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Tropomyosin-mediated Regulation of Cytoplasmic Myosins

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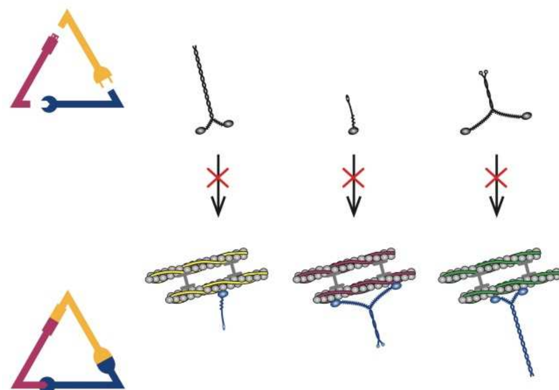
Synopsis: The metazoan actin-based cytoskeleton facilitates an assortment of diverse cellular functions. This is made possible by the members of the tropomyosin multigene family, which at discrete cellular locations form well-defined copolymers with unique functional properties. Here we present a unifying theory in which the tropomyosin isoform associating with the actin defines the surface landscape of the co-polymer to determine the identity and activity of myosin motors that move upon it.



Abstract The ability of the actin-based cytoskeleton to rapidly reorganize is critical for maintaining cell organization and viability. The plethora of activities in which actin polymers participate require different biophysical properties, which can vary significantly between the different events that often occur simultaneously at separate cellular locations. In order to modify the biophysical properties of an actin polymer for a particular function, the cell contains diverse actin binding proteins that modulate the growth, regulation, and molecular interactions of actin-based structures according to functional requirements. In metazoan and yeast cells, tropomyosin is a key regulator of actin-based structures. Cells have the capacity to produce multiple tropomyosin isoforms, each capable of specifically associating as co-polymers with actin at distinct cellular locations to fine-tune the functional properties of discrete actin structures. Here, we present a unifying theory in which tropomyosin isoforms critically define the surface-landscape of co-polymers with cytoplasmic β- or γ-actin. Decoration of filamentous actin with different tropomyosin isoforms determines the identity and modulates the activity of the interacting myosin motor proteins. Conversely, changes in the nucleotide-state of actin and post-translational modifications affect the composition, morphology, subcellular localization, and allosteric coupling of the associated actin-based superstructures.

The capacity to rapidly and appropriately respond to changes in physiological needs is essential for all life on earth. Highly dynamic cytoskeletons facilitate this need by not only providing a reactive framework to promote the distribution of molecules and organelles to precise intracellular locations, but also facilitating the generation of strictly controlled forces. The metazoan actin-based cytoskeleton is formed from different types of linear, bundled and branched filamentous structures that can exist simultaneously at the same cellular location. Their contractile properties and dynamic behavior are attributed to a large number of regulatory proteins (1). Force-generation mediated by the interaction of cytoplasmic myosin isoforms with the different types of actin-based structures is essential for cell motility; modulation of cell shape and polarity; intracellular transport processes; the integration and turnover of channels, pumps, receptors, or translocators in membranes, and also influences the organization of actin filaments itself (2, 3). Actin filament initiation and either concomitant or subsequent association of actin binding proteins, such as the myosin motors leads to the formation of specialized superstructures, such as actin-based contractile networks and fibers that support myosin-dependent transport processes (Fig. 1).

Mammalian cells have the capacity to produce six actin isoforms. All isoforms share more than 92% sequence identity. Four isoforms, $\alpha_{skeletal}$-actin, $\alpha_{cardiac}$-actin, α_{smooth}-actin and γ_{smooth}-actin function primarily within a sarcomere-specific context in skeletal, cardiac, and smooth muscle. The cytoplasmic isoforms β_{cyto}-actin and γ_{cyto}-actin are ubiquitously produced within mammalian cells. Although only subtle sequence variations at the amino-terminus distinguish the cytoplasmic β- and γ-actin isoforms, they cannot substitute for each other. Indeed, the different actin isoforms have a discrete spatial organization within the cell (4, 5). Similarly high-level synthesis of exogenous actins leads to perturbations in normal cell organization and morphology (6-8), and impacts the distribution of the Tropomyosin isoform expression (9). These findings indicate each actin isoform is likely to play a key role in defining the functional properties of the actin polymer, as they have the capacity to show distinct functional interactions with specific myosins (10). However, even accounting for the modulating effects of different nucleotide binding states (11, 12) and post-translational modifications of F-actin (13), two actin isoforms appear insufficient to facilitate functional specificity required of the cytoskeletal polymers. Critically, diversification of the metazoan actin cytoskeleton is determined by interactions with diverse actin binding proteins (14).

