Myosins: Tails (and Heads) of Functional Diversity

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The myosin family of actin filament-based molecular motors consists of at least 20 structurally and functionally distinct classes. The human genome contains nearly 40 myosin genes, encoding 12 of these classes. Myosins have been implicated in a variety of intracellular functions, including cell migration and adhesion; intracellular transport and localization of organelles and macromolecules; signal transduction; and tumor suppression. In this review, recent insights into the remarkable diversity in the mechanochemical and functional properties associated with this family of molecular motors are discussed.

Myosins are actin-dependent molecular motors that use the energy of ATP hydrolysis to move along actin filaments. In the past 20 years many novel members of the myosin superfamily have been identified, with 16 new myosin classes joining founding members of this protein family: Myo1 and 2.* Several recent reviews have discussed the myosins' functions, with particular focus on Myo6, Myo1c, and Myo5, and the role of myosins in sensory functions and the nervous system (15, 16, 18, 45, 50, 95, 107). In this review we summarize the latest developments in the myosin field, with the emphasis on newly identified or substantially expanded intracellular functions of myosins.

Myosin Superfamily: Diversity of Structural Motifs and Mechanochemical Properties

Most myosin heavy chains consist of three distinct regions: an NH_2-terminal motor or head domain, responsible for actin binding and ATP hydrolysis; a neck region containing one or more IQ motifs that bind light chains (calmodulin or other members of the E-F hand family of proteins); and a COOH-terminal tail, which is responsible for cargo binding and/or dimerization of heavy chains. Based on the sequence comparison of myosin head domains, the myosin superfamily is currently divided into ~20 classes; myosins within each class are also similar in terms of their tail domain organization. Many of these classes exhibit a broad range of phylogenet-

ic expression, e.g., Myo1, 2, 3, 5, 6, 7 (note, classes 3 and 6 are only expressed in metazoans); Myo8 and 13 are found only in plants; some are expressed only in vertebrates (Myo10 and 16); and the remaining classes have been identified in only one or a few related species (Myo4, 12, 14, 17) (8, 131). The heavy-chain domain structure of the myosins discussed in this review is shown in FIGURE 1.

Tails of some myosins contain heptad repeat sequences that can form α-helical coiled-coils and allow heavy chains to dimerize, resulting in formation of two-headed motors, whereas other myosins do not contain coiled-coil motifs and have either been shown or presumed to be single headed (Table 1). Some myosin tails contain predicted coiled-coil regions, but their dimerization has not been experimentally confirmed. It is also possible that for some myosins their ability to dimerize may be regulated. For example, full-length Myo6 has been shown to be a monomer but its tail contains a predicted coiled-coil motif, and it has been proposed that this myosin may form dimers in the presence of tail-binding proteins or upon phosphorylation (18).

Other regions of the tail, in addition to coiled-coil domains, may contribute to dimerization of heavy chains. For example, dimerization of Myo7a heavy chains, which contain a short predicted coiled-coil motif, is observed only for full-length constructs, whereas truncated Myo7a constructs containing the coiled-coil region but missing the rest of the tail are monomeric (55). In addition to dimerization motifs, myosin tails also contain a number of conserved protein domains, which are responsible for protein-protein interactions and/or additional functions such as kinase activity or lipid binding. Numerous binding proteins for myosins have been identified, many of which are discussed below, providing important insights

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*Please note, for uniformity of nomenclature and due to space concerns, myosins are designated by abbreviations (Myo + Arabic numeral denoting myosin class), except in the case of class V budding yeast myosins, which are referred to as Myo2p and Myo4p, and *Dictyostelium* class I myosins, referred to as MyoA etc., according to accepted nomenclature.

Table 1. Structural and mechanochemical characteristics of various myosin classes

Myosin	Number of Heads	Processivity	Duty Ratio	Directionality
I	1 (145)	No	Low (34, 93)	Plus
IIa	2 (145)	No	*Low* (64)	Plus
IIb	2	?	*High* (116, 144)	Plus
III	1	*No?* (63)		*Plus* (63)
V	2 (20)	No (106), yes (82)	*Moderate* (i.e., 20%) to high (31)	Plus (20)
VI	1 (76) (does contain coiled-coil)	*Yes as a dimer* (90, 110) No as a monomer (76)	High (30)	*Minus* (148)
VII	1 or 2?			*Plus* (55)
IX	1	*Yes* (56) Yes (100)	High (100)	Plus (91) *Minus* (56)
X	2?		*Moderate* (i.e., 16%) (65)	Plus (52)
XI (higher plants)	2 (135)	Yes (135)	High (135)	

Processivity and duty ratio are shown only for those myosins for which they have been determined experimentally. Italics denote studies that were performed using recombinant myosin constructs rather than native, tissue-purified proteins. Myosins with question marks under NUMBER OF HEADS indicate presence of predicted coiled-coil domains but for which experimental evidence of oligomerization state is lacking. Numbers in parentheses are references.

into the diversity of myosin functions (see Table 2).

Diversity of myosin function is also manifested through significant differences in the motor properties among members of the myosin family. There are several characteristics of a molecular motor

Table 2. Myosin-binding proteins

Myosin	Binding Partners
I	Sucrase-isomaltase (139), CARMIL (60)
III	INAD (149)
V	Melanophilin/Slac-2/MyRIP (41, 154), Rab11 (70), Bmf (102), CaM kinase II (23), BERP (36), microtubules (19), kinesin (reviewed in 69), dynein light chain (7), intermediate filaments (103), She3p (11, 78, 129), Vac17p (57)
VI	Dab2, SAP97, GIPC (reviewed in 18), optineurin (118)
VII	Vezatin (68), harmonin (10), sans (Usher1 syndrome protein) (1), melanophilin/Slac-2c/MyRIP (35, 41)
IX	RhoA (99), BIG1 (GEF for Arf1) (117)
X	Integrin (157), Mena/VASP (134), microtubules (147)
XV	Whirlin (6, 32)

