Multifunctional roles of Tropomodulin-3 in regulating actin dynamics

Tropomodulins (Tmods) are proteins that cap the slow growing (pointed) ends of actin filaments (F-actin). The basis for our current understanding of Tmod function comes from studies in cells with relatively stable and highly organized F-actin networks, leading to the view that Tmod capping functions principally to preserve F-actin stability. However, not only is Tmod capping dynamic, but it also can play major roles in regulating diverse cellular processes involving F-actin remodeling. Here, we highlight the multifunctional roles of Tmod with a focus on Tmod3. Like other Tmods, Tmod3 binds tropomyosin (Tpm) and actin, capping pure F-actin at submicromolar and Tpm-coated F-actin at nanomolar concentrations. Unlike other Tmods, Tmod3 can also bind actin monomers and its ability to bind actin is inhibited by phosphorylation of Tmod3 by Akt2. Tmod3 is ubiquitously expressed and present in a diverse array of cytoskeletal structures, including contractile structures such as sarcomere-like units of actomyosin stress fibers and in the F-actin network encompassing adherens junctions. Tmod3 participates in F-actin network remodeling in lamellipodia during cell migration, and in the assembly of specialized F-actin networks during exocytosis. Furthermore, Tmod3 is required for development, regulating F-actin mesh formation during meiosis I of mouse oocytes, erythroblast enucleation in definitive erythropoiesis, and megakaryocyte morphogenesis in the mouse fetal liver. Thus, Tmod3 plays vital roles in dynamic and stable F-actin networks in cell physiology and development, with further research required to delineate the mechanistic details of Tmod3 regulation in the aforementioned processes, or in other yet to be discovered processes.

Multifunctional Roles of Tropomodulin-3 in Regulating Actin Dynamics

Justin Parreno and Velia M. Fowler*

Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037

*To whom correspondence should be addressed.

Velia M. Fowler, Ph.D.
Department of Molecular Medicine
The Scripps Research Institute
10550 N. Torrey Pines Road
La Jolla, CA 92037

Phone: 858-784-8277
Email: velia@scripps.edu

ORCID IDs: 0000-0001-5277-6255 (JP); 0000-0002-3161-8802 (VMF)

Abstract

Tropomodulins (Tmods) are proteins that cap the slow growing (pointed) ends of actin filaments (F-actin). The basis for our current understanding of Tmod function comes from studies in cells with relatively stable and highly organized F-actin networks, leading to the view that Tmod capping functions principally to preserve F-actin stability. However, not only is Tmod capping dynamic, but it also can play major roles in regulating diverse cellular processes involving F-actin remodeling. Here, we highlight the multifunctional roles of Tmod with a focus on Tmod3. Like other Tmods, Tmod3 binds tropomyosin (Tpm) and actin, capping pure F-actin at submicromolar and Tpm-coated F-actin at nanomolar concentrations. Unlike other Tmods, Tmod3 can also bind actin monomers and its ability to bind actin is inhibited by phosphorylation of Tmod3 by Akt2. Tmod3 is ubiquitously expressed and present in a diverse array of cytoskeletal structures, including contractile structures such as sarcomere-like units of actomyosin stress fibers and in the F-actin network encompassing adherens junctions. Tmod3 participates in F-actin network remodeling in lamellipodia during cell migration, and in the assembly of specialized F-actin networks during exocytosis. Furthermore, Tmod3 is required for development, regulating F-actin mesh formation during meiosis I of mouse oocytes, erythroblast enucleation in definitive erythropoiesis, and megakaryocyte morphogenesis in the mouse fetal liver. Thus, Tmod3 plays vital roles in dynamic and stable F-actin networks in cell physiology and development, with further research required to delineate the mechanistic details of Tmod3 regulation in the aforementioned processes, or in other yet to be discovered processes.

Introduction

Diverse cytoskeletal structures required for cellular processes are formed through the dynamic regulation of actin filament (F-actin) assembly and associations. Fine tuning of F-actin assembly/disassembly is achieved through its interaction with specialized actin binding proteins. There are a host of proteins that nucleate new filaments in linear or branched arrays, stabilize actin by binding along filaments, cap the fast-growing (barbed) end to prevent filament growth, or bind monomeric actin (G-actin) to control available monomer pools (Pollard et al. 2000; Pollard and Cooper 2009). At the slow growing (pointed) end of actin filaments, the tropomodulin (Tmod) family of proteins (~40kDa) cap F-actin to prevent actin monomer association or disassociation (Fowler and Dominguez 2017; Yamashiro et al. 2012). The leiomodins (Lmods) are a group of larger (~65-70kDa) Tmod-related proteins present mainly in striated and smooth muscles. Lmods also bind to the pointed end, but are potent nucleators of new filaments which grow rapidly from their barbed ends, unlike Tmods which inhibit filament growth by capping pointed ends (Fowler and Dominguez 2017). Indeed, Tmods remain the only known F-actin pointed-end capping proteins.

The vertebrate tropomodulin (Tmod) family consists of four isoforms (Tmod1, Tmod2, Tmod3, Tmod4) which share high (~80%) amino-acid sequence similarity in mammals, with somewhat lower similarity in chickens, frogs and zebrafish. Tmods are also present in *D. melanogaster* (*tmod*), *C. elegans* (*unc-94*) and other invertebrates (Yamashiro et al. 2012). Tmods contain two major structural and functional domains that work together to cap F-actin pointed ends (Figure 1A) (Colpan et al. 2013; Fowler and Dominguez 2017; Yamashiro et al. 2012). The N-terminal domain is mostly unstructured and contains an actin binding site (ABS1) flanked by two tropomyosin (Tpm) binding sites (TMBS1 and TMBS2) required for high affinity

Tmod capping of Tpm-coated F-actin pointed ends. The ABS1 spanning residues 58-99 contains a short α-helix (residues 65-75), whereas the TMBS1 (residues 1- 38) and TMBS2 (residues 109-150) are intrinsically disordered, with short portions adopting an α-helical conformation upon binding to Tpm (residue numbering from Tmod1) (Colpan et al. 2013; Fowler and Dominguez 2017). A second actin binding site (ABS2) is contributed by the globular C-terminal domain, consisting of five leucine-rich repeats followed by a nonhomologous α-helix and a C-terminal tail, which allows for F-actin pointed-end capping. Tmod caps pure F-actin pointed ends at submicromolar concentrations (K_d ~0.1-0.2 μm), but caps Tpm-coated F-actin pointed ends at concentrations at least an order of magnitude lower (K_d <20 nm) (Fowler and Dominguez 2017; Weber et al. 1994; Yamashiro et al. 2012). All Tmods have the capacity to bind Tpm and are high affinity caps for Tpm-coated F-actin pointed ends, but Tmod isoforms have preferential Tpm binding partners that influence their capping activity (Colpan et al. 2016; Gokhin and Fowler 2011a; Lewis et al. 2014; Lim et al. 2015; Uversky et al. 2011; Yamashiro et al. 2012; Yamashiro et al. 2014).

Whereas Tmod3 is ubiquitously expressed in mammalian cells and tissues, the expression patterns of Tmod1, 2, and 4 are tissue specific (Cox and Zoghbi 2000; Yamashiro et al. 2012). Tmod1 is expressed in terminally differentiated, post-mitotic cells including striated muscle, red blood cells, lens fiber cells and neurons. Tmod2 and 4 are predominantly expressed in neurons and skeletal muscle, respectively. A great deal of our understanding of Tmods is based on seminal findings for Tmod1 in the red blood cell membrane skeleton (Fowler 1987) and striated muscle sarcomeres (Fowler et al. 1993). In red blood cells, Tmod1 binds to the pointed ends of the short actin filaments in the membrane skeleton, and in striated muscle Tmod1 binds to the pointed ends of the long actin filaments (thin filaments) in sarcomeres. In both F-actin networks,

Tmod1 capping blocks actin monomer association and dissociation at pointed ends to regulate filament length and preserve actin stability (Fowler 1996; Fowler and Dominguez 2017; Gokhin and Fowler 2011c; Gregorio et al. 1995; Moyer et al. 2010). As actin is relatively stable in the red blood cell membrane skeleton and in striated muscle sarcomeres, this has led to the general view that Tmods function principally to regulate F-actin length and stability in highly organized and stable actin cytoskeletal structures. Nevertheless, in both red blood cells and in striated muscle thin filaments, Tmod1 capping is dynamic and actin subunits exchange at filament ends, hinting at functions for Tmods in dynamic actin cytoskeleton remodeling (Fowler and Dominguez 2017; Gokhin and Fowler 2016; Littlefield et al. 2001).

