeletal muscle and erythrocyte tropomyosins. J Biol Chem 1994; 269: 27510–8.
31. Vera C, Lao J, Hamelberg D, Sung LA. Mapping the tropomyosin isoform 5 binding site on human erythrocyte tropomodulin: further insights into E-Tmod/TM5 interaction. Arch Biochem Biophys 2005; 444: 130–8.
32. Kostyukova AS, Hitchcock-DeGregori SE. Effect of the structure of the N terminus of tropomyosin on tropomodulin function. J Biol Chem 2004; 279: 5066–71.
33. Kostyukova AS, Rapp BA, Choy A, Greenfield NJ, Hitchcock-DeGregori SE. Structural requirements of tropomodulin for tropomyosin binding and actin filament capping. Biochemistry 2005; 44: 4905–10.
34. Fowler VM, Greenfield NJ, Moyer J. Tropomodulin contains two actin filament pointed end-capping domains. J Biol Chem 2003; 278: 40000–9.
35. Kong KY, Kedes L. Cytoplasmic nuclear transfer of the actin-capping protein tropomodulin. J Biol Chem 2004; 279: 30856–64.
36. Chereau D, Boczkowska M, Skwarek-Maruszewska A, Fujiwara I, Hayes DB, Rebowski G, Lappalainen P, Pollard TD, Dominguez R. Leiomodin is an actin filament nucleator in muscle cells. Science 2008; 320: 239–43.

37. Krieger I, Kostyukova A, Yamashita A, Nitanai Y, Maeda Y. Crystal structure of the C-terminal half of tropomodulin and structural basis of actin filament pointed-end capping. Biophys J 2002; 83: 2716–25.
38. Lu SY, Symersky J, Li SL, Carson M, Chen LQ, Meehan E, Luo M. Structural genomics of Caenorhabditis elegans: crystal structure of the tropomodulin C-terminal domain. Proteins 2004; 56: 384–6.
39. Chait BT, Kent SB. Weighing naked proteins: practical, high-accuracy mass measurement of peptide and proteins. Science 1992; 257: 1885–94.
40. Dorovkov MV, Beznosov SN, Shah S, Kotlianskaia L, Kostiukova AS. [Effect of mutations imitating the phosphorylation by TRPM7 kinase on the function of the N-terminal domain of tropomodulin]. Biofizika 2008; 53: 943–9.
41. Wagner LM, Fowler VM, Takemoto DJ. The interaction and phosphorylation of tropomodulin by protein kinase C alpha in N/N 1003A lens epithelial cells. Mol Vis 2002; 8: 394–406.
42. Weber A, Pennise CR, Babcock GG, Fowler VM. Tropomodulin caps the pointed ends of actin filaments. J Cell Biol 1994; 127: 1627–35.
43. Littlefield RS, Fowler VM. Thin filament length regulation in striated muscle sarcomeres: pointed-end dynamics go beyond a nebulin ruler. Semin Cell Dev Biol 2008; 19: 511–9.
44. Fowler VM, Vale R. Cytoskeleton. Curr Opin Cell Biol 1996; 8: 1–3.
45. Moyer JD, Nowak RB, Kim NE, Larkin SK, Peters LL, Hartwig J, Kuypers FA, Fowler VM. Tropomodulin 1-null mice have a mild spherocytic elliptocytosis with appearance of tropomodulin 3 in red blood cells and disruption of the membrane skeleton. Blood 2010; 116: 2590–9.
46. Yamashiro S, Speicher KD, Speicher DW, Fowler VM. Mammalian tropomodulins nucleate actin polymerization via their actin monomer binding and filament pointed end-capping activities. J Biol Chem 2010; 285: 33265–80.
47. Craig EM, Van Goor D, Forscher P, Mogilner A. Membrane tension, myosin force, and actin turnover maintain actin treadmill in the nerve growth cone. Biophys J 2012; 102: 1503–13.
48. van Kesteren RE, Carter C, Dissel HM, van Minnen J, Gouwenberg Y, Syed NI, Spencer GE, Smit AB. Local synthesis of actin-binding protein beta-thymosin regulates neurite outgrowth. J Neurosci 2006; 26: 152–7.
49. da Silva JS, Dotti CG. Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci 2002; 3: 694–704.
