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COMMENTARY

Tropomyosin – master regulator of actin filament function in the cytoskeleton

Peter W. Gunning[1,*], Edna C. Hardeman[1,*,‡], Pekka Lappalainen[2,*] and Daniel P. Mulvihill[3,*]

ABSTRACT

Tropomyosin (Tpm) isoforms are the master regulators of the functions of individual actin filaments in fungi and metazoans. Tpms are coiled-coil parallel dimers that form a head-to-tail polymer along the length of actin filaments. Yeast only has two Tpm isoforms, whereas mammals have over 40. Each cytoskeletal actin filament contains a homopolymer of Tpm homodimers, resulting in a filament of uniform Tpm composition along its length. Evidence for this 'master regulator' role is based on four core sets of observation. First, spatially and functionally distinct actin filaments contain different Tpm isoforms, and recent data suggest that members of the formin family of actin filament nucleators can specify which Tpm isoform is added to the growing actin filament. Second, Tpms regulate whole-organism physiology in terms of morphogenesis, cell proliferation, vesicle trafficking, biomechanics, glucose metabolism and organ size in an isoform-specific manner. Third, Tpms achieve these functional outputs by regulating the interaction of actin filaments with myosin motors and actin-binding proteins in an isoform-specific manner. Last, the assembly of complex structures, such as stress fibers and podosomes involves the collaboration of multiple types of actin filament specified by their Tpm composition. This allows the cell to specify actin filament function in time and space by simply specifying their Tpm isoform composition.

KEY WORDS: Actin cytoskeleton, Isoforms, Tropomyosin

Introduction

Tropomyosin (Tpm) is best known for its role in the regulation of contraction of skeletal muscle and the heart. The contraction of these striated muscles involves the synchronized movement of myosin heads that are engaged with actin filaments to produce a net translocation of the myosin thick filament with respect to the actin thin filament (Geeves, 2012; Lehrer and Geeves, 2014). The actin thin filament is composed of three core elements: a double-stranded polymer of actin, two continuous polymers of Tpm running along each side of the actin and the troponin complex, a heteromeric protein complex consisting of troponin T (TnT), troponin I (TnI) and troponin C (TnC), which is located on each Tpm dimer (Lehman and Craig, 2008). In response to a pulse of Ca^{2+} the troponin complex moves the position of the Tpm polymer to facilitate the coordinated engagement of the heads of the myosins in the thick filament with actins in the thin filament.

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The discovery of Tpm in mammalian non-muscle cells in the mid-1970s by Elias Lazarides, naturally, led to the expectation that Tpm has a similar function in these cells compared with its role in skeletal muscle cells (Lazarides, 1975). Immunofluorescence experiments led Lazarides to propose the existence of two populations of actin polymers in non-muscle cells, those that contained Tpm and those that did not (Lazarides, 1976). In hindsight, it is difficult to understand how the discussion of the potential significance of Tpm as a core component of the actin filament in the non-muscle cytoskeleton slowly disappeared from the literature. There are several simple explanations that are likely to account for this. First, Tpms are difficult to work with in protein chemistry experiments because, in solution, they are largely unstructured and have a tendency to oligomerize and/or aggregate. Furthermore, Tpm dimers bind F-actin only with very low affinity (K_d~10^{-3} M) (Wegner, 1979) and their efficient incorporation into an actin filament requires the formation of a head-to-tail Tpm polymer, consisting of homopolymers of Tpm dimers that run along the actin filament (Tobacman, 2008). Finally, Tpms only interact with actin through ionic interactions and, in essence, 'float' above the surface of the actin polymer, making them unlike most other actin-binding proteins (von der Ecken et al., 2015).

The significance of Tpm has been brought into focus by a recent analysis, which showed that evolution has selected for increasing diversity of actin filament composition and that, in the case of fungi and metazoans, Tpms have provided this diversity (Gunning et al., 2015). This Commentary examines the functional consequences of using Tpms to diversify the actin filament composition in the cytoskeleton of fungi and metazoans, and will make the case that Tpms are the master regulators of cytoskeletal actin filament function in these organisms. We focus on four main issues: (i) the mechanism of assembly of actin filaments that contain homopolymers of specific Tpm isoforms, (ii) the physiological function of specific populations of actin filaments that contain different Tpms, (iii) the mechanism by which different Tpms direct different functional outcomes and (iv) how different Tpm-containing filaments contribute to the assembly of large-scale actin-based structures. Where mammalian Tpms are specified in the text, we will use the new nomenclature (see Geeves et al., 2015; supplementary Table 1).

Assembly of specific Tpms into actin filaments

Most fungi and metazoan cells have the capacity to express multiple isoforms of Tpm, resulting from either different gene products or different post-translational modifications. The biophysical properties of each Tpm filament and the specific way in which they interact with actin can differ significantly. This cooperative interaction with the actin polymer is crucial for Tpm function because it regulates interactions with other actin-binding proteins (e.g. myosins and cofilin) (Bryce et al., 2003), as well as the biophysical and/or dynamic properties of the actin filament. Different Tpms are, therefore, able to impart distinct physical properties to different actin filaments and, thereby, dictate their function. As many cell types can express multiple

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Tpm isoforms, it is crucial for the viability, function and mobility of a cell to recruit the appropriate Tpm to an actin polymer at the correct place and time (Bach et al., 2009). Indeed, we know that different Tpms are sorted to different actin filament populations in many systems and cell types, but how this is brought about is not yet fully understood (Vindin and Gunning, 2013).

The weak binding of Tpm dimers to actin can only result in a continuous polymer of Tpm along an actin filament through head-to-tail contacts between individual Tpm dimers (Tobacman, 2008). The Hitchcock-DeGregori group has long supported a model whereby the actin–Tpm interaction is promoted by discrete actin-binding domains that are present in the Tpm molecule (Singh and Hitchcock-DeGregori, 2006), thus restraining the structural conformation of the actin–Tpm complex. In contrast, others support models where the complementary shape and charges at the interface between the coiled-coiled-coil (polymers of the coiled-coil dimer have an intrinsic coiled shape) Tpm polymer and actin filament result in the Tpm dimer associating with actin with sub-micromolar affinity, yet still allowing movement across its surface to regulate interactions with other actin-binding proteins (Holmes and Lehman, 2008). However, the precise nature of the actin–Tpm interaction is likely to remain the subject of controversy for some time to come.