Included are proteins for which direct binding has been demonstrated either biochemically or by using the yeast two-hybrid system. CARMIL, capping protein, Arp2/3, and Myo1 linker; INAD, inactivation no afterpotential D; MyRIP, Myo7a- and Rab-interacting protein; BERP, brain-expressed RING finger protein; SAP97, synapse-associated protein 97; GIPC, G_{α}-interacting protein interacting protein, COOH terminus; GEF, guanine nucleotide exchange factor; VASP, vasodilator-stimulated phosphoprotein.

that may determine its ability to perform specific intracellular functions: duty ratio (the proportion of the ATPase cycle that a motor spends strongly attached to its track), processivity (the ability of a motor to make multiple steps without detaching from the track), the amount of force produced during a single ATPase cycle, and the velocity and directionality of the movement along the actin track (see Table 1). Load may also alter the mechanochemical properties of some myosins, as has been shown for Myo6 (2).
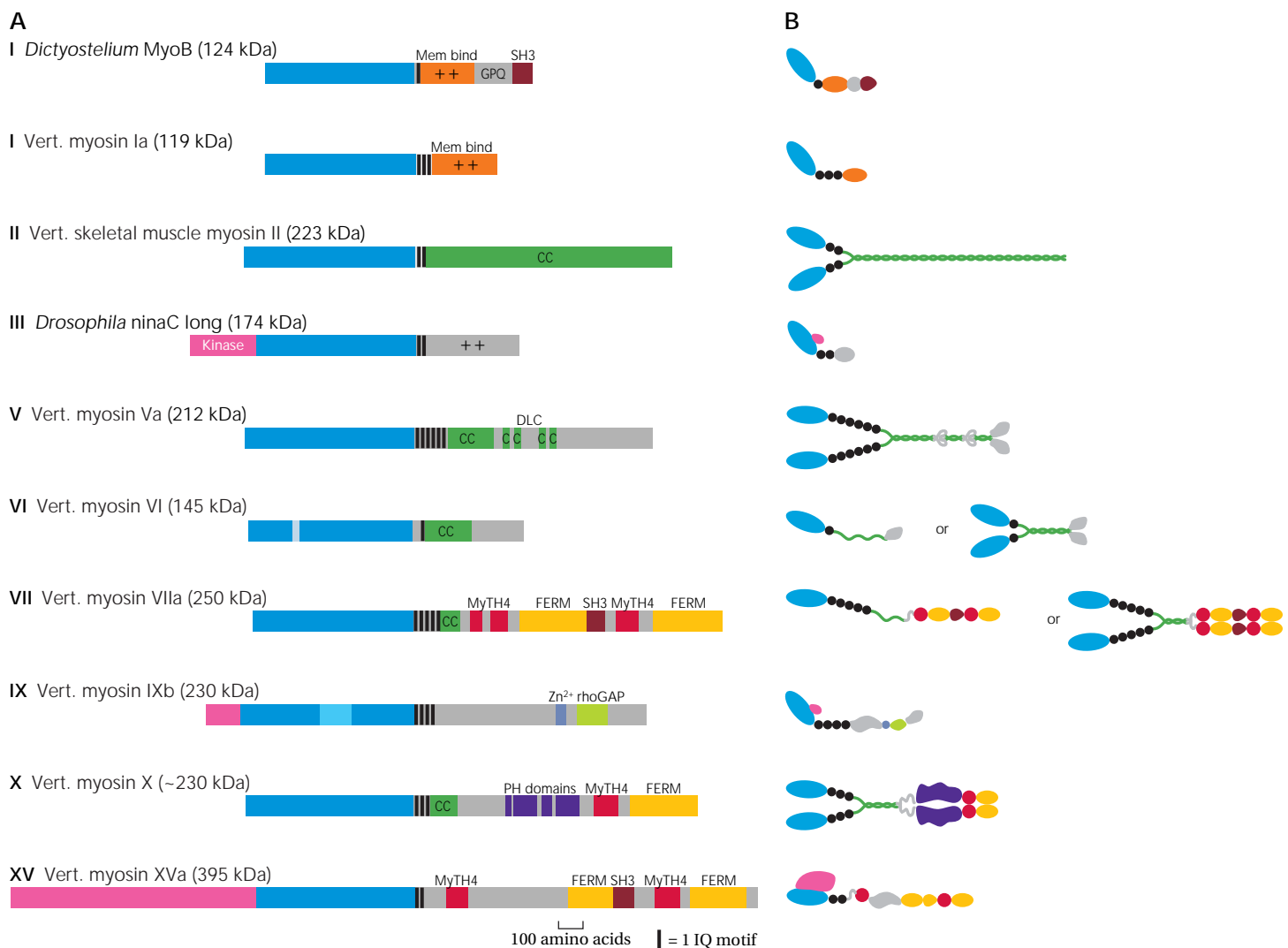
Highly processive motors, such as vertebrate Myo5a (105), are thought to be well suited to the transport of cargo, e.g., vesicles or protein complexes, along actin filaments, because binding of just a few processive motor molecules is sufficient to deliver the cargo to its destination without detaching from the actin track. Factors determining the ability of a motor to support processive movement include its ability to dimerize and its duty ratio. In a two-headed, high-duty-ratio motor such as Myo5, at least one of the two heads is attached to the actin filament at all times, which results in the ability to move processively in a hand-over-hand fashion (138). If processivity of a motor depends on heavy-chain dimerization, it is possible to envision a regulatory mechanism allowing switching from a two-headed, processive motor to a single-headed, nonprocessive one, as has been proposed for Myo6 (18).

