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To the memory of my friend Dr. Robert Makuch who passed away so suddenly

Review

Myosins and pathology: genetics and biology★✪

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This article summarizes current knowledge on the genetics and possible molecular mechanisms of human pathologies resulted from mutations within the genes encoding several myosin isoforms.

Mutations within the genes encoding some myosin isoforms have been found to be responsible for blindness (myosins III and VIIA), deafness (myosins I, IIA, IIIA, VI, VIIA and XV) and familial hypertrophic cardiomyopathy (β cardiac myosin heavy chain and both the regulatory and essential light chains).

Myosin III localizes predominantly to photoreceptor cells and is proved to be engaged in the vision process in *Drosophila*.

In the inner ear, myosin I is postulated to play a role as an adaptive motor in the tip links of stereocilia of hair cells, myosin IIA seems to be responsible for stabilizing the contacts between adjacent inner ear hair cells, myosin VI plays a role as an intracellular motor transporting membrane structures within the hair cells while myosin VIIA most probably participates in forming links between neighbouring stereocilia and myosin XV probably stabilizes the stereocilia structure.

About 30% of patients with familial hypertrophic cardiomyopathy have mutations within the genes encoding the β cardiac myosin heavy chain and both light chains that are grouped within the regions of myosin head crucial for its functions. The alterations lead to the destabilization of sarcomeres and to a decrease of the myosin ATPase activity and its ability to move actin filaments.

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Abbreviations: ERG, electroretinogram.

Myosins are actin-based molecular motors that are ubiquitously expressed as multiple isoforms in all eukaryotic cells. They form both structurally and functionally diverse superfamily that consists of at least 18 distinct families (Berg *et al.,* 2001). The classification is based on the diversity of amino-acid sequences of the globular motor domain, responsible for myosins activities (see Sellers, 1999). The classic two-headed myosins are called conventional myosins and represent myosin II family. The other seventeen families are referred to as unconventional myosins. So far only myosin II isoforms have been found to form filaments. The unconventional myosins characterized so far, despite the fact that some of them are two-headed, do not form filaments and remain monomeric. Myosins consist of two major components – heavy and light chains. Three structural and functional domains can be distinguished within the heavy chain (Figs. 1, 2): (i) the N-terminal, most conserved globular motor domain where actin- and nucleotide-binding sites are located; (ii) the regulatory domain called also the neck, containing the IQ motifs that define the light chain-binding region (it is believed that the number of IQ motifs determines the number of the light chains bound); (iii) the C-terminal tail domain, the most diverse part of the heavy chain that is responsible for the specific myosin functions such as dimerization, filament formation, binding to membranes or targeting to membrane proteins. Interestingly, Korn (2000) observed that a similar classification of the myosin superfamily could be achieved while performing analysis of the amino-acid sequences of myosin tails. While there are specific light chains non-covalently attached to conventional myosin heavy chains (Fig. 1), for most of the unconventional isoforms calmodulin molecules play a role of their light chains (Sellers, 1999).

Myosins are involved in various cellular functions such as cell motility and muscle contraction, intracellular transport of particles and organelles, cytokinesis, endo- and exocytosis, and, probably, gene expression. Generally, the more complicated the organism the higher number of myosin isoforms it contains, representing the higher the number of families (Berg *et al.,* 2001). For example, while there are the isoforms representing three myosin families (I, II and V) in the yeasts *Saccharomyces cerevisiae*, 39 genes encoding myosin heavy chains belonging to at least 12 families (I, II, III, V, VI, VII, IX, X, XV, XVI, XVIII and a yet not classified novel myosin) have been found in humans (Berg *et al.,* 2001).

Taking into account such diversity and complexity within the myosin superfamily, it is not surprising then that mutations within the genes encoding myosin components may lead to alterations in cell function, and in consequence to pathology. In this review the current knowledge on the genetics and, where possible, the molecular mechanisms of human pathologies resulting from mutations within the genes encoding myosin isoforms will be presented.

MYOSINS AND VISION

Myosin III (Fig. 2) from *Drosophila melanogaster* is the product of the *NinaC* gene, that has been originally isolated as visual mutation with an electrophysiological phenotype (Montell & Rubin, 1988). Since then myosins III have been found in the horseshoe crab *Limulus polyphemus*, octopus, and in humans where two myosin III isoforms have been found (IIIA and IIIB) (Dose & Burnside, 2002). *Drosophila NinaC* is expressed as two extensively overlapping mRNAs encoding proteins with molecular mass of 132 kDa (p132) and 174 kDa (p174), differing only by the C-terminal domain (Montell & Rubin, 1988). These two proteins turned out to be the novel myosins that, in addition to the typical motor, neck (containing two IQ motifs) and variable tail, domains contain in their

N-termini a 34-kDa kinase domain (Fig. 2) (Montell & Rubin, 1988). The kinase domain has about 50% identity to the human serine/threonine kinases of the p21-activated kinase (PAK) family (Dose & Burnside, 2000) and has been found to phosphorylate exogenous p132 NinaC and several cytoskeletal proteins such as caldesmon or conventional myosin regulatory light chains (Ng *et al.*, 1996).

