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ARTICLE

Unconventional Myosins and the Genetics of Hearing Loss

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Mutations of the unconventional myosins genes encoding myosin VI, myosin VIIA and myosin XV cause hearing loss and thus these motor proteins perform fundamental functions in the auditory system. A null mutation in myosin VI in the congenitally deaf Snell's waltzer mice (*Myo6*^{sv}) results in fusion of stereocilia and subsequent progressive loss of hair cells, beginning soon after birth, thus reinforcing the vital role of cytoskeletal proteins in inner ear hair cells. To date, there are no human families segregating hereditary hearing loss that show linkage to *MYO6* on chromosome 6q13. The discovery that the mouse shaker1 (*Myo7*^{ash1}) locus encodes myosin VIIA led immediately to the identification of mutations in this gene in Usher syndrome type 1B; subsequently, mutations in this gene were also found associated with recessive and dominant nonsyndromic hearing loss (*DFNB2* and *DFNA11*). Stereocilla of sh1 mice are severely disorganized, and eventually degenerate as well. Myosin VIIA has been implicated in membrane trafficking and/or endocytosis in the inner ear. Mutant alleles of a third unconventional myosin, myosin XV, are associated with nonsyndromic, recessive, congenital deafness *DFNB3* on human chromosome 17p11.2 and deafness in shaker2 (*Myo15*^{sh2}) mice. In outer and inner hair cells, myosin XV protein is detectable in the cell body and stereocilia. Hair cells are present in homozygous *sh2* mutant mice, but the stereocilia are approximately 1/10 of the normal length. This review focuses on what we know about the molecular genetics and biochemistry of myosins VI, VIIA and XV as relates to hereditary hearing loss. Am. J. Med. Genet. (Semin. Med. Genet.) 89:147–157, 1999. **Published 2000 Wiley-Liss, Inc.**†

KEY WORDS: unconventional myosin; deafness; hearing loss; myosin VIIA; myosin VI; myosin XV

INTRODUCTION

Hereditary hearing loss can be caused by mutations of many different genes [Griffith and Friedman, 1999]. Three of these genes, *Myo6, MYO7A,* and *MYO15,* encode the unconventional myosins VI, VIIA, and XV, respectively. Despite not fully understanding the function of these motor proteins in the process of sound transduction, co-segregation of deafness with mutant alleles at these loci indicates that these three molecular motor proteins perform fundamental functions in the auditory system. There may well be other myosin genes expressed in the auditory system that are important for sound transduction [Solc et al., 1995; Hasson et al., 1997]. This review focuses on genetic, molecular, developmental, and biochemical studies of myosins VI, VIIA, and XV that permit us to make genotype–phenotype correlations.

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MYOSINS

Myosins are motor proteins that bind actin and hydrolyze adenosine triphosphate (ATP) to produce force and movement [Mermall et al., 1998]. The myosin motor domain is usually located near the amino terminus, and its major structural elements are highly conserved. The myosin superfamily is now subdivided into 15 classes of proteins based on the degree of sequence divergence of the motor domain and different tail domains [Mermall et al., 1998; Probst et al., 1998; Sellers, 1999]. Con-

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ventional myosins belong to class II myosins and are expressed in muscle and nonmuscle cells. The other 14 classes of nonfilament-forming myosins are referred to as unconventional myosins. Mammals have multiple myosin II genes as well one or more genes from classes I, V, VI, VII, IX, X, and XV.

The tails of myosin II class molecules contain an extensive stretch of coiled-coil α-helix-forming sequences that allow for dimerization of the molecules and the formation of a tail that can self-associate to form filaments. Myosins from other classes (V, VI, VII, VIII, X, XI, and XIII) have shorter regions that are predicted to form coiled coils that may be able to dimerize but not to form filaments. Other classes, such as I, III, IV, IX, XII, XIV, and XV, have no sequence predicted to form a coiled coil, and these molecules are thought to be single-headed and thus function as monomeric motors.

Between the motor and the tail is a neck region with one or more light-chain-binding IQ motifs (Fig. 1). The IQ motif is an α-helical sequence that serves as a binding site for calmodulin or myosin light chains, depending on the myosin. Myosins may have from zero to six IQ motifs. The unique C-terminal tails are divergent in length and sequence among different classes of myosins and are presumed to confer each myosin class with distinct binding properties for cargo transported within the cell and/or for association with membranes (Fig. 1).

Unconventional myosins participate in various functions that include endocytosis, regulation of ion channels, localization of calmodulin, movement of vesicles and particles in the cytoplasm, determinant localization, and anchoring inner ear cell stereocilia [Baker and Titus, 1998; Mermall et al., 1998; Titus, 1998; Huang et al., 1999]. Comprehensive biochemical characterizations of classes I, II, V, and, to a lesser extent, IX molecules have been published [for a review, see Sellers, 1999]. These studies show large differences in the enzymatic properties, actin-translocating ability, and mode of regulation of myosins, both within a class and between classes. Despite this variation, it appears that myosins share a conserved structural design of the motor domain, with most of the differences occurring at surface loops. Thus, the crystal structure of chicken fast skeletal muscle myosin II [Rayment et al., 1993], *Dictyostelium* myosin II [Fisher et al., 1995], chicken smooth-muscle myosin II [Dominguez et al., 1998] and scallop striated myosin II [Houdusse et al., 1999] serve as templates to model other classes of myosins (Fig. 2).

In the remainder of this review, each of the three myosins known to be associated with hereditary hearing loss are discussed. Because none of these myosins have been purified and studied enzymatically, speculation on possible functions and effects of mutations are based on their cellular localization and disease phenotypes and on models and knowledge of better characterized myosins.

Myosin VI

Unconventional myosin VI genes have been identified in a variety of species, including *Caenorhabditis elegans* (HUM-3) [Baker and Titus, 1997], Drosophila (95F myosin) [Kellerman and Miller, 1992], pig (myosin VI) [Hasson and Mooseker, 1994], mouse (*Myo6*) [Avraham et al., 1995], and human (*MYO6*) [Avraham et al., 1997]. The myosin VI tail contains a short segment of predicted α-helix and coiled-coil sequences that should allow for dimerization. The remainder of the tail has no recognizable domains and is presumed to be globular.

The discovery of a null mutation in myosin VI in Snell's waltzer (*sv*) mice ($Myo6^{SV}$) has reinforced the vital role of cytoskeletal proteins in the hair cells of the inner ear, since *sv* mice are congenitally deaf and exhibit the characteristic waltzing and circling behavior of inner ear mutants [Steel and Brown, 1994]. Two Snell's waltzer alleles have been identified, *sv* and se^{SV}. The cloning of the $Myo6^{SV}$ locus was facilitated by the radiation-induced mutation se^{SV} from the Oak Ridge National Laboratory, one of a large collection of mutations used to identify developmentally important genes [Russell, 1971]. While many radiation-induced mutations harbor large chromosomal deletions, homozygous se^{SV} mice are viable as homozygotes. Along with results from a series of genetic crosses, this finding suggested that the se^{SV} phenotype was due to a ~2-cM chromosomal inversion.

Homozygous se^{SV} mice, in addition to being deaf, have skeletal defects and cropped pinnae, as the "short ear" name implies. The proximal break of this inversion is downstream of the se gene and appears to alter the regulation of this gene, which encodes bone morphogenetic protein 5 ($Bmp5^{se}$) [Kingsley et al., 1992; DiLeone et al., 1998]. With regard to the deafness and circling observed in these mice, subsequent cloning of the distal break point of this inversion led to the isolation of the *Myo6* gene based on sequence similarity to the previously identified pig myosin VI [Hasson and Mooseker, 1994; Avraham et al., 1995]. The distal break of se^{SV} is upstream of the coding region of the *Myo6* gene [Avraham et al., 1995, no. 1], however, and is likely to be a regulatory or a position effect mutation affecting *Myo6* expression. The observed reduction in myosin VI protein levels in all tissues of se^{SV} agrees with this hypothesis [Avraham et al., 1995; Bedell et al., 1996].

