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Tails of unconventional myosins

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Abstract. In addition to the conventional myosins (class II) required for processes such as muscle contraction and cytokinesis, the myosin superfamily of actin-based motor proteins includes at least 14 'unconventional' classes. These unconventional myosins are defined by myosin-like head (motor) domains attached to class-specific tail domains that differ greatly from those of myosin-II. The unconventional myosins account for almost two-thirds of the 28 or more myosin genes currently believed to be expressed in humans and 80–90% of the ~10 or more myosin genes expressed in a typical nonmuscle cell. Although these members of the myosin superfamily have not been as intensively investigated as the conventional myosins, unconventional myosins are known or believed to power many forms of actin-based motility and organelle trafficking. The presence of signaling domains such as kinase domains, SH3 domains, PH domains or GTPase-activating domains in the tails of unconventional myosins indicates that these proteins can also be components of signal transduction pathways. Since several classes of the myosin superfamily have been found only in lower eukaryotes or plants (VIII, XI, XIII and XIV), in this review we will focus on the structures and properties of the unconventional myosins found in multicellular animals (excluding classes I and V, which have been reviewed elsewhere recently). Special attention will be focused on the three classes of unconventional myosins that can cause deafness in mouse or humans when mutated. In addition, we discuss the discovery of a pair of intriguing domains, the Myosin Tail Homology 4 (MyTH4) and FERM (band 4.1, Ezrin, Radixin, Moesin) domains, that are present in the tails of otherwise very different myosins as well as a plant kinesin-like protein. Recent progress in the identification of novel unconventional myosins will also be summarized.

Key words. Motor proteins; unconventional myosin; myosin genes; myosin-III; ninaC; myosin-VI; myosin-VII; myosin-IX; myosin-X; myosin-XII; myosin-XV; MyTH4 domain; FERM domain; deafness; shaker; Usher syndrome.

Introduction

The past several years have witnessed explosive growth in our knowledge of the myosin superfamily of actin-based motor proteins, which currently consists of at least 15 distinct classes [1] (reviewed in [2–6]). In general, the heavy chains of unconventional myosins are characterized by an ~80-kDa head, a 'neck' consisting of one or more repeats of the IQ motif, and a class-specific tail [7]. The head is responsible for binding to F-actin, hydrolysis of ATP and production of force, whereas the IQ motifs provide binding sites for calmodulin or calmodulin-like light chains [8]. The tail domains of myosins are believed to be largely responsible for class-specific functions such as bipolar filament formation for the class II myosins or GTPase-activating activity for the class IX myosins. Unlike muscle myosins, many of the unconventional myosins either bind to or localize to cell membranes. The tails of many unconventional myosins include segments predicted to form coiled coils, suggesting that they may form dimers. Until recently it was thought that there was little other structural similarity in the tails of the different classes, each of which is phylogenetically ancient and believed to be associated with conserved, if largely unknown, biological functions [9, 10].

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Although the yeast *Saccharomyces cerevisiae* expresses only 5 myosin genes, from 3 classes (classes I, II and V) [11], humans have at least 28 myosin genes (10 conventional and 18 unconventional) from ~10 classes. Most important, typical nonmuscle cells cultured from vertebrates appear to express only 1 or 2 conventional myosins but upwards of 10 unconventional myosins [12]. While relatively little is known about most of these unconventional myosins, their importance is underscored by the discovery that mutations in unconventional myosins can lead to deafness, blindness, seizures and death [6].

Since other reviews in this series focus on myosins from plants and on studies of myosins from classes I and V, the emphasis here will be on the other unconventional myosins, especially those of multicellular animals. Particular attention will be given to the intriguing protein domains found in the tails of these myosins and to the 'deafness myosins' from classes VI, VII and XV. The identification of several novel unconventional myosins will also be summarized. Wherever possible, experimental data will be reviewed that shed light on both the biological role, as well as the impact on human diseases, for each of the classes considered. We will follow the convention used in Mermall et al. [6] and use Arabic numbers to abbreviate the names of particular myosins (for example, myosin-VIIa will be referred to as M7a).

Class III: ninaC and other myosins with N-terminal kinase domains

The *Drosophila* ninaC (neither inactivation nor afterpotential) myosin was initially discovered in a screen for phototransduction mutants [13] and now provides a model for the function of unconventional myosins in signal transduction. Defects in ninaC, which is a relatively abundant component of the *Drosophila* photoreceptor, lead to electrophysiological defects and retinal degeneration [13]. Within the photoreceptor cells, the larger of the two splice forms of ninaC is located in the rhabdomere [14, 15], a microvillus-like structure that is the site of phototransduction [16]. Like brush-border myosin-I in intestinal microvilli, ninaC appears to form morphological cross-links between the actin filaments at the core of the rhabdomere and the surrounding plasma membrane [16]. NinaC is unusual in that a ~300-amino acid kinase-like domain is located N-terminal to its motor domain [13]; recent experiments with baculovirus-expressed ninaC indicate that this domain is in fact a functional serine-threonine kinase [17]. In addition to its rather divergent kinase and myosin head domains, ninaC has a neck domain consisting of either one or two IQ motifs (depending on the splice form) that bind to calmodulin [18]. NinaC's relatively short tail exists in two alternatively spliced forms, p132 and p174, the larger of which contains a rhabdomere localization signal [14]. Mutations that abolish ninaC's rhabdomere localization also lead to an absence of calmodulin in the rhabdomere, indicating that this unconventional myosin is responsible for transport or localization of this Ca^{++}-signaling molecule [15]. Interestingly, another unconventional myosin, brain myosin-V, has recently been reported to bind to Ca^{++}-calmodulin-dependent kinase [19], and may thus also be involved in anchorage or transport of calmodulin.

NinaC is a component of a remarkable multiprotein signaling complex known as the 'transducisome' [20] or 'signalplex' [21]. This signaling complex is based on the inaD scaffolding protein, which physically associates via its five PDZ domains with most of the key proteins of the phototransduction signaling cascade including rhodopsin, a phospholipase C, the cation channels TRP and TRPL, and a protein kinase C [20, 21]. Recent data indicate that ninaC binds directly to the inaD scaffolding protein and links it to the actin filament at the core of the rhabdomere [22]. Although little is known about the motor properties of ninaC, the possibility that it is a component of a motile signaling complex is extremely intriguing. In this respect it is important to realize that an unconventional myosin might exert force and move over distances much smaller than the micron-scale distances easily observed with light microscopy. The putative adaptation motor myosin-Ib [23], for example, might only need to move a few nanometers to open or close the stretch-activated channels in the microvilli-like stereocilia of hair cells in the inner ear.