Expression of both p174 and p132 is restricted only to photoreceptor cells but p132 is localized to the cytoplasm of the photoreceptor cells and p174 is located at the long actin-rich structures termed rhabdomeres (Hicks & Williams, 1992; Porter *et al.,* 1992). It is believed that NinaC p174 is critical for normal adaptation and termination of photoresponse (Porter & Montell, 1993). Systematic mutagenesis studies of *Drosophila NinaC* have revealed that the kinase and myosin subunits play distinct roles in phototransduction (Porter & Montell, 1993). In flies in which the entire motor domain has been deleted, p174 fails to localize within the rhabdomere – the flies develop retinal degeneration and their electroretinogram (ERG) is altered. Point mutations within the motor domain also lead to the degeneration but the ERG is normal until the degeneration occurs. This suggests that the motor domain may be required for proper trafficking and/or anchoring of p174 to the rhabdomere compartment and the structural integrity of rhabdomeres. In contrast, flies in which the kinase domain has been eliminated exhibit altered ERG but do not develop retinal degeneration. This may indicate that the myosin III kinase subunit is necessary for normal phototransduction but not for the rhabdomere structural integrity (Porter & Montell, 1993). Also, two phosphorylatable serine residues (1316 and 1320) of the p174 C-terminal tail domain seem to be important for stable termination of visual cascade, as their substitution with alanine residues leads to alterations in ERG indicating a defect in deactivation of the flies photoresponse (Li *et al.,* 1998).

The sub-rhabdomeral localization of calmodulin, also critical for phototransduction, depends solely on the presence of myosin III (Porter *et al.,* 1993), despite the fact that there are several other retinal calmodulin-binding proteins in this cell compartment, including the TRP and TRPL ion channels, INAD and calossin (Xu *et al.,* 1998). That observation gave birth to a hypothesis that the control of calmodulin distribution might be a myosin III function. A problem how the myosin/calmodulin interaction might be translated into the functioning of the visual cascade became then intriguing. Most probably calmodulin binds to the NinaC neck IQ motif but fish myosin III contains IQ motifs also in the tail (Dose & Burnside, 2001). There are several possibilities of the involvement of myosin III in calmodulin trafficking: p174 may act as (i) a motor bringing calmodulin molecules to submembrane areas, (ii) a factor anchoring calmodulins bound to its heavy chain to submembrane complexes (iii) and/or a molecule simply storing calmodulin and releasing them during phototransduction. While there is no biochemical data yet on the possible motor functions there are reports justifying the other two possibilities. It has been observed that in the presence of elevated Ca^{2+} ions calmodulins may dissociate from the brush border myosin I heavy chain (Wolenski *et al.,* 1993). Also, it has been reported that *in vitro,* without exogenous calmodulins, the molecules bound to the brain myosin V heavy chain neck domains do activate calmodulin-dependent kinase II (Costa *et al.,* 1999). Montell's group has reported that the C-terminal part of the p174 tail domain interacts with the first out of five PDZ domains of INAD, a 80-kDa protein that is activated by calmodulin and is proposed to play a role of a molecular adapter coordinating phototransduction (Wes *et al.,* 1999). INAD *via* its PDZ domains binds also directly to rhodopsin, protein kinase C and the light sensitive channels – TRP and TRPL (for references see Wes *et al.,* 1999). The interaction of INAD with calmodulin occurs *via* the

region distinct from the PDZ motifs (for references see Wes *et al.,* 1999). The interaction of INAD with myosin III has been found to be critical in enhancing the speed of deactivation of phototransduction (Wes *et al.,* 1999). Based on this, it is believed that INAD, by an interaction with both the phototransduction proteins and myosin III, and therefore with the actin-based cytoskeleton, enables positioning of these proteins in the sub-rhabdomeral compartment and affects the kinetics of the response deactivation (Wes *et al.,* 1999). There is a possibility that myosin III serves as the calmodulin donor to INAD but if and how the NinaC/INAD interaction is regulated and if myosin plays a donor/motor role(s) certainly has to be answered in future studies.

Both human myosins III are expressed in retina and so far no correlation has been made between blindness and mutations within the genes encoding them. Unexpectedly, mutations within the myosin IIIA genes have been associated with nonsyndromic deafness DFNB30 (Walsh *et al.,* 2002). This problem will be elucidated in the section below, devoted to myosins and deafness.

The other human myosin participating in the vision process is myosin VIIA. Over forty mutations within its gene have been linked to USHER1B syndrome, a hearing loss associated with progressive retinitis pigmentosa (reviewed by Rędowicz, 1999). The characteristics of myosin VIIA and its involvement in both the vision and hearing processes are described in the deafness section.

MYOSINS AND DEAFNESS

Genetic deafness is the most common form of sensory disorder in humans. Due to the complex structure of the inner ear, it is believed that as much as 100 genes might participate in the hearing process, and a mutation in just one of them may disturb the inner ear delicate balance, and lead to deafness. The conversion of the mechanical stimuli such as sound and gravity into electrical signals, transmitted to the brain, takes place in inner ear cochlea, and more precisely in the organ of Corti. The organ of Corti (or spiral organ) is composed of highly specialized epithelium formed by the inner and outer hair cells that are supported by various columnar cells. Hair cells on their apical surface have actin-rich stereocilia, inserted into the cuticular plate, that are aligned in characteristic V-shaped clusters. The mechanicals forces cause a sliding of adjacent stereocilia which leads to the opening/closing of transduction channels and depolarization/hyperpolarization of the hair cell membrane.

