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MyTH4-FERM myosins in the assembly and maintenance of actin-based protrusions

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Abstract

Unconventional myosins are actin-based molecular motors that serve a multitude of roles within the cell, contributing to cell shape and function. One group of myosin motors, MyTH4-FERM myosins, plays an integral part in building and maintaining finger-like protrusions, which allows cells to interact with their external environment. Suggested to act primarily as transporters, these motor proteins enrich adhesion molecules, actin-regulatory proteins and other factors at the tips of filopodia, microvilli, and stereocilia. Below we review data from biophysical, biochemical, and cell biological studies, which implicate these myosins as central players in the assembly, maintenance and function of actin-based protrusions.

Keywords

myosin-7; myosin-10; myosin-15; Myo7a; Myo7b; Myo10; Myo15a; filopodia; microvilli; brush border; IMAC; stereocilia; tip-link; hair bundle; hair cell

The physiological role of a differentiated cell is dictated in large part by its morphology, which in turn is controlled by the cytoskeleton. Although actin, microtubule, and intermediate filament networks each contribute to global cell shape control, this mini-review will focus on how cells employ the actin cytoskeleton and its associated motor proteins to create fine surface features, specifically the finger-like protrusions that enable biochemical and physical interactions with the external environment. Three general classes of such protrusions exist: filopodia, microvilli, and stereocilia (BOX 1 and Figure 1). **Filopodia** are highly dynamic and typically substrate-attached features that form on the ventral/basolateral cell surface of migrating cells where they function in detecting and responding to environmental cues. **Microvilli** are found on the apical surface of diverse epithelia, where they function to increase membrane area and thus, solute transport capacity; these protrusions are typically found in great numbers and are packed into highly ordered ensembles, also known as brush borders. **Stereocilia** are microvillus-derived features found on the surface of sensory epithelial cells in the cochlear and vestibular systems, where they are organized into rows of graded height known as ‘hair bundles’ and play a direct role in

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the mechanotransduction process. We use the word ‘protrusion’ herein to refer specifically to these finger-like features.

Box 1

Actin assembly in finger-like protrusions

Filopodia, microvilli, and stereocilia are finger-like plasma membrane protrusions supported by parallel bundles of actin filaments. In each of these cases, bundled filaments are oriented with barbed ends toward the protrusion tip. A general paradigm for actin bundle construction invokes coordinated filament nucleation, elongation, and bundling directly at membrane sites designated for protrusion formation (e.g. the leading edge of a motile cell, or the apical domain of a transporting or mechanosensory epithelial cell). Following assembly, protrusion maintenance and lifetime are controlled by factors that regulate actin filament turnover. While the molecules controlling these events in filopodia are well studied, factors governing the assembly and maintenance of microvilli and stereocilia are still poorly understood. This box highlights what we know about actin filament assembly in specific structures and points out gaps in our understanding that require additional investigation.

Filopodia

Filopodia are dynamic protrusions that extend into the extracellular space and allow cells to probe the physical and chemical features of their environment. These structures are composed of 10–30 bundled actin filaments and can reach up to 10 microns in length [95,96]. In response to extracellular signals, phosphatidylinositol kinases such as PIPK1 α localize to specific sites on the plasma membrane and generate phosphatidylinositol-phosphate PI(4,5)P₂, a critical signaling lipid involved in filopodia assembly [97]. Concurrently, the Rho family GTPase Cdc42 binds to newly synthesized PI(4,5)P₂ at the plasma membrane, and this lipid-protein complex activates multiple proteins involved in filopodia formation such as the Arp2/3 activating proteins WASp and N-WASP [98], formin mDia2 [99], the F-BAR protein TOCA-1 [100], and the I-BAR protein IRSp53, which is critical for localizing VASP to nascent filopodia [101]. Two models for filopodial assembly have been proposed by different groups, although it should be noted that both models are not mutually exclusive and mechanistic details of assembly are probably cell-type specific. The ‘convergent elongation’ model suggests that filopodia emerge from a network of branched actin filaments in the lamellipodia, which are initially nucleated by the Arp2/3 complex [96,102]. Following nucleation, a subset of these filaments are protected from capping at their barbed ends through the action of the actin polymerase VASP [103–105] and formins [106–108], and this promotes their continued elongation. Myo10 dimers are then able to focus these elongating barbed ends at the membrane in a motor domain dependent manner [40]. Once actin filaments are in close proximity to one another at the membrane, fascin recruitment to these filaments leads to assembly of a parallel actin bundle capable of driving and supporting membrane protrusion [109]. A second explanation for filopodial assembly is the ‘tip nucleation’ model [107]. Here, activated formins cluster at the membrane (e.g. mDia2) where they nucleate and elongate actin filaments which are subsequently bundled

