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Review

Myosins: a diverse superfamily

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Abstract

Myosins constitute a large superfamily of actin-dependent molecular motors. Phylogenetic analysis currently places myosins into 15 classes. The conventional myosins which form filaments in muscle and non-muscle cells form class II. There has been extensive characterization of these myosins and much is known about their function. With the exception of class I and class V myosins, little is known about the structure, enzymatic properties, intracellular localization and physiology of most unconventional myosin classes. This review will focus on myosins from class IV, VI, VII, VIII, X, XI, XII, XIII, XIV and XV. In addition, the function of myosin II in non-muscle cells will also be discussed.

Keywords: Myosin II; Myosin IV; Myosin VI–VIII; Myosin X–XV

1. Introduction

Myosins constitute a large superfamily of proteins that share a common domain which has been shown to interact with actin, hydrolyze ATP and produce movement in all cases examined to date (Fig. 1) [1,2]. However, only a few members of the superfamily have been biochemically characterized and it is possible that some myosins may have lost one or more of these features. Myosins are typically constructed of three functional subdomains: (1) the motor domain which interacts with actin and binds ATP, (2) the neck domain which binds light chains or calmodulin, and (3) the tail domain which serves to anchor and position the motor domain so that it can interact with actin. The motor domains are relatively conserved with the exception of several surface loops and the amino-terminus. Light chains and calmodulin bind to a helical sequence termed the IQ motif found in the neck which has a consensus sequence of IQXXXRGXXXR [3]. The number of IQ motifs present in the necks of different myosins can vary between zero and six. The tail domains are the most diverse domains and vary widely in length and in sequence. Functional motifs, such as SH3 domains, GAP domains, FERM domains, and pleckstrin homology (PH) domains are sometimes found in the tails of myosins (Fig. 2). In addition, the tails of many myosins contain coiled-coil forming sequences which allow the molecules to dimerize and produce two-headed molecules.

Phylogenetic analysis, usually of the motor domain, groups myosins into 15 distinct classes, although there are a number of outliers which do not adequately align with any of the established classes [1,2]. In addition, two of the defined classes (IV and XII) are currently comprised of only a single member. Perhaps, in hindsight, they should also have been considered outliers. The nomenclature assigns

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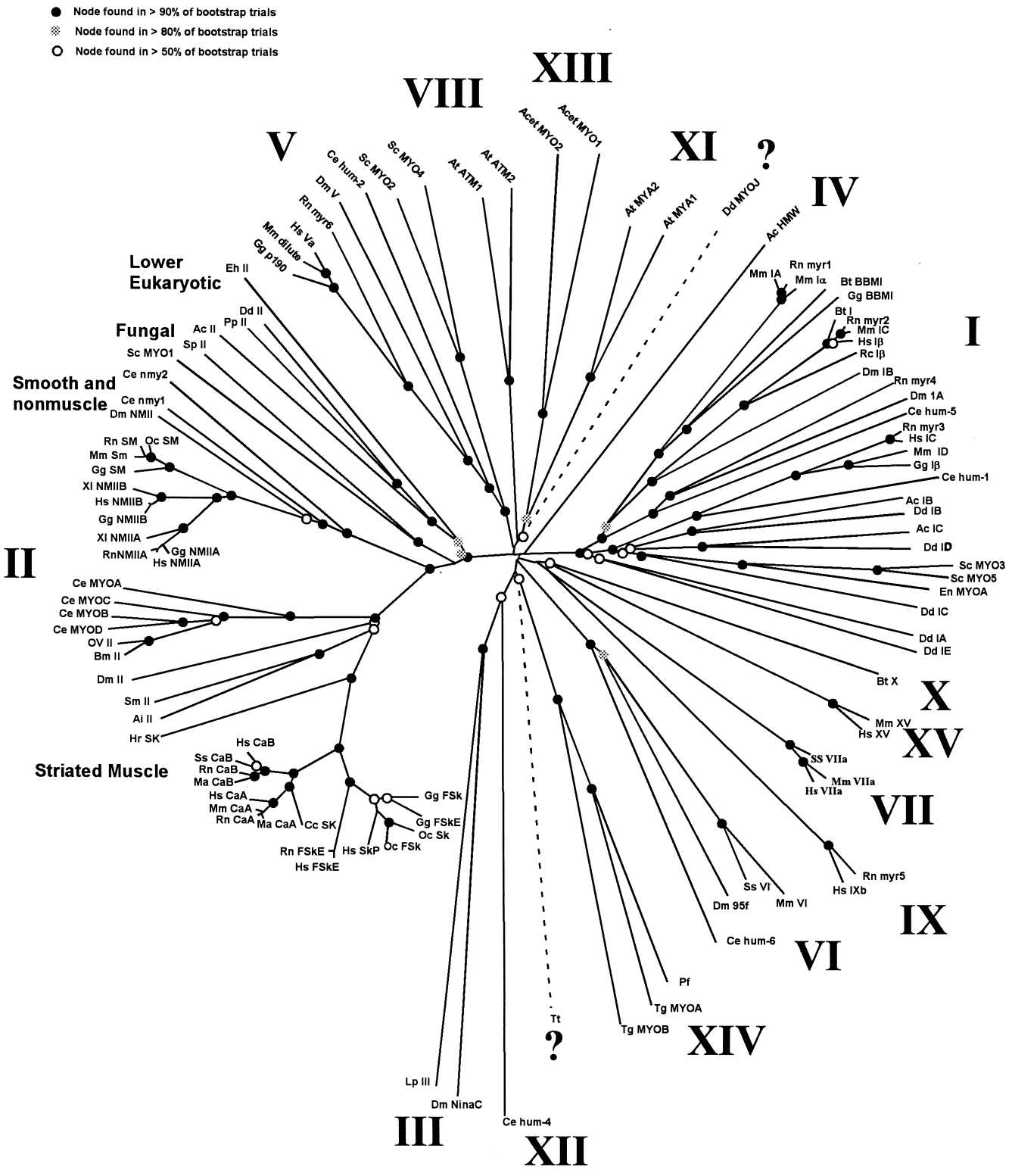
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Dm V
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Hs Va
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Rc Iβ
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Mm IC
Rn myr2
Bt I
Gg BBMI
Bt BBMI
Mm Iα
Mm IA
Rn myr1

Fig. 1. Phylogenetic tree obtained from neighbor joining analysis of the myosin motor domain protein sequences as performed by ClustalW. Branch lengths are drawn to scale in units of percent divergence. The tree is drawn unrooted. Bootstrap resampling (1000 trials) was used to judge the robustness of nodes, and results are indicated on the figure as explained in the key. Abbreviations are: Ac, *Acanthamoeba castellani*; Acet, *Acetabularia cliftonii*; At, *Arabidopsis thaliana*; Ae, *Aequipecten irradians*; Bt, *Bos taurus*; Bm, *Brugia malayi*; Ce, *C. elegans*; Cc, *Coturjix coturnix*; Dd, *D. discoideum*; Dm, *D. melanogaster*; En, *Emericella nidulans*; Eh, *Entamoeba histolytica*; Gg, *Gallus gallus*; Hr, *Halocynthia roretzi*; Hs, *Homo sapiens*; Lp, *L. polyphemus*; Ma, *Mesocritus aureus*; Mm, *Mus musculus*; Ov, *Onchocerca volvulus*; Oc, *Oryctolagus cuniculus*; Pp, *Physarum polycephalum*; Pf, *P. falciparum*; Rc, *Rana catesbeiana*; Rt, *Rattus norvegicus*; Sc, *S. cerevisiae*; Sm, *Schistosoma mansoni*; Sp, *S. pombe*; Ss, *Sus scrofa*;, Tt, *Tetrahymena thermophilus*; Tg, *T. gondii*; Xl, *Xenopus laevis*.
←

class number II to the large family of filament forming conventional myosins which were discovered over 60 years ago and are found in muscles and in the cytoplasm of animal cells. Class I myosins were next discovered and the subsequent classes were numbered in order of the discovery of the founding member of the class. While most family trees are constructed by analysis of the motor domains, analysis of the whole molecule or of the tail domains alone generally gives the same relationships [4].