The first reports about a possible involvement of motor proteins in the hearing process were published a decade ago when Gillespie *et al.* (1993) showed that myosin IC (formerly termed Iβ) from frog sacculus is located at the tips of stereocilia of the hair cells (these cells convert mechanical stimuli into electrical signals) bundles. Those and subsequent studies (Hasson *et al.*, 1997) have led to a hypothesis according to which this monomeric one-headed myosin I (Fig. 2) plays an essential role as an adaptation motor amplifying displacements caused by a mechanical force into maximal channel opening or closing and, as a consequence, electrical signals are generated and transmitted *via* the auditory nerve to the central nervous system. Recently the Gillespie's group has shown that myosin IC interacts with the hair cells receptors and surprisingly the binding occurs *via* the IQ domains, not, as has been previously suggested, *via* the tail domain and, moreover, the binding is negatively regulated by calmodulin (Cyr *et al.*, 2002). Despite intensive research, no mutations within the gene encoding myosin I isoforms have so far linked to hearing loss in humans although the gene encoding the myosin IF isoform is been considered as a candidate gene for nonsyndromic deafness (nonsyndromic means that there are no other symptomps than deafness) DFNB15 (Chen *et al.,* 2001).

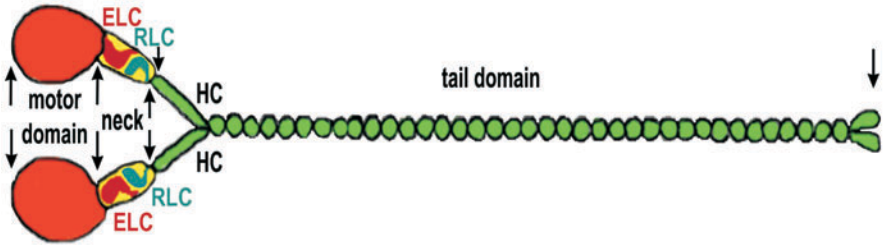

Figure 1. Schematic representation of the structure of conventional myosin.

HC, heavy chain; ELC, essential light chain; RLC, regulatory light chain. In this and the following figure, orange marks the motor domain, yellow, the neck domain (IQ motifs) and green, the tail domain.