In this review, we highlight the function of Tmods in the diverse F-actin networks of non-muscle cells. The developmental expression and cellular functions of Tmod1, 2, and 4 isoforms have been reviewed elsewhere (Fowler and Dominguez 2017; Gokhin and Fowler 2011c; Gokhin and Fowler 2013; Gray et al. 2017; Yamashiro et al. 2012) and will not be discussed in great detail. Rather, here we focus on the role(s) of Tmod3. Apart from being ubiquitously expressed, Tmod3 has unique molecular properties that position it as a multifunctional Tmod with the ability to regulate actin cytoskeletal structures across a broad swath of biological contexts.

Tmod3 is a unique tropomodulin

Despite the similarities in sequence and structure between the Tmod isoforms, Tmod3 has several key differences. In addition to capping to F-actin pointed ends, Tmod3 can also bind G-actin *in vitro* and in cells (Fischer et al. 2006; Yamashiro et al. 2010). *In vitro*, kinetic modeling and steady state polymerization assays indicate that Tmod3 sequesters monomeric G-actin at submicromolar concentrations, similar to Tmod3 capping of F-actin pointed ends. The

ability to sequester G-actin is exclusive for Tmod3 as Tmod2 has only a small effect on steady state monomer levels, and Tmod1 and 4 have no effect. Binding assays with truncated and mutant Tmod3 fragments, as well as cross-linked peptides identified by EDC/sulfo-NHS and mass spectrometry indicate that the G-actin binding site of Tmod3 includes ABS1 and interacts with actin subdomains 2 and 4 (Figure 1A) (Yamashiro et al. 2010). Interestingly, the crystal structure of a Tmod1-gelsolin segment 1-actin complex shows that a Tmod1 ABS1 peptide extends across actin subdomains 4, 2 and 1 (from amino- to carboxy terminal) (Rao et al. 2014), but the structural basis for G-actin binding (Tmod3) versus F-actin capping (Tmod1 and Tmod3) is unclear. Tmod3 has a preference for binding ATP-G-actin (K_d ~0.1 μM) over ADP-G-actin (K_d ~0.6 μM) (Fischer et al. 2006), and the affinity of Tmod3 for ATP-G-actin is comparable to other G-actin sequestering molecules, such as profilin or thymosin β4 (Tβ4) (Figure 1B). Some experiments suggest that Tmod3 may interact better with non-muscle β– or γ–G-actin than with α-skeletal muscle G-actin, but this has not been explored fully (Gokhin and Fowler 2011a; Yamashiro et al. 2014).

Tmod3 is also unique among Tmods in that it is a substrate for Akt2 phosphorylation (Lim et al. 2015) placing it downstream of PI3K mediated activation of PIP2 to PIP3 by insulin receptor signaling (Gonzalez and McGraw 2009). Sequence analysis demonstrates a unique Akt2 consensus motif at Ser71 within the ABS1 α-helix of Tmod3 not present in other Tmods (Figure 1A). Chemical cross-linking of recombinant phospho-mimetic (S71D) or phospho-defective (S71A) Tmod3 to actin followed by SDS-PAGE revealed that formation of Tmod3-actin complexes was reduced for the S71D mutant, suggesting that Akt2 phosphorylation at Ser71 inhibits Tmod3-actin interactions. However, since this assay detects Tmod3 cross-linking

to actin and oligomeric complexes it remains unclear whether Akt2 phosphorylation modulates Tmod3 G-actin binding and/or Tmod3 F-actin capping activity.

In tissues where several Tmods are present, Tmod3 preferentially regulates some F-actin networks via specific binding partners. In skeletal muscle, Tmod3 stabilizes the sarcoplasmic reticulum (SR) and maintains myofibril alignment by capping γ-actin filaments and binding to small ankyrin 1.5 (sAnk1.5), a SR-associated membrane protein at the M line in skeletal muscle (Gokhin and Fowler 2011a). This is unique to Tmod3, as Tmod1, which is also present in skeletal muscle, does not coimmunoprecipitate with sAnk1.5 or γ-actin filaments, or colocalize with sAnk1.5 at the M line. In addition, unlike Tmod1, Tmod3 is present in a complex with Tpm3.1 and Tpm4 that are associated with the γ-actin filaments. Thus, Tmod3 participates in capping γ-actin filament networks in the SR, while Tmod1 caps the α-actin filaments in muscle sarcomeres (Gokhin and Fowler 2011b).

Tmod3 is ubiquitous with the ability to cap Tpm-F-actin and pure F-actin pointed ends and can selectively regulate specialized Tpm-F-actin networks. It can also bind and sequester actin monomers. Furthermore, binding of Tmod3 to actin is modulated by Akt2 phosphorylation. This suggests that Tmod3 has the potential to play multifunctional roles in regulating dynamic actin-based processes. Indeed, accumulating evidence demonstrates Tmod3 is associated with diverse actin cytoskeletal and contractile structures, implicating a broad role for Tmod3 in regulating many cellular processes.

Actin cytoskeletal structures and cellular processes that depend upon Tmod3

Tmod3 is associated with contractile stress fibers

In nonmuscle cells adherent on stiff substrates, F-actin and nonmuscle myosin II assemble into large contractile bundles termed stress fibers, in which NMII bipolar filaments alternate with α-actinin-cross-linked F-actin in periodic arrays of sarcomere-like contractile units (Naumanen et al. 2008; Pellegrin and Mellor 2007). In fibroblasts, super-resolution imaging of GFP-Tmod3 using structured illumination microscopy (SIM) reveals narrow stripes of GFP-Tmod3 flanked by closely-spaced doublets of NMII motor domains at ends of bipolar filaments (Hu et al. 2017) (Figure 2A). The GFP-Tmod3 stripes alternate with broader α-actinin bands that are coincident with F-actin free barbed ends labeled by incorporation of fluorescent-tagged G-actin. This suggests that Tmod3 caps the F-actin pointed ends located in the middle of the sarcomere-like units, similar to Tmod1 in striated muscle sarcomeres (Fowler and Dominguez 2017; Gokhin and Fowler 2011c; Gokhin and Fowler 2013). A function for Tmod3 in contractile actomyosin bundles is suggested by the phenotype of *Tmod3-/-* mouse fetal liver megakaryocytes spreading on collagen I, where the robust F-actin bundles associated with periodic myosin IIA stripes fail to assemble normally (Sui et al. 2015).

A role for Tmod3 in contractile force generation is also suggested by observations from striated muscle. In *Tmod1-/-* mouse skeletal muscles, Tmod3 relocalizes from the sarcoplasmic reticulum-associated γ–actin cytoskeleton to cap the thin filament pointed ends in sarcomeres, where it substitutes for Tmod1 in regulating thin filament lengths (Gokhin and Fowler 2011a; Gokhin et al. 2010). However, *Tmod1-/-* sarcomeres with Tmod3-capped thin filaments (with normal thin filament lengths) produce less force in skinned fiber assays, due to impaired Tpm strand movement and reduced myosin cross-bridge binding to the thin filaments (Ochala et al. 2014). This suggests the unexpected idea that Tmod capping of Tpm-F-actin pointed ends may either permit (Tmod1) or restrict (Tmod3) the azimuthal movement of striated muscle Tpm

strands necessary for thin filament activation and actomyosin contraction. One may speculate that Tmod3 capping of Tpm-F-actin may contribute to activation or inhibition of NMIIs by recruiting different Tpm isoforms (Barua et al. 2014; Clayton et al. 2015; Gateva et al. 2017; Pathan-Chhatbar et al. 2018).

