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Tropomyosin isoforms: divining rods for actin cytoskeleton function

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Actin filament functional diversity is paralleled by variation in the composition of isoforms of tropomyosin in these filaments. Although the role of tropomyosin is well understood in skeletal muscle, where it regulates the actin–myosin interaction, its role in the cytoskeleton has been obscure. The intracellular sorting of tropomyosin isoforms indicated a role in spatial specialization of actin filament function. Genetic manipulation and protein chemistry studies have confirmed that these isoforms are functionally distinct. Tropomyosins differ in their recruitment of myosin motors and their interaction with actin filament regulators such as ADF-cofilin. Tropomyosin isoforms have therefore provided a powerful mechanism to diversify actin filament function in different intracellular compartments.

Introduction

The actin filament system is required to perform a remarkable array of functions in eukaryotic cells, including cytokinesis, cell motility, contractile force, intracellular transport, cell morphology and cell size. Indeed, it is hard to find a cytoplasmic cellular process that does not involve or is not impacted by the actin cytoskeleton. This therefore begs the question of how a single filament system, however exquisitely regulated, can independently perform such an array of functions. It is becoming increasingly clear that actin filaments are not a homogeneous system but rather consist of compositionally distinct filaments arising from the use of isoforms of both actin and tropomyosin (Tm), which are temporally and spatially regulated in most cellular systems. The contribution of actin isoforms has been reviewed elsewhere [1–3], and this review will focus on the role of Tm isoforms in the generation of actin filament functional diversity.

The actin filament consists of a two-stranded helical polymer of actin. Most, but not all, actin filaments contain a Tm polymer running along the major groove in the actin filament. In contrast to the globular structure of actin [4], Tm exists as a rod-shaped coiled-coil dimer that forms a head-to-tail polymer along the length of an actin filament [5,6]. The dimers can be hetero- or homo-dimers. Actin filaments that lack Tm tend to be rapidly treadmilling pools of filaments such as those associated with neuronal growth cone filopodia and the leading edge of mammary adenocarcinoma cells [7–9]. This correlates well with the role of tropomyosin in regulating many properties of actin, including stabilizing actin filaments [10]. The role of tropomyosin in skeletal muscle is well defined, where it plays a central role in regulating muscle contraction [11]. In the relaxed state, Tm obscures the myosin-binding site on actin and the myosin is disengaged from the actin filament. Upon neuronal stimulation of muscle and release of intracellular calcium, the troponin complex (which is bound to Tm) binds to calcium. This leads to lateral movement of Tm within the major groove of the actin filament, uncovering the myosin-binding site of actin that is required for engagement of the myosin head with actin [12,13]. The interaction of myosin heads with the actin filament leads to sliding of the myosin filament with respect to actin and muscle contraction. By contrast, the role of Tm in non-muscle cells is poorly understood.

Generation of Tm diversity by alternative splicing

Mammalian and avian tropomyosins are encoded by four genes α, β, γ and δ (Figure 1) [14]. Historically, the tropomyosins have been divided between two classes, high-molecular-weight (HMW) and low-molecular-weight (LMW), which are ~284 aa and 247 aa in length, respectively. This size difference is generated by the use of alternative promoters, which lead to the inclusion of exons 1a and 2a or 2b (for HMW Tm) or exon 1b (for LMW Tm) at the N-terminus of the protein (Figure 1) [14,15].

In addition to the use of alternative promoters, the genes encoding Tm isoforms also utilize a mutually exclusive alternative internal splice of exons 6a versus 6b and alternative carboxyl termini 9a and 9d in the β-Tm gene, 9a, 9c and 9d in the γ-Tm gene and 9a, 9b, 9c and 9d in the α-Tm gene to generate over 40 isoforms (Figure 1). Alternative splicing is seen with both HMW and LMW isoforms from the α-gene but has thus far only been seen with LMW isoforms from the γ-gene. There are also RNA isoforms where the protein sequence is identical but the 3′ UTR is derived from different exons such as 9a9b versus 9a9d in the γ-Tm gene (Figure 1). In this case, the splice of 9a to 9d creates a frame-shift in 9d and the whole exon now functions as a 3′ UTR [16] (Figure 1).

The alternative exon choices within a gene display much greater sequence divergence than do the corresponding exons between genes [14]. Thus, the N-terminal