in parallel by fascin. In both cases, actin monomers incorporate at the tips of the forming filopodial actin bundles and then treadmill through the bundle to the base at a rate of a few microns per minute [110]. There, the actin depolymerization factor cofilin, which preferentially disassembles fascin bundled actin filaments, mediates the recycling of filopodial F-actin back into the cytosolic G-actin pool [111]. Now that the general players in filopodial actin assembly have been determined, future studies must focus on mechanistic differences that give rise to distinct classes of filopodia in specific biological contexts.

Microvilli

Microvilli are plasma membrane protrusions that extend from the apical surface of epithelial cells that line hollow organs (e.g. gut, kidney, lung, inner ear, and gall bladder). Although much of what we know comes from studies of intestinal microvilli, factors relevant in this system probably control actin assembly in other types of microvilli as well. A single microvillus is supported by a parallel bundle of 30–40 actin filaments [112], which terminates in a subapical network of intermediate filaments known as the ‘terminal web’ [113]. Microvillus length can be remarkably uniform within and between cells (0.5–3 μm depending on the tissue); recent studies also suggest this parameter might be regulated by intermicrovillar adhesion [81^{**},82^{*}]. While it was recently shown that a protein complex containing the F-BAR domain containing protein syndapin-2 and the actin nucleator cordon-bleu regulates microvillar growth [114^{**}–116], the molecules that control the elongation of actin filaments in microvilli remain largely unknown and must be the subject of future studies. In this system, espin [117], fimbrin/plastin [118], and villin [119] contribute to actin filament bundling in a regionalized manner. While villin and espin localize throughout the microvillar actin bundle, fimbrin is enriched in the terminal web where, in addition to bundling actin filaments, it is thought to link the microvillar actin bundle with keratin-19 in the terminal web, eliciting further stabilization of microvilli [120]. Interestingly, mice lacking all three bundling proteins can still form microvilli, providing strong evidence for additional players [121]. The actin binding protein EPS8 is found at the tips of microvilli where it may bundle and cap actin filaments, and thereby control microvillar length [122,123]. Experiments in cultured polarized epithelial cells show that microvillar F-actin treadmills slowly, with actin incorporating at bundle tips and disassembling at the base [124,125], but the development of new model systems may allow investigators to revisit these measurements in primary cultures derived from mice or *in vivo* [126].

Stereocilia

Stereocilia are large microvillus-derived protrusions arranged in rows of graded height on the apical surface of cochlear and vestibular epithelial cells (also known as ‘hair cells’). These protrusions range from 1 micron in length at the proximal end of the cochlea to 100 microns in length in the vestibular epithelium [127]. During hair cell differentiation, stereocilia progress through phases of widening and elongation of their core F-actin bundle to ultimately achieve their final shape. A single mature stereocilium is supported by a bundle consisting of hundreds of actin filaments, which tapers at its base, creating the flexural rigidity required for mechanosensory function. The pointed ends of actin