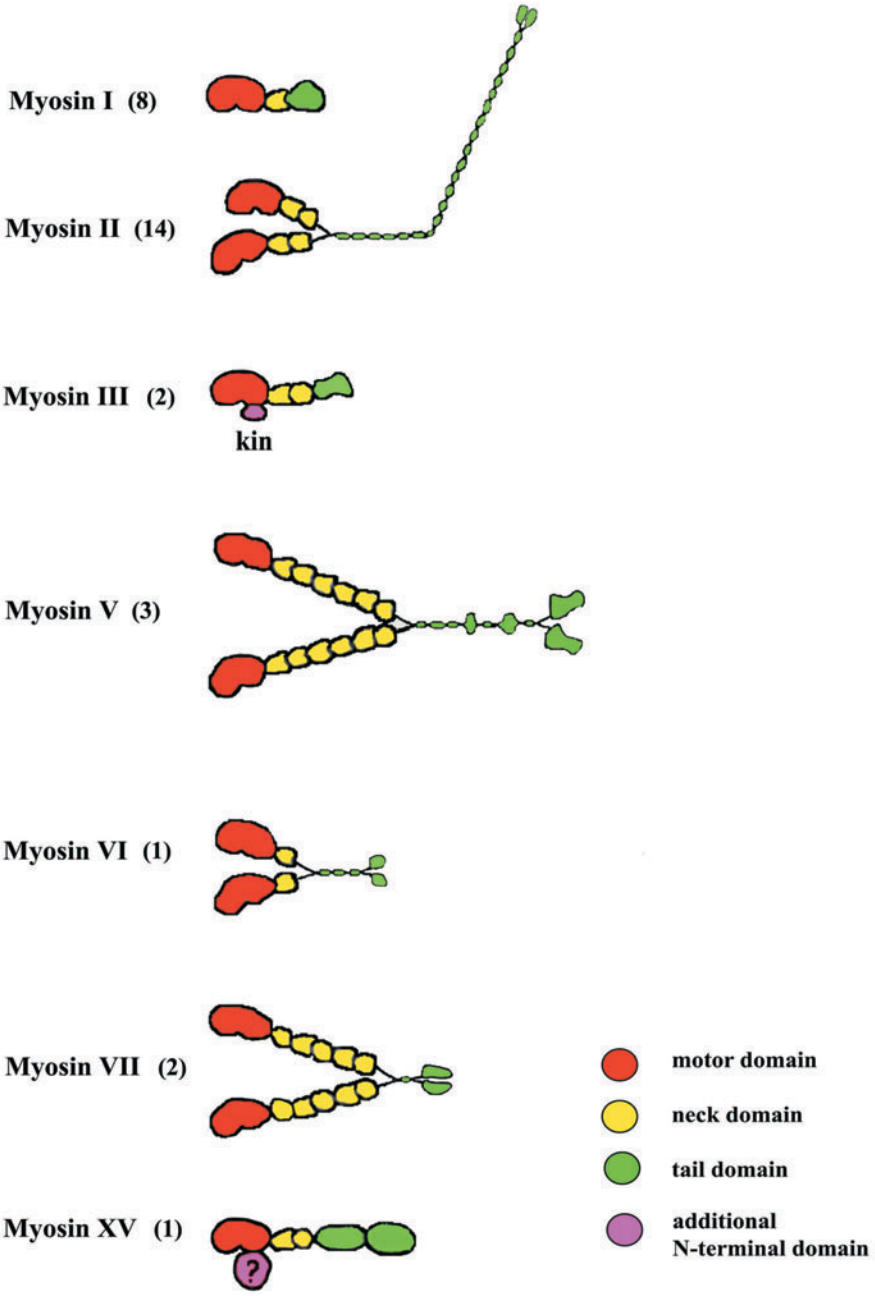

Figure 2. Schematic overall structure of myosin isoforms involved in human pathology.

Numbers in brackets reflect the number of isoforms expressed in humans. The colour code as listed above, and purple, marks the additional N-terminal domains.

Such links were found for myosins: IIA (Lalwani *et al.*, 2000), VI, VIIA, XV (for details see Rędowicz, 1999), and quite recently (and unexpectedly) for myosin IIIA (Walsh *et al.*, 2002). Interestingly, despite the fact that all these myosins are expressed not only in hair cells, the predominant clinical manifestation of their dysfunction is hearing impairment.

Myosin II

Out of 14 genes encoding conventional myosin heavy chains, only the gene *MYH9* encoding nonmuscle myosin IIA is involved in the hearing process. Within last two years there were several reports on the connection between mutations within the myosin IIA gene and May-Hegglin anomaly, Sebastian, Fechtner, Alport-like, and Epstein syndromes (Heath *et al.*, 2001) as well as the autosomal dominant myopathy OMIM 605637 (Tajsharghi *et al.*, 2002). What is clinically common for the latter pathologies are autosomal dominant alterations in platelets, i.e. macrothrombocytopenia with inclusions in leukocytes (except for Epstein syndrome). Patients with Fechtner and Epstein syndromes develop nephritis and cataracts. Additionally, some patients with the platelet anomalies are deaf (Heath *et al.*, 2001). Out of the fourteen mutations detected so far in the myosin IIA heavy chain gene (situated both in the motor domain and the coiled-coil rod) only one, R705H, is linked with the hearing impairment DFNA 17 (Lalwani *et al.*, 2000). This mutation occurs in a highly conserved region of the myosin motor domain that is critical for the proper myosin function. R705 resides in a 16-amino-acid linker region containing two free thiol groups (SH1 and SH2) that play an essential role in the conformational transitions within motor domain coupled to ATP binding. The R705H mutation may alter the architecture of this region and therefore disrupt the mechanical properties of the myosin motor domain (Lalwani *et al.*, 2000). The questions arise of: (i) where myosin IIA is localized in the inner ear, (ii) and what role it may play there. In rat cochlea it is localized throughout the hair cells of Corti organ, in the Reissner membrane (a barrier that forms the upper boundary of the cochlear duct) and in the subcentral region of spiral ligament (the outer wall of cochlear duct). There is no data on the specific role of myosin IIA in the inner ear but the collapse of the Reissner membrane of a proband affected with DFNA 17 indicates that it may be important for the maintenance of cells architecture and cochlear homeostasis (Lalwani *et al.*, 2000).

Myosin III

As it has been mentioned above, myosin III (NinaC) plays an essential role in the vision process in *Drosophila*. It has been believed that human myosin III (Fig. 2) has similar function as its fly counterpart. Surprisingly Walsh *et al.* (2002) has recently reported that one of the two human myosin III isoforms, myosin IIIA, is involved in the hearing process. This isoform is expressed predominantly in the retina and cochlea, where it is localized in hair cells. The authors have linked the progressive, recessive autosomal nonsyndromic hearing loss DFNB30 to three different loss-of-function-mutations in the myosin IIIA heavy chain gene. Two of these mutations, 668X and 1043X, are nonsense and lead to the expression of the protein truncated within the motor domain and the motor/IQ domains junction, respectively. The third one, affecting the splice acceptor of intron 8, results in unstable message and, in consequence, lack of protein expression. Interestingly, none of these mutations affected the vision process which might be explained by the functional redundancy of myosin III family in the human eye. In fact, the other myosin III isoform that is expressed in the retina, IIIB, shares 65% overall identity with the myosin IIIA molecule and may substitute for the loss of myosin IIIA function in this organ (Dose & Burnside, 2002). This isoform, besides the retina ex-

pressed also in the kidney and testis, is a candidate for the causative agent of Bardet-Biedl syndrome (Dose & Burnside, 2002) that is characterized by limb dysmorphism, retinal dystrophy, obesity, male hypogenitalism and sometimes mental retardation and/or diabetes mellitus (Green *et al.*, 1989).

While the mechanism of the involvement of *Drosophila* NinaC in the vision process *via* the interaction with INAD seems to be elucidated (see above) there is no information on the ligands of the myosin IIIA tail domain. Moreover, this part of the molecule is not conserved within the myosin III isoforms sequenced so far (Dose & Burnside, 2002; Walsh *et al.*, 2002). The identification of the protein(s) interacting with the tail domain might help in understanding the role of myosin III in the hearing process.