Tmod3 associates with F-actin at adherens junctions and contributes to epithelial cell morphogenesis

Adherens junctions form the interface between epithelial cells and function to coordinate cell-cell interactions and integrate the actomyosin networks across neighboring cells (Charras and Yap 2018; Lecuit and Yap 2015; Pilot and Lecuit 2005). These E-cadherin-mediated cell-cell junctions are critical to coordinate cellular contraction as well as generate tissue-level tension and patterning to drive developmental morphogenic processes. In mature junctions, F-actin is stabilized by Tpm (Caldwell et al. 2014; McKeown et al. 2014; Weber et al. 2007), and organized with NMII bipolar filaments into a circumferential belt that runs parallel to the plasma membrane, located near the apical portion of polarized epithelial cells (Mege and Ishiyama 2017; Zhang et al. 2005). In primary epithelial cells in tissues, the circumferential belt of NMII and F-actin is organized into striking periodic arrays of sarcomere-like contractile units, similar to the actomyosin stress fibers in fibroblasts (Ebrahim et al. 2013). While these structures have not been studied in a large number of epithelial cell types or in cultured cell lines, they were characterized in organ of Corti epithelial cells and have also been observed in intestinal enterocytes and stomach epithelial cells in tissues.

Tmod3 is present in polarized epithelial cells, where it associates with F-actin at the lateral membranes encompassing the adherens junctions, and in the F-actin rich terminal web

underlying the apical membrane (Cox-Paulson et al. 2014; Cox-Paulson et al. 2012; Guo et al. 2014; Weber et al. 2007) (Figure 2B). In cultured human intestinal epithelial cells (Caco2), knockdown of Tmod3 results in dissociation of Tpm and disassembly of F-actin from lateral membranes, along with disruption of αII-spectrin organization. The disassembly of Tpm, F-actin and αII-spectrin is accompanied by a decrease in epithelial cell height and concomitant expansion of cell apical and basal surface area, but no effects on apical-basal cell polarity. Tmod3 may stabilize the mechanically resilient αII-spectrin-F-actin network (membrane skeleton) that is linked to E-cadherin via ankyrinG and controls lateral membrane biogenesis and cell height (Bennett and Healy 2009). This would be analogous to the function of Tmod1 in stabilizing the spectrin-F-actin membrane skeleton in red blood cells (Moyer et al. 2010).

In *C. elegans* intestinal epithelial cells, loss of UNC-94/Tmod function results in reduced F-actin in the terminal web and a flattened intestinal lumen (Cox-Paulson et al. 2014). Strikingly, the defects due to loss of UNC-94 function are rescued when actomyosin contractility is increased by RNAi depletion of the myosin phosphatase regulatory subunit, *mel-11*. While the nanoscale level organization of Tmod3 along F-actin at adherens junctions or in the terminal web is unknown, it is attractive to consider that Tmod3 caps F-actin pointed ends in sarcomere-like contractile structures of the circumferential belts at adherens junction, similar to Tmod3 in stress fibers of fibroblasts (Figure 2A). We would hypothesize that the absence of Tmod3 would lead to a disorganization or disassembly of these sarcomere-like structures, leading to cellular relaxation. This could explain the reduction in cell height and increase in cell area with Tmod3 knockdown in epithelial cells (Weber et al. 2007), and the flattening of the intestinal lumen in *C. elegans* (Cox-Paulson et al. 2014). A role for Tmod3 in circumferential actomyosin belts also

does not exclude a role for Tmod3 in the spectrin-F-actin membrane skeleton, with both of these F-actin structures contributing to epithelial cell morphology.

Further supporting a role for Tmod in maintaining tissue-level epithelial cell tension at adherens junctions, *C. elegans* UNC-94/Tmod was identified as a synthetic lethal enhancer of a weak loss-of-function allele of *hmp-1*, an α-catenin homologue (Cox-Paulson et al. 2012). HMP-1/α-catenin is a component of the cadherin-catenin complex that protects epidermal adherens junctions from contractile stresses imposed by circumferential actomyosin bundles during embryonic morphogenesis (Vuong-Brender et al. 2018). UNC-94 is localized to the adherens junction in proximity to, but not directly interacting with, HMP-1 (Cox-Paulson et al. 2012; Stevenson et al. 2007). High speed imaging of GFP-JAC1/p120catenin-labeled adherens junctions during embryonic elongation in epidermal morphogenesis demonstrated that, as compared to loss of function for HMP-1 alone, the loss of function for both HMP-1 and UNC-94 resulted in a much greater extent of junctional disruption. Therefore, UNC-94/Tmod is likely recruited to F-actin at adherens junctions to withstand contractile mechanical stresses associated with morphogenesis. A contractile NMII-F-actin network, which requires Tmod3, may provide the necessary intrinsic cellular tension that would be resistant to pulling forces.

Tmod3 associates with lamellipodia F-actin to regulate cell migration

Actin assembly/disassembly dynamics are critical for the extension of the plasma membrane in the lamellipodia at the leading edge of migrating cells (Abercrombie et al. 1970; Carlier and Shekhar 2017; Pollard and Borisy 2003; Pollard and Cooper 2009; Ridley 2011). In brief, the actin-related protein 2/3 complex (Arp2/3), nucleates new actin filaments from the sides of pre-existing filaments to form a branched dendritic network. New ATP- and ADP-P_i rich

filaments grow rapidly at their free barbed ends towards the membrane, until they are capped by heterodimeric capping protein, while older ADP filaments are severed by ADF/cofilin to promote actin disassembly and recycle monomers for new network growth driving lamellipodia protrusion.

While barbed end capping is a positive regulator of actin assembly dynamics in lamellipodia protrusion and cell migration (Carlier and Shekhar 2017; Pollard and Borisy 2003), Tmod3 is a negative regulator of lamellipodia actin assembly and cell migration (Fischer et al. 2003). In cultured human microvascular endothelial cells (HMEC-1), Tmod3 is localized to the F-actin rich lamellipodia of migrating cells (Figure 2A). siRNA knockdown of Tmod3 increases rates of cell migration, while overexpression of GFP-Tmod3 reduces cell migration rates and leads to loss of cell polarity in random migration assays (Fischer et al. 2003). Tmod3 levels are inversely correlated with free pointed ends, and with levels of F-actin, free barbed ends and Arp2/3 levels in lamellipodia. This suggests that increased pointed end capping by Tmod3 and inhibition of F-actin depolymerization could prevent network turnover and reduce the pool of G-actin available for assembly onto barbed ends, thereby decreasing the lamellipodia F-actin network (Fischer and Fowler 2003). Conversely, Tmod3 depletion could reduce capping and enhance F-actin pointed end disassembly, providing more G-actin for network assembly and increasing the lamellipodia F-actin network.

Tmod3 capping of Tpm-F-actin pointed ends may also prevent migration by enhancing the binding of Tpm along F-actin, independently stabilizing F-actin and reducing network turnover (Fischer and Fowler 2003; Ono 2010; Yamashiro et al. 2012). In general, Tpms are also thought to be negative regulators of lamellipodia extension (Blanchoin et al. 2001; DesMarais et al. 2002; Gupton et al. 2005). Microinjection of rabbit skeletal muscle α-Tpm (Tpm1.1) in

motile cells prevents lamellipodia formation by preventing ADF/cofilin severing as well as Arp2/3 branching (Gupton et al. 2005). One particular Tpm isoform, Tpm2.1, is absent from the leading edge of lamellipodia in mouse embryonic fibroblasts (Brayford et al. 2016), and is restricted to more stable populations of F-actin in the lamella, behind the lamellipodia. Due to the roles Tmod3 and Tpm2.1 play in reducing the lamellipodia F-actin network, it may be presumed that Tmod3 reinforces the interaction of Tpm2.1 with F-actin, however, this remains to be tested in cells. Of note, not all Tpm isoforms are negatively correlated with motility and lamellipodia generation. Tpm 1.8/1.9 is present at the leading edge of lamellipodia and enables stabilization and persistence of lamellipodia protrusions (Brayford et al. 2016; Hillberg et al. 2006). Thus, the network arrangement of F-actin may also depend on the spatial distribution of specific Tpm isoforms (Gateva et al. 2017).