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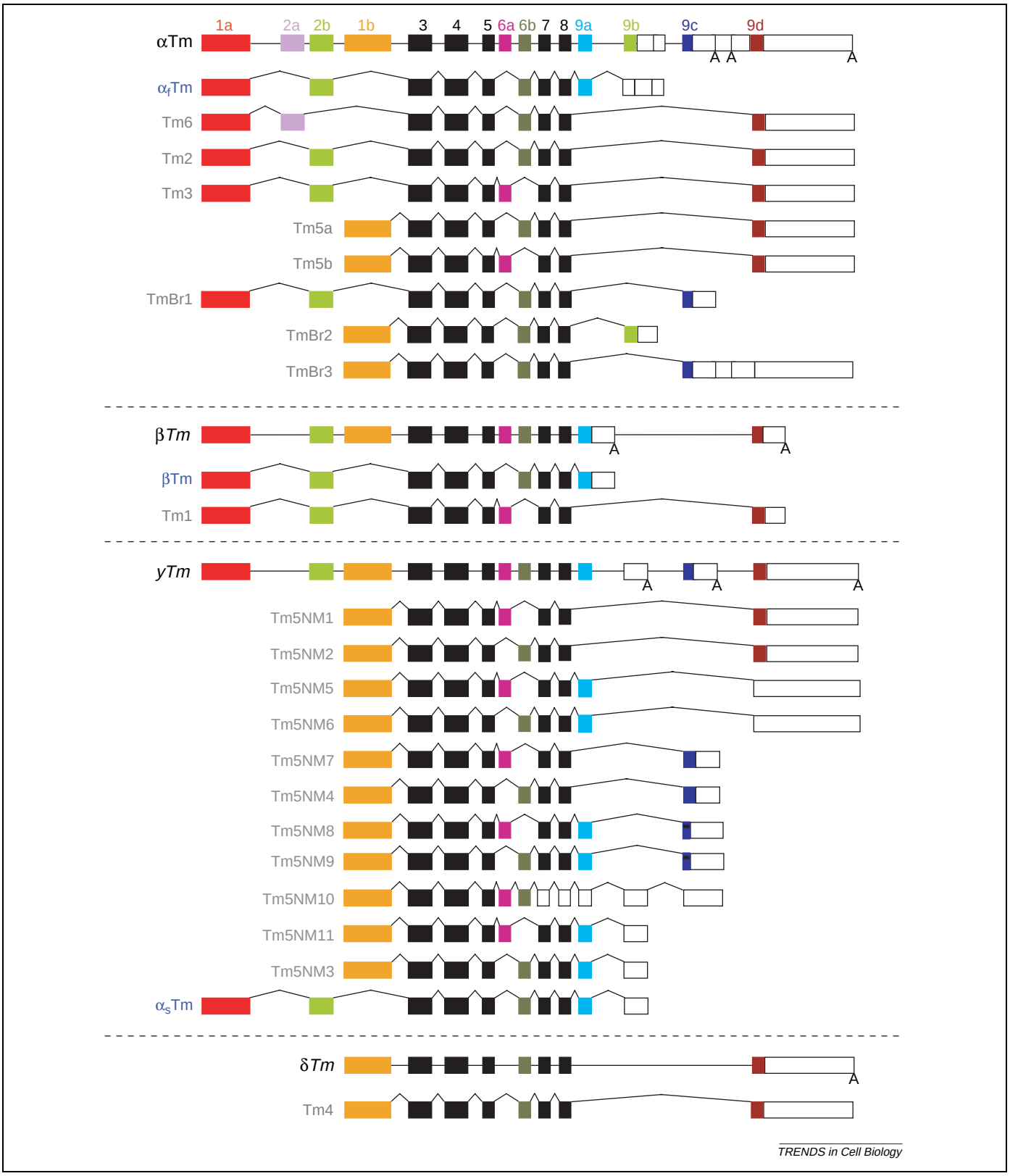

Figure 1. Tropomyosin (Tm) isoform diversity is generated by the use of four genes (α,β,γ,δ) and alternative splicing within at least three genes. Alternative splicing generates multiple products through the use of alternative promoters, resulting in different amino termini, mutually exclusive internal splicing of 6a versus 6b and alternative carboxyl termini. Colour coding is used to indicate that the 1a exon, for example, from the α Tm gene is more similar to the 1a exon from the β Tm and γ Tm genes than it is to the alternative N-terminal 1b exon from the α Tm gene. Not all isoforms generated from these genes are shown, although the existence of those shown has been confirmed by northern blots. In most cases, the isoforms arising from alternative splicing do not contain an exon unique to just one isoform. Rather, the isoforms gain their individuality from a unique combination of exons. This has made it challenging to generate an instructive set of antibodies (see Table 1). 'A' indicates polyadenylation sites, and the altered color size in the 9c exon in Tm5NM8 and Tm5NM9 reflects a frame shift in 9c translation in the 9a–9c splice compared with that in the 8–9c splice. For further details, see [16,21,25].

exons 1a and 1b are very dissimilar within the α- and γ-Tm genes; whereas, the α-, β- and γ- gene 1a exons are very similar, as are the 1b exons from the α- and γ-Tm genes. Thus, it has been comparatively easy to generate antibodies that discriminate between different exon-encoded peptides from the same gene, and these are listed in Table 1.