The situation is complicated further by the fact that the relatively abundant Tpm isoforms that are expressed in a cell can each have different affinities for actin. Affinity for actin is, therefore, probably not the only factor that determines the association of a specific Tpm with actin because it would be very unlikely that weak binding and/or non-abundant Tpms are found to interact with actin *in vivo*. The situation is clearly illustrated in metazoan cells that can express more than eight different Tpm isoforms, many with comparable high affinities for actin (Schevzov et al., 2011). Therefore, the cell is faced with a problem because the highly abundant isoforms with high affinities would bind to all of the actin polymers in an unregulated manner (thus precluding models in which actin-binding sites on the Tpm molecule initiate the actin–Tpm association in a cell that expresses multiple Tpms) and because Tpm levels are limiting compared with those of actin polymers – as shown for yeast and a number of mammalian cell types (McMichael and Lee, 2008; Marguerat et al., 2012; Schevzov et al., 2008; Kee et al., 2015).

A mechanism must, therefore, exist to ensure that highly specific actin–Tpm polymers can be formed in a manner that can be flexibly regulated within time and space. Such a mechanism, of ensuring that the correct Tpm is recruited to the correct actin filament, is likely to vary between cell types, depending on the dynamics of the actin cytoskeleton and the needs of the cell. For example, within a sarcomere, the actin polymer is stable and does not continually grow and shrink as is the case in a growing cell. Lehman and Holmes have proposed a so-called 'Gestalt model' (Holmes and Lehman, 2008), where the most highly abundant and stable Tpm dimer within the muscle cell (usually an αβ heterodimer) associates randomly along the actin filament and grows into Tpm polymers from these seed points. Although this implies a certain spatial synchronicity along an actin–Tpm polymer, this model allows the cell to exchange the Tpm on the stable actin polymers throughout its life. However, this model does not take into account the dynamic situation within growing or migrating cells, where individual actin polymers undergo regular cycles of growth and shrinkage. It is clear that, in these cells, a mechanism must exist to regulate which Tpm binds to the growing actin polymer in order to define Tpm specificity, thereby modulating the functional properties of the actin filament.

A number of models can explain the mechanism for defining Tpm specificity. Although isoform-specific transport to different intracellular locations is one possibility, studies in which cytoskeletal drugs were used have comprehensively ruled this out (Schevzov et al., 1997). It was found that isoform sorting depended on the maintenance of polymeric structures, and that destruction of these structures in response to actin-polymerization- and microtubule-inhibiting drugs dispersed sorted Tpm isoforms throughout the cell (Gunning et al., 1998). Instead, the spatial specificity of Tpms appears to be controlled through localized filament assembly (Schevzov et al., 1997). Similarly, a more recent study ruled out a Tpm transport mechanism that depends upon 'sorting signals' and demonstrated that sorting only functions in the context of actin–Tpm polymer formation, consistent with the notion that a Tpm isoform is being held in place through assembly into isoform-specific polymeric structures (Martin et al., 2010).

There has been growing evidence that actin organizes into polymers of different conformation (Egelman and Orlova, 1995), not only owing to differences in the actin isoforms and the discrete nucleotide-binding states but also through different co-factor interactions (e.g. Galkin et al., 2010). Subtle differences in the shape of an actin polymer can affect the affinity of different actin-binding proteins including, probably, specific Tpm isoforms to ensure the same Tpm isoform associates along the length of a given actin filament. By contrast, the interaction between actin and a specific Tpm is necessary to perpetuate specific structural constraints along the entire length of the actin–Tpm polymer. However this does not address the question of what molecule or event initiates the interaction between a specific Tpm and actin and, thus, determines the functional properties of the actin–Tpm polymer. One class of candidate molecules are actin nucleators (e.g. the Arp2/3 complex, formins or spire proteins), which not only have a preference for specific types of actin (e.g. profilin-bound actin isoforms), but also can define the organization of actin filaments (e.g. branched network or straight filaments). In addition, they also affect the structure of individual actin filaments (Bugyi et al., 2006) and control which stabilizing actin-binding proteins interact with the actin filament (e.g. fimbrin) or with Tpm (Tang and Ostap, 2001; Skau and Kovar, 2010).

Together, these ideas led to the development of a model, in which formins orchestrate the Tpm composition of the actin polymer at the time of nucleation (Fig. 1) (Johnson et al., 2014). This model fits well with previous findings (Martin et al., 2010) because it explains how Tpm sorting can occur in the context of actin–Tpm co-polymerization on a growing actin filament. Furthermore, there has been direct *in vivo* evidence from the fission yeast model system (Johnson et al., 2014). This yeast contains a single Tpm (Cdc8), which exists in either an N-terminally acetylated (80% of total Tpm) or non-acetylated state (Johnson et al., 2014), with the acetylated Tpm associating with actin filaments that are incorporated into the cytokinetic actomyosin ring during mitosis and the unacetylated form associating with more-dynamic actin filaments during interphase (Skoumpla et al., 2007; Coulton et al., 2010). Indeed, forcing the mitotic and interphase formins to switch location, led to a corresponding switch in the acetylated state of Tpms within each actin–Tpm polymer, which – in turn – was shown to redirect the location of the myosin motors in the cell (Johnson et al., 2014). This aligns well with the observation that the formin Dia2 may be responsible for recruiting Tpm4.2 in an isoform-specific manner to actin filament arcs in U2OS cells, which, in turn, appears to recruit MyoIIA to these filaments (Tojkander et al., 2011). Thus, formins have been shown to play a key role in determining the Tpm composition and physical properties of an actin polymer.