Studies of Myo9b indicate that dimerization of heavy chains is not the only possible mechanism for myosin processivity, because this single-headed motor has also been shown to be processive (56, 100). Processivity of Myo9b may be achieved via an

A

I *Dictyostelium* MyoB (124 kDa)

I Vert. myosin Ia (119 kDa)

II Vert. skeletal muscle myosin II (223 kDa)

III *Drosophila* ninaC long (174 kDa)

V Vert. myosin Va (212 kDa)

VI Vert. myosin VI (145 kDa)

VII Vert. myosin VIIa (250 kDa)

IX Vert. myosin IXb (230 kDa)

X Vert. myosin X (~230 kDa)

XV Vert. myosin XVa (395 kDa)

100 amino acids   ▌ = 1 IQ motif

B

**FIGURE 1. Myosin family of molecular motors**
A: structure of the heavy chains of myosin classes discussed in this review. *B*: schematic representations of myosin structures. Class VI and class VII include myosins that may exist in both a dimeric and a monomeric form. Figure is redrawn from Mermall V, Post PL, and Mooseker MS. Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* 279: 527–533, 1998.

electrostatic interaction between actin and a highly basic insert in the Myo9b motor domain, which prevents myosin from diffusing away from an actin filament (56, 100).

Low-duty-ratio motors may be better suited to induce rapid contraction of actomyosin assemblies rather than sustained movement of a cargo along a filament. For these myosins to function efficiently, the probability of their encounters with actin filaments has to be high. This can be achieved by assembling individual myosin molecules into bipolar filaments, as is the case for muscle and nonmuscle Myo2s, or by allowing myosins to act in the regions of high actin-filament concentration, such as brush-border microvilli or the leading edge of the cell, as has been proposed for Myo1s (34, 59). Interestingly, vertebrate nonmuscle Myo2s are represented by both a low-duty-ratio Myo2a (64) and a

high-duty-ratio Myo2b (116, 144), both of which may be expressed in the same cell. This may allow Myo2b to perform functions associated with maintenance of cortical tension, whereas Myo2a may be responsible for rapid contractile movements.

Actin filaments possess an inherent polarity, with plus (or barbed) and minus (or pointed) ends. Although most myosins move toward the plus ends of actin filaments, Myo6 has been shown to be a minus-end-directed motor, and Myo9b may move toward both plus and minus ends, which suggests that its directionality may be regulated (see Table 1). Depending on the orientation of actin filaments within a specific intracellular region, plus- or minus-end-directed myosins may be better able to perform specific functions. For example, many types of actin-rich protrusions (filopodia, microvilli of intestinal brush border, inner-ear hair-cell
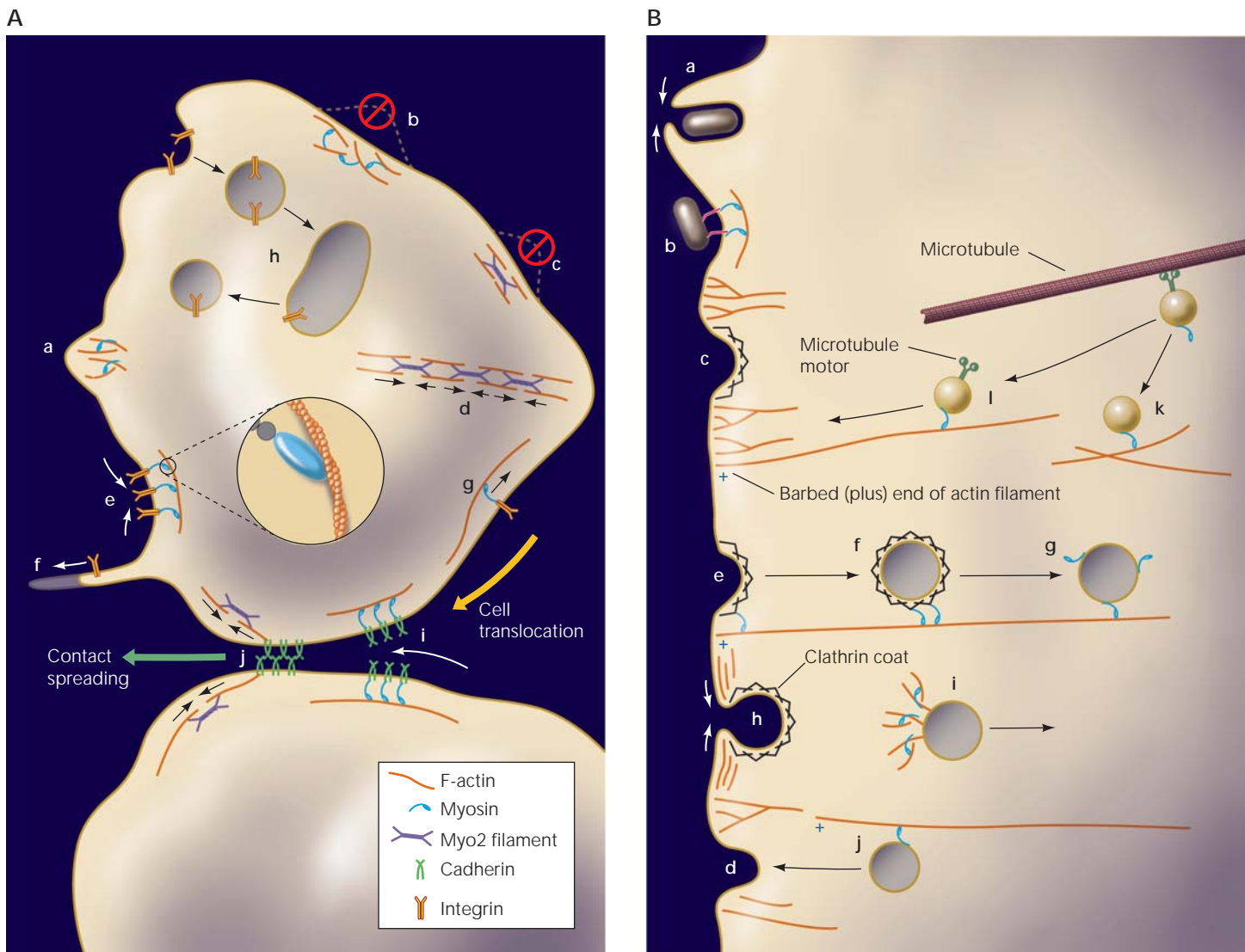
FIGURE 2. Intracellular functions of myosin motors
A: myosin functions in cell motility and cell adhesion. *a*, Lamellipodial protrusion driven by actin polymerization, with Myo1 family members contributing to the activation of actin nucleation by the Arp2/3 complex. *b*, Limitation of lateral extension of lamellipodia by Myo1-dependent cortical tension. *c*, Limitation of lateral extension of lamellipodia by Myo2-driven contractility. *d*, Myo2-dependent tail retraction. *e*, Clustering of cell-substrate adhesion receptors. *f*, Elongation of filopodia due to Myo10-dependent transport of integrins or VASP/Mena proteins to the filopodial tip. *g*, Myosin (e.g., Myo10 or Myo2) acting as a clutch coupling cell-substrate adhesion to the forward movement of the cell. *h*, Cell adhesion receptor endocytosis and recycling. *i*, Clustering of cell-cell adhesion receptors. *j*, Myo2-mediated "zippering up" of cell-cell junctions. *B*: roles of myosins in membrane traffic. *a*, Extension of pseudopods during phagocytosis (Myo1, Myo10). *b*, Adhesion of bacteria/food particles to the cell surface during phagocytosis (Myo7). *c–d*, Reorganization of cortical actin to allow invagination of a clathrin-coated pit (*c*) or fusion of an exocytic vesicle with the plasma membrane (*d*) (e.g., by Myo1). *e*, Contribution of a minus-end-directed myosin (e.g., Myo6) to deformation of plasma membrane during clathrin-coated pit formation. *f–g*, Minus-end-directed transport of clathrin-coated (*f*) and uncoated (*g*) vesicles along actin filaments by Myo6. *h–i*, Membrane scission (*h*) and vesicle propulsion (*i*) driven by Arp2/3-regulated actin polymerization with Myo1 contributing to Arp2/3 activation. *j*, Plus-end-directed transport of exocytic vesicles (e.g., by Myo1). *k–l*, Switch between microtubule-dependent and actin-dependent transport, resulting in vesicle capture and anchoring (*k*) or short-range transport along actin filaments (*l*).