Identifying the inversion break points of se^{SV} mice saved laborious back cross-analysis for positionally cloning the sv gene. But it was the second Snell's waltzer allele, *sv,* a spontaneous mutation that arose at the Jackson Laboratory [Deol and Green, 1966], that provided conclusive evidence that the loss of myosin VI leads to deafness in these mice. A deletion of 130 bp was detected in $Myo6^{SV}$ cDNA, due to a larger, 1.1-kb genomic deletion. A splice site is deleted, leading to "skipping" of an exon and a subsequent frameshift in the coding region, which forms a stop codon in the light-chain-binding (neck) domain. Western blot analysis showed that there is no detectable myosin VI protein produced in any tissues of homozygous sv mice, showing that sv is likely a null allele of *Myo6* [Avraham et al., 1995].

Light microscopy of the cochlea shows progressive loss of hair cells, be-

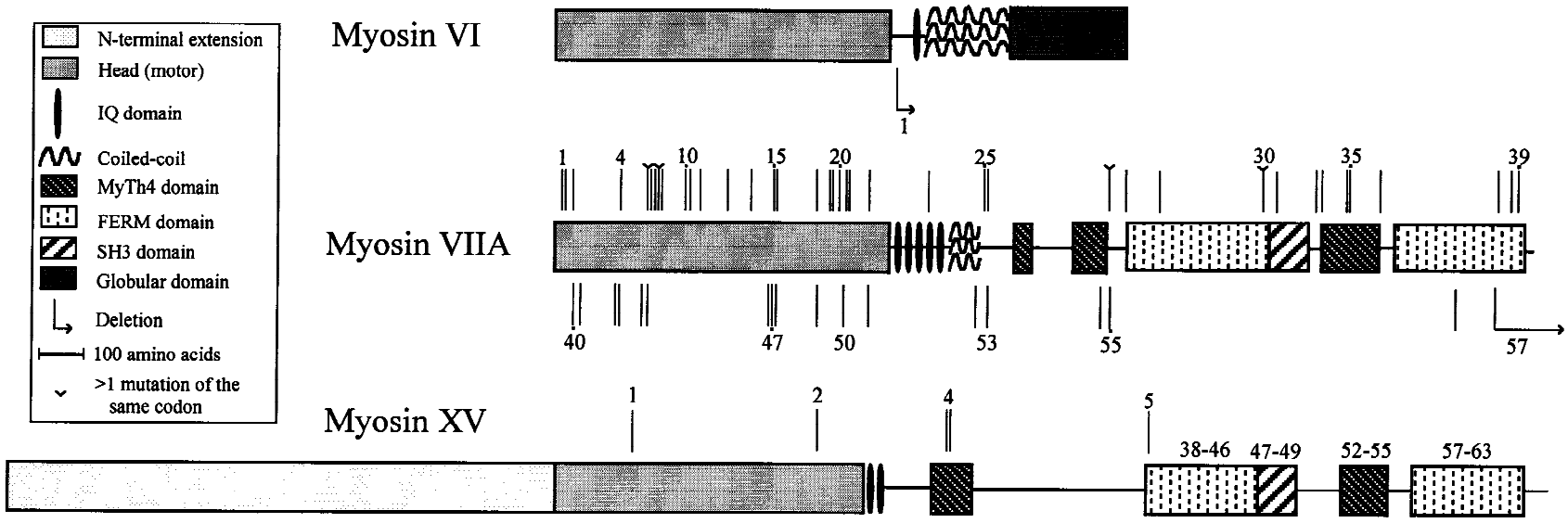

Figure 1. Schematic drawings of the longest reported isoforms of myosins VI, VIIA, and XV, depicting the conserved motor domain, the neck containing one or more IQ motifs, the divergent tails, and the unique N-terminal domain of myosin XV. The amino acid sequence of myosins VI, VIIA, and XV is based on accession numbers U49739, U39226.1, and AF051976, respectively, with the gene structures for myosins VIIA and XV based on the work of Kelley et al. [1997], Liang et al. [1998], and Wang et al. [1998]. The tail domains of myosins VI and VIIA contain a short segment predicted to form a coiled coil that may allow for dimerization to a two-headed molecule. SH3 domains, which are thought to interact with proline-rich sequences, have been found in other myosins, as has the MyTh4 (myosin tail homology) domain, which is of unknown function. The FERM domain [Chishti et al., 1998] (band 4.1-ezrin-moisen-radixin motif, formerly called Talin domain) is also found in other myosins as well as other cytoskeletal protein and may mediate cytoskeletal protein interactions. Many of the point mutations have been shown to map to structural motifs in the tail region of myosins VII and XV. Although no binding partners to mammalian myosins VI, VII, and XV have been identified, it is possible that mutations of the FERM and MyTh4 domains affect binding to a different proteins.

Mutations indicated by a number above the schematic diagram of each myosin are missense or nonsense mutations. Numbered lines below the schematic of myosins VI, VIIA, and XV indicate small deletions, insertions, or splice site mutation. For mutations of myosins VIIA, "U", "A" or "B" to the right of the number indicates USH1B, DFNA11, or DFNB2, respectively. 1U (L16X), 2U (G25R), 3U (C31X), 4U (R150X), 5U (R212H and R212C), 6U (G214R), 7U (Q234X), 8U (R241S), 8 $sh1^{6J}$ (R241P), 9B (R244P), 10U (R302H), 11U (E314X), 12U (Y333X), 13U (A397D), 14U (E450Q), 15 sh1 (R502P), 16U (P503L), 17B (M599I), 18U (C628X), 19U (R634X), 20 atypical USH (L651P cpd. het. w/ R1602Q), 21U (R666X), 22U (R669X), 23 $sh1^{4626SB}$ (Q720X), 24U (A826T), 25U (G955S), 26U (E960X), 27U (R1240Q and R1240E), 28U (A1288P), 29U (R1343S), 30U (R1602Q) and atypical USH (R1602Q cpd. het. w/ L651P), 31U (A1628S), 32U (Y1719C), 33U (R1743W), 34U (Q1798X), 35 $sh1^{26SB}$ (F1800I), 36U (R1861X), 37U (G2137E), 38U (G2163S), 39n $sh1^{3336SB}$ (C2182X), 40B (IVS3-2), 41U (75delG), 42U (IVS5 + 1), 43U (120delC), 44 $sh1^{4494SB}$ (IVS6nt + 2T→A), 45U (ΔD218-I219), 46U (468 + Q), 47U (IVS13-8), 48U (532delA), 49 $sh1^{816SB}$ (IVS16nt-2), 50U (IVS18 + 1), 51U (724delC), 52A (Δ886–888), 53B (IVS24-21), 54B (V1199 + T), 55U (IVS29 + 2), 56U (2065delC), 57U (>2kb deletion); myosin XV, 1 (G1358S), 2 sh2 (C1775Y), 3 (N2111Y), 4 (I2113F), 5 (L2601X). The original description of these mutations are in the references cited in Table I and in Cuevas et al. [1998] and Mburu et al. [1997].