The number of myosin genes present in mammals is conservatively estimated at 25–30 from classes I, II, III, V, VI, VII, IX, X and XV. The entire genome of the budding yeast, *Saccharomyces cerevisiae*, is now sequenced and contains only five myosin genes, two of class I, one of class II and two of class V [5]. The slime mold, *Dictyostelium discoideum*, represents a higher level of organismal complexity. It can exist as a single amoeboid cell or undergo a complex developmental cycle to produce multicellular slugs and fruiting bodies. While the genome of *Dictyostelium* has not been fully sequenced, a large of number of myosins have been identified by a combination of methods. These include seven myosin I genes, a single myosin II gene and four other genes that do not stringently group with the existing classes [4]. One of these groups weakly with myosin V or XI while another groups weakly with myosin VII or X. The two remaining genes do not classify well with any existing class. The sequence of the *Caenorhabditis elegans* genome is mostly complete. To date, there are four genes for muscle myosin II, two for non-muscle myosin II, two for myosin I, one for myosin V, two for myosin VI, one for myosin VII, one for myosin IX and one for myosin XII [6] (M. Titus, personal communication). No class of myosin appears to be universally expressed in all phyla. For example, no myosin II class molecules have yet been found in plants, and several myosins are, so far, found exclusively in plants (VIII, XI and XIII). However, all eukaryotic animal cells examined contain at least one myosin II gene and, usually, multiple myosin I genes. In addition, myosin V genes are found widely, if not universally (particularly if the criteria for relatedness are relaxed somewhat).

The features of myosins from class I, III, V and IX are covered elsewhere in this special volume [7–9] and this article will concentrate on the remaining classes. In addition, it will review the structure of the myosin motor domain as determined by crystallography of several myosin II myosin fragments and what the structure, combined with site-directed mutagenesis, can tell us about the function of the molecule.

2. The myosin II motor domain and its use as a model for other myosins

The myosin II class is also referred to as 'conventional' myosins since this was the only class of myosin known for decades. Members of this class are hexameric enzymes composed of two heavy chains with a molecular weight of 171–244 kDa and two pairs of light chains. The amino-terminal portion of the heavy chains (collectively referred to as the 'head') contains the prototypical motor domain and two IQ motifs in the neck. The carboxyl-terminal half of the heavy chain consists of coiled-coil forming sequence which homodimerizes to form the long rod (or tail) which usually terminates in a short non-helical segment [10–12]. Myosin II molecules have a two-headed structure, due to the dimerization of the heavy chain in the tail. The tails of myosin self-associate to form filaments both in vivo and in vitro at low ionic strength. Such filaments complicate ki-

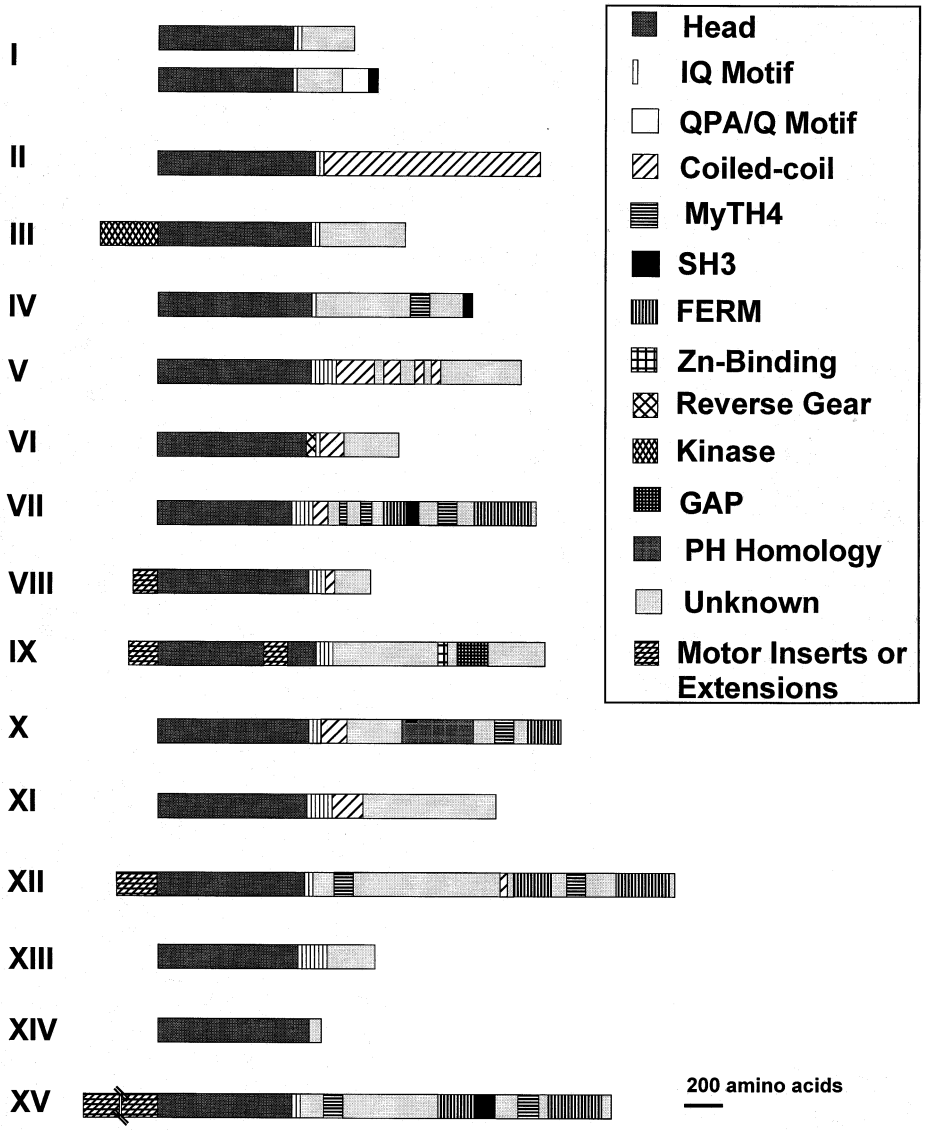

Fig. 2. Schematic representation of the domain structure of myosin superfamily members. The length of the molecules including their various structural motifs is drawn roughly proportional to the number of amino acids. In some cases, members of a particular class may have isoforms with alternatively spliced regions that change the length of the isoform.

netic and biophysical characterization of myosin's enzymatic activity. This complication can be circumvented by the use of proteolytically produced fragments which are soluble and enzymatically active. Myosin II molecules can be proteolytically cleaved into discrete functional domains. One site of cleavage at the junction between the head and the tail produces a soluble fragment termed subfragment one (S1) and the rod which remains a coiled-coil dimer and retains the solubility properties of the parent molecule. S1 binds to actin and nucleotides and contains the two light chains. Another cleavage site of myosin II occurs about 40 kDa into the rod structure and produces two fragments, heavy meromyosin (HMM) and light meromyosin (LMM). HMM, which is soluble even at low ionic strength, contains the head region and a portion of the coiled-coil forming sequence (termed subfragment 2 or S2) which dimerizes to produce a two-headed fragment. LMM retains the solubility properties of the parent