Another way that Tmod3 could interfere with F-actin assembly in lamellipodia would be by Tmod3 sequestration of G-actin (Figure 2A), reducing the available pool of monomers (Fischer and Fowler 2003). Tmod3 could also reduce available monomers by competing for binding with thymosin β4, as shown *in vitro* (Fischer et al. 2006), or with profilin, other G-actin sequestering proteins essential for dendritic actin network assembly and turnover at the leading edge of lamellipodia (Lee et al. 2013; Vitriol et al. 2015). Indeed, the endogenous concentration of Tmod3 in endothelial cells (~0.5 μM) (Fischer et al. 2003) is sufficient for Tmod3 to bind G-actin in cells (K_d ~0.1 μM for ATP-actin) (Fischer et al. 2006). Diffuse Tmod3 staining throughout the cytoplasm also suggests that Tmod3 exists in a soluble pool within motile cells, and fractionation studies indicate ~30-40% of endogenous Tmod3 is associated with the TX-100 insoluble cytoskeleton (Fischer et al. 2003). However, the nanomolar affinity of Tmod3 for Tpm-F-actin pointed ends implies that the amount of cytosolic Tmod3 available to bind monomers and

influence the monomer pool would be limited by the numbers of Tpm-F-actin pointed ends in cells, which has not been determined. Future studies of actin dynamics and F-actin network structure in lamellipodia of cells expressing Tmod mutants with disabled monomer binding, F-actin capping, or Tpm binding sites (Colpan et al. 2013; Gray et al. 2017; Yamashiro et al. 2012) are required to reveal the mechanistic details of Tmod3 regulation of F-actin network assembly in lamellipodia protrusion and cell migration.

Tmod3 regulates cortical actin remodeling during exocytosis

The actin cytoskeleton plays crucial roles in the distal stages of exocytosis at the plasma membrane by tethering vesicles as well as facilitating membrane docking and fusion (Lopez et al. 2009). During vesicle docking and fusion, various actin associated molecules promote assembly of cortical F-actin as well as form a contractile F-actin-myosin network surrounding vesicles to enable their docking and subsequent fusion to the plasma membrane. In this process, ADF/cofilin is required to disassemble F-actin to provide G-actin for actin assembly (Miklavc et al. 2015). F-actin is then polymerized by Wasp activation of Arp2/3 nucleation, forming a branched dendritic network, similar to lamellipodia (Tran et al. 2015; Yang et al. 2014). Furthermore, myosin II is incorporated into the cortical F-actin surrounding exocytic vesicles, where it cross-links F-actin and generates contractile force (Chiu et al. 2010; Chung le et al. 2010; Miklavc et al. 2012).

A role for Tmod3 in exocytosis has been elucidated in insulin-stimulated GLUT4 insertion at the plasma membrane in 3T3-L1 adipocytes (Lim et al. 2015) (Figure 2C). In this system, insulin stimulates Akt2-dependent phosphorylation of Tmod3 at Ser71. shRNA-mediated knockdown of Tmod3 or expression of a Ser71 phospho-defective Tmod3 (Tmod3-

S71D) interferes with cortical F-actin enrichment and prevents GLUT4 insertion at the plasma membrane, reducing glucose uptake. This provides the first evidence that signaling pathways modulate Tmod3 function to alter actin assembly and regulate a cellular function. However, the exact molecular role(s) of Tmod3 during actin remodeling in exocytosis remains unclear. It was suggested that non-phosphorylated Tmod3 sequesters G-actin, based on diffuse cytoplasmic staining for Tmod3 in untreated cells, and the ability of Ser71 phosphorylation to inhibit association of Tmod3 with actin *in vitro*. In this case, Akt2-dependent Ser71 phosphorylation would function to release G-actin from Tmod3 to increase the monomer pool and promote F-actin assembly (Figure 2D). While insulin stimulation does lead to an increase in Tmod3 association with cortical F-actin, it is unclear if phosphorylation promotes this association (Lim et al. 2015). In addition to Tmod3, Tpm3.1 is assembled along cortical F-actin following insulin stimulation (Kee et al. 2015). Binding of Tmod3 binding to Tpm3.1 is important for GLUT4 exocytosis, as cells overexpressing a Tmod3 mutant unable to bind Tpm3.1, with disabled TMBS1 and TMBS2, have reduced GLUT4 insertion to the plasma membrane (Lim et al. 2015). This mechanism may operate *in vivo*, based on studies in white adipose tissue and skeletal muscle of Tpm3.1-overexpressing or Tpm3.1-null mice, which demonstrate alterations in F-actin and increased or decreased insulin-stimulated glucose uptake, respectively (Kee et al. 2015). Tmod3 capping of cortical F-actin pointed ends could promote Tpm3.1 binding, or binding of Tpm3.1 along F-actin could facilitate Tmod3 capping (Yamashiro et al. 2012). Together, Tmod3 and Tpm3.1 likely cooperate to preserve newly assembled cortical F-actin to facilitate vesicle docking and fusion. Tpm3.1 also recruits myosin IIA which cross-links F-actin and provides contractile forces that may aid in vesicle fusion (Chung le et al. 2010; Kee et al. 2015). Thus, Tmod3 may assist in promoting contractile actomyosin networks to facilitate exocytic vesicle

docking and fusion, similar to proposed roles of Tmod3 in stabilizing the contractile actomyosin networks in stress fibers and adherens junctions as discussed above.

Tmod3 associates with the cytoplasmic F-actin mesh required for asymmetric division of mouse oocytes

Asymmetric division is critical in mammalian development. To maintain the maternal components in oocyte maturation, asymmetric divisions occur during meiosis I and II to form totipotent large haploid oocytes and smaller polar bodies. In mouse oocyte maturation, formation of a cytoplasmic F-actin mesh coordinates with cortical F-actin rearrangements to control central positioning of the nucleus in the prophase oocyte, and spindle migration to the cortex that occurs in meiosis I (Almonacid et al. 2014; Almonacid et al. 2017; Namgoong and Kim 2016). Failure to form the F-actin mesh impairs nucleus centering and spindle migration, leading to defects in asymmetric division and polar body formation. Several crucial actin regulators are required for formation of the F-actin mesh and spindle migration in mouse oocytes, including spire and formin actin nucleators, capping protein, Tpm3, cofilin and filamin. Arp2/3 nucleation of actin assembly also drives cytoplasmic streaming of a dynamic actin network that assists spindle migration and maintain the asymmetric position of the spindle at the cortex after the first meiosis (Yi et al. 2011).

Tmod3 capping of F-actin pointed ends is also critical for assembly of the cytoplasmic F-actin mesh in mouse oocytes (Jo et al. 2016) (Figure 2D). Tmod3 protein is present throughout oocyte maturation and is in a punctate pattern in the cytoplasm. siRNA knockdown of Tmod3 results in a reduced cytoplasmic F-actin mesh, accompanied by impaired chromatin migration and aberrant asymmetric division with formation of an abnormally large polar body. Since

Tmod3 depletion in oocytes decreases the F-actin mesh, this suggests that Tmod3 does not function solely by sequestering G-actin, as then Tmod3 depletion would have been expected to lead to an increase in the F-actin mesh, which is not observed. Instead, Tmod3-capping of Tpm-coated F-actin pointed ends is likely to be important, since expression of a truncated Tmod3 lacking the C-terminal F-actin capping domain (which binds to Tpm but not to pointed ends) has a dominant negative effect, reducing the F-actin mesh. In addition, knockdown of both Tmod3 and Tpm3 leads to a greater reduction in F-actin mesh formation than does either alone. By contrast, overexpression of full length GFP-Tmod3 enhances F-actin mesh formation and interferes with asymmetric cell division, indicating that excessive F-actin mesh formation may impede spindle migration. Tmod3 capping of Tpm3-F-actin pointed ends likely stabilizes the F-actin mesh by reducing pointed end depolymerization and protecting filaments from cofilin severing, which would reduce the available pool of monomers for dynamic mesh turnover, similar to Tmod3 in lamellipodia, as discussed above. Future studies will be required to establish how Tmod3 activity is integrated with the other actin regulators to coordinate the complex events of spindle migration and asymmetric division, including actin-dependent cytoplasmic streaming, vesicular movements, and cortical F-actin rearrangements (Almonacid et al. 2014; Almonacid et al. 2017; Namgoong and Kim 2016).

