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INVITED EDITORIAL
Unconventional Myosins, the Basis for Deafness in Mouse and Man

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Myosins are molecular motors that use the energy from ATP hydrolysis to generate force and move along actin filaments. Conventional myosin, or myosin-II, has the specialized ability to form bipolar filaments and is the basis for muscle contraction. Mutations in conventional myosins have been observed in man; dominant cardiomyopathies arise from mutations in β-cardiac myosin-II and other myosin-associated structural proteins (reviewed in Vikstrom and Leinwand 1996). Unlike myosin-II, unconventional myosins do not form bipolar filaments. Instead, these novel myosins serve in intracellular movements along actin filaments, primarily within nonmuscle cells. What the specific roles may be, however, has remained elusive. As described by Adato et al. (1997) in this issue of the *Journal,* as well as by other groups (Weil et al. 1995; Weston et al. 1996; Levy et al. 1997), mutations in one unconventional myosin, myosin-VIIa, lead to Usher disease, a syndromic recessive deafness. Mutations in myosin-VIIa can also lead to nonsyndromic recessive deafness in humans (Liu et al. 1997*b;* Weil et al. 1997) and result in deafness in the *shaker-1* mutant mouse (Gibson et al. 1995). Myosin-VIIa is not the only unconventional myosin that is the basis for deafness phenotypes. The gene for mouse myosin-VI encodes the deafness gene, *Snell's waltzer* (Avraham et al. 1995).

An Introduction to Unconventional Myosins

Myosins share a conserved motor domain. Phylogenetic analysis of the motor domains has divided the family of myosins into 12 classes, 7 of which are present in vertebrates (Mooseker and Cheney 1995; Hasson and Mooseker 1996). The seven vertebrate myosin classes—myosin-I, -II, -V, -VI, -VII, -IX, and -X—each can contain multiple members. For example there are seven different myosin-I genes in the mouse (Hasson et al. 1996). Overall, 26 different myosin genes (10 conventional and 16 unconventional) have been identified, many of which have also been mapped in humans (Hasson et al. 1996; Crozet et al. 1997).

All myosins, in addition to having a motor domain, share a regulatory or light-chain–binding domain. This binding domain is composed of repeat units, termed "IQ motifs," which serve as binding sites for members of the EF-hand family of calcium-binding proteins. For the unconventional myosins studied thus far, the light chain has been shown to be calmodulin. Depending on the number of IQ motifs present, unconventional myosins bind as many as six calmodulin light chains per myosin heavy chain, and, in the few unconventional myosins studied thus far, these calmodulin light chains have been shown to regulate the motor activity in a calcium-dependent fashion.

Although the myosins were divided into classes based on differences in their motor domains, each class also has a distinctive C-terminal tail domain, with a size range of 30–160 kD. The tail domain mediates protein-protein and protein-membrane interactions and gives each myosin isoform a unique function. The tail also participates in dimerization and subcellular targeting. It is generally thought that the tail binds the cargo that the myosin transports within the cell.

Genetic Evidence Implicating Myosins in Inner-Ear Defects

Both *Snell's waltzer* and *shaker-1* mice exhibit head tossing, hyperactivity, and circling behavior, which are due to vestibular dysfunction, and rapid progressive hearing loss accompanied by neuroepithelial degeneration. The genes encoding Snell's waltzer and shaker-1 were both identified by positional cloning. In the case of *Snell's waltzer,* two alleles were available, and one allele, se^{sv}, had a chromosomal inversion spanning the *short ear* (*se*) and *Snell's waltzer* (*sv*) loci. Avraham et al. (1995) identified the breakpoints of the chromosomal inversion and, using an exon-trapping scheme, identified the myosin-VI gene (*Myo6*) as a candidate gene. The second allele, *sv,* was then found to have an intragenic deletion within the myosin-VI gene. This deletion results in a destabilization of the myosin-VI mRNA, as well as in a deletion within the coding sequence. No myosin-VI

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transcript or protein is expressed in *sv* mice. In contrast, as a result of regulatory or position effects of the chromosomal inversion, se^{sv} mice express reduced (10%–25%) levels of myosin-VI RNA and protein (Avraham et al. 1995). These results suggest simply that a minimal level of myosin-VI protein expression is required for the function of the inner ear. They do not, however, suggest any specific function for myosin-VI in this tissue.

