Minireview

Vertebrate Unconventional Myosins*

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Myosins are a large family of structurally diverse mechanoenzymes that bind to F-actin and hydrolyze ATP to produce mechanical force. Phylogenetic analysis of the myosin motor domains has identified 11 distinct classes of myosin, seven of which are expressed in vertebrates (for review see Ref. 1). These seven myosin classes include conventional myosin, or myosin-II, and six more recently identified unconventional myosin classes, myosins-I, -V, -VI, -VII, -IX, and -X (see Fig. 1). At the N terminus, each vertebrate unconventional myosin contains a conserved motor domain that includes both the ATP binding and ATP-sensitive actin binding sites (for review of the structure of the myosin motor domain see Ref. 2). Following the motor is a light chain (LC) binding, regulatory “neck” domain comprised of 1–6 copies of a 24–30-amino acid repeat termed the IQ motif (3). For all vertebrate unconventional myosins characterized to date, this neck domain serves as a binding site for calmodulin (CaM), a member of the EF-hand superfamily of calcium binding proteins. There is preliminary evidence for additional non-CaM LC associated with myosin heavy chains (see below). At the C terminus, each class has a distinct tail domain, which provides sequences that can serve, for example, in dimerization or membrane binding (Fig. 1). There is evidence for roles in protein binding and/or enzymatic activities as well, and it is hypothesized that each tail targets the myosin to its particular subcellular location (for review see Ref. 1; Fig. 1).

Within a single cell or tissue, multiple myosins are present. For example, in a porcine kidney proximal tubule cell line, expression of myosins representing all seven classes has been observed (4). Indeed, upward of 40 different full-length or partial cDNAs have been identified in vertebrates which, by comparison at the amino acid level, can be grouped as 14 distinct unconventional myosins (Table I). As recently confirmed by mapping studies, vertebrates express at least six myosins-I, two myosins-V, one myosin-VI, two myosins-VII, two myosins-IX, and one myosin-X in addition to 10 different isoforms of myosin-II (Table I) (2). We will be summarizing the most recent advances in the study of vertebrate unconventional myosins focusing on the two major approaches taken to better understand the functions of unconventional myosins: traditional biochemical and cell biological approaches and genetic studies.

Myosin-I

All vertebrate myosins-I consist of a 110–130-kDa heavy chain and 1–6 CaM LC (Fig. 1). A comparison of the primary structure of the head domains of known myosins-I indicate that there are at least 4 distinct subclasses (6, 7) (reviewed in Ref. 1), all of which are expressed in vertebrates. The working hypothesis is that each subfamily performs distinct sets of functions; however, there are no genetic studies that firmly establish a function for a vertebrate myosin-I.

Subclass 1, Acanthamoeba Type Myosins-I: Human Myosin-IC, Chicken BB Myosin-IB, Rat Myr3—It has been more than two decades since the initial purification of myosin-I of this subclass from Acanthamoeba (8), and the wealth of information with regard to the biochemical properties, functional domain structure, and subcellular localization of the multiple myosins-I expressed in Acanthamoeba has been reviewed in detail elsewhere (for references see Refs. 1, 9, and 10). Several subclass I myosins have been identified in vertebrates (see Table I). Based on primary structure, as well as functional domain mapping studies of Acanthamoeba myosins-I, the tails of these myosins have been divided into three tail homology (TH1–3) subdomains. The TH-1 domain, positioned immediately after the neck domain is rich in basic residues and may effect binding to acidic phospholipids. A similar basic domain comprises the full tail of the other three known myosin-I subclasses. The TH-2 domain is rich in proline and also either glycine, as in human myosin-IC (Fig. 1), or glycine/alanine and glycine/glutamate, as in Dictyostelium myosins-I. For Acanthamoeba myosins-I-1, it has been shown that this domain exhibits ATP-insensitive actin binding, thus allowing this myosin to cross-link actin filaments. This critical feature has not been established for vertebrate subclass I myosins. The TH-3 domain consists of a Src homology-3 (SH3) domain. In Acanthamoeba, it has been shown that the head domain of this class of myosin-I must be phosphorylated to be active by a specific myosin-I heavy chain kinase at a serine or threonine residue in a conserved portion of the motor domain (reviewed in Ref. 10). None of the known vertebrate myosins-I contain either a serine or threonine at this site (the THDS site (11)).

The expression profile of human myosin-IC (12) and its rat ortholog, Myr3 (13), indicate that this myosin-I is present in a wide range of tissues and cell types. Localization studies have been restricted thus far to cultured normal rat kidney cells, and in these cells, Myr3 exhibits a punctate distribution in the cytoplasm and is concentrated in F-actin-rich, elongate structures at sites of cell contact. Although no subclass I myosin-I has been purified from a vertebrate source, analysis of the ATP-dependent actin binding of Myr3 present in tissue extracts suggests that the TH-3 domain of Myr3 may modulate the interaction of this myosin-I with actin.