Tmod3 regulation of F-actin in embryonic development

Tmod3 is ubiquitous, and has essential functions in many developmental processes, not limited to those mentioned above. For instance, Tmod3 null embryos have severe defects in fetal liver erythropoiesis resulting in anemia and embryonic lethality at E14.5-16.5 (Sui et al. 2014). The absence of Tmod3 profoundly affects definitive erythropoiesis at multiple levels including

reduced progenitors, inability to form erythroblast-macrophage islands and impaired erythroblast cell survival, cell cycle progression, and enucleation (Sui et al. 2014). Tmod3-null erythroblasts demonstrate aberrant F-actin organization that may explain their defects in enucleation, which is an F-actin-dependent cellular process likened to asymmetric division (Ji et al. 2011; Li 2013; Nowak et al. 2017). Additionally, Tmod3 null embryos have macrothrombocytopenia (fewer and abnormally large platelets), due to defects in megakaryocyte morphogenesis and proplatelet formation. Tmod3 null megakaryocytes differentiate normally but have altered demarcation membrane systems with uneven cytoplasmic organelle distribution, producing abnormally large proplatelet buds with variable organelle content and aberrant Tpm4 and F-actin distributions (Sui et al. 2015). It was proposed that Tmod3 capping of Tpm4-F-actin may stabilize the α2-spectrin membrane skeleton that is known to control the formation of the demarcation membrane system, required for normal proplatelet biogenesis (Patel-Hett et al. 2011).

Observation of Tmod3 null mouse embryos at midgestation also shows anomalies in other tissue types, from bone to brain (R.B. Nowak and V.M. Fowler, unpublished), suggesting that Tmod3 is involved in morphogenesis of other specialized cells and tissues during embryonic development. However, whether defects in biological processes within these other tissues are directly due to deletion of Tmod3, or instead to secondary systemic effects has not been investigated (i.e., with tissue-specific knockouts). It is also possible that some of these developmental defects may originate from earlier defects in asymmetric division during oocyte maturation, discussed above. Nevertheless, growing evidence from exploratory expression/biomarker studies demonstrates likely Tmod3 involvement in other specialized cellular phenomena (Lopez-Ubeda et al. 2015; Lu et al. 2017; Paez et al. 2016; Qiu et al. 2010)

and pathologies (Gajbhiye et al. 2017; Gajbhiye et al. 2012; Paez et al. 2016), providing evidence of Tmod3's broad and relatively unexplored roles.

Conclusions

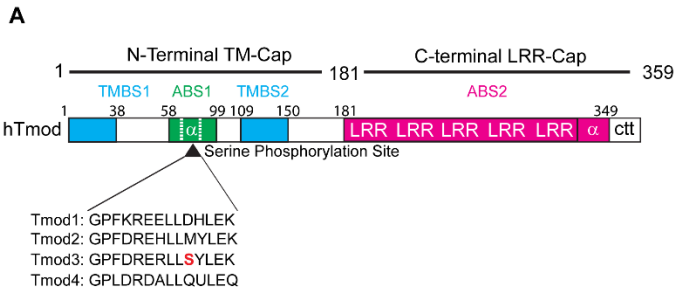
While it is evident that Tmod3 is associated with many different types of actin cytoskeletal networks in cells, and plays vital roles in numerous dynamic biological processes, many questions remain open. For example, it is unclear to what extent Tmod3's functions in complex biological processes of erythropoiesis or megakaryocyte proplatelet formation might be due to Tmod3 regulation of specific types of F-actin networks, such as lamellipodia dendritic networks, cortical F-actin, contractile actomyosin bundles, or membrane-associated spectrin-F-actin networks. Indeed, Tmod3 localization and F-actin network composition and organization in these and other specialized cells during embryonic development are not well-defined. It is possible that specific Tmod3-binding partners may selectively target Tmod3 to different F-actin network structures in these specialized cell types, analogous to sAnk1.5 in the SR of skeletal muscle. The molecular mechanisms of Tmod3 function in these F-actin networks and cellular processes also requires further examination. In comparison to the other, tissue-specific Tmod isoforms, Tmod3 is unique in that it can bind and sequester G-actin. However, the importance of Tmod3 G-actin binding *in vivo* within cells and/or tissues has not been definitively demonstrated. Additionally, we suggested that a competition between Tmod3 and other actin sequestering proteins could be important; reconstituted F-actin assembly systems could help investigate these possibilities. Tmod3 function is linked to Akt2 phosphorylation in adipocytes, but how Akt2 phosphorylation or other signaling pathways may regulate Tmod3 function in other cell types, and whether they affect G-actin sequestering or F-actin capping, is not clear. New specialized

435 tools (i.e. conditional knock-outs/knock-ins/mutant mice) and improved methodology (i.e.
436 Tmod3 visualization using Tmod3-GFP or Tmod3 specific antibodies in cell/tissues with super-
437 resolution imaging) will provide opportunities to achieve a greater understanding of Tmod3
438 regulation and dynamics of F-actin networks in diverse biological processes.

439

440 **ACKNOWLEDGEMENTS:** We are grateful to Roberta B. Nowak for preparation of Figure 2.
441 This work was funded by National Institutes of Health (NIH) Grants R01 HL083464 and R01
442 EY017724 to V.M.F. J.P. was supported by a fellowship from the Natural Science and
443 Engineering Research Council of Canada.

444



B

	K_d (μM)		
	G-actin		F-actin
Protein	ATP	ADP	pointed end
Tmod3	0.13	0.6	0.1-0.2
Profilin	0.1	0.5	-
ADP-Cofilin	8	0.1	-
Thymosin B4	0.1-3.9	80-100	-
Twinfilin	0.47	0.05	-

Figure 1.

445

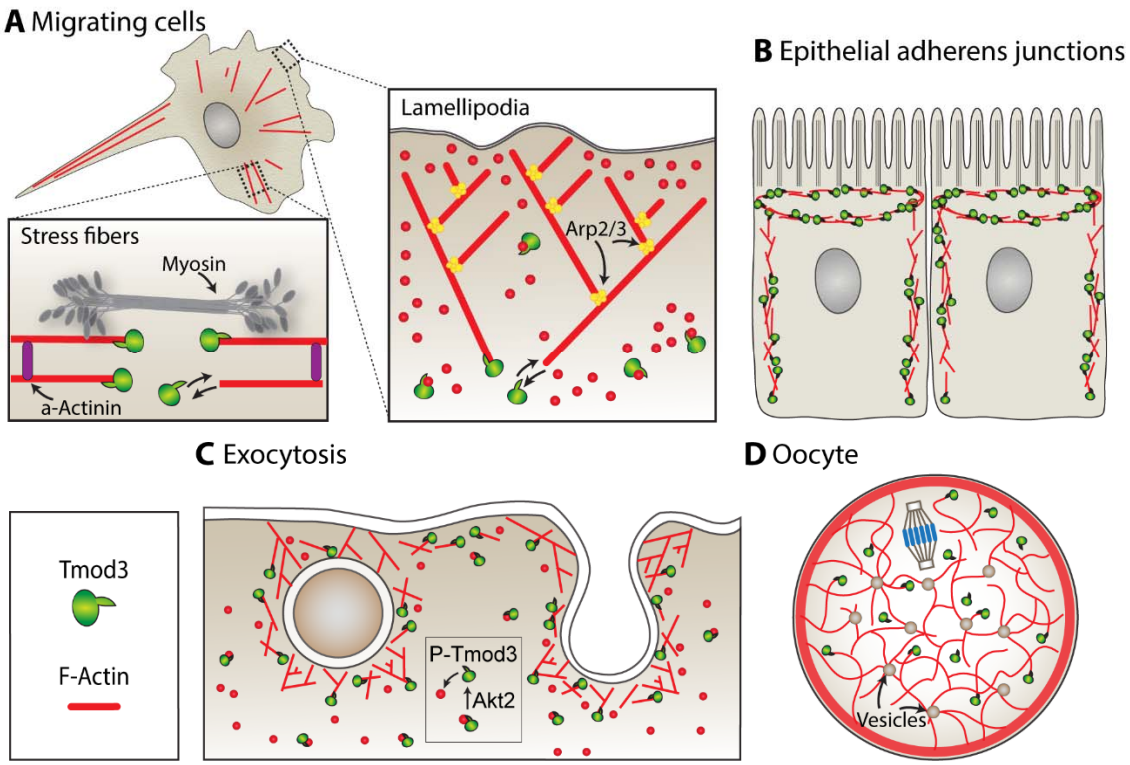


Figure 2.