Tm isoforms are developmentally regulated

Studies using both mRNA and protein analysis have demonstrated widespread regulation of Tm isoform expression during development and between different cell types. All four genes display extensive changes in isoform expression accompanying muscle differentiation [17]. In the brain, development is accompanied by widespread changes in isoform expression, and regional variation in expression is also seen [16,18–20]. For example, the α-isoform α_f-Tm is restricted to cardiac and fast-twitch skeletal muscle, whereas, α_s-Tm is restricted to slow-twitch skeletal muscle [17,21]. By contrast, the α-isoforms TmBr-1 and -3 are restricted to expression in neurons [18,21,22,25]. Most isoforms, nevertheless, are expressed in a range of different cells and tissues, although the pattern of expression differs substantially between different tissues [23,24]. Qualitative and quantitative variation in the Tm content of actin filaments is therefore widespread and has the potential to contribute to specialized actin function in different cell types. Indeed, isoform-specific changes in tropomyosin isoform expression have been commonly associated with cancer, including human primary tumors [26–31]. In particular, reduction of expression of high-molecular-weight tropomyosins is very common in highly malignant cells [29–32] (see below).

Tm isoforms are functionally distinct and perform essential functions

The rescue of transformed cell structure by tropomyosin transfection provided the first evidence that these isoforms are not functionally equivalent. Several studies have demonstrated that the disruption of stress fibre organization that accompanies cell transformation could be reversed by transfection of the HMW isoforms Tm1 and Tm2 [33–36]. Indeed, Boyd *et al.* [33] also demonstrated a striking correlation between loss of tumor-suppressor activity and loss of Tm1. The Helfman laboratory further demonstrated that, although Tm2 and Tm3 could both rescue stress fibre organization in ras-transformed fibroblasts, they were more prominent in the cells expressing Tm2 [37]. As both Tm2 and Tm3 are components of stress fibres, this provided compelling evidence that they do not perform the same role within these structures. In a similar study, the Prasad laboratory demonstrated that Tm1 but not Tm2 could rescue a transformed phenotype [38]. This has led to the proposal that the gene encoding Tm1 is a tumor-suppressor [39]. This ability of Tm1 to rescue the organization of the actin cytoskeleton has been successful with MCF7 breast cancer cells [40] but does not extend to human neuroblastoma cells [41].

Bryce *et al.* [42] used the neuroepithelial cell line B35 to demonstrate functional differences between the isoforms Tm5NM1 and TmBr3. Both isoforms are expressed in neurons, and TmBr3 displaces Tm5NM1 from the axon during maturation [19]. Tm5NM1 increased cell spreading in a dose-dependent manner, promoted stress fibre formation and decreased cell motility. By contrast, TmBr3 induced lamellipodial formation, reduced cell spreading and stress fibre formation and increased cell motility. When TmBr3 was introduced into Tm5NM1 transfectants, it was able to produce an exaggerated TmBr3

Table 1. List of tropomyosin antibodies

Antibody name	Exon specificity	Tm gene	Tm isoform recognition	Refs
TM311	Exon 1a	α, β, γTm	Tm6, 1, 2, 3 α,β, γ muscle Tm	[88]
α/2a	Exon 2a	αTm	sm mus Tm	[24]
anti-rTM9c	Exon9c	αTm	TmBr-1, TmBr-3	[7]
α-9c	Exon9c	αTm	TmBr-1, TmBr-3	[18]
WSα/9c	Exon 9c	αTm	TmBr-1, TmBr-3	[19]
α/9c (Mab)	Exon 9c	αTm	TmBr-3	[20]
α/9d	Exon 9d	αTm	Tm6, 1, 2, 3, 5a, 5b	[8]
Pep3–43	Exon1b	αTm	Tm5a, Tm5b, TmBr-2, TmBr-3	[89]
Anti-TM1	Exon6a	βTm	Tm1	[90]
CG3	Exon1b	γTm	all Tm5NM products	[91]
γ/9a	Exon 9a	γTm	Tm5NM3, 5, 6, 8, 9, 11 α, β, γ muscle Tm	[20]
TC22–4mAb	Exon9c	γTm	Tm5NM4, 7	[92]
γ/9c	Exon 9c	γTm	Tm5NM4, 7	[20]
γ/9d	Exon 9d	γTm	Tm5NM1, 2	[64]
δ-9d	Exon9d	δTm	Tm1, Tm4	[7]
WD4/9d	Exon 9d	δTm	Tm4	[62]
Sarcomeric Tm (CH1)	Exon 9a	α, β, γTm	α, β, γ muscle Tm, does not recognize 9a-containing Tm5NMs	[93]

A large number of antibodies against tropomyosins (Tms) are now available that recognize specific exon-encoded epitopes. Although most of these antibodies recognize multiple isoforms, they have provided sufficient specificity to quantitate isoform expression and visualize isoform sorting. As the specificity of the antibodies has improved, so has the ability to demonstrate differential localization of isoforms. Several of these antibodies, however, do recognize related products from different genes [24]. For example, the TM311 antibody recognizes isoforms that contain the 1a exon from the α-, β- and γ-Tm genes. By contrast, the α/9d antibody only recognizes 9d-containing products from the α- and β- genes, whereas γ/9d only recognizes products from the γ-Tm gene. Most of the antibodies listed in this table are now commercially available. The reference column numbers correspond to the numbered references as used in the main article.

phenotype in which actin cables were recruited into very pronounced lamellipodia. This suggests that Tm isoforms were capable of directing radically different organization of actin filaments in the same cell.