Subclass 2, BB Myosin-I and Myr1/Myosin-Iα—The founding member of this myosin-I subclass is chicken brush border (BB) myosin-I (Fig. 1, Table I). BB myosin-I is expressed in the intestinal epithelia of the large and small intestine, where it comprises the spiral lined microvilli that tether the microvillus actin core to the plasma membrane (reviewed in Ref. 14). In contrast, to BB myosin-I, the other members of this subclass, myosin-Iα and Myr1 (Table I), have been shown to be expressed in a wide range of tissues. Both BB myosin-I and myosin-Iα/Myr1 contain multiple neck-associated CaM LC. Myr1 has been purified from liver (15) and, like BB myosin-I (for review of biochemical studies of BB myosin-I see Refs. 14, 16, and 17), Myr1 exhibits a relatively slow (30–50 nm/s) in vitro motility that is inhibited by calcium.

Since BB myosin-I is among the best characterized vertebrate unconventional myosins, it serves as an excellent test case to determine if in vitro expression systems can be used for detailed structural and functional analysis of these myosins. Thus, it is noteworthy that expressed chicken BB myosin-I, with properties comparable with tissue-purified protein, has been obtained from transfected COS cells (18) and most recently using the baculovirus expression system in Sf9 cells (19).

A fundamental question to address for these and all other unconventional myosins is what role the tail domain plays in subcellular localization. In the case of BB myosin-I, Fodor and Bretscher
myosin classes are shown in and tail domains of vertebrate myosins. IQ repeats are shown as sin-I main is necessary for proper subcellular targeting. Higher levels of cytosolic expression suggesting that the head domain of myosin-V contains a conserved functional docking site. Purified myosin-V is also associated with two as yet unidentified LC distinct from calmodulin. The tail domain of myosin-V is of unknown function but may serve to target this myosin to organelles. Interestingly, sequences similar to the domain of myosin-V were recently uncovered in the non-myosin proteins human AF-6 and its homolog Drosophila Canoe (30), suggesting that the tail of myosin-V contains a conserved functional domain as is seen for many of the other unconventional myosin classes (Fig. 1).

Myosin-V

Myosin-V, unlike myosin-I, are two-headed as they self-assemble via an α-helical coiled-coil portion of the tail (Fig. 1). All myosin-V contain six IQ motifs and have been shown to be associated with multiple CaM LCs. Purified myosin-V is also associated with two as yet unidentified LC distinct from calmodulin. The tail domain of myosin-V is of unknown function but may serve to target this myosin to organelles. Interestingly, sequences similar to the tail of myosin-V were recently uncovered in the non-myosin proteins human AF-6 and its homolog Drosophila Canoe (30), suggesting that the tail of myosin-V contains a conserved functional domain as is seen for many of the other unconventional myosin classes (Fig. 1).

Genetically, myosin-V have been implicated in membrane movements. Mice with mutations in myosin-V exhibit a lightened coat coloring or dilute phenotype. This is due to a defect in transport of the melanosomes, or pigment granules, to the dendrites of the melanocytes, which results in inefficient phagocytosis of the pigment by the keratinocytes of the hair shaft (summarized in Ref. 31). To date, actin-based movement of melanosomes has not been observed, but supporting a role for myosin-V in melanosome movements, preliminary work (32) has shown that myosin-V can be immunoprecipitated from isolated melanosomes from normal animals but not from dilute mice.

Other allelic of dilute termed dilute-lethal, in addition to the lightened coat coloring also produce an abnormal postnatal neurological disorder characterized by convulsions and death by 3 weeks of age (31). No obvious defect in neuroanatomy of these mice has been observed, and the basis for the neurological abnormalities is not understood. Because of the abundant expression of myosin-V within neurons, but not within other cells of the nervous system, it has been proposed that this myosin postulated to be involved in the movement of organelles within neurons.
actin bundles at rates of 0.48 μm/s, a rate very similar to what is seen for chick brain myosin-V. It has been characterized.3 As diagrammed in Fig. 1, myosin-VIIa has an organelle motor in brain. Recent biochemical studies on purified chick brain myosin-VI homologs (36). Using extracts from rat kidney, myosin-VI was shown to bind F-actin in an ATP-sensitive fashion (36). Myosin-VI motility has not been observed yet, however, perhaps due to regulation by phosphorylation of the heavy chain at a conserved threonine residue (TEDS site) similar to what is seen for Acanthamoeba myosin-I (11, 36).