50. Gokhin DS, Lewis RA, McKeown CR, Nowak RB, Kim NE, Littlefield RS, Lieber RL, Fowler VM. Tropomodulin isoforms regulate thin filament pointed-end capping and skeletal muscle physiology. J Cell Biol 2010; 189: 95–U141.
51. Gokhin DS, Fowler VM. Cytoplasmic gamma-actin and tropomodulin isoforms link to the sarcoplasmic reticulum in skeletal muscle fibers. J Cell Biol 2011; 194: 105–20.
52. Sung LA, Fowler VM, Lambert K, Sussman MA, Karr D, Chien S. Molecular cloning and characterization of human fetal liver tropomodulin. J Biol Chem 1992; 267: 2616–21.
53. Ursitti JA, Fowler VM. Immunolocalization of tropomodulin, tropomyosin and actin in spread human erythrocyte skeletons. J Cell Sci 1994; 107: 1633–9.
54. Watkins NA, Gusnanto A, de Bono B, De S, Miranda-Saavedra D, Hardie DL, Angenent WG, Attwood AP, Ellis PD, Erber W, Foad NS, Garner SF, Isacke CM, Jolley J, Koch K, Macaulay IC, Morley SL, Rendon A, Rice KM, Taylor N, Thijssen-Timmer DC, Tijssen MR, van der Schoot CE, Wernisch L, Winzer T, Dudbridge F, Buckley CD, Langford CF, Teichmann S, Göttgens B, Ouwehand WH; Bloodomics Consortium. A HaemAtlas: characterizing gene expression in differentiated human blood cells. Blood 2009; 113: e1–9.
55. Almenar-Queralt A, Gregorio CC, Fowler VM. Tropomodulin assembles early in myofibrillogenesis in chick skeletal muscle: evidence that thin filaments rearrange to form striated myofibrils. J Cell Sci 1999; 112: 1111–23.
56. Lee A, Fischer RS, Fowler VM. Stabilization and remodeling of the membrane skeleton during lens fiber cell differentiation and maturation. Dev Dyn 2000; 217: 257–70.
57. Woo MK, Lee A, Fischer RS, Moyer J, Fowler VM. The lens membrane skeleton contains structures preferentially enriched in spectrin-actin or tropomodulin-actin complexes. Cell motil cytoskeleton 2000; 46: 257–68.
58. Weber KL, Fischer RS, Fowler VM. Tmod3 regulates polarized epithelial cell morphology. J Cell Sci 2007; 120: 3625–32.
59. Sung KL, Yang L, Whittemore DE, Shi Y, Jin G, Hsieh AH, Akeson WH, Sung LA. The differential adhesion forces of anterior cruciate and medial collateral ligament fibroblasts: effects of tropomodulin, talin, vinculin, and alpha-actinin. Proc Natl Acad Sci USA 1996; 93: 9182–7.
60. Fischer RS, Fritz-Six KL, Fowler VM. Pointed-end capping by tropomodulin3 negatively regulates endothelial cell motility. J Cell Biol 2003; 161: 371–80.
61. Cox PR, Fowler V, Xu BS, Sweatt JD, Paylor R, Zoghbi HY. Mice lacking tropomodulin-2 show enhanced long-term potentiation, hyperactivity, and deficits in learning and memory. Mol Cell Neurosci 2003; 23: 1–12.
62. Ito M, Swanson B, Sussman MA, Kedes L, Lyons G. Cloning of tropomodulin cDNA and localization of gene transcripts during mouse embryogenesis. Dev Biol 1995; 167: 317–28.
63. Sussman MA, Sakhi S, Tocco G, Najm I, Baudry M, Kedes L, Schreiber SS. Neural tropomodulin: developmental expression and effect of seizure activity. Dev Brain Res 1994; 80: 45–53.
64. Yao W, Nathanson J, Lian I, Gage FH, Sung LA. Mouse erythrocyte tropomodulin in the brain reported by lacZ knocked-in downstream from the E1 promoter. Gene expression patterns: GEP 2007; 8: 36–46.
65. Yang JW, Czech T, Felizardo M, Baumgartner C, Lubec G. Aberrant expression of cytoskeleton proteins in hippocampus from patients with mesial temporal lobe epilepsy. Amino acids 2006; 30: 477–93.