446

FIGURE LEGENDS

Figure 1. Tmod structural domains and actin dissociation constants. (A) Structure of Tmod highlighting important regions for actin binding and capping as well as Tpm binding (residue numbering from human Tmod1). Tmod3 is unique as it contains a phosphorylation site at Ser71 which is a substrate for Akt2 phosphorylation. The structure schematic is redrawn based on both Yamashiro et al. 2012 (N-terminal CAP and C-terminal CAP terminology) and from Fowler and Dominguez 2007 (updated domain assignment and numbering). (B) Table showing the dissociation constants (K_d) of Tmod3 and select actin binding proteins for G-actin (Carlier et al. 1997; Fischer et al. 2006; Hertzog et al. 2004; Ojala et al. 2002; Pollard et al. 2000; Yu et al. 1993) and of Tmod3 for pure F-actin pointed ends (Fischer et al. 2003; Fischer et al. 2006).

Figure 2. Tmod3 regulates diverse actin cytoskeletal structures. The regulation of these cytoskeletal structures requires multiple actin binding proteins, however, only the minimal number of binding proteins are drawn for simplicity and to highlight the role of Tmod3. (A) Tmod3 is present in stress fibers where it caps F-actin pointed ends within the actomyosin sarcomere-like units (pictured here within a migrating cell). Tmod3 is also present in lamellipodia where it may cap F-actin pointed ends to stabilize filaments, and/or sequester G-actin. By stabilizing the pointed end and/or sequestering actin monomers, Tmod3 acts as a negative regulator of lamellipodia actin assembly. (B) In polarized epithelial cells, Tmod3 is associated with F-actin at lateral membranes and in the apical domain where it may cap F-actin pointed ends in the circumferential actomyosin belt. The stabilization of F-actin by Tmod3 is critical to maintaining the cellular tension required for cell shape as well as for withstanding

mechanical stresses associated with morphogenesis of epithelial sheets during morphogenesis. (C) Tmod3 is associated with the F-actin cortex in adipocytes where it facilitates vesicle docking and fusion during insulin-mediated GLUT4 exocytosis. Insulin-mediated signalling to Akt2 leads to phosphorylation of Tmod3 at S71, which inhibits Tmod3 binding to actin, and releases G-actin for cortical F-actin assembly. (D) Tmod3 is associated with the cytoplasmic F-actin mesh in mouse oocytes. During oocyte maturation, Tmod3 is required for assembly of the F-actin mesh and is critical for proper spindle migration and asymmetric division. These schematics are not drawn to scale.

References

Abercrombie M, Heaysman JE, Pegrum SM (1970) The locomotion of fibroblasts in culture. II. "RRuffling" Exp Cell Res 60:437-444

Almonacid M, Terret ME, Verlhac MH (2014) Actin-based spindle positioning: new insights from female gametes J Cell Sci 127:477-483 doi:10.1242/jcs.142711

Almonacid M, Terret ME, Verlhac MH (2017) Control of nucleus positioning in mouse oocytes Semin Cell Dev Biol doi:10.1016/j.semcdb.2017.08.010

Barua B, Nagy A, Sellers JR, Hitchcock-DeGregori SE (2014) Regulation of nonmuscle myosin II by tropomyosin Biochemistry 53:4015-4024 doi:10.1021/bi500162z

Bennett V, Healy J (2009) Membrane domains based on ankyrin and spectrin associated with cell-cell interactions Cold Spring Harb Perspect Biol 1:a003012 doi:10.1101/cshperspect.a003012

Blanchoin L, Pollard TD, Hitchcock-DeGregori SE (2001) Inhibition of the Arp2/3 complex-nucleated actin polymerization and branch formation by tropomyosin Curr Biol 11:1300-1304

Brayford S, Bryce NS, Schevzov G, Haynes EM, Bear JE, Hardeman EC, Gunning PW (2016) Tropomyosin Promotes Lamellipodial Persistence by Collaborating with Arp2/3 at the Leading Edge Curr Biol 26:1312-1318 doi:10.1016/j.cub.2016.03.028

Caldwell BJ et al. (2014) Tropomyosin isoforms support actomyosin biogenesis to generate contractile tension at the epithelial zonula adherens Cytoskeleton (Hoboken) 71:663-676 doi:10.1002/cm.21202

Carlier MF et al. (1997) Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility J Cell Biol 136:1307-1322

Carlier MF, Shekhar S (2017) Global treadmilling coordinates actin turnover and controls the size of actin networks Nat Rev Mol Cell Biol 18:389-401 doi:10.1038/nrm.2016.172

Charras G, Yap AS (2018) Tensile Forces and Mechanotransduction at Cell-Cell Junctions Curr Biol 28:R445-R457 doi:10.1016/j.cub.2018.02.003

Chiu TT, Patel N, Shaw AE, Bamburg JR, Klip A (2010) Arp2/3- and cofilin-coordinated actin dynamics is required for insulin-mediated GLUT4 translocation to the surface of muscle cells Mol Biol Cell 21:3529-3539 doi:10.1091/mbc.E10-04-0316

Chung le TK et al. (2010) Myosin IIA participates in docking of Glut4 storage vesicles with the plasma membrane in 3T3-L1 adipocytes Biochem Biophys Res Commun 391:995-999 doi:10.1016/j.bbrc.2009.12.004

Clayton JE, Pollard LW, Murray GG, Lord M (2015) Myosin motor isoforms direct specification of actomyosin function by tropomyosins Cytoskeleton (Hoboken) 72:131-145 doi:10.1002/cm.21213

Colpan M, Moroz NA, Gray KT, Cooper DA, Diaz CA, Kostyukova AS (2016) Tropomyosin-binding properties modulate competition between tropomodulin isoforms Arch Biochem Biophys 600:23-32 doi:10.1016/j.abb.2016.04.006

Colpan M, Moroz NA, Kostyukova AS (2013) Tropomodulins and tropomyosins: working as a team J Muscle Res Cell Motil 34:247-260 doi:10.1007/s10974-013-9349-6

Cox-Paulson E et al. (2014) The minus-end actin capping protein, UNC-94/tropomodulin, regulates development of the Caenorhabditis elegans intestine Dev Dyn 243:753-764 doi:10.1002/dvdy.24118

Cox-Paulson EA et al. (2012) Tropomodulin protects alpha-catenin-dependent junctional-actin networks under stress during epithelial morphogenesis Curr Biol 22:1500-1505 doi:10.1016/j.cub.2012.06.025

Cox PR, Zoghbi HY (2000) Sequencing, expression analysis, and mapping of three unique human tropomodulin genes and their mouse orthologs Genomics 63:97-107 doi:10.1006/geno.1999.6061

DesMarais V, Ichetovkin I, Condeelis J, Hitchcock-DeGregori SE (2002) Spatial regulation of actin dynamics: a tropomyosin-free, actin-rich compartment at the leading edge J Cell Sci 115:4649-4660

Ebrahim S et al. (2013) NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry Curr Biol 23:731-736 doi:10.1016/j.cub.2013.03.039

Fischer RS, Fowler VM (2003) Tropomodulins: life at the slow end Trends Cell Biol 13:593-601

Fischer RS, Fritz-Six KL, Fowler VM (2003) Pointed-end capping by tropomodulin3 negatively regulates endothelial cell motility J Cell Biol 161:371-380 doi:10.1083/jcb.200209057

