Unconventional Myosins in Cell Movement, Membrane Traffic, and Signal Transduction

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In the past few years genetic, biochemical, and cytolocalization data have implicated members of the myosin superfamily of actin-based molecular motors in a variety of cellular functions including membrane trafficking, cell movements, and signal transduction. The importance of myosins is illustrated by the identification of myosin genes as targets for disease-causing mutations. The task at hand is to decipher how the multitude of myosins function at both the molecular and cellular level—a task facilitated by our understanding of myosin structure and function in muscle.

Over the past several years, evidence has emerged for the existence of a large superfamily of myosins. To date, at least 13 structurally distinct classes (*1*) of myosin heavy chains have been identified in addition to the well-characterized myosins-II of muscle and nonmuscle cells (Figs. 1 and 2). These classes are empirically defined on the basis of sequence comparisons of their conserved motor or head domains, although nothing is known about the mechanochemical properties of most of these myosins. The motor domains of characterized myosins bind actin in an adenosine triphosphate (ATP)–sensitive manner and generate force through the hydrolysis of ATP. Almost all known myosins consist of this NH_2-terminal motor domain linked to a COOH-terminal tail via a neck domain that serves as the binding site for myosin light chains (*2*). Although these classes are defined by differences in head structure, the tail domains are also characteristic of a given myosin class. A subset of myosins have tail domains with predicted coiled-coil–forming α-helical domains, suggesting that these myosins, like M2, are two-headed, whereas others may be single-headed like M1. As noted below, some myosin tail domains contain structural motifs found in other proteins (Fig. 2), but the functions for myosin tail domains are largely unknown. However, a common assumption is that the tail directs the interaction of a given myosin with its cargo.

Some myosin classes exhibit a broad range of phylogenetic expression, whereas others have been identified in only a single organism thus far (Fig. 1). Budding yeast has only five myosin genes distributed among three classes (I, II, and V), whereas 26 myosin genes in seven classes have been identified thus far in mouse (*3*). Within a given vertebrate cell type, expression of at least a dozen myosins has been documented (*4*), suggesting that there may be a wide range of functions for actin-based motors in the cell (Table 1). In this review we focus on recent evidence for functions associated with the so-called "unconventional" nonmuscle myosins (Table 1). The reader is referred to comprehensive reviews for discussion of M2 functions, biochemistry, and structure (*5*) and for discussion of the biochemistry and structure of unconventional myosins (*6*).

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Myosins in Membrane Traffic and Organelle Movements

The most striking examples of actin-dependent organelle movement occur in plants. These include dramatic cytoplasmic streaming observed in *Characean* algae, stamen hairs, and pollen tubes. An actomyosin system appears to transport vesicles and the male germ unit along pollen tubes (*7*). Although several plant myosin genes have been cloned, the specific myosins involved in these phenomena remain unidentified. Antibodies raised against heterologous myosins react with small particles and organelles in *Lilium longiflorum* pollen tubes; these antigens may participate in transport through the pollen tube (*8*). A myosin morphologically similar to vertebrate M5 has been purified from *Chara* (*9*).

Direct evidence for the involvement of specific myosins in organelle movements is sparse. In the early *Drosophila* embryo, myosin 95F (class VI) has been implicated in critical cargo-transport functions. Class VI myosin has an insert in the head domain and is predicted to dimerize, based on the coil-coil sequence motif in the tail region (Figs. 1 and 2). 95F myosin–associated particles undergo cell cycle–dependent, 95F myosin–dependent directed movement in vivo. Antibody inhibition of 95F myosin blocks the directed transport of these particles and results in misorganization of the cortical actin cytoskeleton (*10*). In mice

Table 1. Potential functions for unconventional myosins discussed in this review.

Potential function	Myosin	Class
Cell growth and development	*Dictyostelium* myoA, B, C	I
	Yeast Myo3p, 5p	I
	Aspergillus MYOA	I
Cell movement	*Dictyostelium* myoA, B	I
RNA transport	Yeast Myo4p	V
Organelle particle movement	Chick brain M5a	V
	Mouse dilute	V
	Yeast Myo2p	V
	Drosophila 95F	VI
Chitin localization	Yeast Myo2p	V
Melanosome transport	Mouse dilute	V
Vacuole inheritance	Yeast Myo2p	V
Endocytosis	*Dictyostelium* myoA, B, C, D	I
	Yeast Myo3p, 5p	I
Exocytosis	*Dictyostelium* myoA, B	I
	Yeast Myo3p, 5p	I
	Aspergillus MYOA	I
Photoreceptor membrane trafficking	*Drosophila* ninaC	III
	Human myosin-7a	VII
Phototransduction	*Drosophila* ninaC	III
Rhabdomere integrity	*Drosophila* ninaC	III
Stabilizing or anchoring stereocilia	Mouse Snell's waltzer	VI
	Human M7a	VII
Hair cell adaptation	M1β	I
Leukocyte differentiation	Human M9b	IX
Signal transduction	Human M9b	IX
	Rat myr5	IX

with mutations in M6, embryogenesis appears to be normal, except for inner ear development (see below).