stereocilia) contain long bundles of actin filaments oriented with their plus ends toward the membrane. Given this arrangement of actin filaments, a plus-end-directed myosin could be used to deliver proteins or organelles toward the tip of the protrusion, whereas a minus-end-directed motor would be necessary to transport cargo inward or maintain membrane invaginations at the base of the protrusions (112).

Myosins in Cell Migration and Adhesion

The first member of a myosin superfamily to be described, muscle Myo2, is responsible for muscle contraction, which it powers via the "sliding filament" mode of action (53, 54). In this model, bipolar myosin filaments induce contraction by forcing two sets of actin filaments of opposite polarity to slide toward each other, causing shortening of the

muscle fibers. Following the discovery that myosins are also present in nonmuscle cells, it was suggested that myosin-dependent contractile activity, similar to that observed in muscle, may also be involved in cell migration and control of cell shape (97). This "sliding-filament" model of cell motility relies on the existence of bipolar myosin filaments and, therefore, can explain only those types of cell motility that involve Myo2, since it is the only known filament-forming myosin. The best examples of nonmuscle sliding-filament-type motility are constriction of the cleavage furrow during cytokinesis (122) and contraction of the apical actin ring associated with adherens junctions in polarized epithelia (51, 61). During cell migration, Myo2 is present in actin stress fibers in the rear of the cell (81, 119) and may be responsible for the forward movement of the cell body and tail retraction. On the other hand, protrusion of the leading edge of the cell is thought to be powered primarily by actin polymerization rather than by myosin-induced contractility (96). However, Myo2-dependent contractility may play an important role in coordination of cell migration and directional motility by preventing formation of lateral pseudopods and limiting pseudopodial/lamellipodial protrusion to the leading edge of the cell.

As an example of the importance of Myo2 in directional movement, a recent study found that Myo2b knockout fibroblasts have numerous, unstable lamellipodia (77) and exhibit high instantaneous velocity and low directional persistence of migration (frequent change of direction). Myo2b appears to be important for limiting formation of lamellipodia to the leading edge of the cell, as well as for sensing mechanical stimuli such as substrate rigidity and pushing/probing forces and for setting direction of migration based on these stimuli. Interestingly, these experiments indicate that Myo2a provides most of the traction forces exerted by a cell on a substrate, whereas the role of Myo2b appears to be to determine/change direction of the traction forces. Myo2b-null cells also exhibit defects in rearward collagen fiber transport along the cell surface and remodeling of 3-D collagen gels (85). Thus, although Myo2b-null cells are able to rapidly, albeit nondirectionally, translocate on two-dimensional substrates, their ability to migrate in 3-D gels, which are more representative of an in vivo environment, may be impaired. Collagen fiber transport occurs via repeated cycles of extension and retraction of lamellipodia that are accompanied by recruitment of Myo2b into extending lamellipodia and rearward movement of Myo2b during retraction. Therefore, Myo2b appears to play an important role in contractile activity of lamellipodia.

Studies in *Dictyostelium* also implicate single-headed, non-filament-forming Myo1 variants in limiting formation of lateral pseudopods. This role is probably accomplished via a mechanism different from that employing Myo2. Myo1 mutants in *Dictyostelium* exhibit decreased velocity of chemotaxis due to increased formation of lateral pseudopods (38, 132). This defect is observed in MyoA, MyoB, and MyoF mutants, with MyoA/MyoB and MyoB/MyoF performing nonredundant functions in cell migration. MyoB also seems to be important for cell response to the waves of chemoattractant cAMP secreted by *Dictyostelium* during starvation-induced cell aggreggation and formation of fruiting bodies (38). Since MyoB is known to bind capping protein, Arp2/3, and Myo1 linker (CARMIL), a regulator of the Arp2/3 activity and binding partner of *Dictystelium* capping protein (60, 109), it may influence pseudopod formation by modulating actin nucleation. Myo1 variants may also act as cross-linkers of the cortical actin meshwork to create surface tension that prevents pseudopod extension (28).