Although class III myosins appear to be absent from *Caenorhabditis elegans*, a new member of the myosin III class has recently been identified in the horseshoe crab *Limulus polyphemus*. This class III myosin, like ninaC, has an N-terminal kinase-like domain and is found in photoreceptors [24]. The *Limulus* class III myosin was also shown to bind calmodulin and to undergo circadian clock-dependent changes in phosphorylation [24]. There are also initial reports of one sequence from human retina (HM3a) and two sequences from fish retina (FM3a and FM3b) that appear to be members of the myosin-III/ninaC class [25]. The more completely sequenced of the two fish homologs includes an N-terminal kinase-like domain, providing strong evidence that class III myosins are indeed present in vertebrates [25].

Class IV: *Acanthamoeba* high molecular weight myosin

The founding and currently only member of the myosin-IV class myosins is a 178-kDa myosin heavy

chain from the soil amoeba *Acanthamoeba castellanii* [26]. The head of this myosin, which was originally considered to be a myosin-I, shares ~38% amino acid sequence identity with conventional myosins. *Acanthamoeba* M4 contains a single IQ motif in its neck domain and is thus expected to bind to a single calmodulin or calmodulin-like light chain. The tail of M4 includes one MyTH4 domain (myosin tail homology 4) [27]. As described below, similar domains are also found in the tails of M7, M10, M12 and M15, although *Acanthamoeba* M4 is the only of these myosins that has been purified. The tail of *Acanthamoeba* M4 ends in an SH3 domain (Src homology 3), a signaling domain that binds to proline-rich motifs and that is also present in the tails of several myosins-I [26]. No interacting proteins are known for *Acanthamoeba* M4, although a proline-rich ligand has been identified for the SH3 domain of *Acanthamoeba* myosin-IC [28]. Preliminary purification data indicate that *Acanthamoeba* M4 has a single head, is approximately 50 nm in length and moves at ~110 nm/s in the sliding filament assay [29]. The biochemical advantages provided by *Acanthamoeba* may make it an attractive system in which to study the properties of a MyTH4-containing myosin.

Class VI: *Drosophila* 95F and a deafness mutation in the *Snell's waltzer* mouse

Class VI was originally described in *Drosophila melanogaster* as an ~140-kDa myosin heavy chain from chromosomal locus 95F [30]. The class VI myosins have relatively short tail domains, and the tail of the *Drosophila* 95F myosin is subject to alternative splicing [30]. An M6 named HUM-3 was recently identified from a survey of unconventional myosins in *C. elegans* [31], and automated analysis of the now virtually complete *C. elegans* genome indicates that a second M6 gene also exists (accession no. AF125462) in this organism. This newly identified M6 shares approximately 60% overall identity with HUM-3 (66% identity in the head domain).

The class VI myosins are one of the three unconventional myosin classes already known to be associated with deafness. Class VI myosins in vertebrates are currently known from full-length sequences in pig [32], mouse [33], human [34] and chicken [35], as well as a partial head sequence from frog [36]. Conserved features of class VI myosins include a head domain with an unusual ~25 amino acid insert near the conserved 'ERNYHIF' sequence [32] and a threonine at the TEDS rule site [37], which suggests that M6 motor activity may be regulated by head domain phosphorylation. The neck domain has an ~52-amino acid region unique to M6 followed by a single IQ motif that appears to bind calmodulin [32]. The tail includes a region predicted to form a coiled coil, thus raising the possibility that the class VI myosins are dimers. The predicted coiled-coil region in vertebrate M6 includes ~192 amino acids [32], which could theoretically form a dimeric stalk of ~30 nm. Although the functions of the globular M6 tail domain remain unknown, M6 has recently been shown to coprecipitate and colocalize with a homolog of the microtubule-binding protein CLIP-170 [38]. Since CLIP-170 is implicated in the anchorage of organelles to microtubules, this provides yet another intriguing example of an interaction between components of the microtubule- and actin-based transport systems.

Mermall et al. [39] used three-dimensional microscopy to demonstrate that the transport of cytoplasmic particles in living *Drosophila* embryos could be inhibited by injection of antibodies to the 95F myosin. Such a role in particle transport might suggest either that M6 is processive or that it functions in an ensemble of multiple motors. The unknown nature of the transported particles also raises the possibility that unconventional myosins may be involved in the transport of nonmembranous particles or complexes so small or nonrefractile as to be invisible without the use of specific tags. In a subsequent study, antibody injection into embryos led to disruption of the actin cytoskeleton and implicated *Drosophila* M6 in the formation of transient cytokinetic furrows [40].

M6 appears to be one of the more abundant and broadly distributed of the unconventional myosins. Relatively high levels of expression are observed in kidney, intestine, testis and brain [32]. In LLC-PK_1 cells, a proximal tubule-derived cell line, M6 constitutes almost 1% of total protein [32]. In cell extracts, M6 cosediments with actin filaments in an ATP-sensitive fashion, as expected for a myosin. In kidney, M6 localizes to the actin-rich apical brush border of proximal tubule cells and is largely insoluble [32]. This is in marked contrast to cultured LLC-PK_1 cells, where M6 is soluble and not associated with actin-rich compartments [32]. The solubility of M6 thus appears to depend on the differentiation status of the cells in which it is found. A recent study reports that M6 localizes to the Golgi apparatus and leading edge of cultured cells and that epidermal growth factor (EGF) treatment triggers phosphorylation of its head domain as well as recruitment into membrane ruffles at the leading edge [35].

One of the most exciting features of M6 has been the discovery that a recessive mutation in M6 is the defect in the *Snell's waltzer* mouse, which suffers from deafness and vestibular problems [33]. These mice exhibit a degeneration of the sensory neuroepithelia required for hearing and balance, but appear to lack defects in other tissues despite the widespread expression of M6. Hearing and vestibular function both involve the detection

of small movements of stereocilia, which are microvillus-like structures with actin filament cores found in highly organized bundles on the apical surfaces of hair cells. The mechanotransduction underlying hearing and balance occurs when bundles of stereocilia are deflected in a particular direction, which pulls open stretch-activated channels on the stereocilia, leading to electrical depolarization of the hair cell. The *Snell's waltzer* mice exhibit a loss of the hair cell stereocilia, and are thus unable to engage in mechanotransduction. Localization studies in hair cells indicate that M6 is especially abundant in the cuticular plate [33, 41], a filamentous meshwork into which the roots of the stereocilia insert that appears to provide them with a foundation for physical support and proper orientation. Within the hair cell, M6 is also found in the 'pericuticular necklace', an organelle-rich region surrounding the cuticular plate [42]. Although the exact cell biological function of M6 remains unclear, the hair cell's requirement for a highly organized apical cytoskeleton appears to make it a very sensitive detector of defects in proteins required for the proper organization of the actin cytoskeleton. Since M6 mutations clearly cause deafness in mice, M6 is a strong candidate to underlie one or more of the numerous forms of recessive nonsyndromic deafness in humans. The human *MYO6* gene has recently been cloned and mapped to chromosome 6q13, which should facilitate the search for mutations in human M6 [34].