The gene encoding myosin-VIIa (*Myo7a*) was identified as a candidate for the *shaker-1* (*sh1*) locus on the basis of proximity to *Omp,* a marker inherited together with the *shaker-1* phenotype in all mice analyzed (Brown et al. 1992). Seven alleles of *sh1* mice are available, and the *Myo7a* mutations were identified in three of these strains (Gibson et al. 1995). The three characterized alleles all have mutations within the N-terminal myosin motor domain and include two missense mutations (*sh1* [original] and $sh1^{6J}$; see below) and one splice-site mutant ($sh1^{816SB}$) (fig. 1). The mouse chromosome 7 region that contains *shaker-1* is homologous to a human chromosome 11 region that includes genes for two types of human deafness, Usher syndrome type 1B (*USH1B*) and nonsyndromic recessive deafness (*DFNB2*). Usher disease is a recessive disorder characterized by the combination of sensorineural hearing loss, vestibular dysfunction, and retinitis pigmentosa. Analysis of Usher patients and DFNB2 families confirmed that *MYO7A* does indeed underlie these two deafness genes (Weil et al. 1995, 1997; Weston et al. 1996; Levy et al. 1997; Liu et al. 1997*b*). During the past 3 years 200 patients have been scanned for mutations in *MYO7A,* and thus far 33 distinct mutations, including missense, nonsense, deletion, frameshift, and splice-site mutations, have been found, some in multiple families (fig. 1). No mutational hot spot has been observed, however, and mutations have now been identified, as described by Adato et al., in the motor, light-chain–binding, and tail domains of the protein.

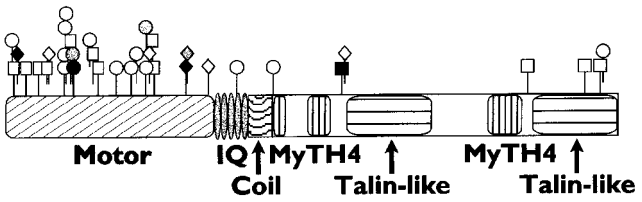

Figure 1 Schematic of the structure of myosin-VIIa, the unconventional myosin defective in Usher syndrome type 1B, nonsyndromic deafness DFNB2, and the *shaker-1* mouse. Domains of myosin-VIIa are described in the text. Mutations identified in myosin-VIIa are placed above their position on the linear myosin-VIIa amino acid sequence. Circles: missense or small in-frame insertions or deletions. Squares: Nonsense mutations and frameshifts (due to either insertions or deletions). Diamonds: Splice site mutations. The positions of the mutations identified in Usher type 1B are shown in the open symbols, in shaker-1 mice are shown in the grey symbols, and in DFNB2 families are shown in the black symbols.

Effects of Myosin-VIIA Mutation on Function

All myosins characterized to date bind to F-actin and bind and hydrolyze Mg^{2+}-ATP both in vivo and in vitro. Myosins have also been shown to exhibit actin-based motility in in vitro assays. These assays require purified protein, however, and, for most of the unconventional myosins, including myosin-VIIa, these sorts of biochemical assays have not been done. Analysis of the mutations found in Usher patients, *shaker-1* mice, and DFNB2 families, however, clearly indicates that the enzymatic activity of myosin-VIIa is critical for its function in the inner ear. The majority of the identified mutations lie in the motor domain (fig. 1), and these mutations include alterations to canonical amino acids found conserved in every myosin class. The crystal structure of the motor domain of chicken skeletal-muscle myosin-II has been solved, and the three-dimensional positions of these canonical amino acids have been identified (Rayment et al. 1993). By means of alignment of the amino acid sequence of myosin-VIIa with the sequence of chicken skeletal-muscle myosin-II, potential locations for the mutations found in the motor domain of myosin-VIIa have been proposed. For example, one of the mutations found in DFNB2, Arg244Pro, has been proposed to be positioned such that it would alter the environment of the nucleotide-binding pocket (Liu et al. 1997*b*).