How the formin defines the Tpm isoform composition of the actin filament is still unresolved (Fig. 1). However, the difference in the

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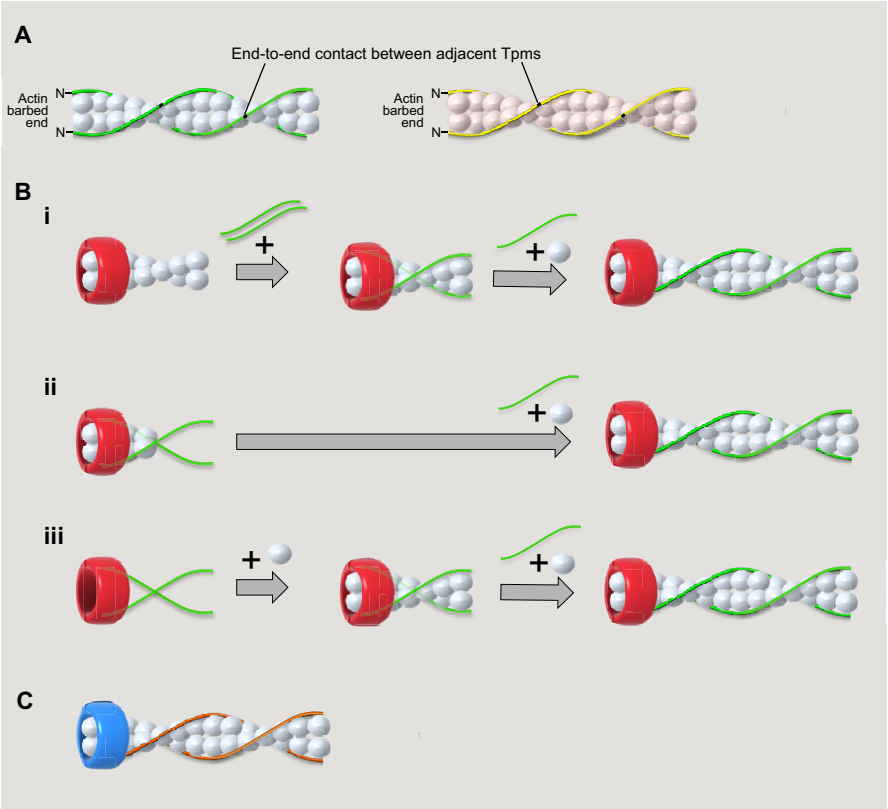

Fig. 1. Models for the formin-directed assembly of actin filaments containing specific Tpm isoforms. (A) In the simplest model that describes the isoform-specific recruitment of Tpms, different actin isoforms (depicted in white and cream) assemble into polymers with different structural conformations. Each then associates with a specific Tpm isoform (depicted in green and yellow). As there is a small number of actin isoforms within a cell (up to three different isoforms depending on the cell type) relative to the Tpms, this mechanism does not explain how all Tpms within one cell are recruited to specific actin structures. (B) In a simple mechanism that has been described previously (Johnson et al., 2014), a formin nucleates the growing actin polymer and then determines the Tpm composition of the actin polymer. The precise temporal order of how the formins, actin and Tpm come together is unclear. We outline three possibilities (i,ii, iii) on how this might happen. (i) Once the formin (red split ring) has initiated the nucleation of an actin polymer of sufficient length, the Tpm isoform (green filament) is recruited through its specific N-terminus to the formin and then integrated onto the formin-actin-polymer structure. (ii) Actin and the specific N-terminus of a Tpm polymer associate directly and simultaneously with a formin. New actin and Tpm subunits are then added in order to polymerize the actin-Tpm cable. (iii) A specific Tpm isoform interacts directly with a specific formin before actin nucleation starts. Each possibility provides a simple mechanism to explain the assembly of specific formin-Tpm-actin-polymer structures, i.e. one type of formin (red) promotes assembly of actin filaments decorated with one Tpm isoform (green). (C) Another type of formin (blue) generates actin polymers stabilized by another Tpm isoform (orange) using the processes described in (B, i-iii). By considering every combination of different actin and formin isoforms within one cell type, the above mechanisms provide sufficient opportunity to recruit different Tpm isoforms to specific actin polymers in order to fine-tune their distinct physical properties, dynamics and functions.

N-terminal acetylation state observed in the study by Johnson et al., 2014 suggests that the N-terminus of the Tpm is key – consistent with the fact that formins are located at the barbed end of actin filaments where they can associate with the N-termini of the Tpm dimer incorporated into the growing actin polymer (Galkin et al., 2011). Consistent with this, many Tpm isoforms that have a sub-micromolar affinity for actin in their non-acetylated state, e.g. the non-muscle tropomyosins Tpm4.2 and Tpm3.1 (also known as Tm4 and Tpm5NM1, respectively) are acetylated at their N-termini *in vivo*, supporting the idea that acetylation also modulates the Tpm N-terminus to promote interactions with a specific actin-binding protein (e.g. formin), or modulates actin-binding in the case of some Tpms, such as budding yeast Tpm1 and Tpm2 or the skeletal muscle Tpms. Such direct interaction between a formin molecule and Tpm is consistent with the variability in the N-termini of most Tpms, which differ significantly with even subtle differences in fission yeast. Furthermore, Tpm itself can regulate the interaction between formins and the growing (i.e. barbed) end of actin (Ujfalusi et al., 2012; Wawro et al., 2007).

As well as interacting with the barbed end of actin, formins can also associate with the lateral surfaces of the actin filament (Bugyi et al., 2006; Martin and Chang, 2006). Therefore, as well as interacting directly with a preferred Tpm, the formin can directly modify the pitch of the actin filament, which could in turn modulate its affinity for specific Tpms (Bugyi et al., 2006; Papp et al., 2006).

Although different mechanisms probably exist to define the Tpm specificity on actin polymers, they all have important implications when it comes to maintaining the N-termini of endogenous Tpm proteins during *in vivo* and *in vitro* studies. For example, placement of a protein tag might disrupt the normal localization and cooperative properties of a Tpm filament with regard to its interactions with other actin-binding proteins such as myosins. Thus, unlike the majority of proteins, fusing tags to any position of the Tpm protein will impact on the regulation and/or function of the protein, and this consequence should be carefully considered when designing experiments that involve Tpms.