Class VII: deafness mutations with MyTH4 and FERM domains

M7a has been cloned from mouse, where it is the product of the *shaker-1* (*sh1*) deafness gene [43, 44], and also from human [27, 45], where it is mutated in Usher syndrome 1B [46], the most common form of hereditary deaf-blindness in human children. M7a has a ~250-kDa heavy chain with a myosin-like head, a neck with five IQ motifs, and a tail that includes ~70 amino acids predicted to form a coiled coil, two MyTH4 domains and two FERM domains (see fig. 1 and tail domain discussion below) [27, 45]. A poorly conserved SH3

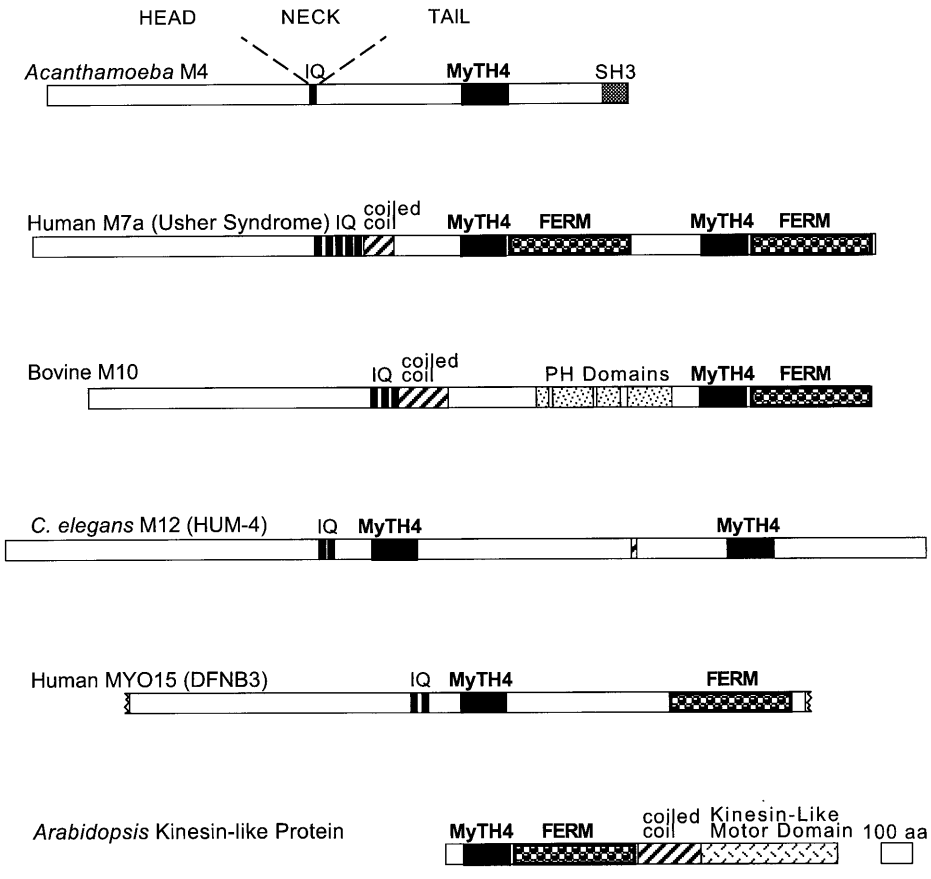

Figure 1. A bar diagram illustrating the presence of the MyTH4 domain and the FERM domain in several different myosins and a kinesin. Other known domains and motifs, such as SH3 domains and the IQ motifs are also illustrated. Note that the MyTH4 and FERM domains are often arranged in tandem and that the exact boundaries of these domains are not yet precisely defined.

domain may also be present in the middle of the tail [44]. A partial head domain sequence for M7a has been cloned from frog [36]. There is also evidence that vertebrates contain a second member of the family, M7b [12, 47].

Class VII myosins also exist in lower organisms. A polymerase chain reaction (PCR) screen originally identified a class VII myosin in *Drosophila* at chromosomal locus 35B [48], and sequence analysis of genomic sequence (accession no. AC002502) indicates that this myosin also has a tail with two MyTH4 domains and two FERM domains. Recent data indicate that *Drosophila* 35B is likely to underlie the *crinkled* mutation, which is associated with developmental abnormalities such as blunt sensory hairs and crinkled wings [49]. In addition, our searches of the *Drosophila* genome provide evidence for a second member of the myosin-VII family in fly (accession no. AC005834; chromosomal locus 28B1–B4). This sequence exhibits approximately 60% identity with the head of the *Drosophila* myosin-VII at locus 35B and contains three well-conserved IQ motifs, two MyTH4 domains and two FERM domains. The *C. elegans* unconventional myosin HUM-6 is also a class VII myosin based both on its head domain similarity and on its tail domain structure [31]. Finally, a partial tail sequence (accession no. U83089) from *Dictyostelium* MyoI [50] contains a MyTH4 and FERM domain, indicating that it shares some features with the class VII myosins.

M7a is responsible for the *sh1* mutation in mice [43], which results in deafness and vestibular dysfunction. Thus, it was exciting when M7a was linked to a human form of deafness, Usher syndrome 1B [45, 46]. This disease is the most common form of inherited recessive deaf-blindness, with symptoms including deafness, loss of vestibular function and progressive retinal degeneration. In addition, patients exhibit abnormal microtubule organization in sperm tail axonemes, photoreceptor cells and nasal cilial cells [46]. As a protein of significant clinical interest, M7a has drawn a great deal of attention and is rapidly becoming one of the better characterized unconventional myosins. The pattern of M7a expression in tissue correlates well with sites most affected in the pathogenesis of Usher syndrome, and mutational analysis is beginning to shed light on the role of M7a in normal cell biology as well as in the disease state.

Myosin VII in development and disease

Localization studies in the inner ear suggest unique and tissue-specific roles for several myosins, including M7a, since antibodies to four different myosins were nonoverlapping in their distributions [104]. M7a expression was specifically localized to the cytoplasm and apical stereocilia of the cochlear inner and outer hair cells, the actin-rich apical domain of retinal pigmented epithelial (RPE) cells, and also in testis, lung and kidney [42]. In another study by Sahly et al. [51], mouse embryos were screened for M7a messenger RNA (mRNA) and protein levels during normal development. Strikingly, expression of M7a was seen only in epithelial cell types, concomitant with the appearance of microvilli and cilia. Earliest expression was detected in the otic vesicles, followed by the olfactory epithelium and liver, then RPE, tongue and other tissues, especially those with microvilli.