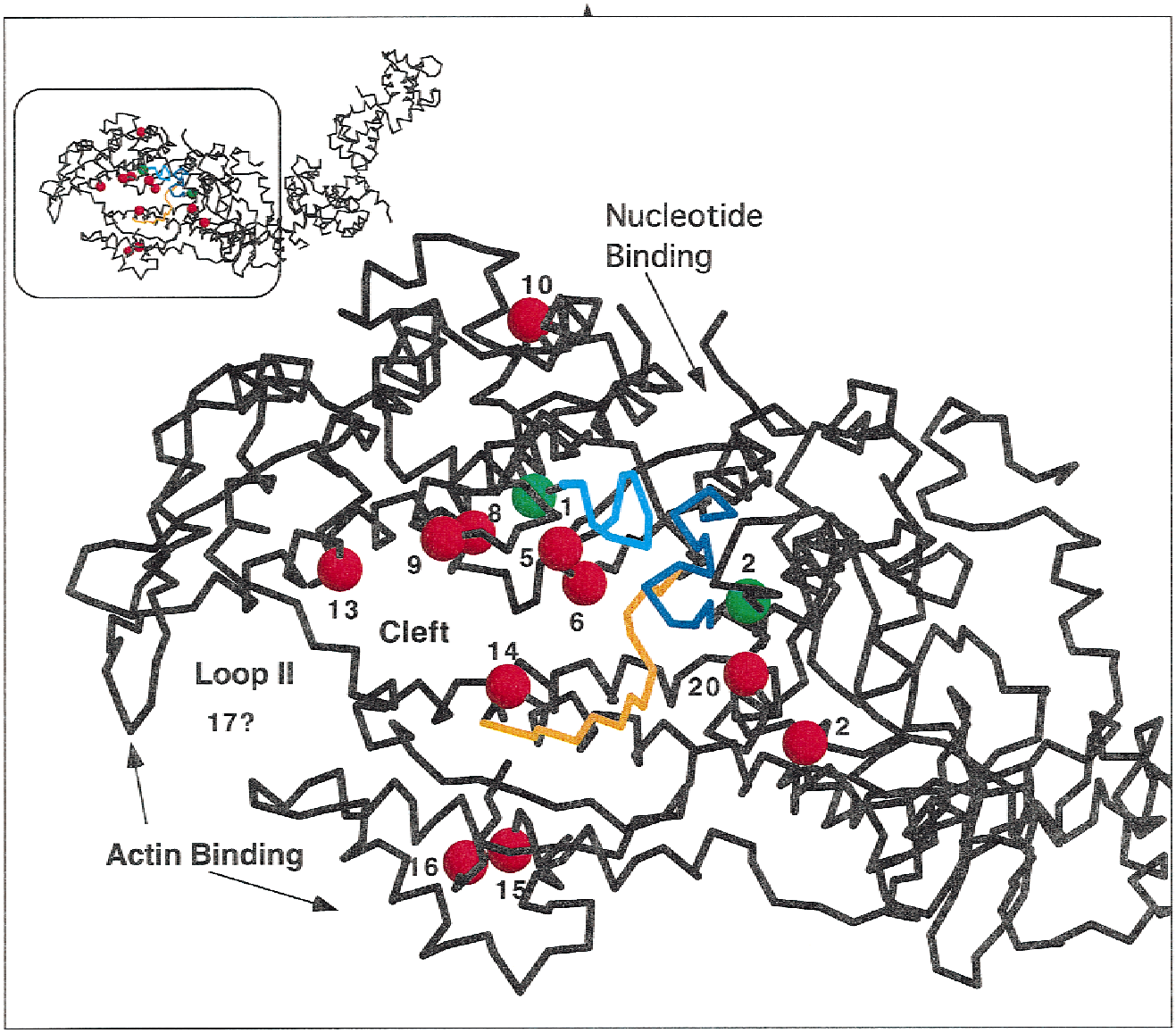

Figure 2. Map of the locations of myosins VII and XV missense mutations of the motor domains on the crystal structure of chicken fast skeletal muscle myosin II [Rayment et al., 1993]. In many cases, these mutations occur at highly conserved amino acids, which serves to validate using a myosin II structure for this mapping strategy. Mutations in mouse or human myosin VII are shown in red, while those of myosin XV are shown in green. The numbering beside each mutation refers to the assignment given in the first column of Table I and in Fig. 1. R241P/S, R244P, and A397D (red balls 8, 9, and 13, respectively) lie on the upper surface of the cleft that separates the upper and lower domains of the tip of myosin. The cleft has been shown to close partially upon binding nucleotide analogues that mimic the transition state [Holmes, 1997]. Another myosin VII mutation, E450Q (red ball 14), lies at the bottom surface of the same cleft. Three of these cleft mutations, R241, A397, and E450, are highly conserved among all myosins. A mutation at the equivalent amino acid to E450 was recovered in the cellular slime mold *Dictyostelium* [Patterson and Spudich, 1996]. In addition, E450 lies at the start of the switch II region (orange line) of myosin, which undergoes a conformational change upon adenosine triphosphate (ATP) hydrolysis. Myosin is predicted to have a broad interaction surface with actin. Three myosin VII mutations lie in putative actin binding sites. The M5991 (17?) mutation lies within a flexible loop (Loop II) at the tip end of myosin, which has been shown to interact with highly charged amino terminus of actin [Chaussepied and Morales, 1988]. The loop was not visualized in the chicken skeletal muscle myosin crystal structure, presumably owing to its flexible nature, thus, this mutation is followed by a questioin mark [Rayment et al., 1993]. The sequence in this loop is highly variable among different myosins and may be a major determinant of the enzymatic properties of myosin [Spudich, 1994]. In the myosin II family, this loop typically has a large number of lysines and glycines. In myosin VII, the loop is shorter and not as highly charged, suggesting that its mode of interaction with actin may be different. Two other mutations, R502P (red ball 15) and P503L (red ball 16) lie on the outer surface of the lower domain of myosin, in a position homologous to amino acids of myosin II that are thought to interact with another domain of actin [Rayment et al., 1993]. The side chain of the homologous chicken skeletal muscle myosin II residue K528 to R502 projects away from the surface and is probably directly available to interact with actin. R212H/C (red ball 5) and G214R (red ball 6) occur at nearly universally conserved residues in the myosin superfamily that constitute the beginning portion of switch I (cyan line), another conserved segment that probably is part of the nucleotide-sensing mechanism of myosin [Holmes, 1997]. These mutations are also very close in linear sequence to two mutations of human β-cardiac myosin that are linked to hypertrophic cardiomyopathy [see review in Sellers, 1999]. The myosin VII missense mutation G25R (red ball 2) lies near the amino terminus of the molecule. This region is not present in several classes of myosin, and there is no sequence homology between the myosin VII class and myosin II class molecules at this region. Therefore, it is difficult to speculate about its function. Two mutations in the motor domain of myosin XV are shown as green balls. G1358S (green ball 1) is located in the switch I region, while C1779Y (green ball 2) is located close in space to the P-loop (dark blue line), a region that interacts with the phosphate groups of ATP. Both of these amino acid residues are highly conserved in the myosin superfamily.

ginning soon after birth, so that by 6 weeks of age, very few hair cells remain [Deol and Green, 1966; Avraham et al., 1995]. Although the precise functions of myosin VI are unknown, several are hypothesized, including membrane trafficking and recycling, cell movement, and endocytosis [reviewed in Mermall et al., 1998]. What remains more of an enigma is the specific role of myosin VI in the sensory hair cells of the inner ear. Indirect immunofluorescence has confirmed myosin VI expression in the hair cells but not in the supporting cells or peripheral cells of the mammalian cochlear and vestibular neurosensory epithelium [Avraham et al., 1995; Avraham et al., 1997; Hasson et al., 1997]. In mouse inner ear hair cells, myosin VI is enriched in the cuticular plate and, in particular, in the pericuticular necklace, a region that sits between the cuticular plate and circumferential actin band [Hasson et al., 1997]. The high concentration of myosin VI at the stereociliary rootlets led to the suggestion that this protein is responsible for maintaining cuticular-plate anchoring of stereocilia rootlets [Hasson et al., 1997].

While degeneration of hair cells is characteristic of acoustic trauma and has been seen in several deaf mouse mutants, scanning electron microscopy of both *sv* alleles has shown a unique pattern of hair cell disorganization [Self et al., 1999]. In 1-day-old homozygote *sv* mice, some of the stereocilia have a disorganized swirling appearance, which by 3 days after birth expands to most hair cells. Transmission electron microscopy shows fusion at the base of the stereocilia at 1 day after birth, and these stereocilia subsequently "zip up" to form giant stereocilia. Degeneration of hair cells takes place at the same time, so that by 20 days after birth only a few large stereocilia are present on each hair cell.