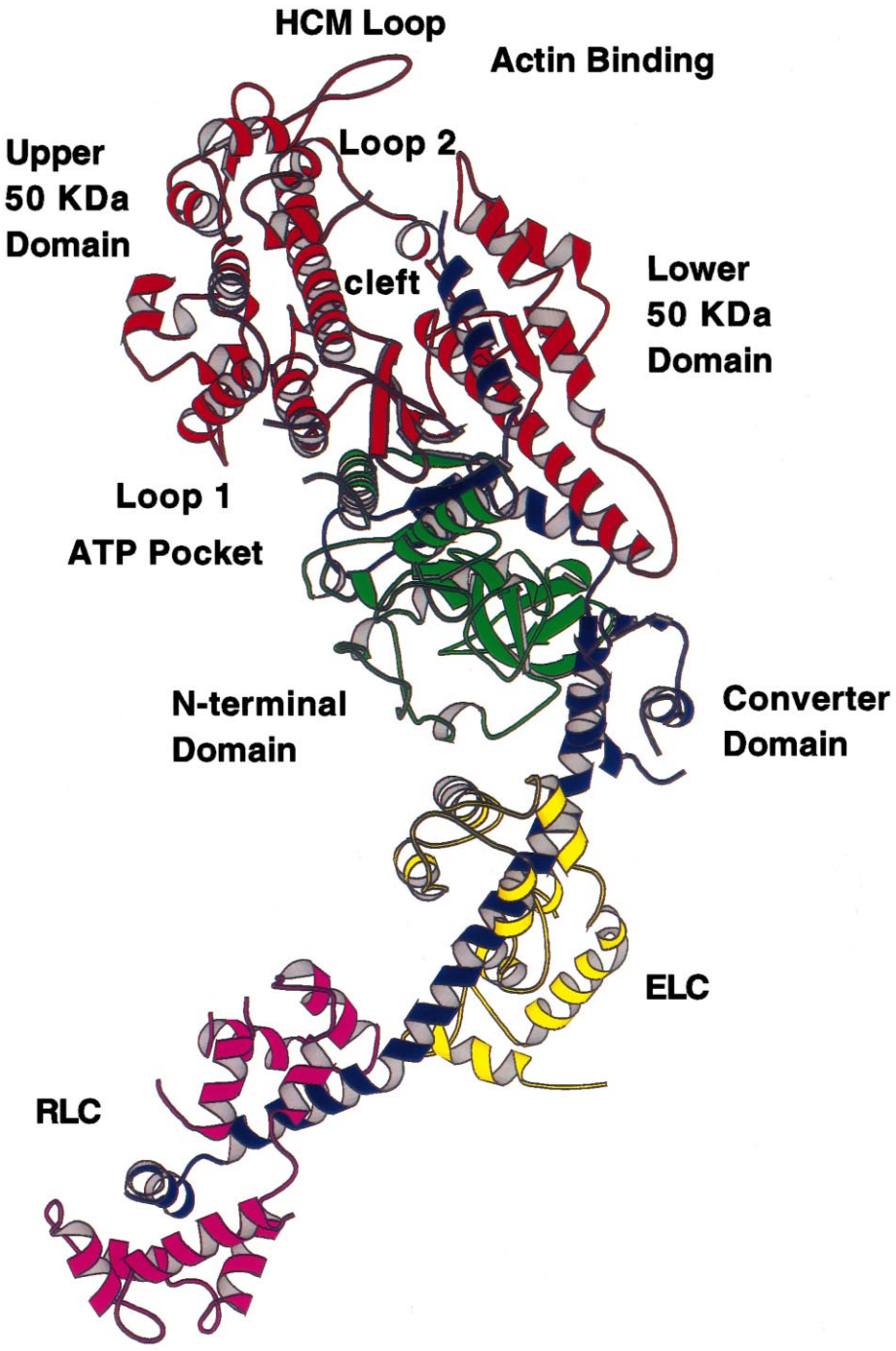

Fig. 3. Three-dimensional structure of chicken fast skeletal muscle myosin S1. The color scheme is as in Rayment et al. [13]. This figure was made with MOLSCRIPT [172].

molecule. Both S1 and HMM have been critical to kinetic, biophysical and structural studies of the myosin molecule.

The three-dimensional structure of fragments of several myosin II molecules has been solved. The fragments used for crystallography were produced by proteolysis or by expression of recombinant molecules. These include the S1 of chicken fast skeletal muscle myosin [13], the S1 of scallop myosin [14], a recombinant fragment of the smooth muscle myosin

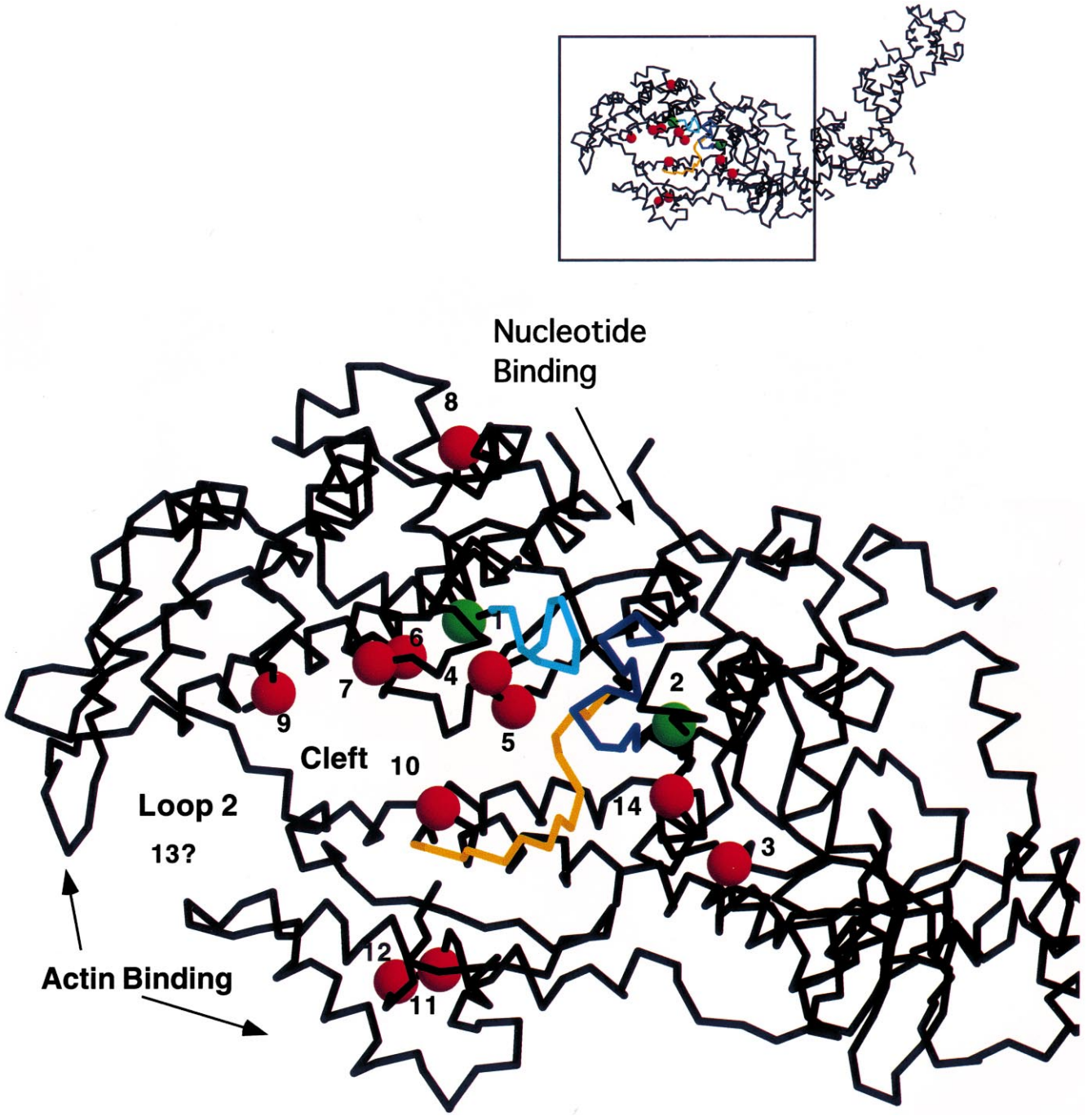

Fig. 4. Missense mutations of myosin VII and XV that give rise to deafness in mouse and human mapped onto the crystal structure of chicken fast skeletal muscle myosin II. Mutations found in humans and mice associated with deafness are mapped onto the three-dimensional structure of chicken fast skeletal muscle myosin S1 [13]. The mutations in green are found in myosin XV while those in red are from myosin VII. The mutations are given the sequence number from their parent myosin backbone with the sequence number for chicken skeletal muscle myosin in parentheses. More information can be found about these mutations in Friedman et al. [157]. Mutations found in myosin XV: (1) G1358S (233); (2) C1775Y (674). Mutations found in myosin VII: (3) G25R (44); (4) R212H/C (245); (5) G214R (247); (6) R241P/S (241); (7) R244P (277); (8) R302H (335); (9) A397D (428); (10) E450Q (476); (11) R502P (528); (12) P503L (529); (13) M599I (Loop 2); (14) L651P (690). The structural elements of the motor domain are colored: Switch I, cyan; Switch II, orange; P-loop, dark blue.

motor domain containing the essential light chain (ELC) [15], a motor domain fragment of *Dictyostelium* myosin [16]. In addition, many of these structures have been solved with one of several different nucleotides or nucleotide analogs bound at the active site.