Taken together, is it clear that the Tpm composition of the actin polymer has a substantial impact upon the physical and functional properties of the actin filament. It is, therefore, crucial that the correct Tpm associates with a specific actin polymer within the cell

at the right place and at the right time. The role formins and other actin-binding proteins have in this is currently the subject of extensive investigations.

Physiological functions of actin filaments that contain different Tpms

The first evidence that Tpms are involved in a range of physiological processes came from elimination of the *Tpm1* gene in budding yeast, which led to defects in cell size and growth, mating and vesicle transport (Liu and Bretscher, 1989, 1992). Since then, a substantial body of evidence points to a variety of specific physiological functions that are regulated by Tpms in an isoform-specific manner and in a variety of organisms at the level of the organism (summarized for the main mammalian Tpm isoforms in Table 1). This supports the notion that the presence of different Tpms defines the functional capability of specific actin filament populations in a variety of cellular and physiological processes, as discussed below.

Embryogenesis

All four mammalian Tpm genes (*Tpm1*, *Tpm2 Tpm3*, *Tpm4*) are expressed in mouse embryonic stem cells (Muthuchamy et al., 1993; Hook et al., 2004). Despite this, it has so far been impossible to delete both copies of the *Tpm3* gene (Hook et al., 2004) because all *Tpm3*-knockout mice embryos died prior to implantation in the uterus. Furthermore, 50% of mice that lack just two isoforms of the *Tpm3* gene failed to survive embryogenesis and it was impossible to generate embryonic stem cells that lack these isoforms in cell culture (Hook et al., 2011). Thus, none of the other three active Tpm genes can compensate for *Tpm3*, indicating a gene-specific function. Tpm3.1 has been shown to regulate organ size and cell proliferation in mice (Schevzov et al., 2015), and another recent study has

Table 1. Summary of the main mammalian Tpm isoforms and their functions

Tpm Isoform	Function	References
Tpm1.6	Rescues transformed cells	Gimona et al., 1996
	Stabilizes stress fibers	Tojkander et al., 2011, 2012
	Inhibits Myo1b and 1c binding	Tang and Ostap, 2001; Kee et al., 2015
Tpm1.7	Forms filipodia with cofilin	Creed et al., 2011
	Inhibits neuronal morphogenesis	Schevzov et al., 2005
	Preferentially binds fascin	Creed et al., 2011
Tpm1.8, Tpm 1.9	Regulates CFTR in the membrane	Dalby-Payne et al., 2003
	Regulates mammary gland differentiation	Zucchi et al., 2001
	Localizes to epithelial cell-cell junctions and cell periphery	Temm-Grove et al., 1998; Schevzov et al., 2011
Tpm1.10, Tpm 1.11, Tpm 1.12	1.10, 1.11, 10.12 Promote MAP2C expression	Curthoys et al., 2014
	1.10 Inhibits neurite arborization and growth cone size	Curthoys et al., 2014
	1.11 Promotes neurite length	Curthoys et al., 2014
	1.12 Promotes neurite formation, arborization, growth cone size and filopodia along the neurite	Curthoys et al., 2014
	1.12 Does not impact Arp2/3-mediated actin assembly	Kis-Bicskei et al., 2013
	1.12 Promotes binding of cofilin	Bryce et al., 2003
Tpm2.1	Reduction associated with transformation	Stehn et al., 2006
	Regulates anoikis	Raval et al., 2003
	Associated with establishing focal adhesions	Tojkander et al., 2011, 2012
	Restores stress fibers in transformed cells	Prasad et al., 1993
Tpm3.1	Expressed in tumor cells	Stehn et al., 2006, 2013
	Regulates glucose uptake	Lim et al., 2015; Kee et al., 2015
	Regulates insulin-stimulated GLUT4 transport to plasma membrane	Kee et al., 2015
	Required for ERK-mediated proliferation	Schevzov et al., 2015
	Regulates cell motility and migration	Bach et al., 2009; Lees et al., 2013
	Preferentially recruits MyoIIA	Bryce et al., 2003
	Strongly inhibits cofilin severing	Bryce et al., 2003
	Inhibits Arp2/3-mediated actin assembly	Kis-Bicskei et al., 2013
	Maintains T-tubule structure	Vlahovich et al., 2009
	Regulates excitation-contraction coupling	Vlahovich et al., 2009
	Regulates contractility and integrity of the epithelial zonula adherens	Caldwell et al., 2014
	Promotes neurite branching	Schevzov et al., 2005
Tpm3.2	Associated with Golgi-derived vesicles	Percival et al., 2004
	Regulates contractility and integrity of the epithelial zonula adherens	Caldwell et al., 2014
Tpm4.2	Expressed in tumor cells	Stehn et al., 2013
	Transiently recruits Myosin II motors	Tojkander et al., 2011, 2012
	Localizes to specialized membranes	Kee et al., 2009b
	Promotes MAP2C expression	Curthoys et al., 2014
	Promotes neurite formation, arborisation, growth cone size and filopodia along the neurite	Curthoys et al., 2014
	Localizes to terminal sarcoplasmic reticulum	Vlahovich et al., 2009
	Associated with myofiber formation, growth and repair/regeneration	Vlahovich et al., 2008

Over 40 potential Tpm isoforms have been described in mammalian cells. Among these are isoforms that are considered to be the main mammalian Tpms (Schevzov et al., 2011). These Tpms are listed with current key information; however, there may be other Tpm isoforms that have important roles in cells. This brief overview is only intended to serve as a 'primer' for those readers new to the field, and the list of functions is not exhaustive.

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reported that a *Drosophila* Tpm also promotes cell proliferation (Goins and Mullins, 2015).