One such actin associating and regulating protein is tropomyosin (Tpm), an α-helical, rod-like, dimeric proteins that can form head-to-tail polymers along the major groove of F-actin. Decoration with Tpm can lead to a significant reduction in the surface area on the filament available for subsequent interactions with myosins. Controlling access to myosin binding sites on F-actin is the basis of the mechanism by which Tpm regulates contraction of striated muscle fibers (15). Here, Ca^{2+}-binding to the sarcomere specific troponin complex triggers a series of conformational changes that are propagated and amplified throughout the troponin complex to affect the positioning of Tpm on the F-actin surface (16-19). As a result Tpm is shifted from a “block” position where it inhibits access to the myosin-binding sites on F-actin, to more open positions, which allow myosin to attach to the filament and produce shortening of the sarcomere (20).

Alternative splicing of transcripts from four different genes (TPM1-4) leads to the expression of more than 40 Tpm family gene products (21). The majority of Tpm isoforms are classified as non-sarcomeric isoforms (22). They function mostly in a cytoplasmic context and, as in smooth muscle cells, without the supporting regulatory action of the troponin complex. Cytoplasmic Tpm isoforms show marked differences in their subcellular localization and tissue-specific interactions with discrete actin-based superstructures (23). These diverse actin-Tpm interactions support a wide range of contractile and transport processes, each driven by either a specific member of the non-muscle myosin-2 family or an unconventional myosin. Thus, elucidating the specific regulatory function of each cytoplasmic Tpm and the mechanisms by which its recruitment and dynamic turnover contribute to the spatial and temporal regulation of discrete myosin-driven complexes is arguably amongst the most important tasks in cell biology.

In this context, it is crucial to understand why the contribution of the troponin complex is limited to the regulation of striated muscle fibers. Kinetic and

functional analyses of reconstituted sarcomeric and cytoplasmic actomyosin complexes show that, in contrast to their sarcomeric counterparts, non-muscle and unconventional myosins operate in a high duty-ratio regime (24). That is they spend a large proportion of their ATPase cycle bound strongly to actin. The extended and dominant contribution of strong actin-bound states to the cross-bridge cycle of high duty-ratio myosins impedes a stringent, troponin-mediated synchronization of myosin motors that act in the context of a bi-polar filament or other type of ordered array. Moreover, an array of high duty-ratio myosins is likely to hold tropomyosin permanently in the "open" position on the actin surface (20). The open nature of the cytoplasmic compartment is a further hindrance. This view provides an explanation for the absence of troponin in non-sarcomeric complexes, but requires an explanation for the occurrence, distribution and role of the different cytoplasmic Tpm isoforms, with the notable exception of branched F-actin networks, many cytoskeletal actin filaments appear to be fully decorated with Tpm isoforms (25-27).

In regard to the regulation of tropomyosin distribution, there is growing evidence that the decoration of cytoplasmic actin filaments with tropomyosin dimers involves a formin-driven co-assembly process. Experiments performed in yeast indicate that specific formin family members dictate which tropomyosin isoform associates with a particular actin filament to modulate its dynamic and functional properties at specific sub-cellular locations (28). It is clear that other actin binding proteins play a role in monitoring the Tpm associated with specific actin polymers (29). The multiple sub-populations of tropomyosin-decorated actin filaments display differentially regulated dynamics and functional properties. They display differences in localization, abundance, dynamics, organization, and mechanical properties and show distinct preferences in their interactions with myosin isoforms (30-32). Preliminary kinetic characterization of prototypic actin-myosin-tropomyosin complexes (A·M·Tpm) shows how the individual rate and equilibrium constants for nucleotide binding to myosin and myosin binding to actin are modulated in the presence of different actin and cytoplasmic myosin isoforms. Dependent on the combination of tropomyosin and nonmuscle myosin-2 isoforms the mean filament velocity was observed to be slower, faster or unchanged in an *in vitro* motility assay, compared to the situation where no tropomyosin is present (33).