In epithelial cells, the LMW Tms 5a and 5b can regulate the activity of a membrane transporter. Dalby-Payne *et al.* [43] observed that Tms 5a and 5b, but not Tms 6, 2, 3 nor 5NM1–11 were enriched at the apical surface and particularly enriched at sites of accumulation of surface cystic fibrosis transmembrane conductance regulator (CFTR). Antisense-induced reduction of Tms 5a and 5b resulted in increased surface expression of CFTR and increased chloride efflux in response to cAMP stimulation. This suggests that Tms 5a and 5b are associated with a population of actin filaments that regulate the insertion and/or retention of CFTR into the plasma membrane.

The isoform specificity of Tm isoform function has been demonstrated in genetic models from yeast to mammals. Wieczorek and coworkers have used a myosin heavy-chain promoter to drive the expression of β-Tm in mouse heart. This led to compensating downregulation of the cardiac α-Tm isoform in some lines to the point where β-Tm became the predominant isoform [44]. Notably, the total muscle Tm pool remained unchanged, thus resulting in what is effectively an isoform substitution. The resulting hearts display extensive characteristics of dysfunction, including thrombus formation in the lumen of both atria and in the subendocardium of the left ventricle, atrial enlargement and fibrosis and diffuse myocytolysis [45].

Transgenic mice in which different skeletal muscle structures are compromised give rise to very different muscle diseases. Introduction of a transgene construct carrying a human-disease-causing mutation in α-Tm slow reproduces the pathology of nemaline myopathy with remarkable accuracy [46]. The mutant α-Tm slow is incorporated into the thin filaments of the contractile apparatus (Figure 2) and reduces the stability of β-Tm in both mice and humans [47]. By contrast, inappropriate expression of cytoskeletal Tm3 in the Z-line-adjacent cytoskeleton (Figure 2) in transgenic mice results in muscular dystrophy [48]. The Z-line-adjacent cytoskeleton appears to have a role in linking the Z-disc region to costameres and is unlikely to play a role in force production, unlike the thin filament of the contractile apparatus [48]. This suggest that dysfunction in different Tm isoforms might compromise the function of different compartments in muscle fibres and lead to different disease phenotypes.

In the budding yeast *Saccharomyces cerevisiae*, elimination of both Tm genes is lethal, and elevated expression of the *TPM2* gene cannot compensate for loss of the *TPM1* gene [49]. At least one essential Tm function in yeast involves vesicle transport from the Golgi to sites of polarized growth [50]. In *Caenorhabditis elegans*, elimination of the third and fourth isoforms of the tmy-1 gene