Morphogenesis

The role of different Tpm isoforms in morphogenesis has been studied most extensively in neurons and has revealed a wide array of morphological roles for Tpm3.1. This isoform promotes increased axon length, growth cone size and dendritic branching, whereas expression of the non-neuronal Tpm1.7 (also known as Tm3) in neurons attenuates neurogenesis (Schevzov et al., 2005). By contrast, knockout of Tpm3.1 and Tpm3.2 (also known as Tm5NM2) resulted in a decrease in the size of growth cones with an increased rate of lamellipodia protrusions (Fath et al., 2010). Loss-of-function mutations in the *Tm2* (also known as *TmII*) gene in *Drosophila* leads to cell autonomous expansion of neuronal dendritic fields (Li and Gao, 2003). Elevated expression of a range of Tpm isoforms in B35 neuroblastoma cells results in a plethora of phenotypes in differentiated cells, which is consistent with an isoform-specific impact on morphogenesis (Curthoys et al., 2014). Tpm1.7 promotes the formation of filopodia in B35 cells (Creed et al., 2011), which is dependent on the availability of active cofilin. In epithelial cells, Tpm3.1 is necessary for the stability of cell–cell junctions, and its deletion in mice greatly reduces the levels of junctional E-cadherin in the kidney (Caldwell et al., 2014). In addition to neurons, Tpm1.9 (also known as Tm5b) was shown to be essential for morphogenesis in a cell model of mammary gland differentiation (Zucchi et al., 2001), and Tpm1.1 (also known as alpha-Tm) is essential for development of the heart (Rethinasamy et al., 1998)

Cell trafficking and cytokinesis

Injection of Tpm1.7 protein into normal rat kidney (NRK) cells led to accumulation of organelles in the perinuclear space, whereas injection of Tpm3.1 had no impact (Pelham et al., 1996). This suggests that specific Tpm isoforms impact on organelle trafficking, which might reflect their differential effect on myosin motors. For instance, Ostap and co-workers have shown that Myo1b, which is involved in organelle transport, does not recognize actin filaments that contain either Tpm1.6 (also known as Tm2) or Tpm3.1 (Tang and Ostap, 2001; Kee et al., 2015; McIntosh et al., 2015). In addition, Tpm1.8 (also known as Tm5a) and Tpm1.9 regulates the recycling of the cystic fibrosis transmembrane receptor (CFTR) (Dalby-Payne et al., 2003). Tpm1.8 and Tpm1.9 are enriched at apical sites, which are also enriched for CFTR, and depletion of Tpm1.8 and Tpm1.9 leads to elevated levels of CFTR in the apical membrane. This is consistent with a role for Tpm1.8 and Tpm1.9 in recycling of CFTR from the plasma membrane. Tpms are also involved in cytokinesis, and a fission yeast Tpm has been shown to be essential for cytokinesis (Balasubramanian et al., 1992). Expression of a mammalian hybrid Tpm results in defects in cytokinesis in Chinese hamster ovary (CHO) cells (Warren et al., 1995). Moreover, the balance between the protein levels of Tpm2.1 (also known as Tm1) and cofilin is important in ensuring efficient cytokinesis in mammalian cells (Thoms et al., 2008).

Skeletal muscle

Deletion of Tpm3.1 and Tpm3.2 in mice impacts on the morphological and functional features of skeletal muscle. Loss of Tpm3.1 from T-tubules results in increased T-tubule dysmorphology (Vlahovich et al., 2009). In addition, altered excitation-contraction-coupling has been observed which is consistent with an altered uptake of Ca^{2+} through the triad, possibly because of changes in T-tubule function (Vlahovich et al., 2009). In addition, expression of the cytoskeletal Tpm1.7, but not of Tpm3.1, in skeletal muscle results in muscular dystrophy (Kee et al., 2009a). This specificity in the effect of cytoskeletal Tpm isoforms mirrors the studies from D. F. Wieczorek's laboratory that demonstrated that isoform switching of striated muscle Tpm isoforms in the heart impacts on cardiac function (Jagatheesan et al., 2010a,b).

Metabolism

The actin cytoskeleton has been implicated in glucose transport for some time (Klip et al., 2014), and two recent studies have demonstrated that Tpm3.1-containing actin filaments regulate glucose uptake in mice in an isoform-specific manner (Lim et al., 2015; Kee et al., 2015). Here, insulin stimulation of Akt was shown to result in phosphorylation of Tmod3, which caps Tpm3.1-containing actin filaments that are involved in actin reorganization at the cell periphery and increased incorporation of GLUT4 into the plasma membrane (Lim et al., 2015). The other study showed that elevated levels of Tpm3.1 in transgenic mice result in increased glucose uptake, whereas the corresponding knockout mouse shows reduced glucose uptake following a high-fat diet (Kee et al., 2015). The authors provided evidence for the existence of at least two populations of actin filaments that are required for insertion of GLUT4 into the plasma membrane, one which contains Tpm3.1 and engages MyoIIA, and a second which lacks Tpm and interacts with Myo1c (Kee et al., 2015).

Cancer

It has been known for several decades that Tpms are sensitive markers of the transformed state of a cell (Hendricks and Weintraub, 1981, 1984; Lin and Leavitt, 1988; Novy et al., 1993). The original observation was that the level of a subset of Tpms is reduced during transformation and correlates with a loss of actin stress fiber bundles (Leonardi et al., 1982; Matsumura et al., 1983; Urbanciková et al., 1984). Subsequent studies have shown that there is a close correlation between the loss of Tpm2.1 from cells and their transformation, and that restoring the levels of Tpm2.1 or Tpm1.6 reverses specific features of transformation (Boyd et al., 1995; Gimona et al., 1996; Bharadwaj et al., 2005). However, the loss of these Tpms is not an absolute predictor of transformation. By contrast, all tumour cells retain Tpm3.1 (Stehn et al., 2006). A role for Tpm3.1 in transformation and, in particular, in cell motility had been first described about a decade earlier (Miyado et al., 1996, 1997). More recent work has confirmed that Tpm3.1 is required for the survival of neuroblastoma in colony-forming assays and regulates cell proliferation through the MAPK pathway (Stehn et al., 2013; Schevzov et al., 2015).