Although Drosophila myosin VI mutants have not yet been identified, the ability to block myosin VI by antibody injection has provided a tool for following the loss of myosin VI in the developing embryo. Actin-based metaphase furrows in the membrane, which are required to separate adjacent mitoses, are not formed with the deep invaginations necessary for proper syncytial blastoderm organization [Mermall and Miller, 1995]. This finding has led to the suggestion that myosin VI may be involved in securing the membrane against surface tension forces, either alone or in conjunction with interacting proteins [Self et al., 1999]. The inability of membranes at the base of the stereocilia to remain distinct from one another in *sv* mice is reminiscent of what is observed in Drosophila developing embryos injected with myosin VI antibodies.

In addition to the inner ear, myosin VI is expressed in many cell types examined, although at varying levels (Avraham et al., 1997, and unpublished observations]. Yet the absence of myosin VI in a homozygous *sv* mouse does not appear to have obvious effects in tissues other than the inner ear. Its high expression in the subapical brush border of polarized epithelial cells implicates this protein in membrane movements, endocytosis, and/or membrane recycling events [Hasson and Mooseker, 1994]. Altered myosin VI function may, therefore, cause more subtle anomalies in nonauditory tissues. For example, there are abnormally fused brush border microvilli in the gut of *sv* mutants. This does not appear to cause any serious repercussions, possibly owing to the fact that cells of the gut turn over at a fast rate, whereas hair cells do not [Self et al., 1999]. Furthermore, myosin VI has been shown to be associated with the Golgi complex and the leading, ruffling edge of fibroblast cells in vitro, suggesting that it plays a role in membrane traffic or secretory and endocytic pathways [Buss et al., 1998]. The lack of obvious phenotypic abnormalities due to the loss of myosin VI in these cells in the *sv* mutant is intriguing and may be due to compensation by other unconventional myosins.

Myosin VI may be regulated by phosphorylation of the motor domain. Amoeboid myosin I is known to be modulated by phosphorylation of a heavy chain kinase [Maruta and Korn, 1977], and, myosin VI is the only other unconventional myosin with a putative phosphorylation site similar to that of amoeboid myosin I. This site is a threonine at position 406 of mouse myosin VI in the motor domain and is conserved between mouse, pig, human, Drosophila, and *C. elegans*. It has recently been confirmed that myosin VI is indeed phosphorylated in the motor domain in mammalian fibroblasts, and p21-activated kinase was shown to be responsible for this phosphorylation in vitro [Buss et al., 1998].

Until now, it was thought that all myosins move along actin towards the 'plus' end of the actin track, where subunits are added at a high rate. However, myosin VI moves along actin towards the 'minus' end of the actin filament [Wells et al., 1999]. This reverse movement is possible due to a 53 amino acid insertion in the converter domain of myosin VI, which is not present in other unconventional myosins. Thus, it appears that myosins provide bidirectional movement, and this may be manifested in transport of vesicles along actin tracks in both directions. Perhaps this reverse movement is essential in the hair cells, providing an explanation of why myosin VI loss appears to affect hearing in *sv* mice.

Evidence that myosin VI may be involved in transporting cargo comes from computational optical sectioning microscopy of rhodamine-labeled myosin VI monoclonal antibody in Drosophila embryos. This experiment documented that myosin VI is involved in actin-based, ATP-dependent transport of cytoplasmic particles [Mermall et al., 1994]. Cellular constituents are thought to be transported by a combination of microtubule-associated and actin-filament transport, with the former operating over long distances and the latter over short distances in the cell. Since coordination of these two systems may be required, efforts have been made to identify microtubule proteins associated with myosin VI. This effort led to the identification of cytoplasmic linker protein-190 (D-CLIP-190) in Drosophila, an orthologue of human CLIP-170, which links endocytic vesicles to microtubules. In Drosophila, D-CLIP-190 co-immunoprecipitates with myosin VI and is thought

to link actin and microtubule cytoskeletons [Lantz and Miller, 1998]. Microtubule and actin-based transport was shown to be coupled through direct interactions between another unconventional myosin, myosin V, and kinesin KhcU [Huang et al., 1999].

Does myosin VI have a role in the human inner ear? To date, more than 60 different loci for inherited nonsyndromic hearing loss have been mapped [Van Camp and Smith, 1999]. However, no families segregating hereditary hearing loss have been ascertained that show linkage to the myosin VI region on chromosome band 6q13 [Avraham et al., 1997, and unpublished observations]. It is possible that homozygosity for *MYO6* mutations is lethal in humans despite nonlethality in the mouse. As a converse example of such a species-specific difference, homozygous *GJB2* frameshift mutations near the beginning of the coding region are likely null mutations in humans and are compatible with life, while a homozygous knockout of *Gjb2* is an embryonic lethal in mice [Gabriel et al., 1998]. An alternative hypothesis is that mutations of *MYO6* associated with hearing loss in humans are rare and will be ascertained in time.

Myosin VIIA

Mutations of myosin VIIA are associated with Usher syndrome type 1B (USH1B) and two forms of nonsyndromic deafness, DFNB2 and DFNA11. Usher syndrome is a clinically and genetically heterogeneous, autosomal recessive disorder comprising sensorineural hearing loss and retinitis pigmentosa, a noninflammatory progressive degeneration of the rods and cones of the retina causing loss of vision. Usher syndrome is estimated to account for approximately 3–6% of congenital profound deafness in children and 50% of deafness and blindness [Vernon, 1969; Boughman et al., 1983; Kimberling and Moller, 1995]. Three clinical subtypes of Usher syndrome are distinguished, based on the onset of retinitis pigmentosa, the severity and onset of hearing loss, and the presence or absence of peripheral vestibular function [Smith et al., 1994]. The USH1 phenotype is congenital profound hearing loss with prepubertal onset of retinitis pigmentosa and profound peripheral vestibular hypofunction. The USH2 phenotype is congenital, stable, severe hearing loss with postpubertal onset of retinitis pigmentosa and normal peripheral vestibular function. The USH3 phenotype includes progressive hearing loss with variable vestibular dysfunction and variable onset of retinitis pigmentosa [Sankila et al., 1995].

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There are at least six unlinked *USH*1 loci, *USH1A–USH1F* [Kaplan et al., 1992; Kimberling et al., 1992; Keats et al., 1994; Wayne et al., 1996; Chaib et al., 1997; Wayne et al., 1997]. Most individuals with USH1 appear to have mutations linked to *USH1B,* which is on chromosome band 11q13.5 [Weston et al., 1996]. The identification of *USH1B* emerged rapidly after the cloning of mouse shaker-1, the first example of the use of a murine strain with hereditary hearing loss to identify a human deafness gene. Orthologues of genes located on human chromosome band 11q13.5 map to mouse chromosome 7 in the region of a mouse mutation causing deafness and vestibular dysfunction—shaker-1 (*sh1*) [Evans et al., 1993]. There are seven alleles of *shaker-1: sh1, $sh1^{6j}$, $sh1^{26B}$, $sh1^{816SB}$, $sh1^{3336SB}$, $sh1^{4494SB}$,* and $sh1^{4626SB}$. Homozygotes for each of the seven *shaker-1* alleles are hyperactive, with head-tossing and circling behavior due to a vestibular defect. *Sh1* homozygotes do not show a normal Preyer reflex in response to a loud noise, presumably as a result of degeneration of the organ of Corti [Gibson et al., 1995]. To identify the *sh1* gene, a 1.4-megabase physical map of the *sh1* interval was constructed. Exon trapping and subsequent cDNA cloning identified the motor domain of an unconventional myosin nearly identical to that encoded by a partial sequence of previously identified human and porcine myosin VIIA sequences. The tail of the predicted full-length message of myosin VIIA encodes a short segment of predicted coiled-coil forming sequence, an SH3 motif, two MyTh4 domains, and two FERM domains (Fig. 1). Myosin VIIA has not been purified from tissues or expressed in vitro, so almost nothing is known of its biochemical and kinetic properties.