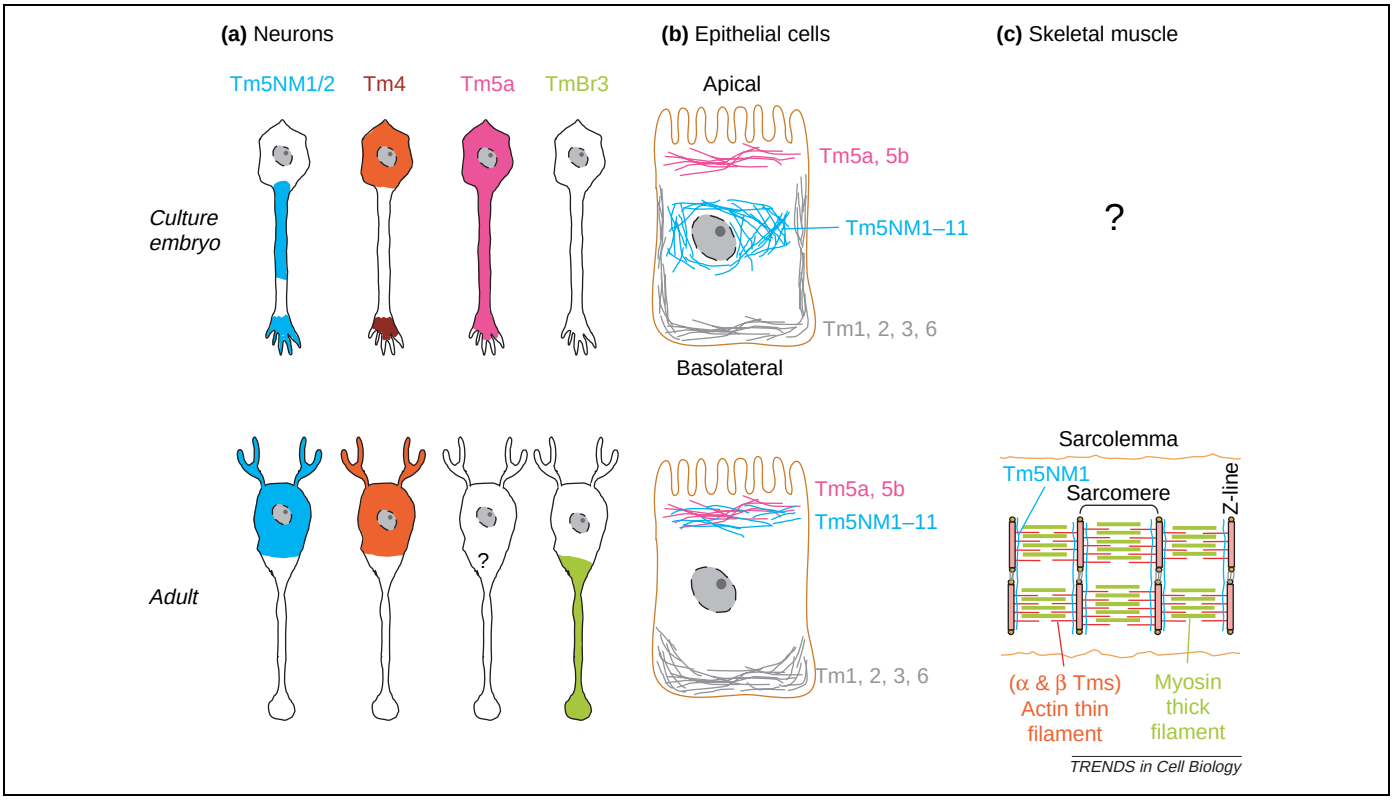

Figure 2. Isoform sorting of tropomyosins (Tms) occurs in different cell types and is developmentally regulated. In embryonic neurons, Tms 5NM1/2, 4 and 5a display quite different sorting patterns, and these undergo profound reorganization in mature neurons [8,19,62]. In particular, Tms 5NM1/2 and 4 are removed from the axonal compartment and replaced by TmBr3. This is evolutionarily conserved between birds and mammals [19]. Similarly, Tm5a/b, Tm5NM1–Tm5NM11 and Tms 1,2,3 and 6 are differentially sorted in epithelial cells in culture and undergo modification in mature epithelium in the gut [43]. Finally, adult skeletal muscle localizes muscle Tm to the thin actin filament of the sarcomere, but Tm5NM1 localizes to a Z-line-adjacent cytoskeleton [48]. Thus, sorting provides an attractive potential mechanism to regulate the structure and function of different actin filament populations in the same cells. The question marks indicate unknown data.

compromise development [51]. Deletion of the only known cytoskeletal Tm genes in *Drosophila melanogaster* results in altered head morphogenesis and altered organization of the striated muscle contractile apparatus [52–54].

Similarly, knockout of only the α-Tm isoform [55] or all α-Tm gene isoforms [56] in mice leads to embryonic lethality at embryonic day 9.5 to 13.5 or day 8.5 to 11.5, respectively. This suggests that one or more cytoskeletal isoforms are required at the earlier time for normal development, and the striated muscle isoform is required shortly after.

The most extreme case of essential Tm gene function has been seen with the mouse γ-Tm gene. Knockout of all cytoskeletal products from this gene eliminates both preimplantation embryo development and embryonic stem cell viability [57]. This occurs despite the activity of three other coexpressed Tm genes. Knockout of the C-terminal 9c alternative exon of the γ-Tm gene leads to compensating use of the alternative 9a exon [58]. This suggests that some degree of functional redundancy might exist within the gene.

Tropomyosin isoforms sort to specific compartments

Tropomyosins display isoform-specific accumulation at a wide range of different intracellular sites. Lin *et al.* [59] were the first to report that isoforms from the γ-Tm gene were enriched at the cell periphery relative to isoforms from the α-Tm gene. Subsequent work has demonstrated that differential localization arises from isoform sorting. Drug studies have demonstrated that the sorting of isoforms to different sites is a dynamic process [8]. In neurons, the exclusion of Tm5NM2 from the growth cone is eliminated by cytochalasin D but rapidly restored upon washout of the drug [8].

The most thoroughly characterized system for sorting is the nervous system [60] and is summarized in Figure 2. Initial axon outgrowth is associated with the sorting of specific isoforms to the growth cone and to the axon shaft [7,8,19,61,62]. With neuronal maturation, additional isoforms are expressed, some isoforms are relocated and the Tm composition of the presynaptic terminal, the axon, the soma and the dendrite are all distinct in the adult brain. More recently, this has been extended to other cell types.

Fibroblasts segregate γ-Tm from α- and β-Tm products virtually completely in early G1 phase cells [63]. As cells approach the restriction point in G1, there is a progressive alignment of products from the α, β and γ genes into parallel stress fibres [63]. However, the γ-Tm isoform Tm5NM-2 does not enter stress-fibres and remains with short Golgi-vesicle-associated actin filaments [64,65]. One or more isoforms carrying the 9a exon from the γ-Tm gene is also associated with a perinuclear compartment and is excluded from stress fibres [24]. Finally, the LMW Tm 5a/b from the α-Tm gene but not HMW tropomyosins from the α- and β-Tm genes are located in stress fibres at the cell periphery and in ruffling membranes [24].