Wolfrum et al. [52] tested the hypothesis that M7a is a common component of cilia by surveying a variety of tissues by immunoelectron microscopy. M7a immunoreactivity was detected in the cochlear stereocilia (specialized microvilli). In addition, M7a was found along the length of the kinocilium (a single true cilium found on the apical surface of the hair cell), especially at the periphery, but not at the kinocilium's basal bodies. The presence of M7a was also demonstrated in olfactory bulb and renal distal tubule cilia, in addition to the previously reported localization in the apical olfactory epithelium and renal proximal tubule (both regions with actin-rich microvilli). M7a was not detected in the axoneme of spermatazoa but was present in biochemical fractions of photoreceptor outer segments enriched in axonemal tubulin. This demonstration of M7a in cilia in addition to microvilli points to additional roles of M7a in these specialized cytoskeletal structures. Although it is thought that M7a is required to maintain the integrity of hair bundles in the inner ear, M7a may also be required developmentally in the kinocilium, which is hypothesized to play a role in organizing the array of stereocilia. M7a may also play dual roles in the photoreceptor and apical microvilli of the RPE. The demonstration of a putatively actin-based motor within the predominantly microtubule-based cilium raises intriguing questions about the nature and significance of actin-based transport in these organelles.

Interestingly, *sh1* mice do not perfectly reflect the disease phenotype seen in humans. Although they exhibit hearing loss and vestibular dysfunction, the *sh1* mice have apparently normal vision and do not undergo retinitis pigmentosa as seen in Usher syndrome IB. Since M7a is detected in both photoreceptors and RPE, both of these cell types could be responsible for the late onset retinal degeneration. It seemed that this disparity was explained by el Amraoui et al. [53], who were able to detect mouse M7a expression only in the RPE. The progressive retinal degeneration that is seen in humans with Usher syndrome IB was therefore thought to be a primary defect in the rods and cones. However, M7a

was detected in both human and rodent photoreceptor connecting cilia by immunoelectron microscopy [54]. Thus, it is still not known why *sh1* mice fail to exhibit retinitis pigmentosa.

Mutational analysis

Of the five loci for Human Usher syndrome type I (USH I), only the gene for *USHIB* has been characterized. *USHIB* encodes M7a, and is clearly the ortholog of the mouse *sh1* gene. Sequence analysis of patients affected by Usher Syndrome IB reveals a tremendous heterozygosity in M7a mutations [46, 55–60]. It is clear that sequence-based prenatal diagnosis like this could now distinguish between syndromic deafness associated with defective M7a and other nonsyndromic forms of hereditary deafness. Moreover, careful analysis of the phenotypes exhibited by patients with particular mutations may prove useful in the study of the function of M7a.

Mutational analysis in mice has already begun to shed light on M7a activity. Seven different mouse mutations for M7a have been described [44]. For example, the original *sh1* mutation is known as $Myo7a^{sh1}$ and contains an arginine-proline missense mutation that results in moderate hearing loss but relatively normal protein levels. $Myo7a^{6J}$ encodes an Arg-145-Pro missense mutation in a highly conserved region of the motor domain. The $Myo7a^{816SB}$ mutation results in a 10-amino acid deletion within the head domain due to inappropriate splicing. Both $Myo7a^{6J}$ and $Myo7a^{816SB}$ have serious effects on M7a activity and result in significantly decreased protein levels: 21% and 6% of normal, respectively [44].

Richardson et al. utilized the $Myo7a^{sh1}$ and $Myo7a^{6J}$ mutants to study the role of M7a in membrane trafficking [61]. They observed that M7a was expressed at high levels in the hair cells of the inner ear and in the kidney proximal tubule (regions that exhibit severe toxicity from aminoglycoside antibiotics such as gentamicin), and they hypothesized that M7a mutations might reduce the accumulation and ototoxic effect of gentamicin on hair cells. Interestingly, hair cells from $Myo7a^{sh1}$ mice were found to accumulate gentamicin, while those from $Myo7a^{6J}$ mice did not, suggesting that the level of M7a protein expression is important and that M7a is involved in some form of endocytic transport. It is still unclear whether this role involves active transport of receptors or other molecules, retention of aminoglycosides within the endocytic pathway, or membrane turnover.

Self et al. [62] studied the effect of three mutations on development and function of cochlear hair cells. In their preparations, $Myo7a^{6J}$ and $Myo7a^{816SB}$ mutants exhibit normal ultrastructural maturation of stereocilia, but highly eccentric placement of the kinocilium and deranged assembly of regular bundles of stereocilia. $Myo7a^{sh1}$ mice, on the other hand, displayed relatively normal rows of stereocilia. All three mutants exhibited decreased physiologic response to sound, although the $Myo7a^{6J}$ and $Myo7a^{816SB}$ mutants were more profoundly impaired. These results reflect the importance of M7a in the normal development of the hair cell bundles as well as postdevelopmental roles in the function of sensory hair cells.

Overall, these findings suggest that M7a is an important component of specialized cytoskeletal structures such as microvilli and cilia. The contributions of M7a to normal cell biology are likely to include important developmental and structural roles as well as active participation in membrane trafficking. Although little is known about nonvertebrate members of class VII, the characterization of human and mouse M7a might suggest possible roles for *C. elegans* HUM-6 and *Drosophila* 28B1–B4 and 35B. Mutations of the 35B myosin have been linked to *crinkled*, a mutant with defects in morphogenesis of a wide variety of actin-based structures [49]. Baker and Titus proposed that the role of HUM-6 in *C. elegans* may include maintenance of specialized cytoskeletal structures underlying the intestinal brush border and specialized chemosensory and mechanosensory neurons [31]. It will be exciting to see if HUM-6 mutations result in defective chemotaxis or sensing of osmolarity.

Is myosin tail homology a MyTH?

A $\sim$110-amino acid region known as the myosin tail homology (MyTH4) domain has been identified [27, 31, 45, 63] in class VII myosins and several other unconventional myosins (M4, M10, M12 and M15) as well as in a microtubule motor protein from plants (kinesin-like calmodulin binding protein; KLCBP) [64, 65] (see figs 1 and 2). Although the precise N- and C-terminal boundaries of this region are difficult to determine because of relatively low sequence identity, the core $\sim$70 amino acids are highly conserved. The tail of M7a includes two MyTH4 domains, the first of which contains an $\sim$90-amino acid insert when aligned with other MyTH4 domains (fig. 2). The MyTH4 domain was initially recognized by sequence similarity search algorithms such as BLAST, but it is now included by 'profile' search utilities such as Pfam [66]. The function of the MyTH4 domain is presently unknown, although its presence in several different putative motor proteins raises the possibility that different motor proteins might share similar mechanisms for targeting or regulation.