filaments in stereocilia bundles are embedded in an actin-rich meshwork referred to as the ‘cuticular plate’, which functions to anchor stereocilia [128]. Specifically tropomyosin, α -actinin, and XIRP2 function to tightly crosslink actin filaments of the cuticular plate [129–131], whereas ACF7 links cuticular plate actin with the underlying microtubule cytoskeleton [132]. While elegant studies from Tilney described in detail changes in stereocilia morphology during development [133–138] (summarized above), our understanding of the molecules that control these precise changes during development and then maintain these structures is lacking. For example, the molecule(s) involved in the nucleation of stereocilia actin filaments are completely unknown. However a number of factors controlling elongation have been identified, including EPS8 [37,91] and espin-1 [139]. Conversely, the bundling proteins fascin-2 [140–142] and fimbrin [143] are dispensable for assembly but required for maintaining normal stereocilia width and height, and TRIOBP, taperin and Fam65b are required for bundling actin filaments specifically in the taper at the stereocilia base where the bundles terminate in the cuticular plate [144,145*]. Additionally, the actin capping protein twinfilin-2 [146] as well as gelsolin [147,148], an actin severing and capping protein, serve to restrict the length of stereocilia by inhibiting actin polymerization at the tips. Although an early study suggested that the actin bundle of stereocilia treadmills [149], subsequent studies agree that stereocilia actin bundles are stable and actin turnover occurs only at the tips of core bundle actin filaments [150*,151*]. With the development of a new system for growing and maintaining hair cells in organoid cultures [152**], as well as advances in stereocilia proteomic analyses that allow for identification of new low abundance proteins and protein complexes [72**], research in the stereocilia field is poised to make key advances in the near future.

Although significant distinctions between filopodia, microvilli, and stereocilia can be made in terms of the density of the protrusions (i.e. number of structures per cell), the shape of the supporting actin bundle (i.e. length, number of bundled filaments), and its mode of anchoring in the cell, these three systems also share some common features. For example, in each case a parallel bundle of actin filaments provides the mechanical rigidity needed for membrane deformation and protrusion. Additionally, all of these structures localize adhesion factors to their distal tips, allowing them to make physical attachments with either the substrate (as is the case of filopodia) or with adjacent protrusions (as is the case for stereocilia and microvilli); such attachments are critical for the organization of these structures. Another theme common to all three classes is the use of actin-based motors, i.e. myosins, for their formation, maintenance, and function. Myosins comprise a large superfamily of ATPases that interact with actin filaments to power motility or generate force for other subcellular applications. In general, myosins contain a highly conserved N-terminal motor (or head domain) where ATP hydrolysis and actin binding occur, a central neck region that binds one or more light chains and acts as a lever arm for force transduction, and a C-terminal tail domain that mediates cargo interactions and, in some cases, auto-inhibition.

MyTH4-FERM myosins as protrusion motors

One specific group of myosin motors implicated in the assembly, maintenance, and function of actin-based finger-like protrusions are the MyTH4-FERM myosins, consisting of Myo7a, Myo7b, Myo10, and Myo15a. Myo7a and Myo15a are found in hair cell stereocilia [1,2], Myo7b is found in microvilli on the surface of transporting epithelial cells in the kidney and gut [3], and Myo10 is found in filopodia on the surface of diverse cell types [4]. These barbed-end directed motors are grouped together because they contain at least one MyTH4-FERM (MF; myosin tail homology 4 - protein 4.1, ezrin, radixin, and moesin) domain in their cargo-binding tail (Figure 2). The distinct MyTH4 and FERM domains form a structural and functional supramodule [5,6]; the FERM domain mediates binding to distinct cargo proteins, whereas the MyTH4 domain functions in microtubule binding [5–8**]. Class 7 and 15 myosins have tandem MF domains separated by a SRC homology 3 (SH3) domain. Myo10 contains Pleckstrin homology (PH) domains followed by a single MF domain. None of these myosins are constitutive dimers; instead they form folded, monomeric structures in solution, as demonstrated with Myo7a and Myo10 [9–11]. All MyTH4-FERM myosins exhibit a high duty ratio [12–18], which is consistent with proposed functions in cargo transport, although other roles such as anchoring or channel gating have also been postulated. Below we discuss in more detail how MyTH4-FERM myosins contribute to the assembly and function of finger-like protrusions.