There is ample indirect biochemical, cytological, and genetic evidence to implicate the class V myosins (M5) in organelle movement. These myosins are two-headed and bind calmodulin and an 8-kD light chain of another potential organelle motor, cytoplasmic dynein (*11*) (Figs. 1 and 2). Chick brain M5a has motor activity and unlike other biochemically characterized myosins has a high affinity for actin in the presence of ATP (*12*); therefore, M5 may remain actin-associated for long periods of time. This would prevent M5a and its cargo from diffusing away from actin at moderate motor densities (*13*).

The subcellular localization of M5a in neurons suggests that this motor is partially organelle-associated. M5a is associated with punctate structures (*14*) and with small organelles that colocalize with both microtubules and actin filaments in growth cones (*15*). The presence of M5a on organelles that associate with both microtubules and actin suggests that this motor can be a passenger on organelles moved by microtubule motors, a phenomenon directly observed for an uncharacterized actin-based motor found on organelles in squid axoplasm (*16*). A role for M5a in synaptic vesicle movement has been suggested by the demonstration that this myosin is present in preparations of purified synaptic vesicles (*17*). However, mice bearing mutations in M5a (see below) exhibit normal numbers and localization of synaptic vesicles (*18*).

Characterization of *dilute* mice, which have mutations in M5a (*19*), also indicates a diversity of potential transport functions for this myosin, including movement (or proper localization) of smooth endoplasmic reticulum (SER) in neurons and pigment granules in melanocytes. The SER does not extend into the dendrites of Purkinje cells of *dilute* mice (*18*) and a presumed *dilute* rat (*20*); this may disrupt regulation of intracellular Ca^{2+} and result in the seizures observed in these animals (Fig. 3).

Although M5a also associates with other organelles in neurons, neurons cultured from *dilute* mice exhibit apparently normal growth cone morphology and motility (*15*), suggesting that M5a within the growth cone may be either nonessential or compensated for by another motor. This result contrasts with evidence for a role of M5a in extension of growth cone filopodia (*21*).

Dilute mice also exhibit abnormal coat pigmentation that results from defects in transfer of pigment granules from the dendritic processes of melanocytes to the keratinocytes of the hair shaft; the melanosomes