In epithelial cells in culture, three discrete zones of sorting are detected. The α-isoforms Tm5a and 5b are localized to the apical surface, whereas, Tm-2 and -3 are associated with the basolateral membrane, and γ-Tm gene products are located in the cytoplasm [43]. A similar distribution is seen in epithelial cells lining the gut, although the γ-Tm gene products become more apical [43]. This is summarized in Figure 2.

Finally, in skeletal muscle, the exon-9a-containing muscle tropomyosins are located in the thin filaments of the contractile apparatus, whereas one known and one novel cytoskeletal Tm from the γ-Tm gene are located external to the sarcomere but adjacent to the Z-line [48] (Figure 2). This correlates with the existence of a γ-actin-based cytoskeleton aligned with the Z-line. It therefore appears that the sorting of Tm isoforms to different intracellular compartments is widespread among different cell types.

Colocation of Tm isoforms in the same compartment might not necessarily mean that they are associated with the same actin filament. Treatment of neurons and fibroblasts with cytochalasin D or nocodazole results in differential relocation of isoforms that were initially colocated in growth cones and stress fibres, respectively [8,63]. While it is possible that different colocated tropomyosins are present together as heteropolymers in the same individual filaments, the results are more easily reconciled with the different isoforms being present as homopolymers in separate, but closely adjacent, actin filaments. In the latter case, the results would be consistent with different Tm-containing actin filaments having different drug sensitivities.

Sorting mechanism and molecular sinks

Isoform sorting is highly regulated but displays geographical flexibility. The location of an isoform in a particular cell type can change during differentiation. Tm5NM1/2 and Tm4 undergo a complete change in targeting during neuronal development (Figure 2) [7,19,62], and Tm5a/b becomes excluded from the growth cone [8]. Sorting information is not conserved in the same exon across the Tm genes. For example, 9c-containing isoforms from the α- and γ-Tm genes sort differently in neurons [19,20], and 9d-containing isoforms from the α- and γ-Tm genes sort differently in early G1 fibroblasts [63]. However, alternative exon choice appears capable of directing alternative sorting. For example, Tm5b and Tm3 that differ only in their N-terminus sort to apical and basolateral compartments, respectively, in epithelial cells [43]. Tm5NM1 and Tm5NM2, which differ only in their use of exons 6a versus 6b, sort to stress fibres and the Golgi, respectively, in fibroblasts [64]. γ-Tm gene isoforms containing the 9c carboxy terminus are found in axons and dendrites, whereas γ-Tm isoforms with a 9d carboxy terminus are excluded from axons [20].

Isoforms do not appear to have an intrinsic sorting signal that specifies a single geographical destination. Higher-order structures most likely play a pivotal role in sorting. Treatment with cytochalasin D is able to rapidly disperse sorted isoforms in neurons [8] and epithelial cells [43], and drug washout restores isoform segregation [8]. These observations are therefore more compatible with a molecular sink model in which isoforms accumulate in structures that have the greatest affinity for that isoform. This would predict that the sites of accumulation of an isoform could change at multiple sites simultaneously and

that fragmentation of microfilament structure would rapidly alter intracellular location because Tm affinity would be changed for the fragmented filament.

A molecular sink model also predicts that actin filament dynamics and the presence of other actin-binding proteins at a specific location might favor accumulation of a specific Tm at that site. For example, Tm5NM1 might accumulate where actin filaments are more stable and myosin II motors are present, whereas TmBr3 might accumulate where filaments are more dynamic and ADF/cofilin is present [42]. Conversely, areas of high filament dynamics and branching might be incompatible with accumulation of any Tm [9].

Multiple mechanisms contribute to differential isoform function

The ability of cells to sort Tm isoforms provides several potential opportunities to regulate actin filament function. At a simple level, sorting provides a mechanism to independently regulate the tropomyosin pool available for microfilament formation at different intracellular sites. To be effective, this would require that the synthesis of tropomyosin be limiting for accumulation of Tm-containing microfilaments. Studies in cell culture suggest that, unlike actin, which appears to regulate its output through a feedback loop [66,67] to maintain a constant pool size, overexpression of Tm usually results in elevated accumulation of tropomyosin [33,37,42]. Although this requires validation in a whole animal model, it suggests that Tm synthesis is limiting for the accumulation of Tm-containing actin filaments.

It is at the level of differentially regulating actin filament function, however, that Tm isoforms are likely to have their greatest impact. *In vitro* analysis of Tm protein interaction with actin and actin-binding proteins already suggests considerable functional differences between isoforms. Early studies indicated that HMW tropomyosins bind with greater avidity than LMW tropomyosins to actin filaments [68]; however, the tightest binding Tm is in fact the LMW Tm5b [69]. Comparative studies suggest that both the amino and carboxy termini can influence the strength of isoform binding [70]. It is predicted that greater avidity would lead to longer, more stable actin filaments.