Movement and localization of MyTH4-FERM myosins in protrusions

The most intuitive function for a myosin motor operating within a protrusion would be transport of materials necessary for assembly and maintenance to the distal tips of these structures, where the dynamic barbed-ends of actin filaments are found. Myo10 is the only MyTH4-FERM myosin where clear evidence for processive motility has been demonstrated in cells, with a velocity of 600 nm/s [19,20]. This is likely due to the fact that its native protrusion, the filopodium, is amenable to time-lapse imaging with high spatial and temporal resolution. Although the protrusions discussed in this review are all supported by parallel actin bundles, each system contains its own unique complement of actin nucleators, elongators, and bundlers (BOX 1). These distinctions may represent important regulatory factors that serve to guide specific myosins to the proper actin networks *in vivo* [21]. Indeed, *in vitro* assays have demonstrated that Myo10 prefers actin bundles as compared to single actin filaments [22–24*]. Track selectivity of Myo10 is also likely mediated by its anti-parallel coiled-coil, which is required for its function in filopodial induction [25]. This structural feature enables a wider range of step sizes [23,24*,26], which provides access to multiple actin binding sites within the bundle; this in turn might allow Myo10 to navigate around obstacles posed by other actin binding proteins found within the filopodium. Through its PH domains, Myo10 binds to and is regulated by its interaction with phosphatidylinositol-3,4,5-triphosphate [10,27], which may act as a method of localized activation since this lipid is enriched in the leading edge of migrating cells, the site of filopodial formation [28–30]. This interaction is thought to relieve auto-inhibition, which in turn, enables dimerization, cargo binding, and motor-driven transport.

In the case of tandem MyTH4-FERM myosins, direct evidence for processive motility has yet to be visualized in cells, although existing evidence does support the idea that at least one aspect of function is a role in transport. Myo15a and Myo7b target to the distal tips of stereocilia and microvilli, respectively, while Myo7a localizes to the upper tip-link density and ankle links of stereocilia [31–33^{***}]. Although monomeric head-neck constructs that lack cargo-binding domains are unable to tip target, forced dimers of Myo7a and Myo7b motor domains do exhibit tip localization in filopodia and microvilli, respectively, demonstrating the potential for processive movement when multimerized [9^{*},33^{***},34]. Motor activity is required for tip enrichment, further suggesting that this is an active process [33^{***},34]. Additionally, a forced dimer of Myo7a is processive *in vitro* [35]. In the case of Myo15a, a full-length construct also moves toward filopodial tips in a manner that requires a functional motor domain [36]. Collectively, these studies implicate motor activity as being critical for the enrichment of MyTH4-FERM myosins at the distal tips of protrusions.

In contrast to Myo10, tandem MyTH4-FERM myosins do not possess well-defined coiled-coil domains. To move processively along parallel actin bundles, these myosins would need to undergo some form of dimerization or oligomerization, which could be regulated by cargo binding. In exogenous cell culture models (e.g. Cos-7 cells), dimerization and localization of full-length Myo7a at filopodial tips is dependent on co-expression with its cargo, suggesting that cargo binding plays a key role in activating transporter function [34]. Additionally, co-expression of Myo15a with its cargo proteins results in tip localization or increased enrichment of the cargo at protrusion tips in both heterologous culture models and endogenous hair cells [34,36–38]. Translocation of Myo15a with its cargoes was also visualized in live Cos7 cells [36,37]. Together these findings point to a motor-dependent targeting mechanism that requires cargo binding for activation, likely through release of auto-inhibition, induction of dimerization/oligomerization, or both. Moreover, while all of these studies implicate motor activity in tip targeting of tandem MyTH4-FERM myosins, they do not rule out a role in cargo anchoring or retention. These more passive mechanisms find some support in recent studies on Myo7b, which demonstrated that a mutant predicted to exhibit defects in force generation was able to target to microvillar tips and partially rescue enrichment of specific cargoes [33^{***}]. However, even in that case, normal motor activity was required for full rescue of cargo enrichment and downstream functions [33^{***}].