66. Sun Y, Dierssen M, Toran N, Pollak DD, Chen W-Q, Lubec G. A gel-based proteomic method reveals several protein pathway abnormalities in fetal Down syndrome brain. J Proteomics 2011; 74: 547–57.
67. Weber A. Tropomodulin increases the critical concentration of barbed end-capped actin filaments by converting ADP middle dot Pi-actin to ADP-actin at all pointed filament ends. J Biol Chem 1999; 274: 34637–45.
68. Kostyukova AS, Hitchcock-DeGregori SE, Greenfield NJ. Molecular basis of tropomyosin binding to tropomodulin, an actin-capping protein. J Mol Biol 2007; 372: 608–18.

69. Kostyukova AS, Choy A, Rapp BA. Tropomodulin binds two tropomyosins: a novel model for actin filament capping. Biochemistry 2006; 45: 12068–75.
70. Fowler VM. Regulation of actin filament length in erythrocytes and striated muscle. Curr Opin Cell Biol 1996; 8: 86–96.
71. Lees-Miller JP, Helfman DM. The molecular basis for tropomyosin isoform diversity. Bioessays 1991; 13: 429–37.
72. Kostyukova AS. Leiomodin/tropomyosin interactions are isoform specific. Arch Biochem Biophys 2007; 465: 227–30.
73. Kong KY, Kedes L. Leucine 135 of tropomodulin-1 regulates its association with tropomyosin, its cellular localization, and the integrity of sarcomeres. J Biol Chem 2006; 281: 9589–99.
74. Merdes A, Brunkener M, Horstmann H, Georgatos SD. Filensin: a new vimentin-binding, polymerization-competent, and membrane-associated protein of the lens fiber cell. J Cell Biol 1991; 115: 397–410.
75. Alizadeh A, Clark J, Seeberger T, Hess J, Blankenship T, FitzGerald PG. Targeted deletion of the lens fiber cell-specific intermediate filament protein filensin. Invest Ophthalmol Vis Sci 2003; 44: 5252–8.
76. Fischer RSS, Quinlan RAA, Fowler VMM. Tropomodulin binds to filensin intermediate filaments. Febs Letters 2003; 547: 228–32.
77. Pappas CT, Bliss KT, Zieseniss A, Gregorio CC. The Nebulin family: an actin support group. Trends Cell Biol 2011; 21: 29–37.
78. Laitila J, Hanif M, Paetau A, Hujanen S, Keto J, Somervuo P, Huovinen S, Udd B, Wallgren-Pettersson C, Auvinen P, Hackman P, Pelin K. Expression of multiple nebulin isoforms in human skeletal muscle and brain. Muscle Nerve 2012; 46: 730–7.
79. McElhinny AS, Kolmerer B, Fowler VM, Labeit S, Gregorio CC. The N-terminal end of nebulin interacts with tropomodulin at the pointed ends of the thin filaments. J Biol Chem 2001; 276: 583–92.
80. Wang K, Knipfer M, Huang QQ, van Heerden A, Hsu LC, Gutierrez G, Quian XL, Stedman H. Human skeletal muscle nebulin sequence encodes a blueprint for thin filament architecture. Sequence motifs and affinity profiles of tandem repeats and terminal SH3. J Biol Chem 1996; 271: 4304–14.
81. Castillo A, Nowak R, Littlefield KP, Fowler VM, Littlefield RS. A nebulin ruler does not dictate thin filament lengths. Biophys J 2009; 96: 1856–65.
82. Gokhin DS, Kim NE, Lewis SA, Hoenecke HR, D'Lima DD, Fowler VM. Thin-filament length correlates with fiber type in human skeletal muscle. Am J Physiol Cell Physiol 2012; 302: C555–65.
83. Gallagher PG, Forget BG. An alternate promoter directs expression of a truncated, muscle-specific isoform of the human ankyrin 1 gene. J Biol Chem 1998; 273: 1339–48.