Myosin VI

Initial evidence that myosin VI (Fig. 2) is involved in the hearing process comes from studies on *Snell's waltzer* mice that were deaf and exhibited typical circling, head-tossing and hyperactivity (reviewed by Rędowicz, 1999). Genetic mapping of these deaf mice revealed that mutations within the gene encoding myosin VI are responsible for these symptoms. Myosin VI is expressed ubiquitously (Berg *et al.*, 2001) and its heavy chains with molecular mass of about 140 kDa contain one IQ motif and form dimers (Fig. 2). In rodent hair cells, myosin VI is localized at the cuticular plate (anchoring stereocilia) and throughout the hair cell body. Two mutations within the myosin VI heavy chain gene were detected in mice, one leading to a 130-bp deletion at the region corresponding to the beginning of the neck region, and the second one causing a 220-kbp inversion of DNA upstream of the known coding region. Both mutations result in significant reduction of functional protein and mRNA expression. Histopathology analysis of the mutant mice has revealed a great dysmorphism of hair cells: they do not contain stereocilia, are pycnotic and degenerate about six weeks after birth (Avraham *et al.*, 1995). So far only one missense, C442Y, mutation has been detected in humans that is associated with nonsyndromic deafness DFNA22 (Melchionda *et al.*, 2001). This mutation affects the conserved cysteine residue that is within the core of the motor domain – replacing it with a larger tyrosine residue probably destabilizes the expressed protein and leads to a partial or complete loss of function (Melchionda *et al.*, 2001). These observations clearly indicate that myosin VI plays an important role in hair cell functioning, probably by maintaining structural integrity of these highly specialized cells. Quite recent studies have shown that myosin VI is a processive motor that moves at 58 nm $\cdot$ s^{-1} towards the minus (pointed) end of actin filaments, opposite to most of the known myosin motors (Wells *et al.*, 1999). Several years ago Mermall *et al.* (1994) have demonstrated that *Drosophila* myosin VI participates in subcellular transport of membrane-containing structures. Those and subsequent studies have led to the proposition that myosin VI is actively involved in receptor mediated endocytosis (Buss *et al.*, 2001). The authors propose that myosin VI participates in (i) spatial organisation of the endocytotic machinery at the base of microvilli; (ii) locomoting of receptors towards the base of microvilli for sequestering and coated pit formation; (iii) invagination of the plasma membrane; (iv) scission of vesicles, and (v) transport of clathrin coated vesicles away from the plasma membrane towards the cell body (Buss *et al.*, 2001). This hypothesis seems to be confirmed by recent studies showing that myosin VI binds to and localizes with Dab2, a signal transduction protein that binds to the clathrin adaptor protein AP-2 (Morris *et al.*, 2002), and SAP97, a protein involved in receptor trafficking (Wu *et al.*, 2002a). Because the cuticular plate of hair cells is the region where very high membrane transporting activity occurs, crucial for the continuous process of stereocilia rebuilding, so it is quite probable then that a

lack of functional myosin VI leads to the impairment of intracellular transport of vesicles, and in consequence to the collapse of stereocilia and dysmorphology of hair cells.

Besides its role in mammals in the hearing process, myosin VI in *Drosophila* is also involved in spermatogenesis (Hicks *et al.*, 1999) and embryogenesis, where it is required for cadherin-mediated border cell migration (Geisbrecht & Montell, 2002).

Myosin VIIA

Numerous mutations within the gene encoding myosin VIIA, one of the two human myosin VII (Fig. 2) isoforms, have been linked to USHER1B syndrome, a profound autosomal recessive hearing impairment associated with balance problems and progressive retinitis pigmentosa leading to blindness (reviewed by Rędowicz, 1999). It has been found that nonsyndromic deafness DFNB2 and DFNA11 are also caused by mutations within the myosin VIIA gene (Weil *et al.*, 1996; Tamagawa *et al.*, 2002). A mouse deafness model, *shaker-1* (*sh1*), with the characteristic animal hyperactivity, head tossing and circling, is a murine counterpart of the USHER1B syndrome (reviewed by Rędowicz, 1999). Interestingly, no vision problems were detected in the mouse, which is probably due to the short life span of the animals, but electroretinographic anomalies were observed in affected animals (Libby & Steel, 2001). The zebrafish (*Danio rerio*) mariner phenotype with its characteristic circling is the orthologous counterpart of the USHER1B syndrome (Ernest *et al.*, 2000). There are two alternatively spliced transcripts of the human myosin VIIA gene that encode proteins differing only in the length of their C-terminal tails. The longer transcript encodes the myosin heavy chain with a molecular mass of about 250 kDa (referred to in the literature as myosin VIIA) that is expressed in the cochlea, retina photoreceptor and epithelial cells, testis and kidney. The shorter transcript encodes the heavy chain with molecular mass of about 138 kDa, expressed exclusively in the testis (reviewed by Rędowicz *et al.*, 1999). No testis abnormalities were observed in male USHER1B patients, although a decreased sperm motility was detected (Hunter *et al.*, 1986).