A Northern blot analysis indicated that the full-length *Myo7a* transcript is approximately 9.5 kb. A limited initial screen of 1,893 bp of the *Myo7a* motor domain of the seven *shaker-1* alleles showed three different mutations of the motor domain in *sh1, $sh1^{6J}$,* and $sh1^{816SB}$ [Gibson et al., 1995]. After the complete sequence of *Myo7a* was determined, mutations in the other four *sh1* strains were identified [Mburu et al., 1997]. Five of the seven *sh1* mutations are in the motor domain, and two are in the tail (Fig. 1). Gibson et al. [1995] argued correctly that mutant alleles of the human orthologue, *MYO7A,* are respnsible for USH1B. Initially, five *USH1B* mutations were found in the portion of *MYO7A* encoding the motor domain [Weil et al., 1995]. Subsequent studies have described a spectrum of mutant alleles associated with USH1B that are distributed across all of the major *MYO7A* protein domains [Weil et al., 1995; Weston et al., 1996; Adato et al., 1997; Levy et al., 1997; Liu et al., 1997a; Liu et al., 1998a] (Fig. 1). Liu et al. [1998a] extended the known phenotypic spectrum associated with *MYO7A* by pinpointing compound heterozygous mutations in two affected sibs with an atypical Usher syndrome phenotype most closely related to that of Usher syndrome type 3.

Nonsyndromic recessive deafness associated with partially penetrant vestibular dysfunction in a family from Tu-

nisia was also mapped to 11q13.5 and designated *DFNB2* [Guilford et al., 1994]. Surprisingly, a locus for nonsyndromic dominant hearing loss also colocalized to the critical interval on 11q13.5 to which *USH1B* and *DFNB2* mapped [Tamagawa et al., 1996]. Hearing loss in this family began in the first decade of life, but after language acquisition, and progressed to a moderate loss by 20–60 years of age. Mutations of *MYO7A* were also found to be the cause of nonsyndromic recessive deafness in two small Chinese families [Liu et al., 1997b] and in the original DFNB2 family from Tunisia [Weil et al., 1997]. In the Tunisian DFNB2 family, an A-to-G transition occurs in the last nucleotide of exon 16, encoding part of the motor domain. This mutation is predicted to result in substitution of isoleucine for methionine (Met599Ile) [Weil et al., 1997] but could also affect the splicing of *MYO7A* mRNA based on its position as the last nucleotide of exon 16. Three additional *MYO7A* mutations in the two small DFNB2 families from China cosegregated with hearing loss (Fig. 1). There is a homozygous Arg244Pro substitution in exon 7 in one family and a compound heterozygote with a splice acceptor site mutation of the motor domain and a frameshift mutation in the other kindred (Table I) [Liu et al., 1997b; Liu et al., 1998a]. Establishing a convincing argument for a connection between the homozygous Arg244Pro mutation and hearing loss is difficult in a small family. The same substitution in a nearby arginine codon (Arg241Pro) is responsible for *sh1*^{6J} [Mburu et al., 1997], and a different substitution, Arg241Ser, is associated with the USH1B phenotype [Janecke et al., 1999] (Fig. 1).

As for DFNA11, all eight affected members of a single family have a 9-bp deletion within exon 23 of *MYO7A* that removes three amino acids but otherwise maintains the correct reading frame. Exon 23 encodes a coiled-coil domain that may allow myosin VIIA to dimerize. The 9-bp deletion is presumed to be dominant, because its gene product is assumed to interact with wild-type myosin VIIA to form a defective dimer [Weil et al., 1997]. Some functional dimers would be predicted to form between wild-type myosin VIIA polypeptides, which might explain the milder hearing loss phenotype in comparison to the profound congenital deafness associated with homozygosity for recessive alleles suspected of causing total loss of functional MYO7A. This explanation for the phenotype in the DFNA11 family is, of course, speculative and does not explain why individuals with this mutation do not have retinitis pigmentosa.

There appears to be no obvious correlation between mutation type (missense, nonsense, frameshift, splice site) and phenotype, whether it be USH1B, DFNB2, or DFNA11 (Fig. 1). The majority of *MYO7A* mutations described to date are in USH1B families and are missense, frameshift, and splice mutations of the head and tail (Fig. 1). There are only four myosin VIIA mu-

TABLE I. Missense Mutations of the Motor Domains of Myosins VIIA and XV

Missense mutation[a]	Phenotype	Exon	Mutation (chick skeletal muscle residue number)	Reference
Myosin VIIA (red)				
2	USH1B	3	G25R (44)	Lévy et al., 1997
5	USH1B	7	R212H (245)	Weil et al., 1995 Weston et al., 1996
5	USH1B	7	R212C (245)	Weston et al., 1996
6	USH1B	7	G214R (247)	Adato et al., 1997 Janecke et al., 1999
8	USH1B	7	R241S (274)	Janecke et al., 1999
8	Shaker-1 (sh1^{6J})	7	R241P (274)	Gibson et al., 1995
9	DFNB2	7	R244P (277)	Liu et al., 1997b
10	USH1B	9	R302H (335)	Weston et al., 1996
13	USH1B	11	A397D (428)	Adato et al., 1997 Epinós et al., 1998
14	USH1B	13	E450Q (476)	Weston et al., 1996
15	Shaker-1 (sh1)		R502P (528)	Gibson et al., 1995
16	USH1B	13	P503L (529)	Weston et al., 1996
17	DFNB2	16	M599I	Weil et al., 1997
20	Atypical USH	18	L651P (690)	Liu et al., 1998a
Myosin XV (green)				
1	DFNB3	9	G1358S (233)	Friedman, unpublished
2	Shaker-2	20	C1779Y (674)	Wang et al., 1998

[a]Colors refer to Figure 2.

tations segregating in three DFNB2 families (R244P, M599I, and a compound heterozygote of IVS3-2 with V1199 + T), and none of them are nonsense or frameshift mutations near the 5′ end of myosin VIIA, which would most likely result in a functional null. The mutations in the three DFNB2 families may have residual myosin VIIA function.

There are data to indicate that some of the *shaker-1* mutations are hypomorphs. The seven *shaker-1* mutant alleles have near wild-type levels of *Myo7a* mRNA but show considerable variation in the amount of mutant myosin VIIA protein. The protein levels range from near normal quantities in the original *shaker-1* strain (a missense mutation, R502P) to less than 1% of the wild-type level in $sh1^{4494SB}$ (splice site mutation) and $sh1^{4626SB}$ (nonsense mutation) (Fig. 1) [Hasson et al., 1997]. This reduction in protein levels may be due to a reduced rate of translation of the mutant mRNAs and/or to protein stability differences. Hasson et al. [1997] extrapolated from these findings in mice and suggested that protein stability differences may explain why one allele of *MYO7A* causes hearing loss alone and another causes hearing loss and retinitis pigmentosa. If this hypothesis is correct, there should be more residual myosin VIIA function in affected individuals from the four DFNB2 families than in USH1B-affected individuals. Testing this hypothesis in humans will be a challenge, since abundant myosin VIIA expression is limited to relatively inaccessible tissues, such as the kidney, lung, retina, testes, and inner ear [Hasson et al., 1995].

If phenotypic variability is not entirely attributable to allelic heterogeneity of *MYO7A,* is genetic background contributing to the very different USH1B and DFNB2 phenotypes? So far there are no examples of the same mutant allele of *MYO7A* associated with USH1B in one family and DFNB3 in another (Fig. 1). Such an occurrence would suggest that the genetic background or environment plays a significant role in the phenotypic outcome of *MYO7A* mutations. Understanding the origin of the extraordinary phenotypic variability of *MYO7A* mutations in humans is of crucial clinical importance. Identification of a modifier gene that permits or prevents blindness or of conditions that stabilize mutant myosin VIIA protein could be helpful in devising therapeutic strategies to forestall the progressive blindness associated with USH1B.

If phenotypic variability is not entirely attributable to allelic heterogeneity of MYO7A, is genetic background contributing to the very different USH1B and DFNB2 phenotypes?