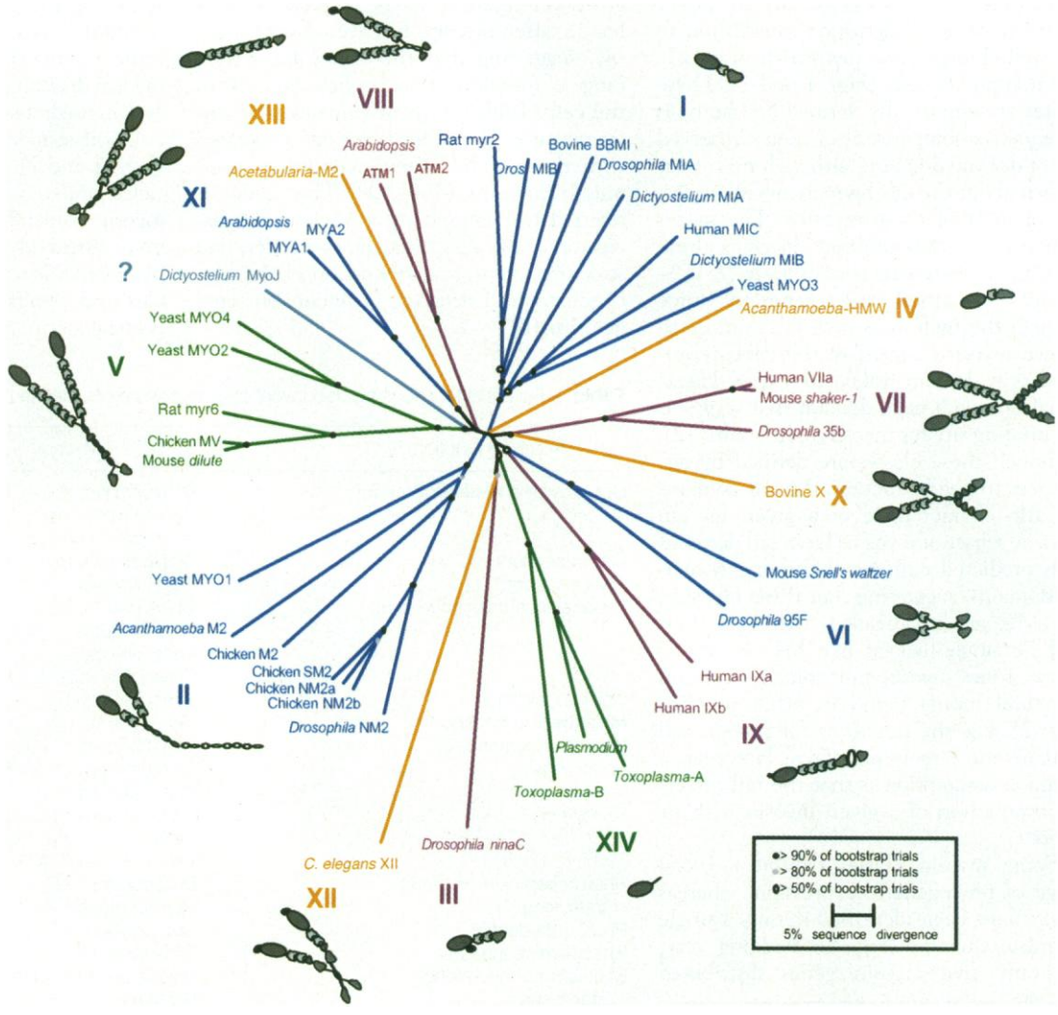

Fig. 1. An unrooted phylogenetic tree of the myosin superfamily based on head domain protein sequences. Myosin classes are shown in roman numerals. Sequence divergence is proportional to the length of the connecting branches. The percentage of times a node occurred out of 1000 bootstrap trials is indicated. Cartoon structures for class I, II, and V are based on experimental evidence, the rest on sequence homologies (Fig. 2). The large globular domains represent heads, smaller globular structures represent neck and tail motifs, and twisted lines denote coiled-coils; see Fig. 2 for more structural detail. The alignment was produced with the default settings of ClustalW (*78*) and the tree created by Drawtree (Phylip software package, http://evolution.genetics.washington.edu/phylip.html). Accession numbers or references for sequences used are as follows: *Acanthamoeba* HMW IV j05678, M2 p05659; *Acetabularia* M2 u94398; *Arabidopsis* ATM1 x69505, ATM2 z34292, MYA1 z28389, MYA2 z34294; bovine BBMI s36618, MX u55042; *Caenorhabditis elegans* XII u66563; chicken M2 p13538, MV q02440, NM2a p14105, NM2b by3402, SM2 p13538; *Dictyostelium* MIA p22467, MyoJ u42409, MIB sp34092; *Drosophila* 35b D. Kiehardt, personal communication, 95F q01989, MIA u07595, MIB u07596, *ninaC* p10676, NM2 u35816; human MIXa (*4*), MIXb u42391, MIC u14391, MVIIau55208; mouse *dilute* x57377, MVI u49739, MVIIa u81453; *Plasmodium* y09693; rat Myr2 s53714, Myr6 u60416; *Toxoplasma* A af006626, Baf006627; yeast MYO1 x57377, MYO2 p19524, MYO3 p36006, MYO4 p32492.

accumulate abnormally in the perinuclear region of melanocytes (*22*). Immunofluorescence studies have shown that M5 partially colocalizes with melanosomes and ER (*23*, *24*). Although the function of M5 in melanosome distribution remains undetermined, data suggest roles for M5 in melanosome delivery to dendrites, tethering of melanosomes in dendritic arbors (*23*, *24*), or both (Fig. 3).

M5a may also have essential functions in cells of the immune system. Recent studies have identified mutations in M5a as the basis for human Griscelli disease (*25*). Similar to *dilute* mice, patients with Griscelli disease have partial albinism and a range of neurological defects; in addition, they exhibit immunological defects including hypogammaglobulinemia and deficient antibody production (*26*).

Mutational analysis of the essential class V myosin encoded by the *Saccharomyces cerevisiae MYO2* gene also suggests a role for M5 in organelle movement. A temperature-sensitive mutation in this gene causes an accumulation of vesicles primarily in the mother cell (*27*) at the restrictive temperature. Myo2p may function in the transport of the yeast vacuole, Chs3p (a chitin synthase), and SEC4 [a small guanosine triphosphatase (GTPase)] because these are mislocalized in *MYO2* mutants (*28*).