The other myosin VII isoform, VIIB, nearly 50% identical to myosin VIIA is expressed primarily in the kidney and intestines (Chen *et al.*, 2001).

Myosin VIIA heavy chain is composed of the typical motor domain, the neck containing five IQ motifs and a long tail portion containing in its C-terminus a short α-helical segment that enables dimerization of the heavy chains (Fig. 2). The remaining part of the tail contains two sequence repeats that are separated by the SH3 (Src-homology) domain, present also in myosin I, IV and XV. Each repeat comprises a MyTH4 (myosin tail homology) domain, found also in myosin IV, X, XII and XV, and FERM (band 4.1/ezrin/radixin/moesin – homology) domain, identified also in myosin X, XV and XVIII. The presence of these domains indicates involvement of these myosins in interprotein and/or protein/lipid interaction (Sellers, 1999; Berg *et al.*, 2001).

Over forty mutations within the myosin VIIA gene that lead to genetically and clinically heterogenous hearing loss and blindness have been detected worldwide in humans (for references and a list of mutations see Rędowicz *et al.*, 1999). The mutations result either in the introduction of a stop codon leading to the expression of truncated, impaired protein (or no protein at all), or in point mutations as well as small in-frame deletion which probably lead to the expression of functionally impaired protein. Mutations within the motor domain are the most common ones (about 30 cases) and lead to the most severe clinical phenotypes, confirming the absolute necessity of this domain for the myosin function. They were detected within or next to the ATP- or actin-binding sites. Two of them, R244P (the residue within a conservative loop forming the ATP pocket) and P503L (the residue

within non-conserved region) have their counterparts in *shaker-1* mice. Much less mutations have so far been identified in the myosin VIIA tail domain. They were found in the neck domain, the coiled-coil region, in both MyTH4 domains, and in the second FERM domain.

Such severe phenotypes in patients with USHER1B syndrome indicate that myosin VIIA plays an important role in the inner ear sensory hairs cells as well as in retina photoreceptor and epithelial cells. The studies on *Dictyostelium discoideum* myosin VII indicate that it is crucial for phagocytosis (Titus, 1999) and mediates the initial binding of cells to substrata (Tuxworth *et al.,* 2001). Initially, the information on the possible role of myosin VIIA has come from its subcellular distribution as well as the morphological changes observed for sensory hair cells of *shaker-1* mice. In hair cells, myosin VIIA is distributed in stereocilia, cuticular plate and cell body (Hasson *et al.,* 1997). In stereocilia it seems to be concentrated at their external sides, in the region corresponding to the horizontal interciliary ankle links. In *shaker-1* mouse the hair cells bundles of stereocilia are severely disorganized indicating that this myosin is important for maintaining the proper arrangement of these actin-rich structures. In the retina, myosin VIIA is concentrated in the connecting cilium of photoreceptor cell and pigment epithelium, where it is associated with melanosomes (Liu *et al.,* 1998). In the pigment epithelium of *shaker-1* mice, defective distribution of melanosomes has been observed (Liu *et al.,* 1998).

Myosin VIIA has been found to be required for aminoglycoside accumulation in cochlear hair cells (Richardson *et al.,* 1997), to participate in opsin transport through the photoreceptor cilium (Liu *et al.,* 1999) and to be responsible for electroretinographic anomalies (Libby & Steel, 2001). It is also important for the normal gating of stereocilia transduction channels (Kros *et al.,* 2002). The C-terminal FERM domain of myosin VIIA binds to vezatin, a protein of the cadherin-catenin adherens junctions (Kussel-Andermann *et al.,* 2000b) and to the regulatory subunit (RIα) of protein kinase A (Kussel-Andermann *et al.,* 2000a). Recently, it has been shown, using antibodies against the C-terminus of myosin VIIA, that microtubule-associated protein (MAP-2B) specifically binds to this myosin isoform, raising the possibility of its interaction with the microtubule-based cytoskeleton (Todorov *et al.,* 2001). Myosin VIIA immunoprecipitated from bovine retinal tissue is associated with calmodulin in a Ca^{2+}-sensitive manner (Udovichenko *et al.,* 2002). An apparent K_d of 10^{-9} M was calculated for the mouse kidney and cochlea isoform (Todorov *et al.,* 2001). The bovine myosin VIIA Mg-ATPase activity is stimulated by F-actin with a k_{cat} of 4.3 s^{-1} and with 7 μM actin required for half-maximal activity (Udovichenko *et al.,* 2002). Immunoprecipitated myosin VIIA has been found to move actin filaments with a velocity of 190 nm $\cdot$ s^{-1}, comparable to the velocities observed for native myosin VA (400 nm $\cdot$ s^{-1}; Cheney *et al.,* 1993) and baculovirus-expressed recombinant myosin IC from adrenal medula (300–500 nm $\cdot$ s^{-1}; Zhu *et al.,* 1996). Because these myosins are believed to act as motors, the authors suggest that myosin VIIA is a *bona fide* actin-based motor, actively participating in the intracellular transport (Udovichenko *et al.,* 2002). This suggestion seems to be confirmed by an interaction of the myosin VIIA tail with MyRIP (myosin-VIIA-and-Rab-interacting protein), a novel Rab effector (El-Amraoui *et al.,* 2002) that seems to be responsible for the proper localization of retinal melanosomes to the actin cytoskeleton and thereby mediates local trafficking of these organelles. A similar mechanism of interaction with Rabs, GTP-binding proteins, has been previously described for myosin V (Wu *et al.,* 2002b).