An understanding of the clinical variability of *MYO7A* mutations may also come from comparative studies of mouse and human myosin VIIA expression patterns in the eye. Weil et al. [1995] pointed out that the lack of retinal degeneration of *sh1* homozygotes resembles the normal retinal phenotype associated with human nonsyndromic deafness DFNB2 and DFNA11 (Table I). Whereas myosin VIIA is found in the pigmented epithelium of the retina as well as the photoreceptor cells of humans, it appears to be expressed only in the pigmented epithelium in the rat, guinea pig, and mouse [Hasson et al., 1995; el-Amraoui et al., 1996; Liu et al., 1998b]. Perhaps another unconventional motor protein can perform the function of myosin VIIA in rodent photoreceptors [Hasson et al., 1997].

The function of myosin VIIA in the auditory system is not understood. There has been speculation that myosin VIIA might be the organ of Corti adaptation motor for the gated channel tip-link tension of the hair cell stereocilia. This hypothesis lacks experimental support, whereas evidence based on ultrastructural localization of myosin-1β at the tip links implicates it as the adaptation motor [Gillespie et al., 1993; Hudspeth and Gillespie, 1994]. The observed pattern of inner ear neuroepithelial degeneration in *sh1* mice and the localization of myosin VIIA to cross-links of adjacent stereocilia and cuticular plate of inner ear hair cells suggests that myosin VIIA is involved in the maintenance of stereocilia integrity [Hasson et al., 1997; Self et al., 1998].

Myosin XV

Mutant alleles of a third unconventional myosin, myosin XV, are associated with nonsyndromic, recessive, profound congenital deafness, DFNB3, in humans and shaker-2 in mice. Using a homozygosity mapping strategy in a genome-wide screen, *DFNB3* was mapped to 17p11.2 in a relatively isolated population in Bali [Friedman et al., 1995; Liang et al., 1998]. Based on conserved synteny, the map position of *DFNB3* suggested that the mouse deafness mutation shaker-2 (*sh2*) on chromosome 11 was its murine orthologue [Friedman et al., 1995; Liang et al., 1998]. Shaker-2 was mapped to a 0.2-cM region [Liang et al., 1998], and a physical map was constructed using bacterial artificial chromosomes (BACs). To identify *sh2,* a functional cloning approach was undertaken that involved injecting BACs that spanned the *sh2* critical interval into the pronucleus of homozygous *sh2* fertilized eggs. These eggs were then transferred to a wild-type foster mother and resulted in one transgenic mouse that did not circle and responded to sound with a normal Preyer reflex. That particular mouse developed from an embryo that had been injected with BAC425p24 (~140-kb insert). This "BAC-rescued" mouse was mated, and correction of the shaker-2 phenotype was correlated with transmission of BAC425p24 in the germ line of his offspring [Probst et al., 1998].

One of two genes encoded in the DNA of BAC425p24 was a novel unconventional myosin, *Myo15* [Probst et al., 1998; Wang et al., 1998]. Both mouse and human myosin XV genes have 66 exons with several isoforms resulting from alternative splicing of myosin XV transcripts [Liang et al., 1999]. In addition to being a very large protein, myosin XV is also exceptional

in that exon 2 encodes ~1,200-amino-acid amino terminal domain. So far, the deduced amino acid sequence of the N-terminal domain of myosin XV shows no obvious sequence motifs that suggest a function of this region. The tail of myosin XV does not contain a sequence that is predicted to form a coiled-coil dimer but does have sequence similarities to the tail of myosin VIIA in terms of its structural domains (Fig. 1).

The *sh2* allele has a G-to-A transition mutation in codon 1779 within exon 20 of *Myo15* that substitutes tyrosine for a highly conserved cysteine (Fig. 2) [Cope et al., 1996; Probst et al., 1998]. To identify mutations in DFNB3 families, *MYO15* exons were sequenced from probands of three consanguineous families from India and in affected individuals from Bengkala segregating neurosensory deafness. Three missense mutations and one nonsense mutation in *MYO15* were identified (Fig. 1) [Wang et al., 1998]. Preliminary data suggest that ~12% of a sample of 85 consanguineous Indian and Pakistani families segregating autosomal recessive, nonsyndromic, neurosensory deafness show evidence of linkage to *DFNB3* [Wilcox, personal communication]. Although mutations in myosin XV may be common in India and Pakistan, the degree to which mutations of this gene contribute to hearing loss worldwide remains to be determined.

Some clues about the role of myosin XV in the inner ear have been obtained from light and electron microscopic studies of tissues from *shaker-2* and *shaker-2* mutant mice. Hair cells are present, but the stereocilia on the inner and outer hair cells are very short, approximately one-tenth of the normal length [Probst et al., 1998]. The stereocilia of the inner hair cells are essential for transducing the physical stimulus of sound within the cochlea to an electrical stimulus that is transmitted to the auditory nerve. The very short stereocilia may explain the lack of hearing in shaker-2 mice, suggesting the role of myosin XV in constructing and/or maintaining the cytoskeleton of stereocilia in hair cells.

CONCLUSIONS

Myosins VI, VIIA, and XV are individually necessary for inner ear hair cell stereocilia integrity. Yet the functions of these molecular motors in the processes of hair cell stereocilia maintenance and sound transduction, or in the other tissues expressing these genes, are largely unknown. These proteins may well have more than one function, since several isoforms are known for each. Different isoforms of nonmuscle myosin II appear to occupy different intracellular positions even within the same cell [Kolega, 1998]. This spatial specificity implies functional specificity for the isoforms.

Despite considerable work, we do not yet know the in vivo cargo moved by myosins VI, VII, or XV.

We surmise that myosins VI, VIIA, and XV, like some of the other members of this superfamily of motor proteins, transport cargo on an actin network and that their tails are associated with unique macromolecular structures. Alternatively, one or more isoforms of these three myosins expressed in the inner ear may occupy a fixed intracellular position and exert force against mobile actin filaments. An analogy would be a motor that scurries up the pole with a flag as opposed to an engine that boosts the whole flagpole.

Despite considerable work, we do not yet know the in vivo cargo moved by myosins VI, VII, or XV. The cargo in each tissue type in which they are expressed may well be different. It is worth noting that the tails of myosin VIIA and myosin XV both have two MyTh4 domains, an SH3 domain, and one or two FERM domains (Fig. 1). Insight into the functions of these three molecular motors in the neuroepithelium of the inner ear might come from the identification of their protein partners by formal genetic screens for enhancers and suppressors of a partial hearing loss phenotype or by two-hybrid screens of inner ear libraries using various regions of these myosins as bait. Furthermore, the genes encoding the protein partners and cargo of these three unconventional myosins are good candidates for "deafness" genes (DFNA and DFNB) that have been mapped but not yet identified. The genes for several nonsyndromic loci have already been cloned [Griffith and Friedman, 1999], and it is possible that some of the recently identified genes for these loci encode protein partners of myosins VI, VIIA, or XV.

Our lack of knowledge regarding the essential role of these unconventional myosins in the inner ear raises a large number of questions. Do particular isoforms of myosins VI, VIIA, and XV co-localize within hair cells? Do myosins VI, VIIA, and XV motor along individually, or do they form a complex of myosin motors to build and secure hair cell stereocilia? Myosins 1β, VI, and VIIA do co-localize at the light microscopic level in the pericuticular necklace of frog inner ear hair cells [Hasson et al., 1997]. Additional light microscopic and ultrastructural studies of the intracellular locations within the mammalian auditory system of each of the isoforms of myosins VI, VIIA, and XV would be helpful in resolving some of these issues.

Our lack of knowledge regarding the essential role of these unconventional myosins in the inner ear raises a large number of questions.

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REFERENCES

Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M, Bonné-Tamir B. 1997. Mutation profile of all 49 exons of the human myosin VIIA gene, and haplotype analysis, in Usher 1B families from diverse origins. Am J Hum Genet 61: 813–821.

Avraham KB, Hasson T, Sobe T, Balsara B, Testa JR, Skvorak AB, Morton CC, Copeland NG, Jenkins NA. 1997. Characterization of unconventional MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice. Hum Mol Genet 6: 1225–1231.