Another class V myosin in yeast, *MYO4* is required for the daughter cell localization of Ash1p, a protein required for mating-type switching (*29*). The expression of Ash1p in daughter cells is a consequence of the asymmetric localization of *ASH1* mRNA (*30*). M5 may transport a factor that anchors ASH1 mRNA to the daughter bud tip or may transport particles containing *ASH1* mRNA (*30*).

Class I Myosins in Membrane Traffic and Cell Locomotion

Recent studies suggest roles for the class I myosins in endocytic and exocytic membrane traffic. The M1s are single-headed and possess tails of various length (Figs. 1 and 2). Much of our understanding of the biochemical and functional properties of these myosins derives from studies of the amoeboid organisms *Acanthamoeba* and *Dictyostelium*. In both of these organisms, multiple M1s are expressed and, on the basis of results of gene knockout studies in *Dictyostelium discoideum*, these myosins may act in concert, or have overlapping functions (*31*). For example, two forms of endocytosis [macropinocytosis (*32*, *33*) and phagocytosis (*33*)] are impaired in cells lacking, and sometimes overexpressing (*34*), certain single or multiple M1 genes (*35*). Similarly, deletion of one of the M1s in budding yeast results in defects in receptor-mediated endocytosis, whereas deletion of both results in defects in fluid-phase endocytosis (*36*). Little is known about the role of M1s in vertebrate endocytic pathways, although localization of M1 to phagocytic cups in macrophages suggests involvement in phagocytosis (*37*).

Class I myosins are also implicated as transporters or regulators of exocytic pathways. In *Dictyostelium*, MyoA and MyoB appear to be involved in secretion of lysosomal enzymes (*38*). A mutation in the only M1 identified in *Aspergillus nidulans* affects secretion (*39*). Although functional evidence is lacking, localization studies with antibodies to M1s suggest that class I myosins may associate with Golgi and secretory granules in vertebrate cells (*40*).

The same knockout strategies used to implicate M1s in endocytic pathways in *Dictyostelium* also reveal a role for M1s in cellular locomotion in this organism. Cells lacking either myoA or myoB exhibit defects in movement (that is, pseudopod extension) (*41*, *42*). myoA, and perhaps myoB, may be necessary to suppress inappropriate or inefficient extension of pseudopods onto substrate, possibly by regulating the strength or stiffness of the actin-based gel in the cell cortex (*42*).

Development in *Dictyostelium* is also impaired by M1 deletions, which may result from defects in the required movements or impairment of signaling pathways necessary for morphogenesis. Cells lacking myoA and myoB or myoB and myoC have abnormal fruiting bodies (*32*). Deletion of certain M1s impairs motility in chemotactic streaming assays (*35*). M1 mutations also affect polarized cell growth in *A. nidulans* and *S. cerevisiae* (*43*). These effects may be the result of secretion defects or secretion-independent effects on morphogenesis.

The examples summarized above provide clear evidence for the involvement of myosins in a variety of membrane-based phenomena, including organelle move-