The myosin motor domain is essentially built of four subdomains connected by flexible linkers [14] (Fig. 3). The amino-terminal subdomain forms an SH3-like motif in myosin II class molecules [15]. This motif is not present in other myosin classes. The amino-terminal subdomain is connected to the upper 50 kDa subdomain which is, in turn, connected to the lower 50 kDa subdomain. The name (50 kDa) for these two subdomains is historical and relates to the fact that there are two proteolytically sensitive surface loops which connect them with the rest of the myosin molecule which when cleaved gives rise to a band on sodium dodecyl sulfate–polyacrylamide gels with a molecular weight of 50 kDa [17]. The upper and lower 50 kDa domains are separated by a cleft which is lined with many conserved residues. This cleft closes slightly upon binding nucleotides and may close more dramatically upon binding actin [14]. These two domains comprise most of the actin binding interface (see [1] for a review of the evidence). Of interest to later discussions of the myosin superfamily is a loop that is found at the surface of the upper 50 kDa domain (residues 403–416 in the chicken skeletal muscle myosin sequence). This loop is the site of the first myosin mutation shown to cause a human disease, hypertrophic cardiomyopathy (HCM), and is sometimes referred to as the HCM loop [18]. The HCM loop is also the location of a phosphorylatable serine (S) or threonine (T) in certain amoeboid myosin I molecules. (For an extensive sequence alignment of myosin motor domains, see [1]). The enzymatic activities of these myosins are dependent upon phosphorylation at this site (see [19] for review). The myosin VI class molecules also have a threonine at this residue [20,21]. Most other myosin superfamily members have a constitutively negatively charged amino acid, either an aspartic acid (D) or a glutamic acid (E) residue, at this site. For this reason, Bement and Mooseker [22] named this the TEDS rule site after the one letter codes for the four amino acids involved.

The nucleotide binding pocket is rather open and does not substantially close upon binding of nucleotide (see [23] for review). It is formed primarily from seven β-strands linked by loops [13]. There are many structural similarities between the nucleotide binding pocket of myosin and those of kinesin, a microtubule-dependent motor and of G-proteins. [24,25]. In each case, there are three conserved sequence elements termed the P-loop, Switch I and Switch II that are involved in nucleotide binding and sensing of the hydrolysis state (ATP or ADP) of the nucleotide.

The fourth subdomain of the motor domain is termed the converter region. A long helix emerges from the converter domain which serves as the binding site of both light chains. The ELC occupies the binding site closest to the converter subdomain while the regulatory light chain (RLC) occupies the second site. The binding sites are highly specific for their respective light chains. The elongated nature of the neck region has given rise to the suggestion that it acts as a lever arm to amplify small changes in the sequence of the motor domain into much larger displacements of actin [14]. In favor of this hypothesis, it was found that there is a correlation with speed of in vitro motility and the length of the neck region from myosin mutagenesis experiments in which binding sites for light chains were either added or subtracted to vary the length of the neck of *Dictyostelium* myosin [26]. In addition, fluorescent probes attached to the RLC of skeletal muscle myosin in skinned fibers show a change in angle upon activation of the muscle [27]. Structural evidence in favor of the lever arm hypothesis comes from crystal forms of a smooth muscle motor domain containing the ELC and from the scallop muscle S1 with different bound nucleotides at the active site [14,15]. A portion of this movement may have been directly visualized in three-dimensional reconstructions of electron micrographs of actin-myosin complexes in the presence and absence of ADP [28,29]. These studies have led to a model for force transduction that supposes that the amino-terminal subdomain and the upper and lower 50 kDa domains remain relatively constrained as to their orientation with actin and that most of the motion is derived from a rigid body movement of the neck. The pivot point or joint for this movement is in the converter subdomain and the movement is controlled by conformational changes in structural ele-

ments that are in communication with Switch II of the nucleotide binding pocket [14].

Alignment of myosin sequences shows two main hypervariable regions that occur at proteolytically sensitive regions of myosin. Interestingly, these regions are also the site of alternative splicing in some myosin molecules (see below). The positions of these hypervariable regions correspond to two portions of the motor domain which were not resolved in the crystal structure probably due to their disordered, flexible nature (Fig. 3). The first of these, termed loop 1, occurs about 25 kDa from the amino-terminus and is located near the nucleotide binding region. Loop 2 occurs about 75 kDa from the amino-terminus and is located in an actin binding interface. Interestingly, binding to actin protects this region from proteolysis [17].

The similarity in the motor domains from these phylogenetically diverse myosin II crystals is remarkable. In fact, alignment of the sequences of these myosins with those of other myosin classes strongly suggests that the myosin II motor domain crystals are a good template for analysis of all motor domains [30]. The alignment reveals that there is a conserved core domain that begins about 80 amino acids from the amino-terminus of the myosin II class molecules and continues to just before the beginning of the long light chain binding helix [31]. Several myosin classes have little or no sequence amino-terminal to the core, while others have large extensions (see Fig. 2 and below). There may also be large extensions at the positions of loop 1 or loop 2 compared to myosin II class molecules. Myosin VI has a unique insert of about 50 amino acids at the converter region which will be discussed later. Finally, the length of the light chain binding helix varies considerably among myosins having anywhere from none to seven IQ motifs. In myosin II molecules, there is usually a 26 amino acid separation between the start of IQ motifs, but in unconventional myosins, the separation can be between 23 and 26 residues [32]. For example, in myosin V molecules, the six IQ motifs are separated by an alternating pattern of 23 and 25 residues. Since the heavy chain target for light chain binding is a helix, the relative contacts between adjacent light chains could be very different depending on the spacing [32]. This could affect the stiffness of the neck, the relative disposition of the heads and possibly the regulation of unconventional myosins.

3. Myosin II

The most unique characteristic of myosin II is the ability to form filaments via the self-association of the rod-like coiled-coil α-helical tail. Bipolar filaments such as those found in sarcomeric muscles are formed by a combination of antiparallel tail interactions at the middle of the filament followed by parallel interactions along most of the filament length. These filaments have a central bare zone which is not populated by motor domains and are designed to pull actin filaments toward there center. Smooth muscle myosins can form side polar filaments which have no central bare zone [33]. These filaments may allow for the extreme shortening that is seen in smooth muscle tissues. The region of the rod necessary for filament assembly has been localized in several cases [34–36]. It is typically a short segment near the carboxyl-terminal one third of the tail. A point mutation at Arg-1889 of *Dictyostelium* myosin is sufficient to disrupt filament assembly by inhibiting the nucleation step [36].