Despite extensive studies on understanding the role of myosin VIIA, the question of how myosin VIIA fulfills its various functions remains open. Based on the data gathered so far there are several possibilities: myosin VIIA (i)

actively participates in transporting protein and/or lipid complexes; (ii) works as a structural constituent of the horizontal link between the adjacent stereocilia, anchoring the cadherin–catenin system to the actin-based cytoskeleton; (iii) acts as a channel-gating motor, and/or (iv), what seems to be quite possible, utilizes all of these mechanisms. Further studies on myosin VIIA will certainly aid in resolving this intrigiung problem.

Myosin XV

Myosin XV (Fig. 2) is another myosin isoform that is involved in the hearing process. Mutations within the genes encoding mouse and human myosin XV are responsible for *shaker-2* syndrome and autosomal recessive nonsyndromic deafness *DFNB3,* respectively (reviewed by Rędowicz, 1999). While there are no visible symptoms other than deafness in DFNB3 patients, *shaker-2* mice exhibit vestibular defects such as head-tossing and circling behaviour. Recently, a mutation within the myosin XV gene was found in a patient with Smith-Magenis syndrome that is usually characterized by multiple congenital anomalies (craniofacial and ocular), hearing loss and mental retardation (Liburd *et al.*, 2001). The syndrome has been previously associated with a 4-Mb interstitial deletion of chromosome 17p11.2, a region that contains the myosin XV gene (see Liburd *et al.*, 2001). Besides being expressed in the cochlea neurosensory cells, myosin XV has been detected in the pituitary gland and in other neuroendocrine cells and tumors including those of adrenal medulla, parathyroid gland, and pancreatic islets (Liang *et al.*, 1999; Lloyd *et al.*, 2001). In mouse inner ear hair cells, myosin XV is found throughout the length of the stereocilia of both the inner and outer hair cells as well as at the level of the cuticular plate and below the level of the apical cell body (Anderson *et al.*, 2000).

The myosin XV heavy chain, encoded by 66 exons, with its molecular mass of about 365 kDa (Liang *et al.,* 1999) is the longest of the myosin heavy chains sequenced so far. In its N-terminus there is a novel, unique proline-rich domain, of about 140-kDa without any sequence similarity to known proteins. The additional domain is followed by the typical motor domain, the neck region with two IQ motifs and the tail region containing a MyTH4, SH3 and two FERM domains (Liang *et al.,* 1999). There is about 90% identity between the mouse and human myosin XV isoforms (Wang *et al.,* 1998; Liang *et al.,* 1999). Berg *et al.* (2001) have identified, in fact a putative human myosin XVB gene, but detailed sequence analysis revealed that it was a transcribed pseudogene (Boger *et al.,* 2001).

Most of the human myosin XV mutations that usually lead to the expression of functionally impaired protein, are located in the tail domain (Wang *et al.,* 1998; Liburd *et al.,* 2001). They were detected both in the MyTH4 domain (D2111Y, I2113F, and T2205I associated with Smith-Magenis syndrome) and the FERM domain (K2601X and Q2716H). Mutations within the motor domain affect its N-terminus (Q1229X and 5′ splice donor site of intron 4-IVS4+1G→T) and the ATP-binding region (G1358S) (Liburd *et al.,* 2001). In *shaker-2* mice, a point mutation in the reactive thiol region of the motor domain, *sh2,* (C1779Y) (Probst *et al.,* 1998) as well as a deletion of the C-terminal FERM domain, $sh2^J$, (Anderson *et al.,* 2000) were found. Stereocilia of the animals with the mutations were abnormal: they were extremely short, although nearly normally arrayed. Moreover, actin-rich 50-μm long protrusions were observed iniating beneath the cuticular plate and extending from the base of each inner but not outer ear, hair cell (Probst *et al.,* 1998). Interestingly, a successful attempt to rescue the *sh2* mutation by an injection of a fragment of the wild type myosin XV cDNA, matching the mutated region, into fertilized mouse eggs has been performed (Probst *et al.,* 1998). This and an other experiment, showing that the *sh2* and $sh2^J$ alleles do not complement each other

(Anderson *et al.,* 2000), confirm that the observed defects are due to mutations within the myosin XV gene.

There are no biochemical data indicative of the possible myosin XV function. Analysis of human and mouse mutations within myosin XV genes detected in deaf individuals shows that both the tail and motor domains are essential for their proper functioning. The subcellular localization of myosin XV in hair cells and alterations in their morphology in *shaker-2* animals may indicate that this isoform plays an important role in maintaining the organization of the sterocilia. The expression of myosin XV in neuroendocrine cells suggests that this isoform may have a role in secretory granule movement and/or secretion (Lloyd *et al.,* 2001). Further studies, including those on the additional N-terminal domain, will certainly help in understanding the mechanism and role of myosin XV in the inner ear and neuroendocrine tissues.