A further complication lies in the finding that tropomyosins can form both homo- and hetero-dimers, and dimer preference will also influence binding affinity [71]. Corbett *et al.* [47] showed that a mutation in α-Tm slow results in a change from heterodimer to homodimer preference in the muscle tropomyosins, potentially destabilizing the thin filament in a form of nemaline myopathy. Perhaps the greatest influence on tropomyosin binding to actin is tropomodulin. Tropomodulin is encoded by a multigene family and simultaneously binds to both the pointed end of actin filaments and to Tm. This stabilizes the actin–Tm interaction [72]. Tropomyosins differ in their binding affinity to tropomodulin, which is turn is likely to impact on the stability of actin filaments containing different tropomyosins [73]. Overexpression studies reveal that the correct stoichiometry of tropomodulin and tropomyosin is required for normal cardiac function [44,74,75], and knockout of cardiac tropomodulin causes failure of cardiac development [76].

Recent data have pointed to very specific differences between Tm isoforms that account at least in part for their differential impact on actin filament function [42] (Figure 3). Tropomyosins can regulate many properties of actin filaments. They increase filament stiffness [77], protect filaments from the depolymerizing effects of ADF/cofilin and gelsolin [78–80] and influence myosin mechanochemistry [81]. Fanning *et al.* [81] demonstrated that the ATPase activities of myosin I and myosin II motors are differentially regulated by the Tm isoform composition of actin filaments. Other studies showed that the Tm5NM1 isoform is able to promote isoform-specific recruitment of a myosin II motor to stress fibres containing this Tm [42]. This is observed both in cell culture and in the cerebral cortex of transgenic mice. In addition, the level of active myosin II is elevated in the cell presumably as a result of increased access of myosin II to actin filaments [42]. By contrast, a Tm that induces lamellipodia, TmBr3, leads to a reduction in active myosin II levels [42]. This is compatible with the observation that Tm isoforms can differentially regulate myosin mechanochemistry in a cell-free system [81] and suggests a possible mechanism to explain the effects of Tm5NM1 and TmBr3 on myosin location and activity. The tropomyosins might be capable of directly regulating myosin access to actin filaments and consequently myosin activity in an isoform-specific manner. Myosins would therefore accumulate where they have their highest affinity interactions with Tm-containing actin filaments. One potential explanation might be that Tm5NM1 and TmBr3 occupy different sites in the major groove of the actin filament, which results in differential access for a myosin motor [83].

Finally, Tm isoforms can regulate ADF-cofilin activity and location in an isoform-specific manner [42]. Tm5NM1 promotes inactivation of ADF-cofilin by phosphorylation and leads to displacement of ADF-cofilin from the cell periphery. By contrast, TmBr3 promotes the association of ADF-cofilin with actin filaments. Immunoprecipitation experiments suggest that ADF-cofilin binds to TmBr3-containing, but not Tm5NM1-containing, actin filaments both in cell culture and whole brain [42]. As ADF-cofilin binding to actin filaments changes the twist of the filament, it is possible that this might also influence Tm binding in an isoform-specific manner [84]. Similarly, Ashworth *et al.* [82] found that ischemia-induced dissociation of Tm from the terminal web actin filaments of renal proximal tubule cells correlates with release of ADF-cofilin into the microvilli and filament destruction.

In conclusion, these studies suggest a reinforcement model of tropomyosin action in which multiple mechanisms promote a common outcome. On the one hand, tropomyosin isoforms can specify motor protein interaction and, on the other, can regulate the activity/interaction with actin-severing proteins. Thus, the impact of Tm5NM1 is to promote filament stability by reducing severing/turnover and driving myosin motor interaction, whereas TmBr3 does the converse (Figure 3). As proposed by Fisher and Fowler [72], this might extend to differences in tropomodulin recruitment, which would further