Myosin II molecules can be subclassified into distinct classes based on sequence analysis of the motor domains (Fig. 1). The sarcomeric myosins from striated and cardiac muscle fall into one group, whereas vertebrate smooth and non-muscle myosins fall into another. A third group is comprised of the myosins from lower eukaryotic species such as *Acanthamoeba*, *Dictyostelium* and *Physarum* and the fourth is from fungi. The fungal myosins are the most dissimilar. The coiled-coil forming sequences in their tails are interrupted by numerous proline residues (see [37] for review). Since no fungal myosins have yet been purified, it is not clear whether they form filaments. One of the *Schizosaccharomyces pombe* myosins appears to bind an EF-hand family protein, Cdc4p. This myosin has one well conserved consensus IQ motif and one that is very degenerate. It is not clear whether it would bind two light chains [38]. This protein, while not stringently grouping with either RLC, ELC or calmodulin, appears to be most similar to ELC. Interestingly, Cdc4p can be phosphorylated in vivo at serine 2 or 6 [39]. Mutation of these resi-

dues to alanines or to aspartates did not affect growth or cytokinesis [39]. There are no direct homologs of the ELC and RLC in the *S. cerevisiae* genome and, instead, a protein with 40% sequence identity to *S. pombe* Cdc4p is present as the best candidate for a light chain [40]. It interacts both genetically and directly by gel overlay assay with the heavy chain of one of the myosin I genes, Myo2. It will be interesting to know if this is also a light chain for the myosin II protein of this species.

The MgATPase activity of vertebrate sarcomeric myosins is constitutively activated by actin. The regulation in these muscles is via the troponin-tropomyosin system [41]. In contrast, the MgATPase activity of most other myosins is regulated in one of three ways. The MgATPase activity of molluscan muscle myosins is greatly activated by calcium binding to the ELC [42,43]. Vertebrate smooth muscle and non-muscle myosins are regulated by phosphorylation of the RLCs by a calcium-calmodulin-dependent enzyme termed myosin light chain kinase (see [44,45] for review). Interestingly, the muscle myosin of *Limulus polyphemus*, the horseshoe crab, is also regulated by light chain phosphorylation [46]. Vertebrate sarcomeric myosins are also phosphorylated on their RLCs, but the effect is only modulatory towards its enzymatic or mechanical properties [47]. However, the phosphorylation of vertebrate striated muscle RLC does disrupt the packing order of the myosin heads against the backbone of the thick filament [48]. The heavy chains of vertebrate smooth muscle and non-muscle myosins are phosphorylated by a variety of kinases. The consequences of these phosphorylations are not well understood [49–51] except for the case of non-muscle myosin IIB, where phosphorylation of the fragments of the heavy chain results in an inhibition of filament assembly [52]. In contrast, the MgATPase activity of myosins from *Acanthamoeba* and *Dictyostelium* myosins is inhibited by heavy chain phosphorylation. The site of phosphorylation differs between the two myosins and the mechanisms by which phosphorylation inhibits the activity of these two myosins are somewhat different (see [19,53] for review). The RLC of *Dictyostelium* is also phosphorylated [54].

Much of our knowledge of the in vivo function of non-muscle myosin II comes from studies in three genetic model systems, *Dictyostelium discoideum*, *S. cerevisiae* and *Drosophila melanogaster*. The single myosin II gene of *Dictyostelium* was ablated by homologous recombination [55]. Surprisingly, cells lacking non-muscle myosin II are viable if grown on a surface where they divide through a process termed traction-mediated cytofission [55–57]. In suspension or on a non-adhesive hydrophobic surface, the myosin II null cells undergo karyokinesis, but not cytokinesis and large multi-nucleated cells are formed [55,58,59]. Under these conditions, the *Dictyostelium* myosin II null cells round up at the start of mitosis, but they do not elongate nor form a cleavage furrow [60]. On an adhesive surface, however, the traction-mediated cytofission events of the myosin null are coupled to mitosis [60,61]. The cells round up, elongate, exhibit polar ruffling and form a cleavage furrow which decreases in width to eventually produce two daughter cells. However, the rate of cleavage furrow constriction is only about half that of wild-type cells [60]. These findings led to the proposal that cytokinesis is driven by two mechanisms [60]. Cytokinesis A is a myosin-dependent restriction of the cleavage furrow and is the only event occurring in *Dictyostelium* cells when they are not grown on an adhesive surface. Cytokinesis B, which occurs only on adhesive surfaces, is myosin-independent and may result from the traction forces generated by polar pseudopods exerting forces on the surface. The studies of cytokinesis in *Dictyostelium* myosin II null cells prompted a reinvestigation of the spatial distribution of myosin II in mitotic cells [62]. This revealed that when grown on a surface, very little myosin II gets targeted to the cleavage furrow. However, when *Dictyostelium* cells are flatten under agar, as is often used for imaging, the mechanical stress results in a major recruitment of myosin to the cleavage furrow [62]. This study concludes that myosin II is not essential for cytokinesis.

Dictyostelium myosin II null cells can migrate and chemotact towards a cAMP gradient, albeit somewhat inefficiently, but do not complete the normal developmental cycle [55,63–65]. They are unable to cap surface receptors or do so poorly [57,66] and there is reduced cortical tension in null cells compared to wild-type [57]. The myosin null cells can also serve as a host for reintroduction of mutated myosin II constructs which can be purified for in vitro biochemical assays [67,68]. Alternatively, the

mutated myosins can be assayed for their ability to restore wild-type phenotypes in such cell behaviors as cytokinesis, motility, chemotaxis and development [69–71]. The ability to perform both in vitro biochemical assays and in vivo studies of mutated myosins constitutes a very powerful combination.

One example of this approach is the study of the regulation of *Dictyostelium* myosin's activity. *Dictyostelium* myosin is phosphorylated at several closely spaced threonine residues near the filament assembly domain in the tail and on the RLC [72,73]. Phosphorylation of the heavy chain sites inactivates the myosin by decreasing the propensity to form filaments in vitro [74]. The role of RLC phosphorylation is not so clear. One study showed that RLC phosphorylation is essential for in vitro motility [54], but subsequent studies showed that RLC phosphorylation is more modulatory, producing only about a 4-fold increase in the actin-activated MgATPase activity [75–77]. To study the effect of heavy chain phosphorylation, a construct which deleted 34 kDa of sequence around the phosphorylation sites in the tail was expressed. This construct restores some cellular function such as growth in suspension, development and capping of surface receptors, but the cells have altered cytoskeletal dynamics [78]. Specifically, myosin does not properly disassemble once recruited to a specific site. In vitro analysis revealed that the mutant myosin forms stable filaments. Site-directed mutagenesis of the phosphorylation sites to alanines gives the overassembly defect [69]. Conversion of the phosphorylatable threonines to aspartates results in phenotypes very similar to those of null cells. The mutated myosin shows lower incorporation into Triton insoluble cytoskeletons and appears to be incapable of driving any contractile event within the cell. Biochemical analysis of filament assembly demonstrates that the alanine mutants form stable filaments whereas the aspartate mutants do not [69]. A subsequent experiment used GFP-tagged myosins bearing the alanine and aspartate mutations. Imaging of living cells demonstrated that the amount of aspartate mutant myosin that enters the cleavage furrow is drastically reduced, whereas the amount of the alanine mutant myosin is much greater than wild-type GFP-labeled myosin [79]. Thus, it appears that as long as myosin can form filaments, it can carry out cellular functions, but inhibition of filament assembly leads to a loss of myosin function and inability to rescue the null phenotype. The major problem with mutants that cannot be phosphorylated is excess assembly in the recruited areas.

An RLC null line was created in *Dictyostelium* which largely phenocopies the heavy chain mutants. However, the interpretation of this experiment is clouded by indications that the RLC-deficient myosin aggregates in the cells [80]. In vitro studies with chicken fast skeletal muscle myosin show that myosin aggregates if the RLC is not present and that myosin missing the RLC does not support in vitro motility of actin filaments even though the actin-activated MgATPase activity is not greatly impaired [81]. Another approach used site-directed mutagenesis of the phosphorylation sites of RLC expressed in the RLC null background. Replacement of the phosphorylatable serine on the RLC with an alanine fully rescues the RLC null phenotype [77]. In vitro studies showed that the non-phosphorylatable RLC mutant myosin had a reduced actin-activated MgATPase activity that was similar to that of myosin with a dephosphorylated RLC [77]. A separate experiment deleted the heavy chain sequence that comprises the binding site for RLC [76]. The mutant myosin functions in vitro (albeit with somewhat altered enzymatic properties) and is capable of rescuing most wild-type phenotypes in vivo. A more detailed study expressed a GFP-tagged myosin lacking both light chain binding sites. The time course of cleavage furrow constriction, of nuclear separation and of cell edge advancement is similar in both wild-type and mutant myosin cells [60]. In combination, these studies support an important regulatory role for myosin heavy chain phosphorylation in *Dictyostelium*, but suggest that RLC phosphorylation may not be so important.