Cell biomechanics

The actin cytoskeleton has been implicated in the regulation of the biomechanical properties of cells (Wakatsuki et al., 2001). The mechanical interaction of myosin II motors with actin filaments has been shown to play a major role in determining the stiffness of the cell cortex (Alenghat and Ingber, 2002). Not surprisingly, Tpm isoforms have been found to regulate cell stiffness in a neuroblastoma cell model (Jalilian et al., 2015). The cancer-associated Tpm3.1 has the greatest ability to increase cell stiffness in this model and its effect depends on myosin II. The ability of Tpms to regulate cell stiffness is isoform dependent and is independent of their level of expression or their effect on actin bundles, suggesting that it reflects an impact on actin organization at a more subtle level (Jalilian et al., 2015). This could be reflected

in differences in the ability of Tpms to influence filament stiffness (Kojima et al., 1994) or flexibility in the presence of cofilin (McCullough et al., 2008).

On the basis of the above studies, it is to be expected that genetic manipulation of Tpms will reveal specific roles for the different Tpm isoforms in many, if not most, physiological processes owing to their ability to uniquely define the functional characteristics of the actin filaments that are engaged in specific processes. It is surprising, however, that no mutations in human cytoskeletal Tpms have been associated with disease, whereas many mutations in muscle Tpms are associated with muscle conditions (Marttila et al., 2014). The development of drugs that target specific Tpm isoforms might open the door to a range of therapeutic opportunities, such as the treatment of cancer (Stehn et al., 2013). The development of drugs that specifically target a subpopulation of actin polymers will also help the identification of the specific Tpm isoforms that underlie particular physiological functions.

Mechanism of function of different Tpm isoforms

As discussed above, different Tpm isoforms have the ability to specify the function of actin filaments by regulating their interactions with myosin motor and actin-binding proteins. The most extensive data concern the interaction with myosins. The initial indication that Tpms can regulate myosin motor activity came from studies investigating the effect of two different Tpms on a myosin II motor *in vitro* (Fanning et al., 1994), and showed that myosin II activity depends on the type of Tpm present. Subsequent work demonstrated that Tpm1.6 was able to account for the intracellular location of Myo1b by excluding its interaction with actin filaments that contain Tpm1.6 (Tm2) (Tang and Ostap, 2001). The ability of Tpms to also prevent Myo1c from interacting with actin filaments has been observed both in other cells and in *in vitro* systems (Kee et al., 2015; McIntosh et al., 2015).

Furthermore, elevated expression of Tpm3.1 (Tm5NM1), but not of Tpm1.12 (TmBr3), resulted in the recruitment of myosin II isoforms to different actin filament populations (Fig. 2) (Bryce et al., 2003). Here, MyoIIA was recruited to actin cables that span the cytoplasm of B35 neuroblastoma cells, whereas MyoIIB was enriched at the cell periphery and found associated with membrane arcs. Several groups have shown that in yeast, the type of Tpm that is associated with actin filaments determines the recruitment of different motors to these filaments (Stark et al., 2010; Clayton et al., 2010; Coulton et al., 2010). A more recent study extended this notion and showed that Tpm enables a class-V myosin motor to move along an actin filament (Hodges et al., 2012).

The function of cofilin at actin filaments is also regulated by Tpms in an isoform-specific manner (Kuhn and Bamburg, 2008). In fact, initially, Tpm had been identified as a competitor of cofilin because it prevents cofilin-mediated severing of actin filaments (Bernstein and Bamburg, 1982). Subsequent genetic studies confirmed this competitive relationship between Tpm and cofilin in *C. elegans* (Ono and Ono, 2002) and yeast (Nakano and Mabuchi, 2006). However, the effect of Tpm on cofilin appears to be isoform-specific because, in contrast to Tpm3.1 that antagonizes cofilin activity in B35 cells, two other Tpms were shown to collaborate with cofilin in the same cells (Bryce et al., 2003; Creed et al., 2011) (Fig. 2). This has led to the suggestion that, depending on the particular isoforms, Tpms and cofilins either compete with each other or act in a cooperative manner (Kuhn and Bamburg, 2008). Similarly, the ability of gelsolin to sever actin filaments is regulated by the type of Tpm that is associated with the target filament. For instance, some Tpms can partially protect actin filaments from severing, whereas other Tpms do not provide such protection (Ishikawa et al., 1989a,b), and this extends to their ability to bring together actin filaments that have been severed by gelsolin to form longer filaments (Ishikawa et al., 1989a). This indicates that