Myosin-VI is ubiquitously expressed, but recent genetic studies suggest that its most critical function is in the maintenance of the cells of the inner ear. Avraham and colleagues (37) were interested in identifying the mouse recessive deafness gene, Snell’s waltzer (sv). sv mice are profoundly deaf due to a complete degeneration of the neuroepithelium of the cochlea and exhibit head tossing and circling phenotypes characteristic of vestibular dysfunction. Positional cloning of sv identified it as the gene for mouse myosin-VI, encoding a polypeptide 91% identical at the amino acid level to porcine myosin-VI (37). Myosin-VI protein was located within the neuroepithelium of the cochlea and was found to be expressed by the inner and outer hair cells of the organ of Corti (37). Within these cells it was seen to be enriched at the cuticular plate, an actin-rich structure at the bases of the stereocilia (37). This location is reminiscent of previous immunolocation studies, which showed that myosin-VI was enriched within the subapical terminal web region of brush border containing polarized epithelial cells (36, 38). These locations have suggested a role for myosin-VI is membrane movements, endocytosis, or membrane recycling events within polarized cells.

**Myosin-VII**

Myosin-VIIa was recently identified as the gene defective in mouse shaker-1, a recessive deafness mutant (39) and in human Usher syndrome type 1B, a recessive disease characterized by congenital deafness, vestibular dysfunction, and retinitis pigmentosa (40). Since these genetic studies, the complete cDNA of human myosin-VIIa has been characterized.3 As diagrammed in Fig. 1, myosin-VIIa has an N-terminal motor, five IQ motifs in its neck domain, and a short coiled-coil domain at the proximal portion of the tail. The remainder of the tail is comprised of two large ~460-amino acid repeats. Each repeat contains two domains, which have been called the MYTH4 domain and the talin-homology domain.3 The MYTH4 domain, for myosin tail homology 4, is shared by myosin-VIIa and Acanthamoeba high molecular weight myosin-IV and is of unknown function. The talin-homology domain, however, is a conserved sequence shared by members of the band 4.1 superfamily and has been shown for both talin and band 4.1 to bind to acidic phospholipids (reviewed in Ref. 41). Sequence homologies suggest that the tail of myosin-VIIa targets the motor to the plasma membrane, as is seen for ezrin, moesin, and other band 4.1 members.

Immunolocation studies have focused on the expression of myosin-VIIa within tissues affected in Usher syndrome. In the cochlea, myosin-VIIa was expressed exclusively by the sensory hair cells of the organ of Corti. Within the inner and outer hair cells, myosin-VIIa is expressed within the stereocilia, as well as within the cuticular plate and throughout the cell body (43). This is distinct from the location of myosin-VI, which was not located to the stereocilia, and myosin-β, the putative adaptation motor, which was specifically located to the stereocilia tips. Since myosin-VIIa is expressed along the entire length of the stereocilia and is potentially a membrane binding protein, myosin-VIIa may serve a structural role, as is seen for brush border myosin-I, tethering the polarized actin filaments of the stereocilia to the plasma membrane.

In the rat retina, myosin-VIIa was expressed exclusively by the pigmented epithelial cells (43). This result suggests that the reti-

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**TABLE I**

| EXAMPLE     | HOMOLOGS & ORTHOLOGS                  |
|-------------|---------------------------------------|
| Myosin-I    |                                       |
| subclass 1  |                                       |
| Human myosin-I | Rat myosin-1                  |
| Chicken BrBb | Human myosin-I                 (*) |
| subclass 2  |                                       |
| Chicken brush border myosin-I | Bovine myosin-I heavy chain |
| Mouse myosin-Ix | Rat myosin-1x |
|            | Human myosin-IxB               (*) |
| Mouse myosin-Ix | F rog myosin-Ix               (*) |
| subclass 3  |                                       |
| Bovine myosin-Iβ | Rat myosin-2 |
| Rat myosin-Iβ | F rog myosin-Iβ               (*) |
| subclass 4  |                                       |
| Rat myosin-γ | Porcine myosin-I               (*) |
| Myosin-V    |                                       |
| Mouse myosin-VI | Mouse Snell’s waltzer            |
|              | Human myosin-VI                (*) |
|              | F rog myosin-VI               (*) |
| Myosin-VII  |                                       |
| Human myosin-VIIa | Mouse shaker-1                  |
|              | Rat myosin-VIIa                (*) |
| Human myosin-VIIb | F rog myosin-VIIa            (*) |
| Myosin-JX   |                                       |
| Rat myosin-5 | Human myosin-JXx               (*) |
| Human myosin-JXx | F rog myosin-JXx        (*) |
| Myosin-X    |                                       |
| Bovine myosin-X | F rog myosin-X                  |
|              | Porcine myosin-VIIb         (*) |

(*) indicates partial cDNA clone. For references see (Ref. 1), except for rat brush border myosin-I (5) and rat myosin-I (22), both of which were recently cloned.