Avraham KB, Hasson T, Steel KP, Kingsley DM, Russell LB, Mooseker MS, Copeland NG, Jenkins NA. 1995. The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for the structural integrity of inner ear hair cells. Nature Genet 11:369–375.

Baker JP, Titus MA, 1997. A family of unconventional myosins from the nematode *Caenorhabditis elegans*. J Mol Biol 272:523–535.

Baker JP, Titus MA. 1998. Myosins: matching functions with motors. Curr Opin Cell Biol 10:80–86.

Bedell MA, Jenkins NA, Copeland NG. 1996. Good genes in bad neighborhoods. Nature Genet 12:229–232.

Boughman JA, Vernon M, Shaver KA. 1983. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chron Dis 36:595–603.

Buss F, Kendrick-Jones J, Lionne C, Knight AE, Cote GP, Luzio JP. 1998. The localization of myosin VI at the Golgi complex and leading edge of fibroblasts and its phosphorylation and recruitment into membrane ruffles of A431 cells after growth factor stimulation. J Cell Biol 143:1535–1545.

Chaib H, Kaplan J, Gerber S, Vincent C, Ayadi H, Slim R, Munnich A, Weissenbach J, Petit C. 1997. A newly identified locus for Usher syndrome type I, USH1E, maps to chromosome 21q21. Hum Mol Genet 6: 27–31.

Chaussepied P, Morales MF. 1988. Modifying preselected sites on proteins: the stretch of residues 633–642 of the myosin heavy chain in part of the actin-binding site. Proc Natl Acad Sci USA 85:7471–7475.

Chishti AH, Kim Ac, Marfatia SM, Lutchman M, Hanspal M, Jindal H, Liu SC, Low PS, Rouleau GA, Mohandas N, Chasis JA, Conboy JG, Gascard P, Takakuwa Y, Huang SC, Benz EJ Jr., Bretscher A, Fehon RG, Gusella JF, Ramesh V, Solomon F, Marchesi VT, Tsukita S, Hoover KB. 1998. The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. Trends Biochem Sci 23:281–282.

Cope MJT, Whisstock J, Rayment I, Kendrick-Jones J. 1996. Conservation within the myosin motor domain: implications for structure and function. Structure 4:969–987.

Cuevas JM, Espinós C, Millán JM, Sánchez F, Trujillo MJ, García-Sandoval B, Ayuso C, Nájera C, Beneyto M. 1998. Detection of a novel Cys628STOP mutation of the myosin VIIA gene in Usher syndrome type Ib. Mol Cell Probes 12:417–420.

Deol MS, Green MS. 1966. Snell's waltzer, a new mutation affecting behaviour and the inner ear of the mouse. Genet Res 8:339–345.

DiLeone RJ, Russell LB, Kingsley DM. 1998. An extensive 3′ regulatory region controls expression of Bmp5 in specific anatomical structures of the mouse embryo. Genetics 148:401–408.

Dominguez R, Freyzon Y, Trybus KM, Cohen C. 1998. Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. Cell 94:559–571.

el-Amraoui A, Sahly I, Picaud S, Sahel J, Abitbol M, Petit C. 1996. Human Usher 1B/mouse shaker-1: the retinal phenotype discrepancy explained by the presence/absence of myosin VIIA in the photoreceptor cells. Hum Mol Genet 5:1171–1178.

Espinós C, Millán JM, Sánchez F, Beneyton M, Nájera C. 1998. Ala397Asp mutation of myosin VIIA gene segregating in a Spanish family with type Ib Usher Syndrome. Hum Genet 102:691–694.

Evans KL, Fantes J, Simpson C, Arveiler B, Muir W, Fletcher J, van Heyningen V, Steel KP, Brown KA, Brown SDM, St. Clair D, Porteous DJ. 1993. Human olfactory marker protein maps close to tyrosinase and is a candidate gene for Usher syndrome type I. Hum Mol Genet 2:115–118.

Fisher AJ, Smith CA, Thoden JB, Smith R, Sutoh K, Holden HM, Rayment I. 1995. X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP.BeFx and MgADP.AlF4. Biochemistry 34:8960–8972.

Friedman TB, Liang Y, Weber JL, Hinnant JT, Barber TD, Winata S, Arhya IN, Asher JH, Jr. 1995. A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. Nature Genet 9:86–91.

Gabriel HD, Jung D, Butzler C, Temme A, Traub O, Winterhager E, Willecke K. 1998. Transplacental uptake of glucose is decreased in embryonic lethal connexin26-deficient mice. J Cell Biol 140:1453–1461.

Gibson F, Walsh J, Mburu P, Varela A, Brown KA, Antonio M, Beisel KW, Steel KP, Brown SD. 1995. A type VII myosin encoded by the mouse deafness gene shaker-1. Nature 374:62–64.

Gillespie PG, Wagner MC, Hudspeth AJ. 1993. Identification of a 120 kd hair-bundle myosin located near stereociliary tips. Neuron 11:581–594.

Griffith AJ, Friedman TB. 1999. Making sense out of sound. Nature Genet 21:347–349.

Guilford P, Ben Arab S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A, Petit C. 1994. A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nature Genet 6:24–28.

Hasson T, Gillespie PG, Garcia JA, MacDonald RB, Zhao Y, Yee AG, Mooseker MS, Corey DP. 1997. Unconventional myosins in inner-ear sensory epithelia. J Cell Biol 137:1287–1307.

Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS. 1995. Expression in cochlea and retina of myosin-VIIa, the gene defective in Usher syndrome type 1B. Proc Natl Acad Sci USA 92:9815–9819.

Hasson T, Mooseker MS. 1994. Porcine myosin-VI: characterization of a new mammalian unconventional myosin. J Cell Biol 127: 425–440.

Hasson T, Walsh J, Cable J, Mooseker MS, Brown SD, Steel KP. 1997. Effects of shaker-1 mutations on myosin-VIIa protein and mRNA expression. Cell Motil Cytoskeleton 37:127–138.

Holmes KC. 1997. The swinging lever-arm hypothesis of muscle contraction. Curr Biol 7:R112–118.

Houdusse A, Kalaboris VN, Himmel D, Szent-Györgyi AG, Cohen C. 1999. Atomic structure of scallop myosin subfragment S1 complexed with MgADP: A novel conformation of the myosin head. Cell 97:459–470.

Huang JD, Brady ST, Richards BW, Stenolen D, Resau JH, Copeland NG, Jenkins NA. 1999. Direct interaction of microtubule- and actin-based transport motors. Nature 397:267–270.

Hudspeth AJ, Gillespie PG. 1994. Pulling springs to tune transduction: adaptation by hair cells. Neuron 12:1–9.

Janecke AR, Meins M, Sadeghi M, Grundmann K, Apfelstedt-Sylla E, Zrenner E, Rosenberg T, Gal A. 1999. Twelve novel myosin VIIA mutations in 34 patients with Usher syndrome type I: confirmation of genetic heterogeneity. Hum Mutat 13:133–140.

Kaplan J, Gerber S, Bonneau D, Rozet JM, Delrieu O, Briard ML, Dollfus H, Ghazi I, Dufier JL, Frezal J. 1992. A gene for Usher syndrome type I (USH1A) maps to chromosome 14q. Genomics 14:979–987.

Keats BJ, Nouri N, Pelias MZ, Deininger PL, Litt M. 1994. Tightly linked flanking microsatellite markers for the Usher syndrome type I locus on the short arm of chromosome 11. Am J Hum Genet 54:681–686.

Kellerman KA, Miller KG. 1992. An unconventional myosin hevy chain gene from Drosophila melanogaster. J Cell Biol 119:823–834.

Kelley PM, Weston MD, Chen ZY, Orten DJ, Hasson T, Overbeck LD, Pinnt J, Talmadge Cr, Ing P, Mooseker MS, Corey D, Sumegi J, Kimberling WJ. 1997. The genomic structure of the gene defective in Usher syndrome type Ib (MYO7A). Genomics 40: 73–79.

Kimberling WJ, Moller C. 1995. Clinical and molecular genetics of Usher syndrome. J Am Acad Audiol 6:63–72.