MyTH4-FERM myosin cargoes and physiological functions

Given the immediate functional insight provided by the identification of binding partners, the search for MyTH4-FERM domain interacting cargoes has been an area of extensive investigation. In the case of Myo10, numerous cargoes have already been uncovered and their physiological functions defined. Overexpression of Myo10 has been shown to induce filopodia formation and elongation [19,39]. Its ability to initiate filopodia formation is intrinsic to Myo10 itself, as a forced dimer construct lacking any cargo-binding tail domains was sufficient to increase the number of protrusions [40]. However, elongation and stabilization of filopodia are linked to molecules that Myo10 transports to the tips. One such factor is Mena/VASP [41], which promotes actin filament elongation by preventing barbed-end capping [42]. Additionally, Myo10 interacts with and transports β -integrins to filpodial tips to mediate cell-substrate adhesion and stabilize protrusions [43]. Myo10 also binds to

transmembrane netrin receptors DCC and neogenin, and disruption of these interactions results in defects of axonal projection and path finding [5,7,44]. These cargoes have been shown to differentially regulate Myo10 activity and induction of filopodia formation, with DCC enhancing basal filopodia elongation and neogenin promoting dorsal filopodia growth [45]. VE-cadherin and N-cadherin are Myo10 cargoes that function in the formation of early endothelial cell-cell contacts and neuronal radial migration, respectively [46,47]. Myo10 also plays a role in endothelial cell migration by transporting BMP6 receptor ALK6 and inducing filopodia formation in a BMP-dependent manner [48]. The fundamental role that this myosin plays in filopodial formation and elongation is conserved in lower eukaryotes; in the social amoeba *Dictyostelium*, MyTH4-FERM myosin, Myo7, targets to and promotes the growth of filopods [49]. Apart from its role in filopodia formation, Myo10 also interacts with tubulin in the context of nuclear anchoring, centrosome positioning, and spindle assembly [50–52]. Upregulation of Myo10 is associated with increased breast cancer cell invasion and metastasis, as well as poor prognosis [53,54].

Myo7a cargo binding interactions are critical for the normal mechanosensory function of stereocilia that extend from the apex of inner ear hair cells. Some studies have localized this MyTH4-FERM myosin to the upper end of the ‘tip-link’ structure [32], which physically connects the tip of one stereocilium to the side of its taller neighbor. Tip-links are *trans* heterophilic complexes composed of cadherin-23 (CDH23) and protocadherin-15 (PCDH15) [55]. PCDH15 localizes to the lower tip-link where it interacts with TMC1 and TMC2, which have been implicated as components of the mechanotransduction (MET) channel [55–58]. CDH23 localizes to the upper portion of the tip-link [55]. At upper tip-link densities, Myo7a uses its N-terminal MyTH4-FERM domain to interact directly with the ankyrin repeat protein, SANS [6,59], which in turn interacts with the actin-bundling PDZ scaffolding protein, harmonin/USH1C (isoform b) [59,60]. The Myo7a/SANS/USH1C complex interacts with the cytoplasmic domain of CDH23 through the N-terminal PDZ domains of USH1C [59,61–65]. Although the only direct cargo binding interaction in this case is with SANS, Myo7a is indirectly responsible for the localization of USH1C [62]. Mutations in Myo7a are responsible for several genetic hearing impairments, including Usher syndrome type 1B (USH1B) [66], autosomal dominant non-syndromic deafness (DFNA11) [67], and autosomal recessive non-syndromic deafness (DFNB2) [68]. Type 1 Usher syndrome patients also develop retinitis pigmentosa and progressive vision loss. Expression of mutant variants of Myo7a in mouse models result in a range of morphological and physiological defects with abnormal inner ear function [69–71]. Mutations that have profound effects on protein stability and expression result in structural and organizational defects of the hair bundle. Although stereocilia still grow and form rows of graded height, they become increasingly disordered after birth, with abnormalities in orientation and position [71]. This eventually leads to the degeneration and reabsorption of some or all of the stereocilia. Additionally, without Myo7a, hair bundles must be deflected beyond their physiological operating range to open the MET channel [69]. More recently, it was shown that Myo7a interacts and colocalizes with PDZD7 at the ankle-link region of stereocilia [72**]. This structure couples stereocilia at their base to maintain hair bundle morphology during development [73,74]. Together, these data suggest that the primary role of Myo7a is not in the formation or elongation of stereocilia per se, but rather in the positioning and