Intriguingly, new results support the view that different types of actin-Tpm cofilaments, which contain different cytoplasmic Tpm isoforms, have different preferential interactions with myosins. Here, the Tpm appears to act as a selective filter, modulating myosin recruitment to actin-based superstructures. Structural analysis of an A·M·Tpm rigor complex shows how myosin defines and dominates the stereospecific contacts within the A·M·Tpm-complex. The A·M, M·Tpm, and A·Tpm contact areas comprise 1,800, 300, and 210 Å^2, respectively (34). All contact areas involve well-defined electrostatic and hydrophobic interactions (Fig. 2). Based on these results, it is possible to begin to relate known differences in their interactions (35) to the structural features of individual myosin and tropomyosin isoforms. For example, Tpm3.1 has been described to determine the trafficking of GLUT4 glucose transporter vesicles to the plasma membrane by balancing the amount of cortical F-actin that can engage with different myosin isoforms. Consistent with this, Tpm3.1-decoration of F-actin enhances the colocalization of non-muscle myosin-2A (NM-2A), while simultaneously limiting the recruitment of Myo1c (36). Optical trap experiments show that the detachment of NM-2A from Tpm4.2-decorated actin filaments is strongly delayed in the presence of resisting forces. As a result, NM-2A will stay anchored to Tpm4.2-decorated filaments and move in a processive manner under high-load conditions. In addition, Tpm4.2 appears to synchronize the power-stroke of NM-2A heads that are attached to the same filament (37).

We propose a model where well-defined combinations of cytoplasmic myosin, actin, and Tpm isoforms form the framework of stress fibers, transverse arcs and other actin-based superstructures that support the different types of motile activities required in the formation of cell-surface extensions, adhesion, organelle and vesicle transport, migration, and cytokinesis (Fig. 3). We regard these actin-based superstructures as allosterically coupled systems, where trigger events such as a post-translational modification, ligand-binding, or a switch in protein isoform affects the function and stability not only of the altered component, but of the entire A·M·Tpm-complex. Approaches targeted at the development of drugs that alter the contractile and motile properties of a particular myosin are thus more informative when they are performed with accurate allosteric awareness, i.e. when the correct actin-based superstructures are assayed (38). Moreover, the detailed analysis of prototypic function-defining A·M·Tpm-complexes with known cellular functions promises to reveal insights in the code that defines structure-function relationships in tropomyosin-regulated actomyosin-based contraction and the integration of cellular signaling events.