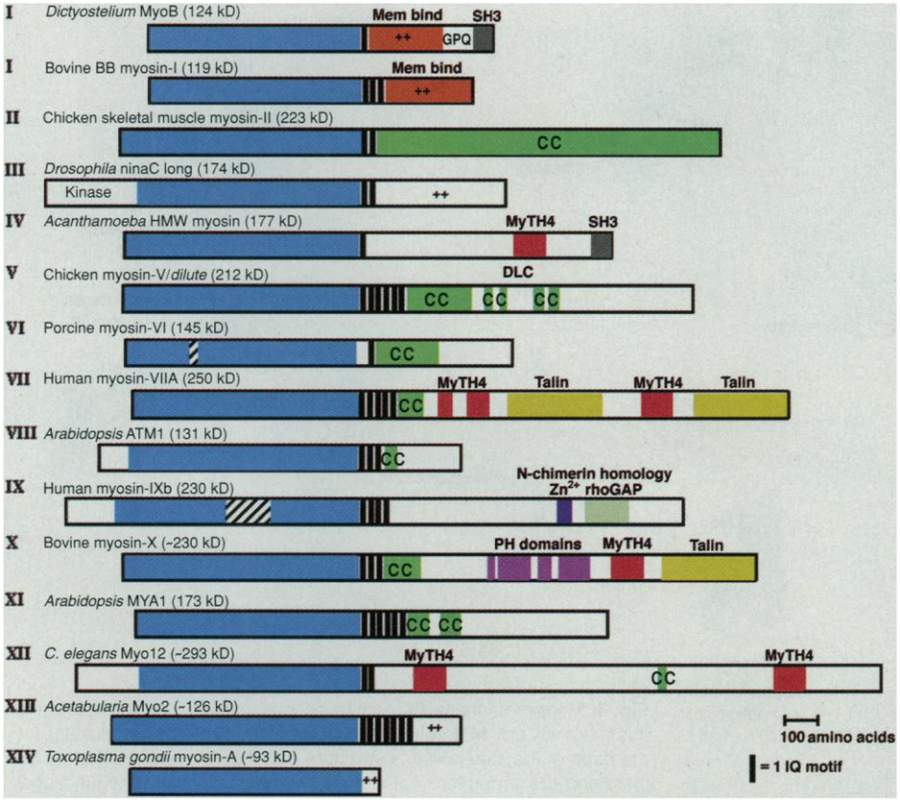

Fig. 2. Schematic diagram demonstrating the diversity of myosin structure. Shown are examples from each of the myosin classes. The motor domains are colored blue, with inserts shown as diagonally hatched boxes. Colored boxes in the tail domains represent different regions predicted by sequence homology: GPQ denotes a glycine-, proline-, and glutamine-rich region; ++, positively charged regions; SH3, SRChomology 3 domains; CC, coiled-coil domains; MyTH4, myosin tail homology 4 domains; DLC, presumptive dynein light chain binding domain; talin, talin homology domain; Zn^{2+}, zinc binding domain; rhoGAP, rho GTPase-activating protein domain; and PH, pleckstrin homology domain. See Fig. 1 for sequence accession numbers.

ments and cellular locomotion; however, the mechanism underlying the role of any given myosin in these processes remains largely unknown. Do myosins, as appears to be the case in certain plant cells, directly move organelles along actin tracks either in concert with or independent of microtubule-based membrane traffic? Do myosins facilitate docking, and subsequent movement, of tail-bound cargo on actin-rich cortical arrays? Finally, do myosins provide mechanochemical input into the multiple vesicle fission or fusion events that drive membrane traffic in the cell?

Myosins in Sensory Function

Myosins are essential in a number of sensory systems, including those of hearing, balance, and vision. One of the more divergent myosins known to play a role in sensory function is the class III myosin encoded by *Drosophila ninaC*. The *ninaC* proteins are unusual in that a predicted kinase domain is found NH_2-terminal of the motor domain (*44*) (Figs. 1 and 2). *NinaC* null flies display two phenotypes: electrophysiological aberrations suggesting a role in phototransduction as well as age- and light-dependent degeneration of photoreceptor cells.

NinaC encodes two alternatively spliced protein isoforms, p174 and p132. The myosin and kinase domains of the p174 *ninaC* isoform have separable functions. Deletion of the kinase domain produces defects in the electrophysiological response to light; in addition to this defect, deletion of the myosin domain results in retinal degeneration and loss of p174 localization from the microvilli of the rhabdomere (*45*) (the site of phototransduction in Arthropods) (Fig. 3). An expressed *ni-*