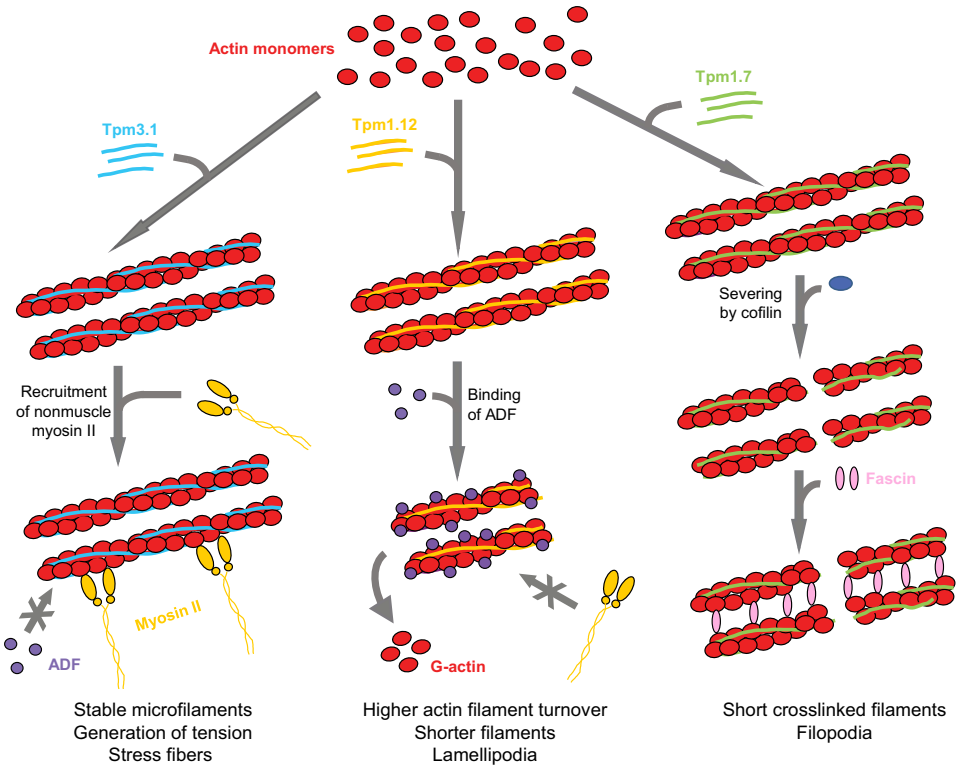

Fig. 2. Tpm isoforms regulate the interactions of actin filaments with actin-binding proteins. Tpm 3.1, Tpm 1.12 and Tpm1.7 have different impacts on the association of myosin II, ADF/cofilin and fascin with actin filaments (Bryce et al., 2003; Creed et al., 2011).

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Tpms are able to confer quite different functional properties onto actin filaments.

The actin-bundling protein fascin is also differentially affected by different Tpm isoforms. Elevated expression of Tpm1.7 promotes the formation of filopodia in B35 cells that is paralleled by elevated levels of fascin (Creed et al., 2011) (Fig. 2). This suggests that fascin becomes more stable if it is more efficiently incorporated into actin filament structures (i.e. less likely to be degraded if it is associated with actin) and correlates with the finding that fascin preferentially associates with Tpm1.7, compared with the closely related isoform Tpm1.6 (Creed et al., 2011). Thus, a change in the number of actin filaments that contain a specific Tpm might lead to an increase in the levels of actin-binding proteins that bind to filaments with this particular Tpm, because this association might reduce their turnover kinetics.

Tpm itself can also regulate actin polymerization through two main means. First, Tpm mechanically stabilizes actin filaments, thereby promoting the formation of longer actin filaments by reducing the probability of severing during elongation (Hitchcock-DeGregori et al., 1988). Second, Tpm decreases the rate by which actin is removed from the pointed end of the actin filament, i.e. the end from which actin depolymerization usually occurs (Broschat et al., 1989). In addition, Tpm collaborates with tropomodulins in an isoform-specific manner to further stabilize the pointed end of the filament. Tropomodulins bind to the pointed end of an actin filament, and to both actin and the associated Tpm (Fowler, 1990; Vera et al., 2000; Rao et al., 2014). The observed differences in the binding affinities of different Tpm isoforms to tropomodulins contribute to the fine-tuning of the stability of the pointed end of an actin filament (Fischer and Fowler, 2003).

Finally, Tpm supply is limiting for the determination of steady-state actin polymer levels in the cell. Elevated expression of Tpm3.1 in mouse brain results in increased levels of actin polymer in the growth cones of primary neurons (Schevzov et al., 2008). Similarly, elevated levels of Tpm3.1 and Tpm1.7 result in increased actin polymerization in mouse fat pads, whereas knockout of Tpm3.1 results in a corresponding decrease in actin polymerization within fat pads (Kee et al., 2015). Therefore, the level of expression of a specific Tpm isoform is expected to determine the number of actin polymers in a tissue that contain this isoform. In line with this, Tpm4.2 has been shown to be a limiting factor for actin polymers that are associated with podosomes (McMichael and Lee, 2008).

Thus, Tpms provide actin filaments with very different capabilities in terms of the way in which individual filaments are crosslinked, are susceptible to severing and engage with different myosin motors. This allows a cell to pick and choose different filament capabilities when establishing cytoskeletal structures.

Role of Tpm-containing filaments in cytoskeletal structures

The functional diversity of cellular actin filament populations is well exemplified by the roles of Tpms in stress fiber assembly. Stress fibers are the main contractile actomyosin bundle in many non-muscle cell-types, where they have an important role in adhesion, morphogenesis and mechanosensing. On the basis of their protein compositions and interactions with focal adhesions, stress fibers can be divided into at least three categories. Dorsal stress fibers are non-contractile actin bundles connected to focal adhesions at their distal ends, whereas transverse arcs are contractile, myosin-II-containing bundles that are not directly associated with focal adhesions. These two structures serve as precursors for the third, ventral stress fibers, which are thick, myosin-II-containing actin bundles linked to focal adhesions at both their ends (Tojkander et al., 2012) (Fig. 3).

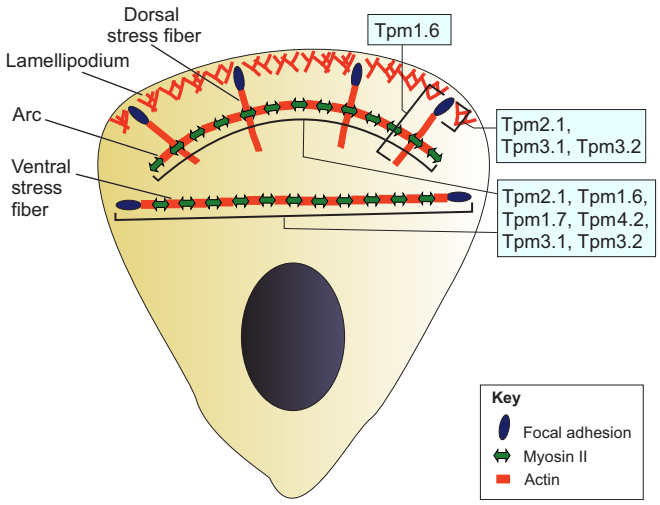

Fig. 3. Localization of Tpm isoforms within the stress fiber network. Tpm1.7 and Tpm4.2 are absent from focal adhesion-anchored dorsal stress fibers. Tpm2.1, Tpm3.1 and Tpm3.2 localize to focal adhesions, whereas Tpm1.6 decorates the entire length of dorsal stress fibers. All main Tpm isoforms are present in contractile transverse arcs and ventral stress fibers, where they colocalize with myosin II. The figure was modified from Tojkander et al. (2011).