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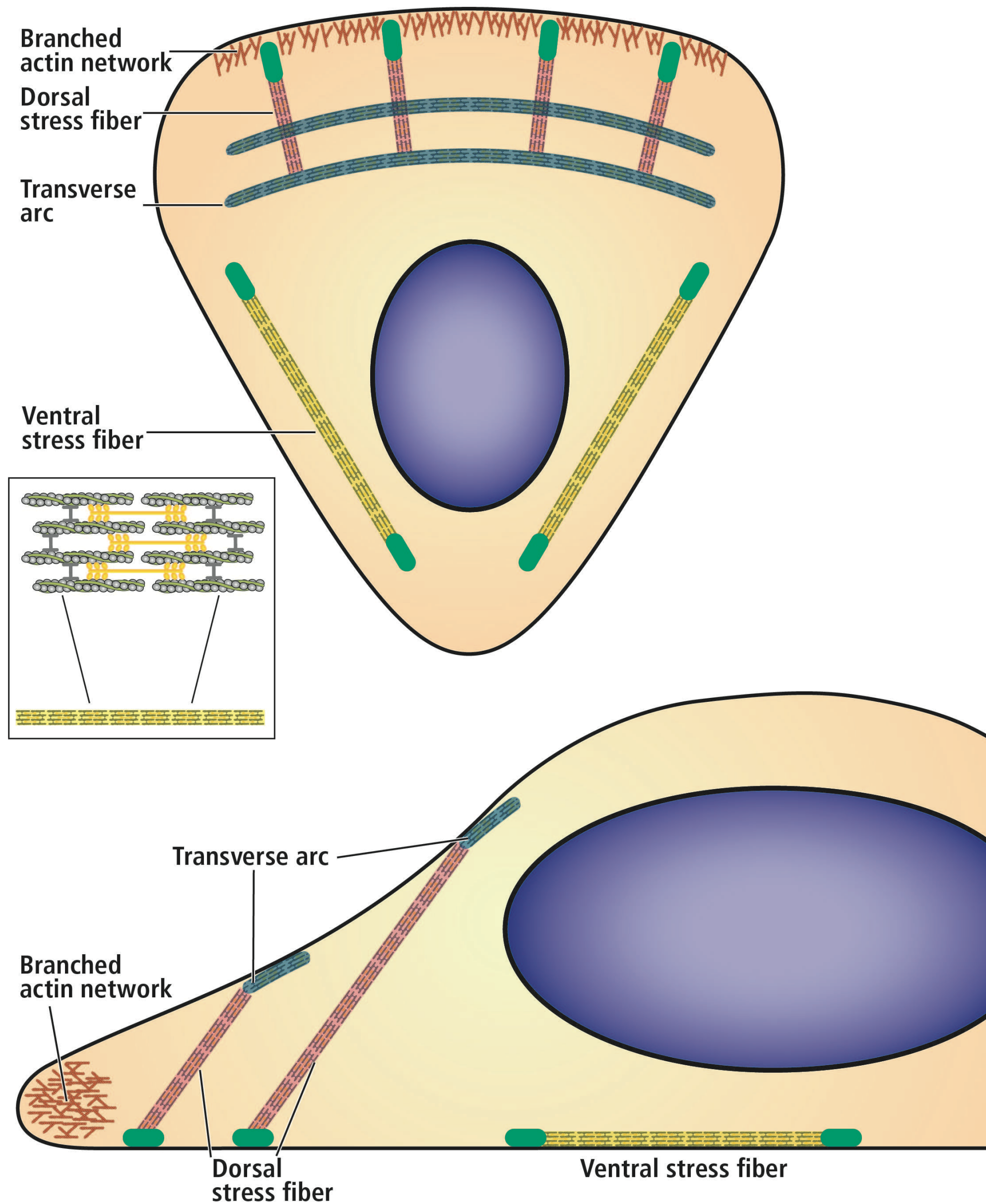

Figure 1: Localization of actin-based superstructures. A subset of the actin-based structures in a migrating cell is shown including the branched actin network in the front of the cell, dorsal and ventral stress fibers, and transverse arcs. Focal adhesions at the end of stress fibers are shown in green. The different coloring of dorsal and ventral stress fibers and transverse arcs indicates their decoration with different Tpm isoforms. The boxed insert shows an enlarged segment of a stress fiber, with bipolar nonmuscle myosin-2 filaments, actin-Tpm copolymers, and actin crosslinking proteins contributing to the architecture of stress fibers.