Ironically, the most exciting recent experimental evidence regarding the function of the MyTH4 domain comes from studies of the *Arabidopsis* kinesin-like

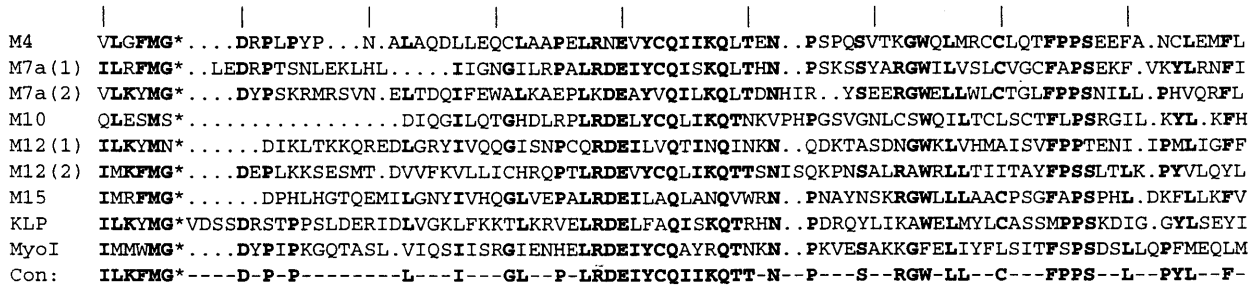

Figure 2. Alignment of MyTH4 domains in the highly conserved region of roughly 90 amino acids. The alignment was generated using the pileup algorithm of the GCG sequence analysis package and edited manually to reflect the insertion of roughly 94 amino acids in the first MyTH4 domain of mouse M7a (asterisk). Consensus was determined by a plurality of four and is indicated in bold. Abbreviations, GenBank accession numbers, and amino acids (aa) included in alignment are as follows: M4, *Acanthameoba* High Molecular Weight myosin I, # A23662, aa 1288–1366; M7a, mouse myosin-VIIa # U81453, aa 1045–1216 and 1777–1858; M10, bovine myosin-X, # U55042, aa 1572–1646; M12, *C. elegans* myosin-XII/HUM-4, # Z66563, aa 1112–1192 and 2312–2395; M15, mouse myosin 15, # AF053130, 889–969; KLP, *Arabidopsis thaliana* kinesin-like calmodulin-binding protein, # AF002220, aa 142–228; and MyoI, *Dictyostelium* MyoI, # U83089 partial cDNA sequence aa 142–224. For a more extensive set of MyTH4 alignments see the pfam collections of domain alignments and bar diagrams (http://pfam.wustl.edu).

calmodulin-binding protein [67]. In order to address the significance of calmodulin-binding on motor function, the authors studied microtubule binding properties of the kinesin motor domain of KLCBP in the presence and absence of nucleotide and/or calmodulin. In the process, they generated fusion constructs covering various domains of the protein, two of which corresponded roughly to the MyTH4 domain (amino acids 12–262) and the MyTH4-FERM domains (amino acids 12–503). Results of cosedimentation assays indicated an ATP-independent microtubule binding site within the N-terminal tail domain. The smaller fusion protein ($\sim$ MyTH4) was sufficient for microtubule binding, although the larger fusion protein ($\sim$ MyTH4-FERM) bound more effectively perhaps due to greater stability of the fusion construct. The MyTH4 domain, which was included in both N-terminal tail constructs, may thus constitute a binding site for microtubules. It is important to note, however, that neither fusion construct was intended to represent the 'MyTH4 domain', and both included some amino acids not generally considered part of the MyTH4 domain that may have mediated microtubule binding. Nevertheless, the possibility that MyTH4 domains in unconventional myosins bind microtubules is extremely interesting and may provide yet another example of an interaction between microtubule- and actin-based transport systems.

A FERM association with membranes

Immediately following each MyTH4 domain in M7a is an $\sim$ 300-amino acid domain similar to the membrane association domains of talin and other members of the band-4.1/ERM family [27, 31, 45, 63]. This domain, now referred to as a FERM domain [68], is found in many proteins including filopodin, merlin, ezrin, radixin, moesin, the tumor suppressor NF 2 and certain tyrosine phosphatases [69–72]. FERM domains are believed to be involved in membrane attachment either directly via binding to phospholipids or through association with integral membrane proteins [68].

The arrangement of MyTH4 and FERM domains in various unconventional myosins is intriguing for several reasons. First, the unusual tandem arrangement of MyTH4 and FERM domains is conserved in M7, M10 and KLCBP, but not in M15, in which the MyTH4 and FERM domains are separated by roughly 500 amino acids. The pairing of MyTH4 and FERM domains may have important consequences for the functions of these proteins, but this role is as yet unknown. Second, the conserved spacing between MyTH4 domains of M7a, M12 and M15 suggests that the positioning may be important. That is, although M12 does not contain recognizable FERM domains, its MyTH4 domains are separated by roughly the same number of amino acids as those of M7a. Additionally, the placement of the MyTH4 and FERM domain of M15 correspond roughly with the position of the first MyTH4 domain and second FERM domain of M7a, respectively. Again, it seems natural that the positional relationships between MyTH4 and FERM domains must have an impact on function.

Possible SH3 domain

The presence of a putative SH3 domain between the two MyTH4-FERM repeats in the mouse M7a tail was reported by Mburu et al. [44]. Profile-scanning algorithms such as Pfam [66] also detect an SH3 domain at this location in human M7a. Although not confirmed by experimental data, this finding raises the possibility of further protein-protein interactions. Many M1s have SH3 domains, and like M7, *Acanthamoeba* M4 has both a MyTH4 domain and an SH3 domain. These similarities suggest common roles for these domains in unconventional myosins and pose interesting avenues for future study.

Class IX: myosins with GAP activity

Two genes for class IX myosins are known to exist in mammals, M9a and M9b [47]. M9b was characterized first, and has been cloned from human [73] and from rat [74], where it is called myr 5. The head of this ~230-kDa myosin has a novel ~60-amino acid N-terminal extension and an ~120-amino acid insert within the surface loop 2, which is thought to form myosin's major contact site with actin [73, 74]. The neck of M9b contains four IQ motifs, and the tail lacks regions predicted to form coiled coil and may thus exist as a monomer [73, 74]. The tail contains a zinc-binding C_6H_2 motif, which may function as a site for protein-protein or protein-lipid interactions [73, 74]. The defining feature of the M9 tail, however, is the presence of a GAP (GTPase activating protein) domain for the rho family of small GTP-binding proteins [73, 74]. The recently cloned ~301-kDa M9a/myr 7 also has a head with an N-terminal extension, as well as an even larger insert at loop 2 [75]. M9a/myr 7 has a neck with six IQ motifs, and its tail includes a region predicted to form a coiled coil. The M9a/myr7 tail also includes both a zinc-binding C_6H_2 motif and a GAP domain for rho proteins [75]. Both M9s appear to be alternatively spliced [73, 74, 76]. Genomic sequence from *C. elegans* indicates that a class IX myosin, HUM-7, also exists in this species (accession no. AF067217) [31].