Whereas the actin cytoskeleton, both through actin polymerization and actomyosin contractility, provides the main driving force for cell motility, it is also involved in establishment and maintenance of cell adhesion. Cytoplasmic domains of transmembrane cell adhesion receptors (integrins in the case of cell-extracellular matrix contacts and cadherins in the case of cell-cell contacts) interact with actin-binding proteins that link them to actin filaments (49). Due to this close association of adhesion receptors with the actin cytoskeleton, actin-dependent motors are likely to play an important role in cell adhesion. Myosins may bind to cell-adhesion receptors and transport them relative to the actin-rich cortex or to the bulk of actin filaments in the cell body. This can lead to the following outcomes: concentration of adhesion receptors at the leading edge or sites of cell-cell contact assembly (121, 123); formation of clusters of adhesion receptors, which increases the strength of cell adhesion and activates intracellular signaling pathways that promote contact assembly (47, 87, 88); and net forward translocation of the leading edge as a result of rearward transport of adhesion receptors, with myosin acting as a clutch (127). In addition, tension exerted by myosin may promote detachment of adhesion receptors from the substrate in the rear of the cell, which is necessary for efficient translocation (25). Recycling of cell-adhesion receptors, which plays an important role in cell migration and cell adhesion (13, 17), may also involve myosins through their functions in endocytosis and/or exocytosis.

Involvement in cell adhesion has been demonstrated for Myo7 and Myo10, two myosins whose

tails contain band four-point-one, ezrin, radixin, moesin (FERM) and myosin tail homology 4 (MYTH4) domains. FERM domains typically mediate binding of cytoskeletal proteins to cytoplasmic domains of transmembrane proteins (42), and the FERM domain of Myo10 has been shown to bind integrins (157). Myo10 overexpression enhances formation of filopodia and promotes integrin recruitment into filopodia (157). Myo10 may act as a clutch linking elongating actin filaments to matrix-bound integrins, or it may stabilize filopodia by promoting concentration of integrins at the filopodial tip and establishing strong adhesion to the substrate.

"Myo6 functions are important for maintaining the integrity of cell monolayers and cell shape, possibly due to its role in regulation of E-cadherin stability."

Filopodia induction by Myo10 expression may also depend in part on its involvement in the delivery of Mena/VASP proteins to the cell edge (see below).

The MYTH4- and FERM-domain-containing region of the Myo7a tail binds vezatin (68) and harmonin (10), two proteins that interact with components of cell-cell junctions (cadherins and catenins) and are involved in formation of lateral links between stereocilia in the inner ear hair bundles. Although both vezatin and Myo7a localize to adherens junctions in epithelial cells (125), no overt disruption of adherens junctions assembly has been detected in cells expressing a Myo7a dominant-negative tail construct; however, this construct prevents cadherin-dependent entry of *Listeria* into epithelial cells (125). Sensory defects in Ushers' syndrome (Myo7a, harmonin, and cadherin mutations in humans) have been attributed to defects in formation of links between stereocilia in the inner ear. Therefore, at least some types of cadherin-dependent adhesive interactions in mammalian cells rely on Myo7a functions, and it remains to be determined whether Myo7a also plays a role in modulation of adherens junction organization.

In *Dictyostelium* amoebae, Myo7 appears to function in both cell-cell and cell-substrate adhesion, as well as in attachment of food particles to cell surface receptors during phagocytosis (136). As a result of defects in adhesion, Myo7-null cells are deficient in cell migration (the leading edge loses contact with the substrate for prolonged time periods). Myo7 is also localized to filopodial tips in *Dictyostelium*, and its role in filopodia formation may be analogous to that of Myo10 in mammalian cells.

Another myosin that has been shown to play a role in both cell migration and interactions with cell adhesion proteins is Myo6. In *Drosophila,* Myo6 appears to be necessary for E-cadherin and β-catenin stabilization, which is important for border cell migration in the ovary (43). This function may be related to the role of Myo6 in endocytosis, because endocytosis and recycling of adhesion receptors may be necessary for their proper localization and stability. A recent study has extended research on the role of Myo6 in migration of epithelial sheets in *Drosophila* by implicating it in epithelial cell migration during dorsal closure (86). Myo6 ablation causes a disruption and folding of epithelial sheets; therefore, Myo6 functions are important for maintaining the integrity of cell monolayers and cell shape, possibly due to its role in regulation of E-cadherin stability. Myo6 is also involved in migration of human ovarian carcinoma cell lines; thus its role in cell migration may not be limited to *Drosophila* epithelia (156).

Myo1c has long been known to be involved in the adaptation response in the inner ear hair cells (closure of mechanosensitive ion channels during sustained stimulation) (45). Recently, it has been shown that this motor also interacts with cadherin 23, a component of the tip links between hair cell stereocilia (124). The function for a myosin motor in the process of adaptation is thought to be to transport the channel along the length of deflected stereocilium, which modulates tension applied to the channel. Interaction of Myo1c with cell-adhesion molecules forming tip links suggests that tip-link protein complexes may serve to connect Myo1c to mechanosensitive channels, so that the tip links, together with associated ion channels, may be translocated along the actin bundles in stereocilia. It is important to note that Myo7a may also play a role in adaptation, because electrophysiological studies on hair cells from Myo7a-mutant mice revealed significant defects in the adaptation response (67).

A recent study found that nonmuscle Myo2a is necessary for cell-cell adhesion in mouse embryo (22). Myo2a in normal embryos and embryonic stem cells localizes to cell-cell contacts. Embryos lacking Myo2a die by embryonic day 7.5 and exhibit disorganization of visceral endoderm, an extraembryonic cell layer responsible for nutrient uptake and transport as well as spatial patterning within an embryo (9). These defects can be traced in vitro to the loss of E-cadherin and β-catenin localization to cell-cell junctions, accompanied by the loss of cell-cell attachment (manifested by cell shedding and lack of strong attachment in vitro).