One of the most exciting findings by Adato et al. is the identification of a missense mutation in the light-chain–binding domain of myosin-VIIa. The mutation, Ala826Thr, alters the fourth IQ motif (fig. 1). Although Ala826 is not one of the canonical amino acids conserved in all calmodulin-binding motifs, comparison with the database of calcium-dependent calmodulin-binding motifs shows that this position is always occupied by a hydrophobic residue such as Ile, Val, or Ala. Alteration of Ala826 to a polar Thr residue may therefore compromise this calmodulin-binding site, leading to an alteration in light-chain regulation of the motor. Alternatively, because the light-chain–binding region may act as a lever arm critical for the movement of myosin along actin (Uyeda et al. 1996), the removal of a light-chain–binding region could compromise the rigidity of the lever arm, changing the power stroke of the myosin motor and altering its motile properties.

A second exciting finding is the identification of mutations in the tail domain of myosin-VIIa. As shown schematically in figure 1, the tail of myosin-VIIa consists of a direct repeat containing two elements. The proximal element, the MyTH4 domain, is a sequence motif found in myosin-VIIa, -X, and -XII and in *Acanthamoeba* high-molecular-weight myosin-IV (Chen et al. 1996; Weil et al. 1996). The distal element, the talin-like domain, is a

region with significant homology to the N-terminus of talin and with limited homology to the N-termini of other members of the band-4.1 superfamily of actin-binding proteins (Chen et al. 1996; Weil et al. 1996). In talin and band 4.1, this domain binds to acidic phospholipids and mediates protein-protein interactions. Therefore, it is thought that the talin-like motifs serve to target this myosin to the plasma membrane. Adato et al. have identified three mutations that would truncate the C-terminal talin-like domain. Even more convincing, Levy et al. (1997) have identified a missense mutation in this C-terminal talin domain, Gly2137Glu, and have proposed that this mutation alters the ability of myosin-VIIa to associate with membrane ligands. No such ligands have yet been identified for myosin-VIIa.

This sort of mutation-function argument is complicated by the possibility that the mutations identified affect the stability of the myosin RNA or protein. Many disease mutations that introduce stop codons lead to mRNA destabilization; the *sv* mouse, for example, has an intragenic deletion that leads to essentially no expression at the RNA (and hence the protein) level (Avraham et al. 1995). More intriguing is the question of whether missense mutations or small in-frame insertions or deletions would affect protein stability. Recent analysis of the seven *shaker-1* alleles shows that point mutations can indeed have a drastic effect on myosin-VII protein stability (Hasson et al. 1997*b*). All *shaker-1* alleles exhibit normal myosin-VIIa mRNA levels, but expression of the myosin-VIIa protein varies, from wild-type levels in the allele *sh1* [original] to 20% of normal levels in $sh1^{6J}$ and to 1% of normal levels in the alleles $sh1^{4494SB}$ and $sh1^{4626SB}$. Interestingly, both *sh1* [original] and $sh1^{6J}$ have Arg→Pro missense mutations in the motor domain, but these mutations have vastly different effects on protein stability.

The results of *shaker-1* mutations on myosin-VIIa protein stability seem to correlate with the potential effects of the mutations on enzymatic activity. For *sh1* [original], a strain that expresses wild-type protein levels, the Arg502Pro mutation, on the basis of homologies, lies in a disordered surface loop near the actin-binding face (Hasson et al. 1997*b*). This position would not be expected to affect enzymatic activity, and, in fact, Arg502 is immediately adjacent to a Pro residue, suggesting that an Arg502Pro mutation might have only a minor effect on protein structure. In contrast, the $sh1^{6J}$ Arg241Pro missense mutation alters a canonical amino acid that, on the basis of homology, lies near the ATP-binding pocket (Hasson et al. 1997*b;* Liu et al. 1997*b*). This mutation would be expected to significantly affect the enzymatic activity of the myosin and is observed to result in an 80% decrease in myosin-VIIa protein expression.