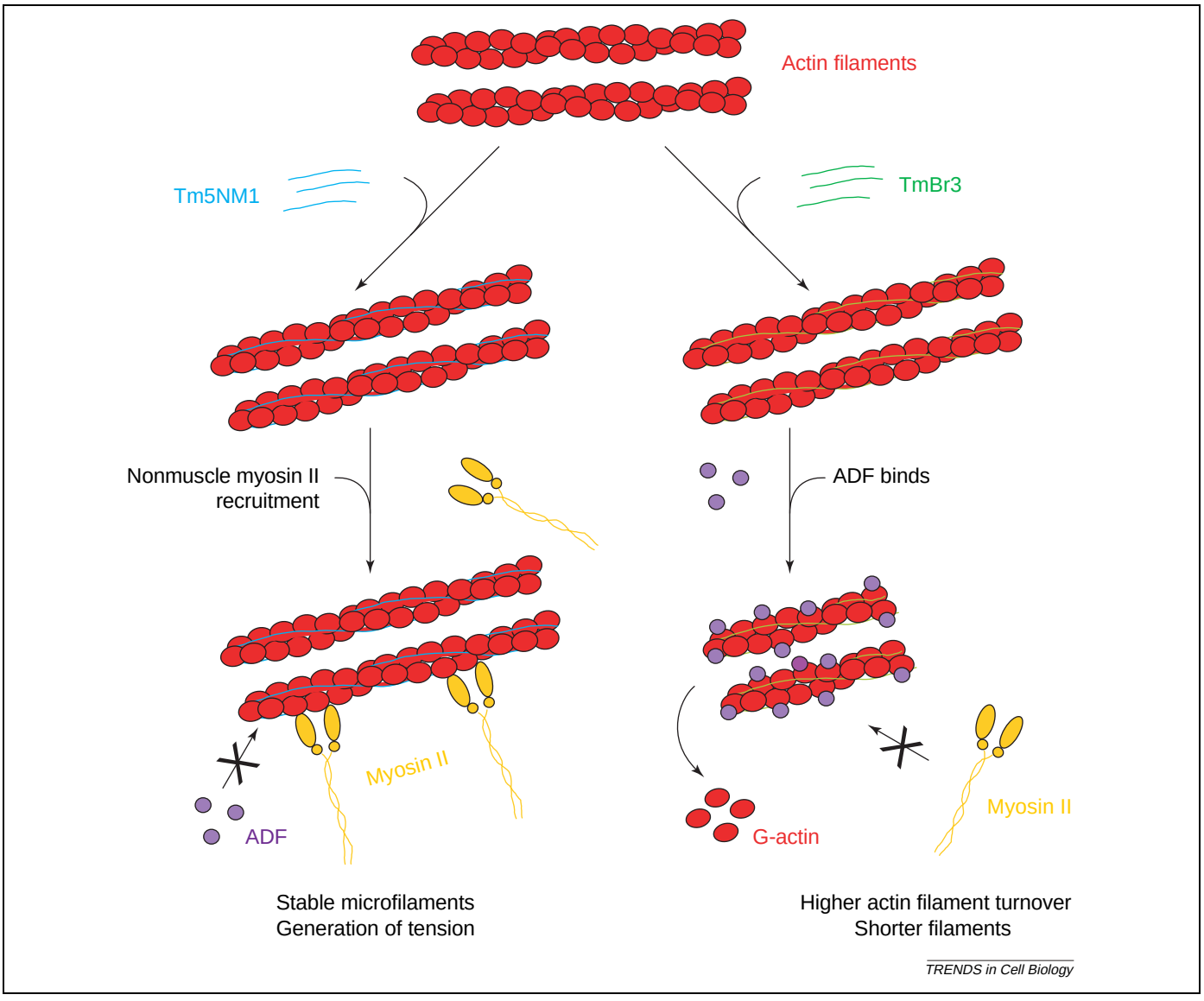

Figure 3. A reinforcement model for tropomyosin-directed regulation of actin filament function. When Tm5NM1 binds to actin filaments, they become permissive for myosin II interaction, and this can lead to myosin II recruitment to these filaments. Simultaneously, Tm5NM1 eliminates ADF binding to the filaments, which results in more-stable filaments engaged in contractile activity. By contrast, binding of TmBr3 is permissive for ADF binding, which promotes severing of the filaments and greater turnover. In parallel, myosin II interacts poorly, if at all, and the result is noncontractile, rapidly remodeling filaments. In both cases, multiple mechanisms reinforce the final outcome.

reinforce filament stability. Tropomyosins are therefore remarkably well positioned, both in space and along the microfilament, to define the dynamic and mechanical properties of actin filaments related to specific functional requirements at specific intracellular sites. From an evolutionary point of view, this is a remarkably economical strategy for promoting the spatial diversification of actin filament function. Perhaps not surprisingly, isoform sorting has been observed in a broad range of protein families [85]. Since isoform generation is now recognized as a major contributor to the generation of biodiversity [86], it appears that spatial specialisation of protein function based on isoform sorting may have played a significant role in this process [87].

Concluding remarks

Tropomyosin isoforms are generated from four distinct genes by alternative splicing. The more than 40 isoforms are both quantitatively and qualitatively regulated in development between different cell types and in disease states such as cancer. The tropomyosins are functionally distinct and perform at least some essential functions. Extensive intracellular isoform sorting results in spatially distinct populations of actin filaments based on tropomyosin composition. Isoform sorting therefore provides a mechanism to both spatially control the pool size of specific tropomyosin-containing actin filaments and to confer functional differences between the different filament populations. The functional differences arise from the ability of tropomyosins to regulate myosin motor and ADF/cofilin interactions with actin filaments in an isoform-specific manner.

Future work will define additional mechanisms of tropomyosin isoform-specific function and the principles underlying sorting of isoforms. Antibodies and tagged tropomyosins will reveal additional compartments in different cell types, and the use of these tools will allow systematic analysis of discrete actin filament populations.

Genetic manipulation of tropomyosins will provide an opportunity to dissect the function of these specific actin filaments. Such studies will address the role of tropomyosin in regulating the diversity of function of the actin cytoskeleton and how this integrates with signaling systems that regulate actin filament organization and dynamics. Finally, the development of drugs that target specific tropomyosin isoforms might provide an opportunity to target clinically relevant populations of actin filaments in a less-toxic manner than achieved with drugs interfering directly with actin.

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