Studies on cultured human osteosarcoma cells revealed that they express at least five Tpm isoforms that are functionally non-redundant; depletion of any one of these isoforms led to drastic defects in stress fiber assembly and/or myosin II recruitment (Tojkander et al., 2011). The Tpm isoforms also display partially distinct localization patterns within the stress fiber network, suggesting that they specify the different actin filament populations that are needed for stress fiber assembly (Fig. 3). Although all isoforms are present in ventral stress fibers and transverse arcs, where they display a periodic pattern and colocalize with myosin II, their distribution in stress fiber precursors differs. Tpm1.7 and Tpm4.2 are absent from dorsal stress fibers. Tpm1.6 decorates the entire length of dorsal stress fibers, whereas, Tpm2.1, Tpm3.1 and Tpm3.2 localize only to the dorsal stress fiber segment that overlaps with focal adhesions (Tojkander et al., 2011). It is also interesting to note, that several formin proteins, including Dia1, Dia2, Daam1 and FHOD1, are linked to stress fiber assembly (Hotulainen and Lappalainen, 2006; Ang et al., 2010; Tojkander et al., 2011; Schulze et al., 2014). Thus, it is possible that distinct formin-Tpm pairs specify different actin filament populations within the stress fiber network, similarly to what has recently been demonstrated in fission yeast for other types of actin filament structure (Johnson et al., 2014) (Fig. 1).

In addition to the stress fiber network, different Tpm isoforms display specific functions in other actin-driven processes, such as in bone remodeling by osteoclasts. The latter are giant multinucleate cells, which contain actin-based adhesion structures that are involved in bone-degradation processes. Osteoclasts express several Tpm isoforms that have distinct subcellular localization patterns. Tpm1.6 and Tpm3.1 display diffuse localization throughout the interior of the cell, whereas Tpm4.2, Tpm1.8 and Tpm1.9 localize to actin-rich adhesion structures, such as the podosomes and the sealing zone (McMichael et al., 2006). RNA interference studies further demonstrated that Tpm1.6 contributes to osteoclast morphogenesis and motility, whereas Tpm4.2 is involved in assembly and/or maintenance of adhesion structures (McMichael and Lee, 2008; Kotadiya et al., 2008).

Specific Tpm isoforms are also involved in the dynamics of intracellular organelles. For example, Tpm3.2 localizes to the Golgi complex, whereas its close homolog Tpm3.1 is present in the stress fiber network (Percival et al., 2004) and, upon insulin stimulation, localizes to the cortical actin network (Kee et al., 2015). Interestingly, specific formins – FMNL1 and INF2 – control the integrity of the Golgi complex and might induce the assembly of actin filament foci in the peri-Golgi region (Colón-Franco et al., 2011; Ramabhadran et al., 2011). Moreover, a splice variant of INF2 that contains a C-terminal prenyl group localizes to the endoplasmic reticulum (ER) where it promotes the assembly of myosin-II-containing actin filament structures that are involved in ER-mediated mitochondrial fission (Korobova et al., 2013, 2014). In the future, it will be interesting to examine the localization of Tpm isoforms in Golgi- and ER-associated actin filament foci, in order to reveal whether Tpm3.2 and/or some other Tpm isoforms together with certain formins, indeed, specify the functions and protein compositions of these structures.

Conclusions and perspectives

As discussed here, Tpms allow fungi and metazoans to assemble compositionally distinct actin filaments by defining their preferred interactions with actin-binding and myosin motors. From an evolutionary viewpoint, in a complex milieu that contains many actin-binding proteins and myosin motors there is a clear advantage in building individual filaments that have the ability to discriminate between all potential interactions to efficiently deliver the desired functional outcome (Michelot and Drubin, 2011). In this regard, homopolymers of specific Tpm isoforms would generate 'fidelity of function' along the entire length of the actin filament that allows it to act as 'the unit of function' as is the case in striated muscle (Holmes and Lehman, 2008). Interactions of a given actin filament with actin-binding proteins and myosin motors are expected to be homogeneous along its entire length. This would also allow multiple filaments with different functional capacities to collaborate both in the same location and during the same process by contributing to different functional roles. Examples for such collaborations are, as discussed above, the assembly of stress fibers (Tojkander et al., 2011) and the regulation of glucose uptake (Kee et al., 2015).

One main question remains; why choose the option of multiple Tpm isoforms rather than that of multiple actin proteins (Gunning et al., 2015)? The answer could be that the existence of many different Tpms provide both fine-tuning and fidelity of actin filament function in the cytoskeleton. It is instructive to remember that the contractile apparatus in metazoan striated muscles evolved from the less-advanced fungal cytoskeletons. Thus, the key principle of coordinated filament function, which is essential for contractile tissue, was most probably already in place within the cytoskeleton of these less-advanced species.

The ability of a cell to generate actin filaments with distinct functional capabilities, simply by specifying the Tpm isoform composition of the filament, has been used widely to regulate cell and organ physiology. Genetic manipulation of mice has revealed a remarkable lack of functional redundancy between Tpm isoforms, which allowed us to identify the roles of different types of actin filament in a variety of physiological processes. The ability to link specific Tpm isoforms to distinct physiological processes opens the door to therapeutic intervention. The recent development of isoform-specific anti-Tpm drugs for the treatment of cancer demonstrates the feasibility of targeting the actin filament while avoiding the problems associated with targeting actin (Stehn et al., 2013). It is expected that further knowledge regarding Tpms will provide new mechanistic insights into the functional specialization of the actin filament in addition to therapeutic developments. The era of the generic actin filament has finally passed.

Competing interests

P.W.G. is a member of the Board of Novogen, a company that is commercializing anti-tropomomyosin drugs for the treatment of cancer.

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Supplementary material

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