Kimberling WJ, Moller CG, Davenport S, Priluck IA, Beighton PH, Greenberg J, Reardon W, Weston MD, Kenyon JB, Grunkemeyer JA. 1992. Linkage of Usher

syndrome type I gene (USH1B) to the long arm of chromosome 11. Genomics 14:988–994.

Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG, Jenkins NA. 1992. The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGFβ superfamily. Cell 71:399–410.

Kolega J. 1998. Cytoplasmic dynamics of myosin IIA and IIB: spatial "sorting" of isoforms in locomoting cells. J Cell Sci 111:2085–2095.

Lantz VA, Miller KG. 1998. A class VI unconventional myosin is associated with a homologue of a microtubule-binding protein, cytoplasmic linker protein-170, in neurons and at the posterior pole of *Drosophila* embryos. J Cell Biol 140:897–910.

Lévy G, Levi-Acobas F, Blanchard S, Gerber S, Larget-Piet D, Chenal V, Liu XZ, Newton V, Steel KP, Brown SD, Munnich A, Kaplan J, Petit C, Weil D. 1997. Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type IB. Hum Mol Genet 6:111–116.

Liang Y, Wang A, Probst FJ, Arhya IN, Barber TD, Chen KS, Deshmukh D, Dolan DF, Hinnant JT, Carter LF, Jain PK, Lalwani AK, Li XC, Lupski JR, Moeljopawiro S, Morell R, Negrini C, Wilcox ER, Winata S, Camper SA, Friedman TB. 1998. Genetic mapping refines DFNB3 to 17p11.2, suggests multiple alleles of DFNB3, and supports homology to the mouse model shaker-2. Am J Hum Genet 62:904–915.

Liang Y, Wang A, Belyantseva IA, Anderson DW, Probst FJ, Barber TD, Miller W, Touchman J, Jin L, Sullivan SL, Sellers JR, Camper SA, Lloyd RV, Kachar B, Friedman TB, Fridell RA. 1999. Characterization of the human and mouse unconventional myosin XV genes responsible for hereditary deafness and Shaker 2. Genomics 61:243–258.

Liu XZ, Hope C, Walsh J, Newton V, Ke XM, Liang CY, Xu LR, Zhou JM, Trump D, Steel KP, Bundey S, Brown SD. 1998a. Mutations in the myosin VIIA gene cause a wide phenotypic spectrum, including atypical Usher syndrome. Am J Hum Genet 63: 909–912.

Liu X, Ondek B, Williams DS. 1998b. Mutant myosin VIIa causes defective melanosome distribution in the RPE of shaker-1 mice. Nature Genet 19:117–118.

Liu XZ, Newton VE, Steel KP, Brown SD. 1997a. Identification of a new mutation of the myosin VII head region in Usher syndrome type I. Hum Mutat 10:168–170.

Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, Brown SD. 1997b. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nature Genet 16:188–190.

Maruta H, Korn ED. 1977. *Acanthamoeba* cofactor protein is a heavy chain kinase required for actin activation of the Mg^{2+}-ATPase activity of *Acanthamoeba* myosin I. J Biol Chem 252:8329–8332.

Mburu P, Liu XZ, Walsh J, Saw D Jr., Cope MJ, Gibson F, Kendrick-Jones J, Steel KP, Brown SD. 1997. Mutation analysis of the mouse myosin VIIA deafness gene. Genes Funct 1:191–203.

Mermall V, McNally JG, Miller KG. 1994. Transport of cytoplasmic particles catalysed by an unconventional myosin in living *Drosophila* embryos. Nature 369:560–562

Mermall V, Miller KG. 1995. The 95F unconventional myosin is required for proper organization of the *Drosophila* syncytial blastoderm. J Cell Biol 129:1575–1588.

Mermall V, Post PL, Mooseker MS. 1998. Unconventional myosins in cell movement, membrane traffic, and signal transduction. Science 279:527–533.

Patterson B, Spudich JA. 1996. Cold-sensitive mutations of *Dictyostelium* myosin heavy chain highlight functional domains of the myosin motor. Genetics 143:801–810.

Probst FJ, Fridell RA, Raphael Y, Saunders TL, Wang A, Liang Y, Morell RJ, Touchman JW, Lyons RH, Noben-Trauth K, Friedman TB, Camper SA. 1998. Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene. Science 280:1444–1447.

Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, Milligan RA. 1993. Structure of the actin–myosin complex and its implications for muscle contraction. Science 261:58–65.

Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA, Wesenberg G, Holden HM. 1993. Three-dimensional structure of myosin subfragment-1: a molecular motor. Science 261:50–58.

Russell LB. 1971. Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. Mutat Res 11:107–123.

Sankila EM, Pakarinen L, Kaariainen H, Aittomaki K, Karjalainen S, Sistonen P, de la Chapelle A. 1995. Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. Hum Mol Genet 4:93–98.

Self T, Mahony M, Fleming J, Walsh J, Brown SD, Steel KP. 1998. Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. Development 125:557–566.

Self T, Sobe T, Copeland NG, Jenkins NA, Avraham KB, Steel KP. 1999. Role of myosin VI in the differentiation of cochlear hair cells. Dev Biol 214:331–341.

Sellers JR. 1999. Protein profiles: myosin. London: Oxford University Press.

Smith RJ, Berlin CI, Hejtmancik JF, Keats BJ, Kimberling WJ, Lewis RA, Moller CG, Pelias MZ, Tranebjaerg L. 1994. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 50:32–38.

Solc CF, Durfler RH, Duyk GM, Corey DP. 1995. Molecular cloning of myosins from the bullfrog saccular macula: a candidate for the hair cell adaptation motor. Auditory Neurosci 1:63–75.

Spudich JA. 1994. How molecular motors work. Nature 372:515–518.

Steel KP, Brown SDM. 1994. Genes and deafness. Trends Genet 10:428–435.

Tamagawa Y, Kitamura K, Ishida T, Ishikawa K, Tanaka H, Tsuji S, Nishizawa M. 1996. A gene for a dominant form of non-syndromic sensorineural deafness (DFNA11) maps within the region containing the DFNB2 recessive deafness gene. Hum Mol Genet 5:849–852.

Titus MA. 1998. Coming to grips with a multitude of myosins. Trends Cell Biol 8:171–172.

Van Camp G, Smith RJH. 1999. http://dnalab-www.uia.ac.be/dnalab/hhh.

Vernon M. 1969. Usher syndrome—deafness and progressive blindness. Clinical cases, prevention, theory and literature survey. J Chron Dis 22:133–151.

Wang A, Liang Y, Fridell RA, Probst FJ, Wilcox ER, Touchman JW, Morton CC, Morell RJ, Noben-Trauth K, Camper SA, Friedman TB. 1998. Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. Science 280:1447–1451.

Wayne S, Der Kaloustian VM, Schloss M, Polomeno R, Scott DA, Hejtmancik JF, Sheffield VC, Smith RJ. 1996. Localization of the Usher syndrome type ID gene (Ush1D) to chromosome 10. Hum Mol Genet 5: 1689–1692.

Wayne S, Lowry RB, McLeod DR, Knaus R, Farr C, Smith RJH. 1997. Localization of the Usher syndrome type IF (Ush1F) to chromosome 10. Am J Hum Genet 61:A300.

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Levilliers J, Weston MD. 1995. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60–61.

Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, Petit C. 1997. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nature Genet 16:191–193.

Wells AL, Lin AW, Chen L-Q, Safer D, Cain SM, Hasson T, Carragher BO, Milligan RA, Sweeney HL. 1999. Myosin VI is an actin-based motor that moves backwards. Nature 401:502–508.

Weston MD, Kelley PM, Overbeck LD, Wagenaar M, Orten DJ, Hasson T, Chen ZY, Corey D, Mooseker M, Sumegi J, Cremers C, Moller C, Jacobson SG, Gorin MB, Kimberling WJ. 1996. Myosin VIIA mutation screening in 189 Usher syndrome type 1 patients. Am J Hum Genet 59:1074–1083.