MYOSIN II AND FAMILIAL HYPERTROPHIC CARDIOMYOPATHY

Six genes encoding cardiac sarcomeric proteins have been so far associated with familial hypertrophic cardiomyopathy. These proteins are: cardiac troponin T, cardiac myosin-binding protein C, α-tropomyosin and the β cardiac myosin heavy chain as well as both the regulatory and essential myosin light chains (reviewed by Malik & Watkins, 1997). Hypertrophic myopathy is a clinically heterogenous disorder that is characterized by increased left ventricular mass and, histologically, by myofibrillar and myocyte disarray. Affected individuals may present dyspnea, palpitations and presyncope, although many are asymptomatic. The clinical course of the disease is variable, with the most serious consequences such as sudden death at young age, stroke and heart failure. The majority of cases are familial with autosomal dominant pattern of inheritance. There are also reports that the hypertrophic cardiomyopathy phenotype has been observed in patients with mutations in mitochondrial tRNA genes (reviewed by Malik & Watkins, 1997).

It was estimated that over 30% of patients with familial hypertrophic cardiomyopathy have mutations within genes encoding β cardiac myosin heavy chain (*MYH7*) and both regulatory and essential myosin light chains (Fig. 1) (reviewed by Malik & Watkins, 1997). Over fifty mutations within these genes have been detected so far (for references and list of mutations see: Sellers, 1999) and most of them are located in the myosin head and are grouped within four regions important for myosin function, i.e. (i) the actin- and (ii) ATP-binding sites (4 and 12 mutations, respectively), (iii) the so-called reactive thiol groups region (9 mutations) and (iv) the neck domain, in the sites of the interaction between the heavy and both types of light chains (10 mutations). Seven mutations have also been found in the S2 domain, the coiled-coil part of conventional myosin heavy chains, connecting the head domain and light meromyosin (LMM), the region employed in filament formation (Fig. 1). So far no mutations within the LMM region have been detected. Mutations within the light chains (two detected within the essential and five within the regulatory ones) affect the regions of their interaction with the heavy chain (Fig. 1), and several of them are associated with a rare phenotype termed midcavity obstruction, characterized by thickening of the left midventricular chamber, particularly of the papillary and surrounding muscles. For example, two substitutions (M149V and R154H) within the essential myosin light chain and at least two substitutions (A13T and G22K) within the regulatory light chain have been linked to this pattern (reviewed by Sellers, 1999). Interestingly, the A13 is only two residues amino-terminal to the phosphorylatable serine thus the mutation may affect the conformation of the phosphorylation site (reviewed by Sellers, 1999). There are cases in

which more than one mutation was found in one patient. For example, both missense (R870H) and nonsense (R54X) mutations were detected in a 16-year-old Japanese boy, but only the missense one seems to cause the disease (Nishi *et al.*, 1995).

Mutations within the subunits of cardiac myosin have variable penetrance and rates of survival. Some of them seem to be asymptomatic and some cause the most severe phenotype. For example, the R403Q mutation, introducing a charge change in the residue within the actin-binding site, is associated with a poor outcome with over 50% mortality by the age of 45 years. Interestingly, the R403W mutation, affecting the same residue and causing a similar charge change, results in a much milder course (reviewed by Sellers, 1999). In the majority of cases, mutations within the β-cardiac myosin components result in expression of functionally altered proteins. Studies on myosins isolated from biopsies obtained from patients, on recombinant myosin fragments engineered to mimick the known mutations within the cardiac myosin genes as well as on transgenic mice, have shown that the activities such as the actin-activated Mg-ATPase and ability to move actin filaments are usually largely decreased in the mutated proteins (reviewed by Sellers, 1999). The clinically most severe missense mutation R403Q has the largest (about 80%) inhibiting effect on these myosin activities. Some of the mutations, as for example R719Q and E743D have the opposite effect and such mutant myosins move actin filaments much faster than the native protein (reviewed by Sellers, 1999; Malik & Watkins, 1997).

While the mechanism of the familial hypertrophic cardiomyopathy caused by mutations within the genes encoding β-cardiac myosin heavy chain as well as both the regulatory and essential light chains seems to be elucidated many questions still remain unanswered. First of all, how a mutation leads to a particular phenotype and why the clinical pattern may be different in patients bearing the same mutation. Another question is how to use the knowledge gathered so far in developing gene therapy of the disease. Certainly, a transgenic mouse model and more detailed genotype/phenotype correlations within members of the affected families may help to resolve these intriguing problems.

MISCELLANEA

For several years mutations within the gene encoding myosin VA (Fig. 2) were believed to be responsible for the Griscelli syndrome, a rare autosomal recessive disease characterized by partial albinism with variable immunodeficiency, leading to hemophagocytic lymphohistiocytosis (Pastural *et al.*, 1997). The prognosis is poor, the average age at death is about 5 years, mainly due to an infiltration of the central nervous system. However, it turned out that some substitutions are simply the result of polymorphism (Pastural *et al.*, 2000). Recent reports point to mutations within the gene encoding the GTP-binding protein Rab27a as the cause of this disease (Menasche *et al.*, 2000; Pastural *et al.*, 2000). Rab27a, along with melanophilin, functions as an organelle receptor for myosin VA (Wu *et al.*, 2002b; Menasche *et al.*, 2000). Thus, impairment of the interaction between myosin VA, playing a role of an organelle-transporting motor, and Rab 27a disturbs the intracellular transport and, in consequence, causes the disease.

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