Fischer RS, Yarmola EG, Weber KL, Speicher KD, Speicher DW, Bubb MR, Fowler VM (2006) Tropomodulin 3 binds to actin monomers J Biol Chem 281:36454-36465 doi:10.1074/jbc.M606315200

Fowler VM (1987) Identification and purification of a novel Mr 43,000 tropomyosin-binding protein from human erythrocyte membranes J Biol Chem 262:12792-12800

Fowler VM (1996) Regulation of actin filament length in erythrocytes and striated muscle Curr Opin Cell Biol 8:86-96

Fowler VM, Dominguez R (2017) Tropomodulins and Leiomodins: Actin Pointed End Caps and Nucleators in Muscles Biophys J 112:1742-1760 doi:10.1016/j.bpj.2017.03.034

Fowler VM, Sussmann MA, Miller PG, Flucher BE, Daniels MP (1993) Tropomodulin is associated with the free (pointed) ends of the thin filaments in rat skeletal muscle J Cell Biol 120:411-420

Gajbhiye R et al. (2017) Panel of Autoimmune Markers for Noninvasive Diagnosis of Minimal-Mild Endometriosis Reprod Sci 24:413-420 doi:10.1177/1933719116657190

Gajbhiye R et al. (2012) Identification and validation of novel serum markers for early diagnosis of endometriosis Hum Reprod 27:408-417 doi:10.1093/humrep/der410

Gateva G et al. (2017) Tropomyosin Isoforms Specify Functionally Distinct Actin Filament Populations In Vitro Curr Biol 27:705-713 doi:10.1016/j.cub.2017.01.018

Gokhin DS, Fowler VM (2011a) Cytoplasmic gamma-actin and tropomodulin isoforms link to the sarcoplasmic reticulum in skeletal muscle fibers J Cell Biol 194:105-120 doi:10.1083/jcb.201011128

Gokhin DS, Fowler VM (2011b) The sarcoplasmic reticulum: Actin and tropomodulin hit the links Bioarchitecture 1:175-179 doi:10.4161/bioa.1.4.17533

Gokhin DS, Fowler VM (2011c) Tropomodulin capping of actin filaments in striated muscle development and physiology J Biomed Biotechnol 2011:103069 doi:10.1155/2011/103069

Gokhin DS, Fowler VM (2013) A two-segment model for thin filament architecture in skeletal muscle Nat Rev Mol Cell Biol 14:113-119 doi:10.1038/nrm3510

Gokhin DS, Fowler VM (2016) Feisty filaments: actin dynamics in the red blood cell membrane skeleton Curr Opin Hematol 23:206-214 doi:10.1097/MOH.0000000000000227

Gokhin DS et al. (2010) Tropomodulin isoforms regulate thin filament pointed-end capping and skeletal muscle physiology J Cell Biol 189:95-109 doi:10.1083/jcb.201001125

Gonzalez E, McGraw TE (2009) The Akt kinases: isoform specificity in metabolism and cancer Cell Cycle 8:2502-2508 doi:10.4161/cc.8.16.9335

Gray KT, Kostyukova AS, Fath T (2017) Actin regulation by tropomodulin and tropomyosin in neuronal morphogenesis and function Mol Cell Neurosci 84:48-57 doi:10.1016/j.mcn.2017.04.002

Gregorio CC, Weber A, Bondad M, Pennise CR, Fowler VM (1995) Requirement of pointed-end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes Nature 377:83-86 doi:10.1038/377083a0

570 Guo Z, Neilson LJ, Zhong H, Murray PS, Zanivan S, Zaidel-Bar R (2014) E-cadherin interactome complexity
571 and robustness resolved by quantitative proteomics Sci Signal 7:rs7
572 doi:10.1126/scisignal.2005473
573 Gupton SL et al. (2005) Cell migration without a lamellipodium: translation of actin dynamics into cell
574 movement mediated by tropomyosin J Cell Biol 168:619-631 doi:10.1083/jcb.200406063
575 Hertzog M et al. (2004) The beta-thymosin/WH2 domain; structural basis for the switch from inhibition
576 to promotion of actin assembly Cell 117:611-623
577 Hillberg L, Zhao Rathje LS, Nyakern-Meazza M, Helfand B, Goldman RD, Schutt CE, Lindberg U (2006)
578 Tropomyosins are present in lamellipodia of motile cells Eur J Cell Biol 85:399-409
579 doi:10.1016/j.ejcb.2005.12.005
580 Hu S et al. (2017) Erratum: Long-range self-organization of cytoskeletal myosin II filament stacks Nat Cell
581 Biol 19:258 doi:10.1038/ncb3479
582 Ji P, Murata-Hori M, Lodish HF (2011) Formation of mammalian erythrocytes: chromatin condensation
583 and enucleation Trends Cell Biol 21:409-415 doi:10.1016/j.tcb.2011.04.003
584 Jo YJ, Jang WI, Kim NH, Namgoong S (2016) Tropomodulin-3 is essential in asymmetric division during
585 mouse oocyte maturation Sci Rep 6:29204 doi:10.1038/srep29204
586 Kee AJ et al. (2015) An actin filament population defined by the tropomyosin Tpm3.1 regulates glucose
587 uptake Traffic 16:691-711 doi:10.1111/tra.12282
588 Lecuit T, Yap AS (2015) E-cadherin junctions as active mechanical integrators in tissue dynamics Nat Cell
589 Biol 17:533-539 doi:10.1038/ncb3136
590 Lee CW, Vitriol EA, Shim S, Wise AL, Velayutham RP, Zheng JQ (2013) Dynamic localization of G-actin
591 during membrane protrusion in neuronal motility Curr Biol 23:1046-1056
592 doi:10.1016/j.cub.2013.04.057
593 Lewis RA, Yamashiro S, Gokhin DS, Fowler VM (2014) Functional effects of mutations in the
594 tropomyosin-binding sites of tropomodulin1 and tropomodulin3 Cytoskeleton (Hoboken)
595 71:395-411 doi:10.1002/cm.21179
596 Li R (2013) The art of choreographing asymmetric cell division Dev Cell 25:439-450
597 doi:10.1016/j.devcel.2013.05.003
598 Lim CY, Bi X, Wu D, Kim JB, Gunning PW, Hong W, Han W (2015) Tropomodulin3 is a novel Akt2 effector
599 regulating insulin-stimulated GLUT4 exocytosis through cortical actin remodeling Nat Commun
600 6:5951 doi:10.1038/ncomms6951
601 Littlefield R, Almenar-Queralt A, Fowler VM (2001) Actin dynamics at pointed ends regulates thin
602 filament length in striated muscle Nat Cell Biol 3:544-551 doi:10.1038/35078517
603 Lopez-Ubeda R, Garcia-Vazquez FA, Romar R, Gadea J, Munoz M, Hunter RH, Coy P (2015) Oviductal
604 Transcriptome Is Modified after Insemination during Spontaneous Ovulation in the Sow PLoS
605 One 10:e0130128 doi:10.1371/journal.pone.0130128
606 Lopez JA et al. (2009) Identification of a distal GLUT4 trafficking event controlled by actin polymerization
607 Mol Biol Cell 20:3918-3929 doi:10.1091/mbc.E09-03-0187
608 Lu Y, Ye Y, Bao W, Yang Q, Wang J, Liu Z, Shi S (2017) Genome-wide identification of genes essential for
609 podocyte cytoskeletons based on single-cell RNA sequencing Kidney Int 92:1119-1129
610 doi:10.1016/j.kint.2017.04.022
611 McKeown CR, Nowak RB, Gokhin DS, Fowler VM (2014) Tropomyosin is required for cardiac
612 morphogenesis, myofibril assembly, and formation of adherens junctions in the developing
613 mouse embryo Dev Dyn 243:800-817 doi:10.1002/dvdy.24115
614 Mege RM, Ishiyama N (2017) Integration of Cadherin Adhesion and Cytoskeleton at Adherens Junctions
615 Cold Spring Harb Perspect Biol 9 doi:10.1101/cshperspect.a028738