M9b is widely distributed in vertebrate tissues [73, 74], but it is particularly abundant in cells of myeloid origin, especially peripheral blood leukocytes, where it constitutes ~0.01% of total protein [77]. In undifferentiated human leukocytes, M9b colocalizes with F-actin in the cell periphery, whereas its localization in differentiated cells is more cytoplasmic and perinuclear [73]. M9b is also found in association with phagosomes [78]. Differences in the existing human M9b and rat myr 5 sequences appear to be due to alternative splicing at their C-termini. Using antibody-mediated tethering in a motility assay, M9b was shown to be capable of translocating actin filaments at the relatively low velocity of ~15 nm/s [77].

Both the M9b/myr 5 GAP domain and the intact protein exhibit specific GAP activity for rho, but little or no GAP activity for rac or cdc42 [74, 77, 79]. Since it is the active, GTP-bound form of rho that is involved in the formation of stress fibers and adhesion plaques, M9b would be expected to act as a negative regulator of rho activity [74, 77]. Evidence for potent regulatory activity by a class IX myosin in cultured cells was provided by Muller et al. [79], who reported that expression of M9b/myr 5 in insect cells induced striking morphological changes, such as the formation of thin processes. A set of deletion construct controls confirmed that this effect was due specifically to the presence of the GTPase-activating domain [79]. This activity was abolished by mutation of a single conserved arginine residue to a methionine (M1695) [79]. Strikingly, overexpression of full-length myr 5 protein or its rho-GAP domain (but not the M1695 mutant) in mammalian cells induced cell rounding and loss of actin stress fibers and focal contacts [79]. M9a/myr 7 is particularly abundant in brain and testis, and like M9b exhibits GAP activity specific for rho [75]. It might thus be expected to play a role as a negative regulator of rho-mediated actin rearrangements in neurons [75].

Class X: a myosin with PH domains

M10 was originally identified from a PCR screen to identify novel myosins in the inner ear that might be involved in sensory transduction [36]. A full-length complementary DNA (cDNA) sequence was obtained from a bovine aorta library (accession no. U55042), and phylogenetic analysis of this sequence's head domain suggests that it constitutes a novel class of the myosin superfamily. The deduced amino acid sequence indicates that bovine M10 consists of 2052 amino acids with a molecular weight of ~236 kDa. Given the 35% level of amino acid identity with the head domain of human skeletal muscle myosin, it is likely that M10 can function as a molecular motor. A neck domain consisting of three IQ motifs provides putative binding sites for calmodulin or calmodulin-like proteins, and the following region of predicted coiled-coil suggests M10 forms dimers. M10 also contains a MyTH4 domain followed by a FERM domain as seen in unconventional myosins from classes IV, VII, XII and XV.

The unique feature of M10 is the presence of three repeats of the pleckstrin homology (PH) domain. This ~100-amino acid domain was originally identified in the protein kinase C substrate pleckstrin, but has since been found in many other proteins involved in signal transduction [80–83]. M10 is the only protein known

where one PH domain (the second one) is inserted into the variable loop region of another (the first), and only the second protein reported to contain three PH domains. If M10 forms dimers, it would be predicted to expose six PH domains for interactions with membranes or other proteins. Two other motor proteins have been found to contain a PH domain. One of these is the microtubule motor unc-104/KIF1a [84], which is required for transport of synaptic vesicle precursors. A newly discovered and as-yet unclassified unconventional myosin from *Dictyostelium*, MyoM, also contains a tail with a PH domain [85].

The PH domains: a role in membrane binding and signal transduction?

Like SH2 and SH3 domains, PH domains are found primarily in proteins involved in signal transduction pathways. Early on, several PH domains were shown to bind directly to membranes containing the signaling lipid phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] [86], while flanking regions close to PH domains were found to synergistically bind membranes and the $\beta\gamma$-subunits of trimeric G-proteins [87]. This is an intriguing idea because it might provide a mechanism for regulating the membrane binding activity of PH domains. Perhaps PH domains will be found to exhibit other protein-protein interactions. A recent short report raises the possibility that the PH domain of protein kinase B (PKB) might bind myosin-II in vitro [88]. However, myosin-II did not bind recombinant full-length PKB or immunoprecipitate full-length PKB expressed in cell culture.

In the past several years, interest has shifted toward binding of PH domains to membrane-bound phosphatidylinositol moieties other than $PI(4,5)P_2$. Several labs have investigated binding characteristics of bacterially expressed PH domains through a variety of techniques including a biosensor probe [89], pull-down using tritiated water soluble phosphoinositides followed by competition assay [90], and dot-blot assay coupled with titration calorimetry [91]. In each case, it is clear that some PH domains exhibit preference for binding to a particular phospholipid or class of phospholipids, whereas some are less specific. PH domains that bind phosphatidylinositides generated by phosphatidylinositol-3-kinase (PI3K), such as phosphatidylinositol-3,4-bisphosphate and phosphatidylinositol-3,4,5-trisphosphate, raise the exciting hypothesis that PH domains may function to localize proteins to the plasma membrane upon receptor stimulation and PI3K activation. Recently, green fluorescent protein (GFP) has been used to directly visualize localization of a variety of PH domain fusion proteins [88, 92].

Although the function of the M10 PH domains remains unclear, it has been proposed that the second PH domain of M10 might bind to products of PI3K on the basis of similarity to other such PH domains. Isakoff et al. [93] used an in vivo assay in yeast to screen several PH domains, including the second PH domain of M10, for binding to products of PI3K. This result suggests that M10 may be targeted to a membrane by PI3K activity, but it is still unknown under what conditions this occurs in vivo, if at all. The binding preferences of M10's other two PH domains remain unknown. A dimeric M10 would thus contain at least 10 candidate domains for membrane or protein interaction: 6 PH domains, 2 MyTH4 domains and 2 FERM domains. This makes it a good candidate to form part of a multicomponent signaling complex.