84. Hannappel E. Beta-thymosins. Ann N Y Acad Sci 2007; 1112: 21–37.
85. Bernstein BW, Bamburg JR. ADF/cofilin: a functional node in cell biology. Trends Cell Biol 2010; 20: 187–95.
86. Rho SB, Chun T, Lee S-H, Park K, Lee J-H. The interaction between E-tropomodulin and thymosin β-10 rescues tumor cells from thymosin β-10 mediated apoptosis by restoring actin architecture. FEBS Letters 2004; 557: 57–63.
87. Yamashiro S, Cox EA, Baillie DL, Hardin JD, Ono S. Sarcomeric actin organization is synergistically promoted by tropomodulin, ADF/cofilin, AIP1 and profilin in C. elegans. J Cell Sci 2008; 121: 3867–77.
88. Bryce NS, Schevzov G, Ferguson V, Percival JM, Lin JJ, Matsumura F, Bamburg JR, Jeffrey PL, Hardeman EC, Gunning P, Weinberger RP. Specification of actin filament function and molecular composition by tropomyosin isoforms. Mol Biol Cell 2003; 14: 1002–16.
89. Behrmann E, Muller M, Penczek PA, Mannherz HG, Manstein DJ, Raunser S. Structure of the rigor actin-tropomyosin-myosin complex. Cell 2012; 150: 327–38.
90. 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.
91. Spiering D, Hodgson L. Dynamics of the Rho-family small GTPases in actin regulation and motility. Cell Adh Migr 2011; 5: 170–80.
92. Wong JS, Iorns E, Rheault MN, Ward TM, Rashmi P, Weber U, Lippman ME, Faul C, Mlodzik M, Mundel P. Rescue of tropomyosin deficiency in Drosophila and human cancer cells by synaptopodin reveals a role of tropomyosin alpha in RhoA stabilization. EMBO J 2012; 31: 1028–40.
93. Perrin BJ, Ervasti JM. The actin gene family: function follows isoform. Cytoskeleton (Hoboken) 2010; 67: 630–4.
94. Vlahovich N, Kee AJ, Van der Poel C, Kettle E, Hernandez-Deviez D, Lucas C, Lynch GS, Parton RG, Gunning PW, Hardeman EC. Cytoskeletal tropomyosin Tm5NM1 is required for normal excitation-contraction coupling in skeletal muscle. Mol Biol Cell 2009; 20: 400–9.
95. Schevzov G, Bryce NS, Almonte-Baldonado R, Joya J, Lin JJ, Hardeman E, Weinberger R, Gunning P. Specific features of neuronal size and shape are regulated by tropomyosin isoforms. Mol Biol Cell 2005; 16: 3425–37.
96. Fath T, Agnes Chan YK, Vrhovski B, Clarke H, Curthoys N, Hook J, Lemckert F, Schevzov G, Tam P, Watson CM, Khoo PL, Gunning P. New aspects of tropomyosin-regulated neuritogenesis revealed by the deletion of Tm5NM1 and 2. Eur J Cell Biol 2010; 89: 489–98.
97. Schevzov G, Fath T, Vrhovski B, Vlahovich N, Rajan S, Hook J, Joya JE, Lemckert F, Puttur F, Lin JJ, Hardeman EC, Wieczorek DF, O'Neill GM, Gunning PW. Divergent regulation of the sarcomere and the cytoskeleton. J Biol Chem 2008; 283: 275–83.
98. Had L, Faivre-Sarrailh C, Legrand C, Mery J, Brugidou J, Rabie A. Tropomyosin isoforms in rat neurons: the different developmental profiles and distributions of TM-4 and TMBr-3 are consistent with different functions. J Cell Sci 1994; 107(Pt 10): 2961–73.
99. Fifkova E, Delay RJ. Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. J Cell Biol 1982; 95: 345–50.
100. Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GC, Kasai H. The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 2008; 57: 719–29.
101. Landis DM, Reese TS. Cytoplasmic organization in cerebellar dendritic spines. J Cell Biol 1983; 97: 1169–78.
102. Sussman MA, Bilak M, Kedes L, Engel WK, Askanas V. Tropomodulin is highly concentrated at the postsynaptic domain of human and rat neuromuscular junctions. Exp Cell Res 1993; 209: 388–91.
103. Guven K, Gunning P, Fath T. TPM3 and TPM4 gene products segregate to the postsynaptic region of central nervous system synapses. Bioarchitecture 2011; 1: 284–9.

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