During establishment of new cell-cell junctions by cultured epithelial cells, Myo2a is associated with contractile arcs of actin filaments that form along the edges of a nascent contact and may promote contact spreading (66). Adhesion defects observed in Myo2a knockouts may be related to decreased contractility of actin bundles during contact formation. Alternatively, Myo2a-mediated tension applied to adherens junctions via apical actin bundles may be necessary for steady-state maintenance of cell-cell contacts.

Myosins and Signal Transduction

As already discussed above for Myo1c and Myo7a, myosins can also play important roles in cell-signaling pathways (reviewed in Ref. 5). Perhaps the most striking examples are Myo9 family members, which contain an active GTPase activation protein (GAP) domain in their tail domain for the G protein Rho (99, 108). Moreover, the GTP exchange factor (GEF) for Arf, BIG1, has recently been identified as a binding protein for Myo9b (117). Overexpression of Myo9 in cultured cells phenocopies expression of dominant-negative Rho (89). Myo9b may play a role in the cytoskeletal remodeling during uptake/infection of cells by the pathogenic bacteria *Shigella* (48). However, for the most part, the functions for Myo9b (for example in regulating Rho-dependent remodeling of the actin cytoskeleton) have yet to be elucidated. It is interesting to note that Myo9b is most highly expressed in highly motile cells such as leukocytes (151). In contrast, however, the other Myo9 in vertebrates, Myo9a, is most highly expressed in brain (21). It will be of great interest to determine if Myo9a is associated with the highly dynamic arrays of actin within synapses (79).

Myo3, an unusual myosin containing an active protein kinase domain at its NH_2 terminus (63), has long been implicated in phototransduction in *Drosophila*, including regulation of intracellular localization of calmodulin in *Drosophila* photoreceptors (98). Recent studies show that this myosin is responsible for the transport of two vision-related signaling proteins, arrestin and $G_q\alpha$, from the cell body to the rhabdomeres in *Drosophila* photoreceptors (27, 72). Myo3-dependent transport requires interaction of arrestin with phosphoinositides (72), suggesting that the mechanism of arrestin transport may involve myosin-dependent movement of phosphoinositide-containing vesicles. In addition to interacting with phosphoinositides, the Myo3 tail also binds F-actin (74) and the adapter protein inactivation no afterpotential D (INAD) (149). Disruption of Myo3-INAD interaction leads to a delay in photoreceptor deactivation (149). Genetic studies link human Myo3 with

defects in both hearing and vision (33, 142).

In addition to its multiple roles in membrane traffic and cargo transport (see below), Myo5a has been shown to play a role in regulating apoptosis. One of the light chains of Myo5a, dynein light chain 2 (DLC2, which is also a subunit of the microtubule motor dynein), binds proapoptotic protein Bmf and sequesters it to actin filaments. Binding of Bmf to Myo5 is regulated by cell attachment to the extracellular matrix, so that cell detachment leads to the release of Bmf and onset of programmed cell death (102).

Myo18b has recently been implicated as a potential tumor suppressor based on the observations that it exhibits reduced expression in a large number of ovarian tumors; mutations in Myo18b were observed in a subset of these tumors (155).

Myosins in Membrane Traffic

Myosins have been implicated in various types of membrane traffic, including endo- and exocytosis and intracellular transport of a variety of organelles. Unlike microtubules, which serve as tracks for long-range organelle transport, actin filaments typically provide tracks for short-range organelle movements (69, 113). Additionally, in many cell types actin filaments form dense cortical meshworks, which may impede centripetal transport of endocytic vesicles and/or fusion of exocytic vesicles or may serve as a scaffold to which organelles can be anchored by myosins. In agreement with these properties of the actin cytoskeleton, the roles that have been proposed for myosins include transport of endo- and exocytic vesicles through the actin-rich regions, short-range, local delivery of organelles, and capture and retaining of organelles in specific subcellular regions (50, 69, 152). Since the majority of actin filament plus ends are thought to be facing the plasma membrane (26), minus-end-directed myosins may be expected to participate in internalization of endocytic vesicles, whereas plus-end-directed myosins may be involved in exocytosis. However, there are examples of plus-end-directed myosins being involved in endocytosis, suggesting that a subpopulation of actin filaments with the inverse orientation may exist in certain cell types and/or that a different mechanism of internalization may be involved. Additionally, myosins may promote endo- or exocytosis by inducing local reorganization of actin meshworks to allow the passage of vesicles or promote actin-dependent constriction and scission of the necks of membrane pits. Myo1, 5, and 6 family members have long been implicated in membrane traffic (for reviews of earlier work, see Refs. 50, 69, and 153), whereas recent studies have provided evidence for the role of Myo7 and Myo10 in phago-

cytosis and for involvement of myosins in exocytosis.

Myo6 is well positioned for a role in endocytosis, because its minus-end-directed movement along actin filaments may promote centripetal transport of endocytic vesicles toward early endosomes. Several recent studies have further dissected the role of Myo6 in endocytosis, clarifying its functions in various cell types. Whereas Myo6 in polarized epithelial cells associates with clathrin-coated vesicles and may be involved in transport of endocytic receptors down the surface of microvilli or invagination of clathrin-coated pits (50), in nonpolarized cells Myo6 is recruited to endocytic vesicles following the loss of clathrin coats (3). Displacement of Myo6 using a dominant-negative (headless) construct delays transferrin uptake and slows down centripetal migration of uncoated vesicles and their delivery to pericentriolar endosomes (3). Thus, in nonpolarized epithelial cells, Myo6 may help vesicles traverse dense actin meshworks at the cell periphery. This hypothesis was confirmed using live imaging of green fluorescent protein-Myo6-labeled vesicles, which indicated net inward movement of vesicles, expression of motor-dead Myo6 (which inhibited vesicle migration), and application of actin-depolymerizing drugs (which promoted transferrin trafficking to early endosomes) (4).