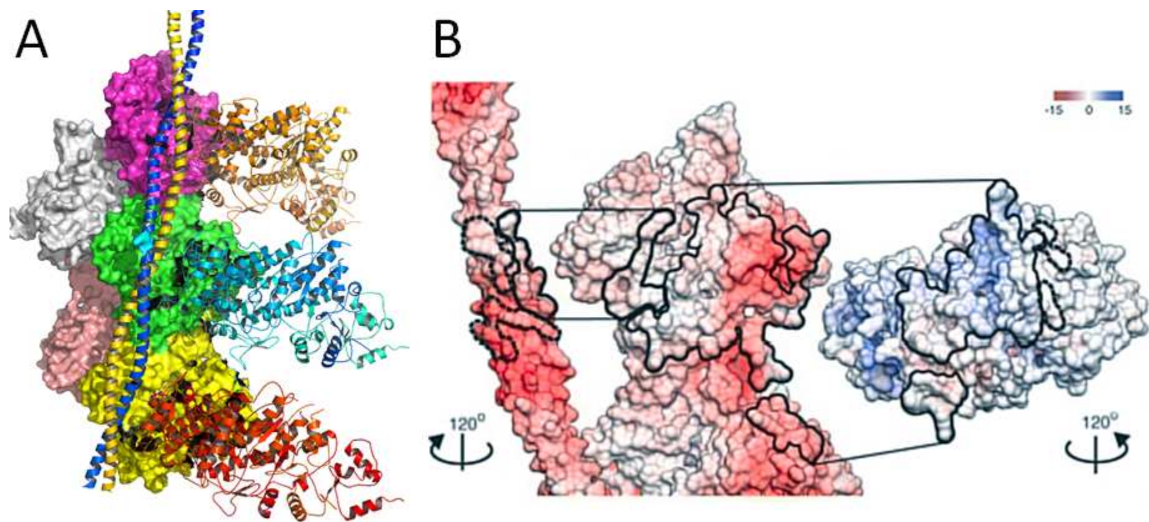

Figure 2: Structure of the A-M-Tpm complex and contact areas within the complex. (A) Overview of the A-M-Tpm complex. Three myosin motor domains and the Tpm filament are shown in ribbon representation. Five actin subunits are shown in surface representation. (B) Binding interface between F-actin, Tpm and the myosin motor. Calculated surface electrostatic potential at pH 7.2 is illustrated. Positive-charge density is colored blue, and negative-charge density is red. Tpm is rotated 120° clockwise und shifted to the left and myosin is rotated 120° counterclockwise and shifted to the right. Panel B was modified from Behrmann et al. (2012).

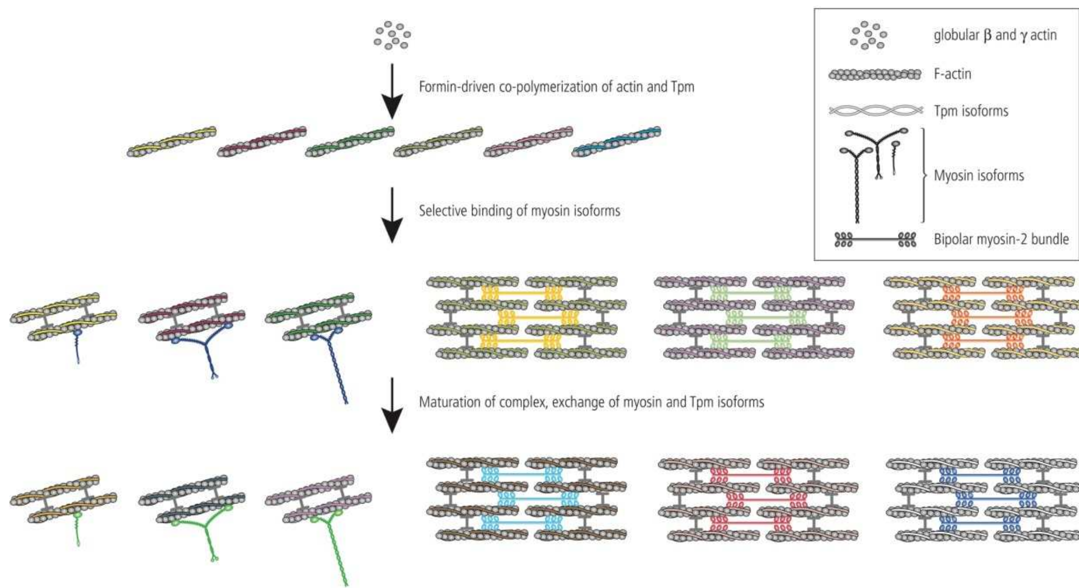

Figure 3: Formation and maturation of actin-based superstructures. Small activated GTPases, F-BAR proteins and other factors including direct interactions with $PI(3,5)P_2$-rich membrane domains recruit formins to specific membranes, where they mediate the isoform-specific formation of actin-Tpm copolymers. The resulting populations of β_{cyto} and γ_{cyto}-actin filaments that are decorated with different Tpm isoforms show clear preferences in their interactions with myosin isoforms. The maturation of the initial filament structures is promoted by post-translational modifications, changes in the nucleotide-state of actin, and protein binding events. This leads to the exchange of actin-bound Tpm isoforms, which in turn promotes a change in the interactions with myosin isoforms and contractile properties.