Similar 'rescue' experiments have been used to study the recruitment of myosin to the cleavage furrow during cytokinesis. A major question is how does myosin get to the cleavage furrow. Several models have been proposed to explain myosin's well orchestrated localization during mitosis: (1) cortical flow generated by contraction of the cortex brings the myosin and actin to the cleavage furrow [82], (2) a gradient of kinases or phosphatases creates a corresponding gradient of active myosin filaments leading to the cleavage furrow, and (3) a myosin

binding protein targets myosin to the cleavage furrow. The kinase model is weakened by the above described myosin heavy chain phosphorylation sites mutagenesis experiments which show that myosin mutants which cannot be phosphorylated still become targeted to the cleavage furrow [69,76–79,83]. Several recent experiments demonstrate that myosin's contractile activity per se is not necessary for targeting to the cleavage furrow. A myosin lacking the motor domain accumulates in the cleavage furrow as does a myosin carrying a mutation in the motor domain that inactivates its actin-activated MgATPase and in vitro motility [84,85]. These experiments eliminate a cortical flow model requiring myosin II activity [86], but do not rule out such a model if other mechanisms such as unconventional myosins generate the driving force for the flow. The myosin binding protein model has been indirectly tested in the many truncation and deletion experiments that have been performed. Since headless myosin targets to the cleavage furrow, the putative binding site cannot be in the head [85]. Similarly, many of the tail deletions either rescue cytokinesis in suspension or localize in the cleavage furrow [78,84,87]. A recent study specifically addressed this question with a series of tail deletions as well as the production of chimeric myosins containing the *Dictyostelium* motor domain and tails from chicken skeletal muscle myosin [84]. All these constructs localize to cleavage furrow and partially or completely rescue cytokinesis in suspension. Thus, a putative myosin binding protein targeted to a specific area on myosin probably does not exist, but rather the ability to form a normal filamentous structure may be critical. In this regard, it is interesting that *Dictyostelium* myosin mutations that do not form filaments do not rescue cytokinesis [55,69,88].

The *Dictyostelium* model system has also been useful in detecting mutations critical to myosin function using mutagenesis screens. Many of the mutations lie within the motor domain although some are in the tail. One screen identified a number of mutations that line the cleft separating the upper and lower 50 kDa domains [89]. Another phenotypic screen has identified a series of conditional mutants with very different molecular phenotypes, some of which affect the binding of ATP and others affect the coupling between ATP hydrolysis and movement of actin [89]. Several are in the actin binding interface [90] while another is in the rod region where it greatly disrupts filament assembly by interfering with the nucleation event independently of altering phosphorylation of the heavy chain [88].

The role of the single *S. cerevisiae* myosin II in mitosis has been explored by ablation experiments which show cytokinesis defects, although the exact phenotype of the mutant was controversial [91,92]. After the first study which showed a cytokinesis defect, a later study showed that the major defects in the myosin II null cells were in chitin deposition to the septum of the cell wall between the mother and bud. Two recent studies revisited cytokinesis in yeast using GFP-tagged myosin II expressed in the null background. Myosin localizes in a contractile ring at the site of the mother-bud junction and the diameter of the ring decreases with time until only a spot of myosin fluorescence is observed [93,94]. There are two myosin heavy chain genes, *Myo2* and *Myp2*, in *S. pombe* [95,96]. Deletion of Myo2p is lethal due to an apparent cytokinesis defect [95]. Deletion of Myp2p is not lethal, but does show cytokinesis defects under conditions of limiting nutrients [96]. GFP derivatives of both of these myosins localize to the contractile ring [95–97].

Drosophila has a single non-muscle myosin II heavy chain gene that can be alternatively spliced at the amino-terminus and at loop 1 [98,99]. It is the product of the *zipper* locus which is embryonic lethal due to a failure to complete dorsal closure [100]. The *Drosophila* non-muscle RLC and ELC genes have also been cloned [101,102]. The RLC gene is the locus of the *spaghetti squash* (*sqh*) gene which is embryonic lethal due to extensive failure to complete cytokinesis [101]. The analysis of both the *zipper* and *sqh* mutation is complicated by the contribution of maternal protein which is sufficient to allow the embryos to proceed through certain developmental events [100,101]. In order to study the role of non-muscle myosin II in later development, an RLC transgene with a heat shock promoter in the *sqh* background was created [103]. Flies will develop into normal adults provided they are heat-shocked periodically to provide the RLC needed for functional myosin II activity. Heat shock can be withheld at different developmental stages to determine the requirement of myosin in various processes [103]. An-

other study used a genetic trick in which RLC null germ line cystoblast ovarian cells could be created within an otherwise RLC wild-type fly [104]. These two developmental studies, along with others that analyzed mutant embryos or used antibody injection techniques to disrupt myosin function, strongly support a role for non-muscle myosin in oogenesis. Multiple defects in this process can be detected including defects in ring canal structure, in migration of follicle cells and in dumping, the process late in oogenesis whereby the nurse cells rapidly transfer their contents to the oocyte [103,104]. Myosin II is required for nuclear migration, cellularization, dorsal closure, imaginal disc formation and cell sheet movement [100,103,105]. The *Drosophila* RLC contains two phosphorylation sites for myosin light chain kinase. Mutation of these sites to alanine in germline cystoblasts does not rescue oogenesis, but conversion of the primary site to glutamate allows for successful completion of oogenesis, supporting a regulatory role for RLC phosphorylation in this myosin [104].

There are two non-muscle myosin II genes in *C. elegans*. One of these, nmy-2, is important in establishing embryonic polarity [106]. Injection of nmy-2 antisense RNA into the ovaries of adult worms causes embryonic partitioning defects and is associated with mislocalization of PAR proteins which are a family of proteins required for early asymmetrical divisions. The PAR-1 protein, a putative Ser/Thr protein kinase, immunoprecipitates with the tail of NMY-2 from whole worm extracts. Antisense injection that targets the nmy-1 gene expression does not give an embryonic lethal phenotype [106].

Vertebrates have two non-muscle myosin heavy chain genes, termed non-muscle myosin IIA and IIB [107]. Most cells express relatively equal amounts of both of these myosins, with a few exceptions such as platelets and chicken intestinal epithelial cells which have only non-muscle myosin IIA and neuronal tissues which express predominantly non-muscle myosin IIB [108,109]. In tissue culture cell models, RBL2H3 cells express only IIA whereas Cos cells express only IIB (Dr. Robert Adelstein, personal communication). Myosin IIA has a higher actin-activated MgATPase activity and moves actin faster in vitro than does myosin IIB [110]. The non-muscle myosin IIB gene contains exons which are alternatively spliced in neuronal tissue [111,112]. The alternative splicing introduces longer sequences at loop 1 or loop 2 or both. The significance of this splicing is unknown as the spliced and unspliced loop 1 isoforms have relatively similar enzymatic activities [113]. The smooth muscle myosin gene is also alternatively spliced at loop 1 which introduces seven more amino acids. In this case, the longer isoform translocates actin about 2.5 times faster than the shorter isoform in vitro [114].