Another recent study uncovered the role of the adaptor protein Dab2 in mediating Myo6 recruitment to clathrin-coated vesicles and found that, although interaction of Myo6 with uncoated vesicles does not require Dab2, association of Myo6 with clathrin-coated vesicles can be observed only in cells expressing large amounts of Dab2 (29). The role of Dab2 in Myo6 recruitment and the possibility that this interaction may be regulated, proposed by Dance et al. (29), may explain why in some cell types Myo6 interacts with both clathrin-coated and uncoated vesicles but in others is found only on uncoated vesicles. One specific example of a receptor that is internalized via a Myo6- and Dab-2-dependent endocytic pathway in polarized epithelia is CFTR (128).

"Myo6-dependent endocytosis may play an important role in regulation of synaptic plasticity."

In addition to Dab2, the Myo6 tail also binds two adaptor proteins: G_α-interacting protein interacting protein, COOH terminus (GIPC), which can interact with a variety of transmembrane receptors, and SAP97, which interacts with AMPA-type glutamate receptors at the synapses (50, 94). Myo6 binding to Dab2 is necessary for recruitment of GIPC to clathrin-coated vesicles (29), where GIPC may serve as a scaffold for assembly of signaling complexes associated with the tails of transmembrane receptors. Neurons from Myo6-deficient *Snell's waltzer* mice exhibit defects in AMPA-receptor endocytosis at postsynaptic densities and decreased number of synapses; therefore, Myo6-dependent endocytosis may play an important role in regulation of synaptic plasticity (94). Constitutive endocytosis of transferrin is not impaired in Myo6-mutant mice (94), suggesting that Myo6 is involved only in endocytosis of certain types of receptors in neurons and that this specificity may be mediated by specialized adaptor proteins such as SAP97.

The importance of Myo1 variants in endocytosis was first suggested by studies in *Dictyostelium* amoeba and has recently been emphasized by a study showing that in budding yeast, Myo1 may be directly involved in vesicle scission from the cell membrane (58). This is probably accomplished through Myo1-mediated assembly and reorganization of actin filaments, because Myo1 in both budding and fission yeast has the ability to activate actin nucleation by the Arp2/3 complex and promote assembly of actin filaments at the sites of endocytosis (37, 71, 73). On the other hand, mammalian Myo1 variants do not contain protein domains that could be involved in the activation of the Arp2/3 complex, and therefore in these cells the role of Myo1 in endocytosis may be more indirect, although it is theoretically possible that SH3-domain-containing Myo1s may interact with Arp2/3 through intermediary proteins functionally similar to CARMIL, an Arp2/3 activator and binding partner of the *Dictyostelium* MyoB (60). Interestingly, although a truncated Myo1a construct has been previously shown to inhibit endocytosis in mammalian cells (104), this dominant-negative effect may not reflect the direct involvement of Myo1 family members in endocytosis but instead result from the loss of Myo6 from the apical membrane, similar to that observed in Myo1a knockout enterocytes (137).

The role of Myo5 in vesicular trafficking is well established. There are three isoforms of Myo5 expressed in humans, Myo5a–c (111), and they have been implicated in movement of various types of organelles: melanosomes, phagosomes, smooth ER, endocytic, and recycling vesicles (reviewed in Refs. 69 and 105). Most Myo5-associated organelles also interact with microtubule-dependent motors, and Myo5 activity may counteract fast microtubule-dependent movement of organelles by capturing them at the periphery of the cell and inducing them to undergo short-range

actin-dependent movements (14, 69, 152). Myo5 isoforms are targeted to their specific cargoes via interactions of their globular tails with various members of the Rab family of small G proteins. Interactions of Myo5 with Rabs may be indirect; for example, interaction between Myo5a and Rab27a, a melanosome-specific protein, is mediated by melanophilin, which binds to both Myo5a and Rab27a (reviewed in Ref. 69). Myo5a, which is predominantly expressed in neurons and melanocytes, plays an important role in regulating melanosome distribution and in organelle transport in neurons (reviewed in Ref. 15). Mutations in Myo5a, Rab27a, and melanophilin are associated in mice with light coat color (due to abnormal pigment distribution) (80, 84, 150) and abnormalities in synaptic function (75) and in humans with Griscelli syndrome, a rare inherited disease that is characterized by defects in hair pigmentation and by neurological and immune system disorders. Detailed genetic characterization of patients with Griscelli syndrome indicates that patients with Myo5a mutations exhibit only neurological symptoms and pigmentation defects, whereas individuals with Rab27a mutations are also characterized by immune system abnormalities (uncontrolled activation of macrophages and lymphocytes) (83, 120). Myo5b has been implicated in recycling of transferrin receptor and muscarinic acetylcholine receptor (70, 141). Expression of dominant-negative Myo5b tail constructs results in perinuclear accumulation of these receptors, as may be expected when centripetal transport by microtubule-dependent motor dynein is not balanced by Myo5 (70, 141). Interestingly, however, a recent study employing a chemical inhibition strategy that locks Myo5b in an inactive, ADP-bound state indicates that this approach to inhibiting Myo5b leads to an opposite result: plasma membrane enrichment of transferrin receptor (101). The authors proposed that the rigor binding of chemically inhibited Myo5b to the actin filaments prevents centripetal transport of endocytic vesicles and promotes accumulation of transferrin receptor at the periphery of the cell. Myo5c is predominantly expressed in epithelial cells, and experiments using Myo5c dominant-negative tail construct implicate this motor in recycling of transferrin receptor but not in its endocytosis (111).

A lot of progress has also been made recently with regard to dissecting the role of the class V myosin Myo2p in vacuolar inheritance in yeast. Studies from Weisman and colleagues identified vacuolar protein Vac17p as a receptor for Myo2p and found that Vac17p is proteolytically degraded in the bud following the delivery of the vacuole from the mother cell into the budding daughter cell (57, 130). Degradation of the myosin receptor may

serve to ensure one-way flow of myosin cargo as well as provide an additional means of regulation of myosin-dependent intracellular transport. It remains to be determined whether this regulatory mechanism is also utilized in mammalian cells, because it has been suggested that proteolytic degradation of melanophilin may play a role in regulation of melanosome transport (40).