maintenance of functional tip-links and ankle links. Although not discussed in depth in this review, Myo7a also regulates cargo transport in photoreceptors [75,76] and melanosome transport in retinal pigmented epithelium through complex formation with adaptor molecules MyRip and Rab27a [34,77–80].

Within microvilli, Myo7b interacts with components of an adhesion complex that bears a striking resemblance to the tip-link complex found in hair cells. This intermicrovillar adhesion complex (IMAC) consists of two scaffolding proteins, ANKS4B and USH1C (isoform a), and two protocadherins, protocadherin-24 (PCDH24/CDHR2) and mucin-like protocadherin (MLPCDH/CDHR5) [81^{••},82[•]]. The protocadherins form a *trans* heterophilic interaction between adjacent microvilli, and play a critical role in brush border assembly and organization [81^{••}]. IMAC components are enriched at the distal tips of microvilli, and this localization is essential for its function [33^{••},81^{••},82[•]]. Myo7b has been shown to directly interact with ANKS4B through its MF1 domain, and USH1C through its MF2 domain [81^{••}–83^{••}]. Interactions between the protocadherins and Myo7b have also been mapped to the MF2 domain [81^{••}]. Additionally, USH1C binds directly to ANKS4B, CDHR2, and CDHR5 [81^{••}–83^{••}]. The multitude of interactions between IMAC components provides a potential mode for higher order oligomerization of Myo7b into transport competent ensembles. Loss of any single IMAC component through knockdown or knockout disrupts microvillar clustering and brush border formation [33^{••},81^{••},82[•]]. In the absence of Myo7b, IMAC components become diffusely localized along the microvillar axis, indicating that an important function of this motor is to enrich IMAC components at microvillar tips [33^{••}]. Interestingly, type 1 Usher syndrome patients with large deletions in USH1C, the only shared component of the tip-link and IMAC, also suffer from enteropathies [84]. Analysis of intestinal tissue from USH1C knockout mice also revealed defects in brush border morphology [81^{••}]. Although previous studies provide clear evidence indicating that Myo7b plays a role in microvillar organization and packing, this motor might also play an indirect role in controlling the length of these protrusions by positioning CDHR2 at the distal tips. Indeed, knockdown of CDHR2 results in disheveled microvilli and a loss of length uniformity [81^{••}].

Myo15a localizes to the tips of stereocilia and is required for stereocilia elongation and staircase formation [31]. The expression and targeting of Myo15a coincides with the onset of staircase development in the hair bundle, suggesting a potential role in regulation of actin polymerization. Whirlin, a PDZ scaffolding protein, also localizes to stereocilia tips [36,38] and colocalizes with Myo15a [36]. Myo15a directly binds to and translocate whirlin to filopodial tips in cultured cells [36,38]. Additionally, epidermal growth factor receptor pathway substrate 8 (Eps8) localizes to stereocilia tips and directly interacts with both Myo15a and whirlin [37]. In mice lacking Myo15a, both whirlin and Eps8 are absent from stereocilia [36,37]. Because Eps8 is an actin bundling and capping protein [85], these findings might offer a molecular rationale for how Myo15a modulates the actin dynamics. Indeed, Myo15a enhances Eps8 tip accumulation and protrusion elongation in both filopodia and stereocilia [37]. These data suggest that Myo15a forms a complex with whirlin and Eps8 to transport these cargoes to the distal tips and promote stereocilia growth. Myo15a was originally identified as the causative gene for human autosomal recessive non-syndromic deafness, DFNB3 [86,87]. In mouse models, mutations in the motor or tail domain of

Myo15a results in short hair bundles and profound hearing loss [88,89]. Consistent with its potential function in localizing the proteins whirlin and Eps8, knockout of either cargo phenocopies the Myo15a knockout with decreased stereocilia length and function [36,37,90,91]. Mutations in these cargo proteins also lead to autosomal recessive non-syndromic deafness [90,92]. These data support a role for Myo15a in building stereocilia by localizing actin-regulatory proteins to the tips.