**Myosin-VI**

Compared with myosins-I and -V much less is known of the biochemistry of myosin-VI. Sequence analysis of myosin-VI revealed that the 145-kDa heavy chain has a 200-aa coiled-coil domain in its tail, which could allow for dimerization (Fig. 1). In agreement with the presence of a single IQ motif in the neck domain, coimmunoprecipitation studies showed that myosin-VI was associated with CaM (36). The neck domain of myosin-VI is unusual, however, as there is a 50-aa linker between the end of the motor domain and IQ motif (Fig. 1). It is unknown if this linker serves as a binding site for a novel LC. The tail domain of myosin-VI is unique, and it is remarkably conserved among myosin-VI homologs (36). Compared with myosins-I and -V much less is known of the actin filament assembly, respectively. This treatment cultured in low doses of nocodazole and cytochalasin, inhibitors of movement of particles within the growth cones of neurons ratbrushbordermyosin-I(5) and ratmyosin-I (35). In striking contrast to conventional myosins and BB myosin-I, indicate that the ATP-dependent actin binding and ATPase properties of this motor are consistent with that of an organelle motor (35). In striking contrast to conventional myosins and BB myosin-I, the steady state Mg-ATPase of myosin-V is maximally activated at very low F-actin concentrations, with a KATPase of ~1 μM actin. It will be critical to determine if these steady state measurements indicate that myosin-V can remain associated with an actin filament for longer periods than conventional myosin during its duty cycle, a critical feature for a motor expected to translocate organelles.

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3 Chen, Z.-Y., Hasson, T., Kelley, P. M., Schwender, B. J., Schwartz, M. F., Remer, M., Kimble, J., Moore, T. A., and Corey, D. P. (1996) Genomics, in press.
nitis pigmented observed in Usher patients is due to a defect in this cell layer. This is quite unique, as all previously identified retinitis pigmentosa genes are defects within the phototransduction machinery (44). Within the retinal pigmented epithelial (RPE) cells, myosin-VIIa was enriched at the apical plasma membrane (43). On the apical surface of RPE cells are villi, actin-rich structures involved in phagocytosis of spent photoreceptor outer segments. This phagocytosis is essential for photoreceptor viability, as defects in this process have been shown to lead to retinal degeneration in the RCS rat (45). In these animals, cellular debris accumulates in the intercellular space between the outer segments and the RPE layer, leading to photoreceptor cell death. Perhaps myo-

**Myosin-IX and -X, Links to Cell Signaling Pathways**

Myosin-IX motors are unusual in that they have an N-terminal extension, which is distinct from all other myosins as well as an insert of 150 aa in the actin binding loop (reviewed in Ref. 1), suggesting that the class IX myosins may have unusual mechanochemical properties. Also of interest is a striking tail domain, which contains a chimerin-like apposition of a zinc-binding motif and a GTPase-activating protein (GAP) domain (Fig. 1). This sequence homology suggests that myosin-IX may serve as GAP proteins for the Rho/Rac family of Ras-like GTP-binding proteins. As reviewed recently (46), assays performed using bacterially expressed fusion proteins containing domains from Myr5, a rat myosin-IX, have shown that the tail does indeed bind zinc, but, unlike chimerin, the Myr5 GAP domain preferentially activated the GTPase activity of Rho, and only marginally activated Rac1 (47). Rac and Rho have both been shown to be critical in the membrane reorganizations involved in the induction of membrane ruffling and the assembly of focal adhesions in fibroblasts. Myosins-IX may therefore form a direct link between the actin cytoskeleton and the Rho-dependent signaling pathway (reviewed in Ref. 48).

Recently a human class IX C DNA was identified, myosin-IXb (49). Myosin-IXb is highly homologous to Myr5, except at the C-terminal 100-aa tip of the tail suggesting that myosin-IXs are alternatively spliced. Expression of myosin-IXb was characterized in a myelocytic cell line HL-60, where it was shown that myosin-IXb exhibited an increase in expression level and a redistribution in location upon differentiation of these cells from promyelocytic to macrophage states (49). This suggests a role for myosin-IXb in the G-protein-coupled actin-based changes in cell shape observed upon differentiation in myelocytic lineages.

Another unconventional myosin with a likely association with G-protein pathways is myosin-X, the most recently identified vertebrate myosin class. First identified in frog (50), myosin-X has now been completely cloned from cow (4) and is shown schematically in [Fig. 2](#fig2). Myosin-X has now been completely cloned from cow (4) and is shown schematically in [Fig. 2](#fig2). Myosin-X has now been completely cloned from cow (4) and is shown schematically in [Fig. 2](#fig2).

**Conclusions**

Clearly, actin-based motilities are critical for the proper function and development of such diverse cell types as sensory hair cells, melanocytes, retinal epithelia, and neurons. In addition to their presumed functions in motile processes, the studies described here highlight the potential roles of unconventional myosins in signal transduction and in direct mechanochemical regulation of membrane channels.

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4 D. P. Corey and R. E. Cheney, unpublished results.