Miklavc P, Ehinger K, Sultan A, Felder T, Paul P, Gottschalk KE, Frick M (2015) Actin depolymerisation and crosslinking join forces with myosin II to contract actin coats on fused secretory vesicles J Cell Sci 128:1193-1203 doi:10.1242/jcs.165571

Miklavc P, Hecht E, Hobi N, Wittekindt OH, Dietl P, Kranz C, Frick M (2012) Actin coating and compression of fused secretory vesicles are essential for surfactant secretion--a role for Rho, formins and myosin II J Cell Sci 125:2765-2774 doi:10.1242/jcs.105262

Moyer JD et al. (2010) Tropomodulin 1-null mice have a mild spherocytic elliptocytosis with appearance of tropomodulin 3 in red blood cells and disruption of the membrane skeleton Blood 116:2590-2599 doi:10.1182/blood-2010-02-268458

Namgoong S, Kim NH (2016) Roles of actin binding proteins in mammalian oocyte maturation and beyond Cell Cycle 15:1830-1843 doi:10.1080/15384101.2016.1181239

Naumanen P, Lappalainen P, Hotulainen P (2008) Mechanisms of actin stress fibre assembly J Microsc 231:446-454 doi:10.1111/j.1365-2818.2008.02057.x

Nowak RB et al. (2017) Tropomodulin 1 controls erythroblast enucleation via regulation of F-actin in the enucleosome Blood 130:1144-1155 doi:10.1182/blood-2017-05-787051

Ochala J, Gokhin DS, Iwamoto H, Fowler VM (2014) Pointed-end capping by tropomodulin modulates actomyosin crossbridge formation in skeletal muscle fibers FASEB J 28:408-415 doi:10.1096/fj.13-239640

Ojala PJ, Paavilainen VO, Vartiainen MK, Tuma R, Weeds AG, Lappalainen P (2002) The two ADF-H domains of twinfilin play functionally distinct roles in interactions with actin monomers Mol Biol Cell 13:3811-3821 doi:10.1091/mbc.e02-03-0157

Ono S (2010) Dynamic regulation of sarcomeric actin filaments in striated muscle Cytoskeleton (Hoboken) 67:677-692 doi:10.1002/cm.20476

Paez AV et al. (2016) Heme oxygenase-1 in the forefront of a multi-molecular network that governs cell-cell contacts and filopodia-induced zippering in prostate cancer Cell Death Dis 7:e2570 doi:10.1038/cddis.2016.420

Patel-Hett S et al. (2011) The spectrin-based membrane skeleton stabilizes mouse megakaryocyte membrane systems and is essential for proplatelet and platelet formation Blood 118:1641-1652 doi:10.1182/blood-2011-01-330688

Pathan-Chhatbar S, Taft MH, Reindl T, Hundt N, Latham SL, Manstein DJ (2018) Three mammalian tropomyosin isoforms have different regulatory effects on nonmuscle myosin-2B and filamentous beta-actin in vitro J Biol Chem 293:863-875 doi:10.1074/jbc.M117.806521

Pellegrin S, Mellor H (2007) Actin stress fibres J Cell Sci 120:3491-3499 doi:10.1242/jcs.018473

Pilot F, Lecuit T (2005) Compartmentalized morphogenesis in epithelia: from cell to tissue shape Dev Dyn 232:685-694 doi:10.1002/dvdy.20334

Pollard TD, Blanchoin L, Mullins RD (2000) Molecular mechanisms controlling actin filament dynamics in nonmuscle cells Annu Rev Biophys Biomol Struct 29:545-576 doi:10.1146/annurev.biophys.29.1.545

Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments Cell 112:453-465

Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement Science 326:1208-1212 doi:10.1126/science.1175862

Qiu J et al. (2010) Gene expression profiles of adipose tissue of high-fat diet-induced obese rats by cDNA microarrays Mol Biol Rep 37:3691-3695 doi:10.1007/s11033-010-0021-6

Rao JN, Madasu Y, Dominguez R (2014) Mechanism of actin filament pointed-end capping by tropomodulin Science 345:463-467 doi:10.1126/science.1256159

Ridley AJ (2011) Life at the leading edge Cell 145:1012-1022 doi:10.1016/j.cell.2011.06.010

Stevenson TO, Mercer KB, Cox EA, Szewczyk NJ, Conley CA, Hardin JD, Benian GM (2007) unc-94 encodes a tropomodulin in Caenorhabditis elegans J Mol Biol 374:936-950 doi:10.1016/j.jmb.2007.10.005

Sui Z et al. (2014) Tropomodulin3-null mice are embryonic lethal with anemia due to impaired erythroid terminal differentiation in the fetal liver Blood 123:758-767 doi:10.1182/blood-2013-03-492710

Sui Z, Nowak RB, Sanada C, Halene S, Krause DS, Fowler VM (2015) Regulation of actin polymerization by tropomodulin-3 controls megakaryocyte actin organization and platelet biogenesis Blood 126:520-530 doi:10.1182/blood-2014-09-601484

Tran DT, Masedunskas A, Weigert R, Ten Hagen KG (2015) Arp2/3-mediated F-actin formation controls regulated exocytosis in vivo Nat Commun 6:10098 doi:10.1038/ncomms10098

Uversky VN, Shah SP, Gritsyna Y, Hitchcock-DeGregori SE, Kostyukova AS (2011) Systematic analysis of tropomodulin/tropomyosin interactions uncovers fine-tuned binding specificity of intrinsically disordered proteins J Mol Recognit 24:647-655 doi:10.1002/jmr.1093

Vitriol EA, McMillen LM, Kapustina M, Gomez SM, Vavylonis D, Zheng JQ (2015) Two functionally distinct sources of actin monomers supply the leading edge of lamellipodia Cell Rep 11:433-445 doi:10.1016/j.celrep.2015.03.033

Vuong-Brender TTK, Boutillon A, Rodriguez D, Lavilley V, Labouesse M (2018) HMP-1/alpha-catenin promotes junctional mechanical integrity during morphogenesis PLoS One 13:e0193279 doi:10.1371/journal.pone.0193279

Weber A, Pennise CR, Babcock GG, Fowler VM (1994) Tropomodulin caps the pointed ends of actin filaments J Cell Biol 127:1627-1635

Weber KL, Fischer RS, Fowler VM (2007) Tmod3 regulates polarized epithelial cell morphology J Cell Sci 120:3625-3632 doi:10.1242/jcs.011445

Yamashiro S, Gokhin DS, Kimura S, Nowak RB, Fowler VM (2012) Tropomodulins: pointed-end capping proteins that regulate actin filament architecture in diverse cell types Cytoskeleton (Hoboken) 69:337-370 doi:10.1002/cm.21031

Yamashiro S, Gokhin DS, Sui Z, Bergeron SE, Rubenstein PA, Fowler VM (2014) Differential actin-regulatory activities of Tropomodulin1 and Tropomodulin3 with diverse tropomyosin and actin isoforms J Biol Chem 289:11616-11629 doi:10.1074/jbc.M114.555128

Yamashiro S, Speicher KD, Speicher DW, Fowler VM (2010) Mammalian tropomodulins nucleate actin polymerization via their actin monomer binding and filament pointed end-capping activities J Biol Chem 285:33265-33280 doi:10.1074/jbc.M110.144873

Yang W et al. (2014) Arp2/3 complex regulates adipogenesis by controlling cortical actin remodelling Biochem J 464:179-192 doi:10.1042/BJ20140805

Yi K, Unruh JR, Deng M, Slaughter BD, Rubinstein B, Li R (2011) Dynamic maintenance of asymmetric meiotic spindle position through Arp2/3-complex-driven cytoplasmic streaming in mouse oocytes Nat Cell Biol 13:1252-1258 doi:10.1038/ncb2320

Yu FX, Lin SC, Morrison-Bogorad M, Atkinson MA, Yin HL (1993) Thymosin beta 10 and thymosin beta 4 are both actin monomer sequestering proteins J Biol Chem 268:502-509

Zhang J, Betson M, Erasmus J, Zeikos K, Bailly M, Cramer LP, Braga VM (2005) Actin at cell-cell junctions is composed of two dynamic and functional populations J Cell Sci 118:5549-5562 doi:10.1242/jcs.02639