As discussed above, Myo7 and 10 share structural and functional similarities. Both of these myosins have been implicated in phagocytosis (24, 44, 136). The mechanisms underlying defects in phagocytosis in Myo7 mutants in *Dictyostelium* amoeba and in mammalian cells appear to be quite distinct from each other. In *Dictyostelium* Myo7-null cells, the defect in phagocytosis is related to adhesion defects (i.e., lack of adhesion of food particles to the cell surface) (136), whereas in RPE cells from mouse Myo7a mutant (*Shaker-1*) decreased phagocytosis of rod outer segments results from a defect in the centripetal transport of phagosomes that prevents fusion with lysosomes (44). The contribution of Myo10 to phagocytosis appears to result from its role in pseudopod formation by macrophages (24).

Myosins have also been shown to play a role in exocytosis and secretion, possibly by promoting delivery of exocytic vesicles to the plasma membrane and/or inducing reorganization of cortical actin to allow membrane fusion. Myo1c promotes insulin-stimulated exocytosis of glucose transporter GLUT4 in adipocytes (12), whereas Myo2b knockdown using antisense RNA decreases the rate of calcium-stimulated exocytosis in fibroblasts (133). Myo5-mediated transport of exocytic vesicles is involved in chromaffin cell secretion (114) and insulin secretion by pancreatic β-cells (140). Roles for Myo1, Myo2, and Myo6 in delivery of vesicles from the Golgi apparatus to the plasma membrane as well as in structural organization of the Golgi have been proposed by several groups based on the association of these myosins with the Golgi membranes and a decrease in secretion from Golgi to the plasma membrane in *Snell's waltzer* fibroblasts (18, 39, 126). A recent study identified optineurin, a Golgi-associated protein, as a Myo6 binding partner that mediates its interaction with the Golgi apparatus and found that knockdown of optineurin expression leads to disruption of Golgi organization and exocytosis (118).

Myosins in Protein, RNA, and Organelle Localization

In addition to playing important roles in the intracellular transport of membrane-bound organelles, myosins are involved in determining intracellular localization of proteins, multiprotein complexes,

RNA, and nonmembranous organelles.

Myo1a, a major myosin of the intestinal brush border, has been shown to be involved in localizing sucrase-isomaltase, an intestinal enzyme involved in disaccharide digestion, to the brush border of intestinal epithelial cells (137, 139). The cytoplasmic domain of sucrase-isomaltase binds to the tail of Myo1a. The mechanism responsible for sucrase-isomaltase localization involves interaction of Myo1a with lipid-raft-associated sucrase-isomaltase. Myo1a is also the major calmodulin-binding protein within the brush border and thus by default is required for localization of high concentrations of calmodulin within the microvilli of the brush border, the site of dietary uptake of Ca^{2+}. The brush border cytoskeleton is destabilized in the Myo1a knockout, perhaps because of the loss of microvillar calmodulin and the resultant poor Ca^{2+} buffering that may activate the Ca^{2+}-dependent actin-severing activity of the microvillar core protein villin (137).

Two myosins, Myo5a and Myo10, have recently been shown to interact with microtubules via their tails (19, 147). Myo10-microtubule interaction is important for cortical anchoring of the nucleus and meiotic spindle positioning in *Xenopus* oocytes (147). In addition to directly binding to microtubules, Myo5a also interacts with microtubule-dependent motor kinesin and with intermediate filaments and may serve as a linker between actin-, microtubule-, and intermediate filament-based cytoskeletal systems (69, 103, 146). In another example of coordination between actin and microtubules, Myo2 has been implicated in centrosome separation (115). Cortical contraction that requires Myo2 activity separates and positions centrosomes following nuclear-envelope breakdown. The authors posited that centrosome separation occurs first via activity of dynein motors that interact with the nuclear envelope. Following nuclear-envelope breakdown, final positioning of centrosomes depends on interaction of astral microtubules with the actin-based cell cortex and on cortical Myo2 activity.

As discussed above, Myo10 is involved in transport of integrins and Mena/VASP proteins to the tips of filopodia (134, 157). Both cell-substrate adhesion via integrins and actin filament elongation due to the anticapping activity of Mena/VASP may contribute to the ability of Myo10 to promote formation of filopodia.

In a curious parallel to Myo10's role in filopodia elongation, another MYTH4- and FERM-domain-containing myosin, Myo15a, has been found to be necessary for elongation of stereocilia of the inner ear hair cells (143). The functions of Myo15a in stereocilia elongation involve delivery of a protein called whirlin, which binds to the SH3-MYTH4-FERM-domain-containing region of the Myo15a tail, to the tips of stereocilia, where whirlin may regulate actin dynamics and actin filament elongation (6, 32, 62). In addition to regulation of stereocilia elongation, Myo15a interaction with whirlin may also play a role in cohesion of stereocilia (32).

In addition to protein and organelle localization, class V myosins are also involved in RNA localization. In budding yeast, the class V myosin Myo4p affects the transport and localization of a number of specific mRNAs from the mother cell to the bud via its direct interaction with She3p, one subunit of a multiprotein complex that interacts with specific sequences within the 3′-untranslated region of the transported mRNAs (reviewed in Ref. 46). In neurons, Myo5a has been shown to be part of a polyribosome-associated mRNA/protein (mRNP) complex containing Puralpha, mStaufen, and fragile X protein (92).

Conclusion

Based on the studies summarized above, it has become clear that within a given cell, multiple myosins are expressed with distinct but often overlapping subcellular localizations and potentially overlapping functions. This review highlights the considerable progress made toward unraveling the complexities of myosin functions at the cellular level. However, a major challenge in the future will be to assess how these functions contribute to physiological functions at the tissue, organ, and organism levels in metazoan species, including vertebrates. ∎

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