Myo15a also plays a major role in differential length regulation of stereocilia in different hair bundle rows. More recently, two Myo15a isoforms have been identified to serve differential roles in hair bundle morphology [93**]. Isoform 1 contains a long N-terminal extension and targets to the tips of the shorter stereocilia rows [93**]. Conversely, the isoform lacking this extension, isoform 2, localizes to the longest stereocilia row and is sufficient to target the cargo proteins Eps8 and whirlin [93**]. Specific knockout of isoform 1 unexpectedly resulted in normal hair bundle development [93**]. However, shortly after birth, mutant mice developed severe hearing loss, resulting from the degeneration of the mechanosensing shorter rows [93**]. This study revealed distinct isoform functions for building the hair bundle: initial development of stereocilia that is isoform 2 dependent and maintenance of the mature structure which requires isoform 1 [93**]. What determines the differential localization and cargo binding remains unknown, and is an intriguing question for further investigation.

Conclusions

Although significant advances have been made in understanding how MyTH4-FERM myosins function within finger-like protrusions, many unanswered questions still remain. For example, Myo10 seems to be the only mammalian MyTH4-FERM myosin that functions in protrusion formation *per se*. Existing models suggest that the two motor domains of Myo10 might function as a bundle initiator, by bringing actin filaments together for anti-capping and stable bundling by fascin [40]. This type of bundle initiating activity may be specific to Myo10 given the mechanism of filopodial bundle formation, where filaments originate from a dense meshwork of branched actin (BOX 1 and Figure 1). The tandem MyTH4-FERM myosins (class 7 and 15) appear to function only in protrusion elongation and organization. Whether this is related to the fact that stereocilia and microvilli emerge from the cell using very different mechanisms than filopodia remains an open question (BOX 1). Apart from Myo10, how do the tandem MyTH4-FERM motors come together to form units capable of processive motility? Although evidence suggests that dimerization is sufficient, the number of motors within a functional unit or complex *in vivo* remains unknown. Other models suggest that directed processive motion might not be the whole story. For example, Myo7b and Myo10 might use motor activity to bias diffusion and promote tip ward movement along parallel actin bundles, as suggested in recent studies [33**,94**]. A final question relates to the apparent selectivity that MyTH4/FERM myosins demonstrate toward specific actin populations within the cell. Does this selectivity result from binding to pre-localized cargoes (lipids or proteins) and thus localized activation, or the inherent capacity for these motors to recognize actin tracks with specific features? The latter possibility finds support in previous studies on Myo10, which selectively targets fascin-bundled actin filaments like those that support filopodia [22]. The extent to which class 7

and 15 myosins are structurally tuned to move within microvilli and stereocilia remains to be determined. New approaches that combine CRISPR-based endogenous-tagging of these motors with state-of-the-art super-resolution imaging systems should enable investigators to tackle questions along these lines in live cells.

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Figure 1.

Finger-like membrane protrusions featured in this review: (A) filopodia, (B) microvilli, and (C) stereocilia. The relevant MyTH4-FERM myosins and their cargoes are listed in each case.

Figure 2.

Domain diagram of the MyTH4-FERM myosins. Numbers indicate amino acids. Figure depicts isoform 1 for Myo15a; isoform 2 (2307 amino acids) does not include the N-terminal extension. *Hs*, *Homo sapiens*; IQs, IQ calmodulin- or light chain-binding motifs; SAH, stable alpha helix; anti-CC, anti-parallel coiled coil; PEST, proline, glutamate, serine, threonine peptide sequence for protein degradation; PDZ, post synaptic density protein (PSD95), Disc large homolog 1 (Dlg1), and zonula occludens-1 (ZO-1).