Myosin IIA and IIB localization varies between different cell types. In general, both isoforms are found in stress fibers in stationary cells, but myosin IIB is often also found in the cell cortex [110,115,116]. In several polarized migrating cell types, myosin IIB is found at the leading edge [110,117,118]. The non-muscle myosin IIB gene has been knocked-out in mice by homologous recombination [119]. Heterozygotes are fully functional, but homozygotes die in utero or within a few hours of birth as a result of profound developmental defects in the heart and show hydrocephalus of the brain. It is interesting that cardiomyocytes express only non-muscle myosin IIB and not IIA [109,120,121]. The heart shows atrial-septal defects which mirror tetralogy of Fallot in humans. The fact that the mice get this far along in development suggests that myosin IIB is not essential for cytokinesis, although it is found at the cleavage furrow in dividing cells.

Other higher eukaryotic model systems suggest that non-muscle myosin II is important in generating forces within cells. The closure of puncture wounds in *Xenopus* oocytes involves an actomyosin-generated purse string [122]. Interestingly, myosin arrives at the site of the wound before actin. A similar multicellular purse string can be seen during dorsal [100]. Myosin filaments were directly observed by electron microscopy in fish epidermal keratocytes [123]. These rapidly migrating cells develop broad leading lamellipodia that contain a dense meshwork of mostly unipolar actin filaments. Short bipolar myosin filaments are observed in the lamellipodia with the highest density seen near the cell body border. The dynamics of this actomyosin network are not consistent with a sarcomeric contraction model of keratocyte motility, but rather with a dynamic network contraction model where the forces for motility are generated in the zone between the cell body and the lamellipodia [123].

Recently, non-muscle and smooth muscle myosin II has been shown to be phosphorylated by several kinases in various signal transduction pathways. The RLC of smooth muscle myosin is phosphorylated by Rho kinase at the same site phosphorylated by myosin light chain kinase, which may provide for a calcium-independent pathway for activation of smooth muscle [124]. Rho kinase also phosphorylates myosin phosphatase and inhibits its activity [125]. This would also have the effect of increasing the level of activation of smooth muscle. However, the overall picture is not so clear. Another kinase in the Rho pathway, P21-activated kinase, phosphorylates myosin light chain kinase and decreases its activity which would decrease the level of myosin activity [126]. Recently, non-muscle myosin II has been shown to be phosphorylated on the heavy chain in PC12 cells following stimulation of the Rac pathway [127]. If the heavy chain phosphorylation destabilizes filaments [52] and is thus inhibitory, this would provide a mechanism for the Rac opposition of the Rho pathway.

4. Myosin IV

Myosin IV has thus far only been found in *Acanthamoeba* [128]. It links very loosely with myosin I class molecules and was first termed HMW myosin I (high molecular weight). The conserved motor domain is followed by a single IQ motif and a tail lacking any coiled-coil forming sequence. There is a MyTH4 domain in the tail which has homology to similar domains in the tails of myosin VII and XV. A preliminary study showed that it binds to actin, hydrolyzes ATP and moves actin filaments in vitro [129].

5. Myosin VI

Myosin VI is a two-headed myosin with a single IQ domain [20,21]. Its tail has a short segment predicted to form a coiled-coil, but is otherwise unremarkable. The motor domain is unique in two regions. There is a 25 amino acid insertion, which by comparison with myosin II crystal structures should be present in a loop at the surface of the upper 50 kDa domain, and another insertion of about 50 amino acids just before the IQ motif. Similar to the lower eukaryotic myosin I molecules, all myosin VI molecules have a threonine at the TEDS rule site (amino acid 406 of the mouse sequence). The motor domain of myosin VI is phosphorylated in fibroblasts and p21-activated kinase phosphorylates the protein in vitro [130]. While myosin VI has not yet been purified from tissue, a fragment corresponding to the motor domain and the IQ motif has been expressed in Sf9 cells via recombinant baculovirus infection [131]. Interestingly, the myosin VI fragment translocates actin filaments in the opposite direction than that shown for other myosins. This is a result of the 50 amino acid insertion which probably alters the converter subdomain. Cryo-electron microscopic three-dimensional reconstructions of decorated actomyosin VI complexes show that the light chain binding region projects to the pointed end of the actin filament [131]. Myosin VI is the only myosin to have this particular insert at the converter region and, thus, may be the only pointed end motor.

Myosin VI has been identified in pigs [21], mice [132], chickens [130,134], humans [133], *Drosophila* [20] and in *C. elegans* [6]. The *Drosophila* myosin VI gene is found at locus 95F [20]. The protein is associated with particles in syncitiitial blastoderms which undergo cell cycle-dependent movements in which transient membrane furrows are produced between adjacent mitotic spindles [135]. Microinjection experiments with antibodies show that myosin VI is essential to early development [136]. The injected embryos show aberrant nuclear position and morphology. In addition, the normal rearrangement of the actin cytoskeleton during mitotic cycles is disrupted. Myosin VI may be required to produce normal actin-based transient membrane septa found in embryos. *Drosophila* myosin VI is also involved in transport of particles from nurse cells into oocytes during oogenesis [137].

In mice, myosin VI is encoded by the *Snell's waltzer* deafness gene [132]. The mutant mice are congenitally deaf and exhibit waltzing and circling behavior [138]. There are two alleles of the *Snell's waltzer* locus. One, termed (sv), is a result of a splice site deletion, which leads to a skipped exon and a frameshift in the coding region. This results in premature termination of the protein around the single IQ motif

[132] and effectively creates a null mutation as no protein is detected in any tissues by Western blotting. The mutant mice show a progressive loss of cochlea hair cells which begins at birth. By 6 weeks, very few hair cells remain [66]. In addition, the membranes of adjacent stereocilia fuse to form giant stereocilia [139].

Myosin VI is found at several locations in the hair cells of the ear. It has a prominent localization at the cuticular plate at the base of the stereocilia which serves to anchor the stereocilia to the soma [140]. It is particularly enriched in the pericuticular necklace region between the circumferential actin band and the cuticular plate. In addition, it is found diffusely in the cell body of hair cells. In frog inner ear hair cells, it is also present at low levels in the stereocilia. Hasson et al. [140] have proposed that myosin VI may be responsible for the anchoring of the stereocilia rootlets. In this regards, it is interesting that myosin VI is also found in the terminal web of polarized intestinal epithelial cells which also have actin-rich microvilli where it may serve a similar function [21,141].

Myosin VI is also expressed in most other tissues and cell types [21]. It is localized to the Golgi complex and to the leading edge of fibroblasts suggesting that it plays a role in membrane trafficking [130]. However, the *sv* mutant *Snell's waltzer* mice show no obvious phenotypes, besides those described above, which suggests that the membrane trafficking roles of this myosin are either not essential or are redundant with other myosins or with microtubule-dependent motors. In this regard, it is interesting that *Drosophila* myosin VI interacts with D-CLIP-190 (cytoplasmic linker protein-190) [142]. This protein is an orthologue of human CLIP-170 which links endocytotic vesicles to microtubules [143].

6. Myosin VII

Myosin VII has been found in mouse [144], pig [145], human [146,147], *Drosophila* [148] and in *C. elegans* [6]. Two myosin VII genes, termed VIIA and VIIB, have been localized in mouse [146]. Myosin VIIA has a conserved motor domain followed by five IQ motifs. A short predicted coiled-coil motif in the tail probably allows for dimerization to form a two-headed structure. The tail region contains two FERM domains (formerly termed talin homology domains) which have been implicated in cytoskeletal protein interactions in other systems, two MyTH4 domains and an SH3 domain. The function of the MyTH4 domains is unknown, but similar domains are also found in myosin IV and myosin XV.

Mutations in myosin VIIA genes are responsible for hereditary deafness in both mouse and human [144,149–152]. In humans, myosin VIIA mutations are associated with Usher syndrome type 1B (USH1B), an autosomal recessive disease with sensorineural hearing loss and retinitis pigmentosa that gives rise to gradual blindness. It is the most common form of deafness-blindness. In addition, two forms of non-syndromic deafness, DFNB2 and DFNA11, are also caused by myosin VIIA mutations [150,152]. Myosin VIIA is the locus of the *shaker1* gene in mice [144]. Mice that are homozygous for any of the seven *shaker1* alleles exhibit deafness, hyperactivity and head tossing. Interestingly, the *shaker1* mice do not experience retinitis pigmentosus [144]. In this respect, the phenotype of these mice more closely parallels that of the human non-syndromic deafnesses which are not associated with retinitis pigmentosus. A possible reason for the lack of associated blindness may be the short lifespan of mice.