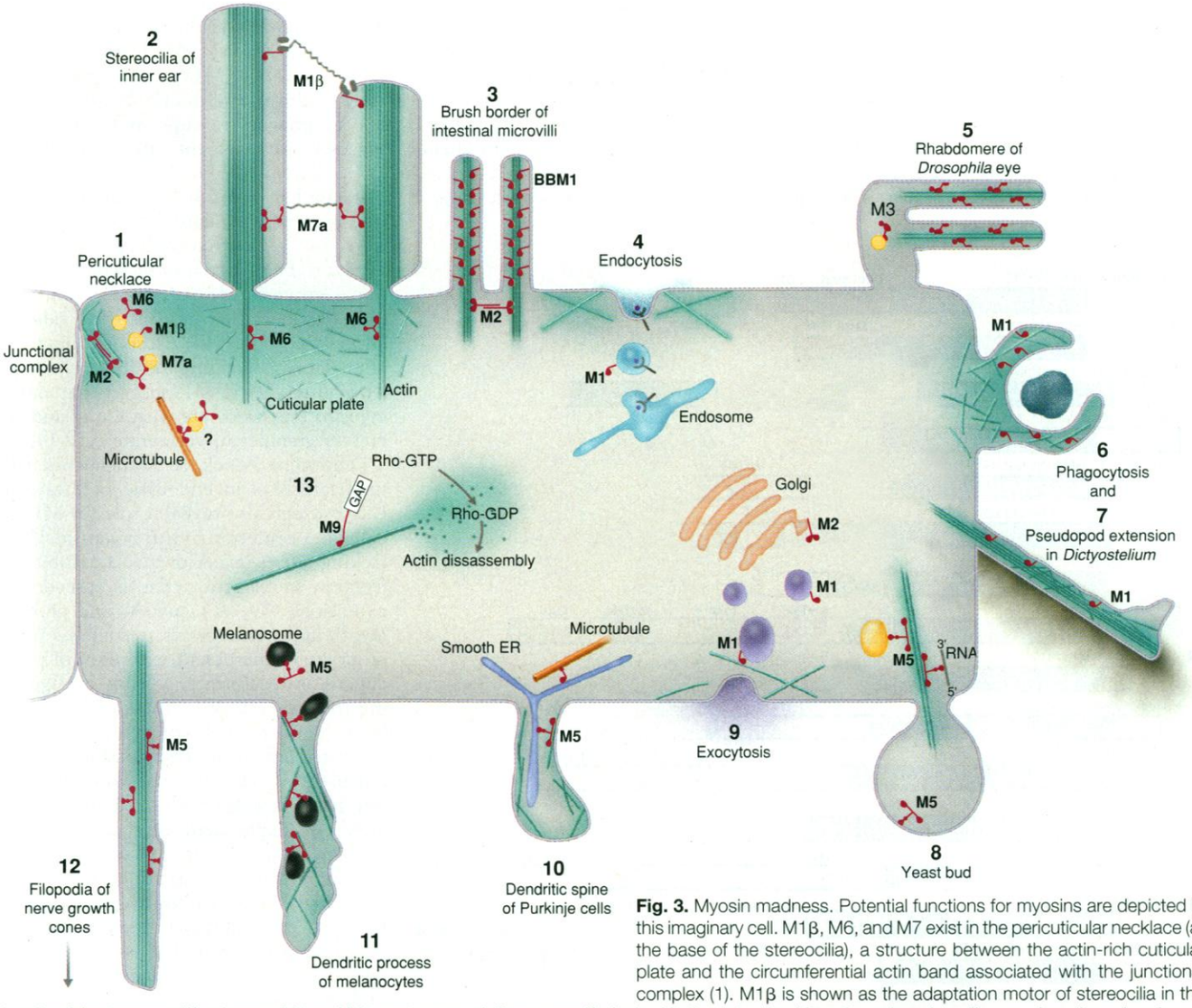

Fig. 3. Myosin madness. Potential functions for myosins are depicted in this imaginary cell. M1β, M6, and M7 exist in the pericuticular necklace (at the base of the stereocilia), a structure between the actin-rich cuticular plate and the circumferential actin band associated with the junctional complex (1). M1β is shown as the adaptation motor of stereocilia in the hair cells of the inner ear (2), whereas M6 and M7 anchor or stabilize stereocilia (or both). Brush border M1 tethers the microvillar core bundle to the plasma membrane of intestinal microvilli (3). M2 exists in the terminal web (3) and may serve to cross-link actin core bundles of the microvilli. M1s may assist endocytosis (4) in yeast, *Dictyostelium*, and vertebrate cells. NinaC (M3) is required for rhabdomere integrity (5) and phototransduction in the *Drosophila* eye. M1s may play a role in phagocytosis (6) in *Dictyostelium* and macrophages in addition to pseudopod (7) extension in *Dictyostelium*. In yeast, M5s may support organelle and RNA transport (8). M2 may assist vesicle budding from the trans Golgi network (9); M1s may participate in yeast secretion and *Dictyostelium* exocytosis (9). M5 may transport smooth ER through dendritic spines of Purkinje cells (10) as well as transport melanosomes though the dendritic processes of melanocytes (11). M5 may assist extension of filopodia of nerve growth cones (12). M9b is a rhoGAP, which inactivates rho and possibly modulates actin organization (13). These structures are not drawn to scale.

*nina*C kinase domain phosphorylates a number of substrates, including expressed p132 (*46*); however, the kinase activity of expressed whole or endogenous M3 is unknown. Both *nina*C isoforms bind calmodulin light chains; the localization of calmodulin in photoreceptor cells is dependent on these proteins, and mutation of the *nina*C calmodulin-binding neck domain results in abnormal photoresponse (*47*). The structure of the actin-based rhabdomere microvilli is disrupted in p174 null and in p174 myosin- and calmodulin-binding domain mutants (*48*). These results suggest that M3 is required for localization of calmodulin and M3 kinase in the photoreceptor, without which normal phototransduction cannot occur; in addition, the myosin domain of M3 also appears to play a role in the structural integrity of the rhabdomere.

Although the loss of the p132 form of *nina*C does not affect the photoresponse or rhabdomere integrity, ultrastructural studies suggest that p132 may function in membrane transport or regulation in the photoreceptor cell. Photoreceptor cells rapidly turnover membrane at the microvilli (*49*). In flies lacking p132, multivesicular bodies accumulate in the cytoplasm (*48*), suggesting that p132 plays a role in photoreceptor membrane traffic.