Class XII: a 307-kDa myosin from *C. elegans* with two MyTH4 domains

Class XII is defined by the *hum-4* gene, which was discovered in *C. elegans* (along with homologs for several other myosins including M5, M6 and M7 [31]). The HUM-4 head domain is highly divergent and exhibits only $\sim$20% identity with the heads of other myosins. In addition, the head exhibits an $\sim$150-amino acid N-terminal extension [31]. Sequence analysis of HUM-4 predicts two IQ motifs, a short region that may form a coiled coil and two MyTH4 domains in the tail [31]. HUM-4 is predicted to have a molecular weight of $\sim$307 kDa, making it the largest myosin heavy chain currently known. The genetic advantages provided by the essentially complete *C. elegans* genome should facilitate analysis of this newly discovered myosin.

Class XV: a new class of deafness myosins with MyTH4 and FERM domains

In addition to class VI and VII myosins, class XV was recently discovered as the third unconventional myosin associated with deafness. The mouse *shaker-2* (*sh2*) gene, which causes severe deafness and vestibular dysfunction when mutated, was suspected as a model for human recessive nonsyndromic deafness DFNB3 on the basis of conserved synteny and phenotype [94]. In order to address this question, Probst et al. utilized an in vivo complementation strategy to identify a bacterial artificial chromosome (BAC) containing 140 kb of *shaker-2* genomic sequence that rescued the *sh2* phenotype in transgenic progeny of *sh2*/*sh2* parents [1]. Analysis of this sequence for

potential coding regions predicted a novel unconventional myosin which they isolated by PCR and designated *Myo15* (accession no. AF053130). A roughly 5-kb mRNA was detected in mouse brain and kidney by Northern blot, and transcripts were detected in mouse liver and inner ear by reverse transcriptase polymerase chain reaction (RT-PCR). Furthermore, screening of *shaker-2* mice revealed a cysteine-to-tyrosine substitution at a highly conserved residue in the myosin actin binding domain, suggesting disruption of motor function in *shaker-2* mutants [1]. Wakabayashi et al. later identified the *sh2* gene product by using a positional

A: Unconventional myosins of selected lower eukaryotic organisms

CLASS [a]	*S. Cerevesiae* *	*Dictyostelium*	*C. elegans* *	*D. melanogaster*
I [b]				
"SH3"	[S76960] *myo3*	[P34092] MyoB		
	[Q04439] *myo5*	[L35323] MyoC	[U52515] HUM-1	
		[L16509] MyoD		
"Short"		[P22467] MyoA		
		[L06805] MyoE		
		[L35319] MyoF [c]		[U07596] 61F "Myo1B" [d]
		[AB017909] MyoK	[X75564] HUM-5 [e]	[U07595] 31DF "Myo1A" [e]
III	None		None	[J03131] 28A "Nina-C"
V	[P19524] *myo2*	[U42409] MyoJ	[U52516] HUM-2	[AF003826] 43CD
	[M90057] *myo4*			
VI	None		[U52517] HUM-3	[X67077] 95F
			[AF125462] HUM-8 [f]	
VII	None		[U80848] HUM-6	[AC002502] 35B "Crinkled" [h]
				[AC005834] 28B1-B4 [i]
IX	None		[AF067217] HUM-7 [g]	
X	None		None	
XII	None		[Z66563] HUM-4	
XV	None		None	
Other		MyoG (unpublished)		[AC004728] 29C3-D1 [j]
		[L35320] MyoH [c]		
		[U83089] MyoI [c]		
		MyoL (unpublished)		
		MyoM (unpublished)		

B: Unconventional myosins of selected vertebrate organisms

CLASS [a]	Human	Mouse	Other
I [k]			
Subclass 1	*MYO1E* [U14391] "Myosin-IC"	*Myo1e* (ESTs)	[X74815] Rat myr3
("SH3")	*MYO1F* [X98411] "Myosin-IE"	*Myo1f* [X97650]	[X70400] Chicken Myosin IB
Subclass 2	*MYO1A* [AF009961] Brush border myosin-I	*Myo1a* [AF009960] Brush border myosin-I	[U25148] Rat brush border myosin-I
	MYO1B [L29138] "Myosin-IB"	*Myo1b* [P46735] "Myosin-1 alpha"	[X68199] Rat myr1
Subclass 3	*MYO1C* [X98507] "Myosin-I beta"	*Myo1c* [U96723] "Myosin-1 beta"	[X74800] Rat myr2
Subclass 4	*MYO1D* [AB018270] KIAA0727 [c,l]	*Myo1d* [C45438] "Myosin-1 gamma"	[X71997] Rat myr4
III	(unpublished)		(unpublished)
Va	*MYO5A* [Y07759] "Griscelli's Syndrome"	*Myo5a* [Q99104] "Dilute"	[S19188] Chicken Myosin-V
b	*MYO5B* (ESTs)	*Myo5b* [M55253] "GAD"	[U60416] Rat myr6
c	*MYO5C* (unpublished)	*Myo5c* (ESTs)	
VI	*MYO6* [U90236]	*Myo6* [U49739] "Snell's Waltzer"	[Z35331] Pig Myosin-VI
VIIa	*MYO7A* [U55208] "Usher Syndrome 1B"	*Myo7a* [U81453] "Shaker-1"	
b	*MYO7B* (unpublished)		
IXa	*MYO9A* [AJ001714]		[AJ001713] Rat myr7
b	*MYO9B* [U42391]	(ESTs)	[X77609] Rat myr5
X	*MYO10* [AB018342] KIAA0799 [c,m]	(ESTs)	[U55042] Bovine Myosin-X
XII			
XV	*MYO15* [AF051976] "DFNB3"	*Myo15* [AF053130] "Shaker-2"	
Other	[AB020672] KIAA0865 [c]		

cloning approach, and they also noted the cysteine-to-tyrosine substitution in *sh2* mice by sequencing RT-PCR products [95]. However, analysis by Northern blot revealed ~5-kb transcripts in heart and skeletal muscle but not in brain or kidney, contrary to the previous findings of Probst et al. [1].

To confirm the hypothesis that *shaker-2*/*Myo15* is the mouse ortholog of the gene mutated in DFNB3, Wang et al. identified human *MYO15* and demonstrated mutations in DFNB3 patients [63]. Starting from fragments of human sequence generated using PCR primers for predicted mouse exons, they acquired partial coding sequence of M15 using RACE (rapid amplification of cDNA ends) and RT-PCR. A roughly 4.4–5.2-kb mRNA was detected in adult and fetal brain by Northern blot, and an RNA dot-blot indicated expression in several other tissues. The authors then predicted exons using a human genomic clone (accession no. AF051976) and deduced the domain structure of M15. The predicted protein was 42% identical to *C. elegans* HUM-6 and 41% identical to M7a. M15 contains a MyTH4 domain similar to those found in other unconventional myosins (classes IV, VII, X and XII as discussed above) and a FERM domain similar to those of M7a and M10. Strikingly, two different families of DFNB3 patients were shown to have mutations in the MyTH4 domain, further stressing its importance in sensory transduction in the inner ear. Thus, it was demonstrated that mutations in human M15, the ortholog of mouse *shaker-2*/*Myo15*, cause nonsyndromic deafness DFNB3; further study should indicate the role of M15 in auditory hair cell function and the general importance of M15 to human deafness.