Myosin VIIA may be involved in stereocilia integrity and in membrane trafficking in the inner ear hair cells. It is found in cross links of adjacent stereocilia and in the cuticular plate [139,140]. The mutation results in progressively disorganized stereocilia in mice. The protein is also found in the pigmented epithelium of the retina and in photoreceptor cells where it is postulated to play a role in phagocytosis and may function in the transport of opsin [153–156].

Nine missense mutations for human myosin VIIA and two for mouse myosin VIIA have been described that lie within the motor domain (see [157,158] for review) (Fig. 4). The mutations can be mapped onto the structure of chicken skeletal muscle myosin S1 [13]. Examination of the localization and nature of the mutations reveal insights into possible molecular abnormalities. Mutations at three of these sites, R241P/S, R244P and A397D, are located on the upper surface of the cleft that separates the upper and lower domains at the tip of the myosin head

while a fourth mutation, E450Q, lies at the bottom surface of the same cleft and is at the start of the Switch II region. Three of these amino acids, R241, A397 and E450, are highly conserved among myosin superfamily members. Several myosin VIIA mutations lie in putative actin binding regions. The M599I mutation lies within loop II at the tip end of myosin which is thought to interact with the negatively charged amino-terminus of actin. Recall that this loop was not seen in the crystal structure, probably due to its flexible nature. In myosin II molecules, this loop is longer and more highly charged than it is in myosin VIIA, suggesting that the function of the loop may be somewhat different in the latter myosin. Two other mutations, R502P and P503L, lie on the outer surface of the lower domain of myosin in regions that are thought to interact with actin in myosin II. Mutations in two highly conserved residues, R212H/C and G214R, lie at the start of the Switch I segment. A final motor domain mutant, G25R, lies at the amino-terminus in a region with little conservation among myosin superfamily members. Myosin VIIA has yet to be purified from tissue or expressed in Sf9 cells so there is no information on the enzymatic properties of the wild-type protein, nor on the effect of these mutations on activity. Some of the mutations affect the level of protein expression in mice which may also contribute to the abnormality of the tissue [140].

7. The plant myosins: classes VIII, XI and XIII

Myosin VIII, first described in the plant *Arabidopsis*, has a predicted molecular weight of 131 000 Da [159]. There is a 90 residue amino-terminal extension of the motor domain, four IQ motifs and a short segment of predicted coiled-coil forming sequence. Another partially sequenced *Arabidopsis* myosin VIII has been reported [160]. Virtually nothing is known about its structure or function.

Myosin XI was also first identified in *Arabidopsis* [161]. This myosin has many structural features similar to myosin V, including six IQ motifs and a tail with segments of coiled-coil interspersed with non-helical segments.

Two myosin XIII genes have been reported, termed MYO1 and MYO3 (accession numbers O04146 and O04145). While their motor domains are quite similar and they both have very short tails, they differ in the number of IQ motifs found in the neck region. MYO1 has 3–5 IQ motifs, whereas MYO3 has 5–7.

Some alga species, such as *Nitella* and *Chara*, show very rapid translocation of vesicles on oriented actin cables [162]. Several groups have reported partial purification of a myosin from *Chara* that translocates actin filaments at rates up to 60 μm/s [163–165]. Interestingly, one of the groups rotary shadowed this myosin and found that its head length is similar to that of conventional myosin II, suggesting that none of the plant myosins cloned to date encodes this myosin [165].

8. Myosin X

Little is known about the function and localization of myosin X. The protein was first identified in a PCR screen from a frog inner ear library, but the transcript does not appear to be very abundant in this tissue and there is no known role for myosin X in hearing [166]. The tail of myosin X has short stretches of predicted coiled-coil forming sequence and is dimeric. There is also a PH domain which is also found in spectrin, dynamin, various GAPS and kinases that interact with signal transduction pathways and the cytoskeleton. [167]. Three IQ motifs follow the motor domain. Recently, a recombinant fragment of myosin X corresponding to an HMM-like fragment has been co-expressed with calmodulin in Sf9 cells using baculovirus [168]. The MgATPase activity is markedly activated by actin with a V_{max} of 10 s^{-1} and a K_{ATPase} of 5 μM at 37°C. It moves actin filaments at a rate of 0.18 μm/s in the in vitro motility assay. There is no activation of either activity by calcium.

9. Myosin XII

This myosin was identified in *C. elegans* by the genome project [6]. Its motor domain sequence is even more divergent from the consensus than that of myosin III. The heavy chain is very large (300 000 kDa) with an amino-terminal extension of

200 amino acids, two IQ domains and a very short sequence predicted to form coiled-coil. However, unlike most myosin classes, the putative coiled-coil forming region is not juxtaposed to the last IQ, but is situated in the carboxyl-terminal one third of the tail. Whether myosin XII is a two-headed myosin will await purification. There are two MyTH4 domains in the tail.

10. Myosin XIV

Two members of the myosin XIV class were found in the parasites *Toxoplasma gondii* and *Plasmodium falciparum* [4]. Three transcripts were found in the former which probably includes one case of an alternatively spliced gene. The myosins are the simplest in composition containing a motor domain with no IQ motifs and only a very short tail. It is not known whether these myosins participate in the infectious process.

11. Myosin XV

This recently discovered class of myosins was discovered in a search for the human gene responsible for DFNB3, a recessive, non-syndromic profound congenital deafness [169]. The mouse ortholog is the locus at the *shaker2* gene in mice which is also associated with deafness [170]. Myosin XV is the largest myosin heavy chain found to date with its longest transcript having a deduced size of 3530 amino acids with a calculated molecular weight of 395 000 Da [171]. The gene encoding the protein has 66 exons. The second exon of both the mouse and human myosin XV encodes a proline-rich domain (of about 1200 amino acids) found at the amino-terminus that has no obvious sequence homology to other proteins. The tail of myosin XV does not have any regions predicted to form coiled-coils. However, there are similarities to the tail of myosin VII in that there are two MyTH4 domains, two FERM domains and an SH3 domain. Several of the exons appear to be alternatively spliced, including the large exon 2 and exon 8 which is 6 nucleotides in length and encodes two amino acids that are found in loop 1 by homology with chicken skeletal muscle myosin [171]. Two mutations in the motor domain of myosin XV have been described. C1775Y is located close in space to the P-loop and G1358S is located in the Switch I region [169].

The morphology of the *shaker2* mice reveals some insight into the function of myosin XV. Immunofluorescent staining shows that myosin XV is present in the cell body and stereocilia. In the *shaker2* mice, the stereocilia of the inner and outer hair cells are only about one tenth of the normal length [170]. The protein is found in the cell body and stereocilia of both the inner and outer hair cells. The only other tissue which shows a significant amount of message for the protein is the pituitary [171].

12. Conclusions

Higher organisms clearly have a plethora of myosin genes. Only a few myosins have as of yet been studied in detail, but it is clear that they are involved in many cellular processes where unique functions are performed. One of the best studied cases to date in the auditory system, where myosins from class I, VI, VII and XV have been shown or postulated to play critical roles [140]. In addition, myosin V is certainly involved in neurotransmission in this tissue. It is also becoming increasing clear that myosins provide an intimate link between signal transduction pathways and the cytoskeleton. Myosins may be a target for direct or indirect regulation of their motor activity by signal transduction kinases or these pathways may affect the localization of myosin or target association of myosin with other proteins or factors within the cell.

Challenges remain to finish the search for myosin genes in humans and in model genetic systems, to purify or express recombinant myosin molecules that have yet to be studied in vitro, to localize the tissue and intracellular distribution of myosins and to screen for involvement in human disease or for animal model systems with which to study myosin function.

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