Recently, class VI and VII myosins have been identified as deafness genes (*50*, *51*), although it is critical to note that the expression of both M6 and M7a is not limited to the cells and tissues affected by mutations in these motors (*52*). Class VII myosins are predicted to have two heads; the tail domain shares repeated conserved regions with other myosins: MyTH4 and talin-like domains (Figs. 1 and 2). In mouse, M6 has been identified as the *Snell's waltzer* gene; the cochlear and vestibular neurosensory epithelium of *Snell's waltzer* mice degenerates soon after birth (*53*). In the inner ear, M6 immunofluorescence is concentrated within the hair cells. The hair cells, specialized sensory cells with actin-rich sensory stereocilia at their apical domain, carry out auditory and vestibular transduction. Within hair cells, M6 is concentrated in the actin-rich cuticular plate, a structure that anchors the bases of stereocilia into the cytoplasm, and in the rootlet actin filaments that descend from the stereocilia into cuticular plate (*54*) (Fig. 3). These findings suggest that M6 may play a role in stabilizing the basal attachment of stereocilia and may actively resist mechanical dislocation of stereocilia (*54*). The human homolog of M6 has recently been cloned (*55*); its role in human deafness remains to be determined.

Unlike M6, M7a has been linked to both rodent and human deafness. Mutations in the genes encoding M7a are responsible for mouse *shaker-1*, human Usher syndrome type IB, human nonsyndromic recessive deafness (DFNB2), and human autosomal dominant nonsyndromic deafness (*56*). *Shaker-1*, like *Snell's waltzer*, mice are deaf and have vestibular defects. In auditory and vestibular organs, M7a is concentrated in the cell body of hair cells and in stereocilia. In frog saccular hair cells, M7a is concentrated in a band corresponding to basal linkages that cross-link stereocilia, but in mammals the cross-links and M7a are distributed along the length of the stereocilia (Fig. 3). These data and the degradation of hair cells in *shaker-1* mice suggest that M7a is important for the integrity of the hair cell bundle (*54*).

In contrast to *shaker-1* mice, Usher syndrome patients are blinded by retinitis pigmentosa. M7a is expressed in both the retinal pigmented epithelium (RPE) and photoreceptor cells (*57–59*). M7a may play a role in phagocytosis of photoreceptor membrane disks by the RPE. M7a may associate with synaptic vesicles in photoreceptors (*58*) or maintain a diffusion barrier between the inner and outer photoreceptor segments (*59*).

Some myosins may play a more direct role in sensory transduction. Auditory and vestibular transduction is dependent on the action of a stretch gated-cation channel that is modulated or adapted to maintain sensitivity to changes in mechanical stimuli. This process of adaptation may be facilitated by a myosin motor restoring resting tension (*50*, *60*). M1β is the most likely adaptation motor (*50*, *61*). M1β is localized to the tip of the stereocilia (*54*, *62*), the site of adaptation. Moreover, calmodulin antagonists block adaptation, and M1β does contain calmodulin light chains and is regulated by Ca^{2+} (*63*); however, this is a common feature of essentially all the vertebrate unconventional myosins characterized thus far.

In addition to the localizations discussed above, M1β, M6, and M7a are all found in the pericuticular necklace, a structure found between the actin-rich cuticular plate and the circumferential actin band associated with the junctional complex (*54*) (Fig. 3). This structure is rich in membrane vesicles, is surrounded by microtubules, and may represent a shuttle zone between microtubule- and actin-mediated transport. The pericuticular necklace may serve as a myosin reservoir, or myosins may function to interconnect the circumferential actin band and the actin-rich cuticular plate (*54*).

Class I, III, VI, and VII myosins have roles in sensory function (Table 1). M3 and M7 are required for vision, whereas M6, M7, and perhaps M1 are required for hearing. These studies have identified potential roles for myosins beyond that of cargo-transporter, including roles such as regulators of ion channels and localizers of other important molecules including calmodulin.

Myosins and Signal Transduction

With the exception of the role of *nina*C in phototransduction, little is known about the participation of unconventional myosins in signal transduction. However, such participation seems likely for certain myosins given the presence of domains implicated as either enzymatic regulators or as effectors of protein-protein interactions in a variety of signaling cascades.

Class IX myosins from humans (M9b) and rat (myr5) are predicted to be single-headed myosins that contain a GTPase-activating protein (GAP) domain within their tails (Figs. 1 to 3) (*64*, *65*) that stimulates the intrinsic GTPase activity of rho, hastening the conversion from the active rho-GTP state to the inactive rho-GDP (guanosine diphosphate) state (*66*, *67*). M9b is an active motor, although it is one of the slowest myosins characterized to date (15 nm/s). The rho family of GTP-binding proteins has been implicated in the regulation of actin-based motile processes (*68*). In the cell, M9b may serve to inactivate rho in order to allow actin remodeling to occur.