Other myosins on the horizon

Several novel myosins have been identified in the past few years in lower eukaryotes. These include a family of short-tailed myosins (class XIV) identified in the parasitic protozoans *Toxoplasma gondii* [96] and *Plasmodium falciparum* [97], the causes of toxoplasmosis and malaria. An unusual and highly divergent myosin-like sequence (~20% identity to other myosin heads) attached to a chitin synthase-like 'tail' domain has been reported from the fungus *Aspergillus* [98]. Although this sequence contains the well-conserved 'GESGAGKT' residues at the P-loop/ATP binding site, sequence analysis of its tail indicate several regions predicted to form transmembrane domains. A partial sequence from the

Figure 3. Unconventional myosins arranged by class and organism to facilitate comparison among species. The selection of organisms is intended to reflect several model systems in which a large number of unconventional myosins are now known to exist. We use the nomenclatural conventions established for each organism: yeast *myo2–5*, *Dictyostelium* MyoA–M, *C. elegans* HUM-1–7 (heavy chain of an unconventional myosin), *Drosophila* by chromosomal location and name where applicable. As per the guidelines for human and mouse gene nomenclature, unconventional myosin genes from human and mouse are indicated by '*MYO*' and '*Myo*', respectively. Previous names given to each protein are included, as well as the disease name where applicable. Rat entries follow the 'myr' (myosin in rat) convention. Myosins from classes IV, VIII, XIII and XIV, thus far found only in lower eukaryotes or plants, were not included in this comparison. Accession numbers are indicated by brackets to unambiguously identify protein entries, cDNA entries or genomic sequences.

* Since the *S. cerevesiae* and *C. elegans* genome sequences are nominally complete, this table contains all known unconventional myosins in these species. Shading indicates classes not present in the selected organisms.

[a] Placement of lower eukaryotic myosins on the same line simply implies membership in a similar class, whereas placement of vertebrate myosins on the same row implies orthologous proteins, especially between human, mouse and rat.

[b] We have divided the class I myosins among lower organisms by the presence or absence of the SH3 domain. Some class I myosins fit the classification scheme for vertebrate class I myosins [3] and will be identified by footnote.

[c] Partial sequence.

[d] *Drosophila* 61F has been placed in vertebrate subclass 3 [3].

[e] *C. elegans* HUM-5 and *Drosophila* 31DF have been placed in vertebrate subclass 4 [3].

[f] Automated exon recognition for *C. elegans* genomic sequence (see text for details).

[g] Automated exon recognition for *C. elegans* genomic sequence (see text for details).

[h] Full genomic sequence for the 35B8–C1 region (see text for details).

[i] Manual and computer-based exon recognition for *Drosophila* genomic sequence from 28B1–B4 (see text for details).

[j] Manual and computer-based exon recognition for *Drosophila* genomic sequence from 29C3–D1 region (see text for details).

[k] Unfortunately, nomenclature of vertebrate class I myosins is unnecessarily confusing due to multiple synonyms. However, uniform mouse and human gene names have recently been assigned [47, 103]. For this table, human and mouse gene symbols will be followed by the GenBank accession number in brackets and the original name of the protein encoded by the gene in quotation marks. Since class I unconventional myosins are reviewed elsewhere, we will not discuss them in detail.

[l] Human KIAA0727, mouse myosin-I gamma and rat myr4 have 97–99% amino acid identity. KIAA0727 maps to the centromeric portion of chromosome 17, the predicted region for the human ortholog of mouse *Myo1d* [47]. Thus, KIAA0727 likely represents human *MYO1D*. The previous report that a human myosin-ID protein fragment (accession no. L29140) represents MYO1D [103] is probably erroneous because this fragment has only 58% amino acid identity to rat myr4 and maps to different chromosomal locations than KIAA0727.

[m] Human KIAA0799 represents a partial coding sequence corresponding to the tail region of bovine M10. However, the reported sequence is truncated before the stop codon of the bovine sequence; this artifact is probably due to incorporation of an intron. When the intron is edited out, high percent identity resumes through the 3′ untranslated region.

ciliate *Tetrahymena thermaphila* also appears to constitute a novel myosin [99]. A divergent myosin head sequence has also been obtained from the brown algae *Fucus* [G. Lu and R. S. Quatrano, personal communication]. In the slime mold *Dictyostelium*, a partial tail sequence (accession no. U83089) from MyoI [50] shows that this myosin shares characteristics with the class VII myosins, whereas MyoK is clearly a class I myosin with a very short tail [100]. The initial report of *Dictyostelium* MyoM indicates that the tail domain of this interesting new myosin, like M10, contains a PH domain [85]. The current tally of myosins in *Dictyostelium*, a model system for their study, is thus 1 conventional myosin and 13 unconventional myosins (see fig. 3).

Several new myosin sequences have also been identified in multicellular animals (see also the discussions of specific classes above). Searches of *Drosophila* genomic sequences coupled with exon recognition programs lead us to conclude that *Drosophila* contains a novel myosin head sequence at chromosomal locus 29C3-D1 (accession no. AC004728). The head domain predicted from this sequence is not closely related to other myosins, sharing at most $\sim 29\%$ identity with the heads of other myosins. This sequence appears to have a ~ 100-amino acid N-terminal extension on its head, a single IQ motif and a relatively short tail. There is also preliminary evidence for the existence of several new members of the myosin superfamily in vertebrates. A third member of the class V family, tentatively called myosin-Vc, has been reported in humans [101]. Searches of the vertebrate database also reveal a partial cDNA sequence that appears to encode a novel unconventional myosin from humans (accession no. AB020672). The partial head domain of this sequence exhibits a maximum of $\sim 27\%$ identity with the head domains of myosins from other classes and is thus rather divergent from known myosins. The deduced sequence of this new myosin from humans is characterized by a neck with one IQ motif and by a proline-rich tail. Patel et al. [102] have also reported a novel myosin from vertebrate (rat) brain that is expressed in granule cell neurons. The full extent of myosin diversity will soon be revealed by the completion of various genome sequencing projects, marking the end of an era of myosin discovery and heralding a renewed effort to define the functions of these intriguing motor proteins. We anticipate that the rapid expansion in myocin number witnessed over the preceding years will be surpassed by many exciting tales of unconventional myosins in the years to come.

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