Overexpression of M9b in NRK and HeLa cells causes a rounded morphology with loss of actin filaments and cell contacts (*67*). M9b expression has also been examined in cells that express M9b endogenously. During induced differentiation of HL-60 cells into macrophage-like cells, expression of M9b is up-regulated and it relocates from the actin cortical array to the cytoplasm, where it is concentrated in the perinuclear region (*64*). It will be of interest to determine if M9b is required for the considerable remodeling of the actin cytoskeleton that accompanies this differentiation.

In amoeboid cells, M1 heavy chain kinase (MIHCK) associates M1 to rho family signal cascades. The motor domain of amoeboid class I myosins is maximally activated by phosphorylation of a conserved serine or threonine within the actin contact site, the TEDS site (*69*). *Acanthamoeba* and *Dictyostelium* MIHCK share sequence similarity with the p21-activated kinase (PAK)/STE20 family of proteins from mammals and yeast, kinases that are activated by cdc42 and rac (*70–72*). PAK1 phosphorylates and activates *Acanthamoeba* M1 as does MIHCK (*71*). Similarly, rac1 increases autophosphorylation of *Dictyostelium* MIHCK, which in turn increases kinase

activity on *Dictyostelium* M1D (*72*). It remains to be seen if myosins with a phosphorylatable serine or threonine at the TEDS site (class I myosins from *Saccharomyces*, *Aspergillus*, and class VI myosins) are also phosphorylation-regulated and are substrates for the PAK/STE20 family of kinases. In mouse fibroblasts, microinjection of activated PAK1 induces formation of membrane ruffles and filopodia (*73*), suggesting that PAK1 regulates the organization of the actin cytoskeleton.

Src homology 3 (SH3) domains, found in the amoeboid-type class I myosins and *Acanthamoeba* high molecular weight M4 (Figs. 1 and 2), mediate protein-protein interactions in signal transduction pathways, presumably by binding to proline-containing motifs (PXXP) (*74*). *Dictyostelium* MIHCK has multiple proline-rich sequences (*72*); it will be interesting to learn whether these facilitate binding to the SH3 domain of M1. Recently, an *Acanthamoeba* protein (Acan125) was found to associate with the SH3 domain of *Acanthamoeba* M1C and to colocalize with the myosin on organelles (*75*). It is unknown whether Acan125 binds SH3 domains of other myosins.

Recent studies examining the effects of overexpression of *Dictyostelium* MyoB (a M1 with both an SH3 domain and a phosphorylatable residue) suggest that both sites are critical for M1 function. Cells overexpressing myoB show defects in membrane transport and morphogenesis; however, cells overexpressing myoB that lack the SH3 domain or the phosphorylatable residue appear to function normally (*34*). Furthermore, myoB lacking the SH3 domain or the phosphorylatable residue cannot rescue myoB deletion defects (*76*).

Class I and IX myosins are the strongest candidates for myosins involved in rho-mediated signal transduction pathways. Other myosins (such as bovine M10) have signal transduction motifs, but it is unknown whether these domains are functional.

Conclusions

There has been an explosion in the number of recognized myosin classes, myosin types found within a given cell, and cell types containing myosins. However, the details of how myosin molecules transduce force and the roles of myosins in cell and organism function is not understood. Although mutational and biochemical analyses have placed some myosins within functional pathways, most myosins must still be purified and characterized both biochemically and structurally. Until such studies are done, we cannot assume that a given myosin is comparable to muscle myosin—or even that it is an active molecular motor. The potential insights such studies can provide into function are highlighted by the unusual actin-binding properties observed for M5, and the striking adenosine 5′-diphosphate–induced structural changes observed in the neck domain of brush border M1 (*77*). Specific myosin-interacting molecules must be identified before the detailed functions of myosins can be understood. As the examples of M6 and M7a mutations demonstrate, myosin expression in a given cell type does not indicate participation of that myosin in an essential function, as only a limited subset of cell types in which these motors are expressed are adversely affected. This underscores the importance of assessing myosin function in the context of the organism in order to make sense of the multitude of myosins.

REFERENCES AND NOTES

1. We denote myosin classes with roman numerals and refer to specific myosins with arabic numerals.
2. The neck domain is also referred to as the regulatory domain. It consists of one or more 24–amino acid–long IQ (Ile-Gln) motifs that serve as the binding site for myosin light chains. To date, only the class XIV *Toxoplasma* myosin-A lacks a neck region [M. B. Heintzelman and J. D. Schwartzman, *J. Mol. Biol.* 271, **139** (1997)].
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