It will be interesting to determine whether protein-stability differences are the basis for myosin-VIIa mutations leading to two distinct phenotypes. As diagrammed in figure 1, the mutations observed in Usher patients are not obviously different from the mutations observed in DFNB2 patients and shaker-1 mice, yet Usher patients exhibit prepubertal blindness. In addition, DFNB2 patients exhibit a variable onset of deafness, which is distinct from the profound sensorineural hearing loss observed in Usher patients at birth. Liu et al. (1997*b*) speculate that the mutated myosin-VIIa in DFNB2 patients is more stable and therefore is capable of minimal functioning allowing for a longer lifetime of the sensory epithelium and retina. This argument cannot, however, explain why shaker-1 mice, many alleles of which express essentially no myosin-VIIa protein, exhibit no retinal dysfunction (Hasson et al. 1997*b*). It was originally postulated that the difference between shaker-1 mice and Usher patient phenotypes was due to a difference in expression of the myosin-VIIa protein in the retina (El-Amraoui et al. 1996), but recent high-resolution immunolocalization studies have shown that there is no apparent difference, in myosin-VIIa expression in the retina, between the mouse and humans (Liu et al. 1997*a*). Therefore, this issue remains to be resolved.

Roles for Unconventional Myosins in the Inner Ear

Unique actin domains within the inner ear may help explain the essential role of unconventional myosins in this tissue (Gillespie et al. 1996). The sensory cell of the inner ear, the hair cell, has a series of actin-based projections on its apical surface, called "stereocilia," that are required to convert mechanical forces such as sound waves and gravity into electrical signals. The stereocilia are linked together into a bundle that is deflected by these mechanical forces. A specialized linkage, the tip link, joins the tip of each stereocilium to its next-highest neighbor. Stretching of this linkage on bundle deflection leads to the opening or closing of stretch-gated transduction channels. Opening of transduction channels causes an influx of endolymphatic K^+ and Ca^{++} ions, which leads to depolarization of the hair cell and to neurotransmitter release. After opening, the channels are reset, in a process termed "adaptation," whereby the tension on the tip link is modulated by movement of the transduction apparatus up or down the actin filaments of the stereocilium. On the basis of sensitivity to inhibitors, it is thought that a myosin is involved in this adaptation process. Both myosin-VI and myosin-VIIa are expressed exclusively by the sensory hair cells and had been suggested as possible components of the adaptation motor (Avraham et al. 1995; Hasson et al. 1995). More-recent studies, however, have suggested that myosin-VI and myosin-VIIa serve other functions in the hair cell and that a different unconventional myosin,

myosin-Iβ, is a better candidate for the adaptation motor.

By means of indirect immunofluorescence and immunoelectron-microscopy techniques, these three unconventional myosins were localized within the inner-ear epithelia of guinea pigs, frogs, and mice and were found to have distinct subcellular locations both within the hair cell and, surprisingly, within the stereocilia bundle (Hasson et al. 1997*a*). Of these three proteins, myosin-Iβ is the only myosin that was enriched at the tips of the stereocilia, the site of the adaptation process. Myosin-VIIa was found along the length of the stereocilium in mammals but is found in a band toward the bottoms of the stereocilia at the basal tapers in frogs. At the basal tapers the number of actin filaments decreases dramatically, from several hundred to several dozen in each stereocilium. This difference, in myosin-VIIa location, between mammals and frogs is consistent with an association of myosin-VIIa with the linkages that join adjacent stereocilia to their neighbors. In mammals these linkages occur along the entire length of the stereocilium, whereas in the frog these linkages are positioned only at the basal tapers. Myosin-VIIa may therefore participate in the assembly of stereocilia into a bundled array and may maintain stereocilium rigidity during the dynamic movements of the bundle.

In contrast to myosin-VIIa and myosin-Iβ, myosin-VI is not localized to the stereocilium but is present in the cuticular plate, an actin-rich region below the bundle in the apical domain of the hair cell (Hasson et al. 1997*a*). The cuticular plate anchors the stereocilia into the cell cytoskeleton; actin filaments at the root of each stereocilium penetrate and connect with the cuticular plate. Purified-hair-cell bundles from the frog contain myosin-VI, but immunolocalization studies show that this myosin is tightly associated with the rootlet actin filaments, suggesting a role for this myosin in the anchoring of the stereocilia bundle into the cuticular plate.

In addition to these locations, all three unconventional myosins were localized to a new subcellular domain of the hair cell, termed the "pericuticular necklace" (Hasson et al. 1997*a*). Found between the zona adherens and the cuticular plate, the pericuticular necklace is rich in membrane vesicles and is the site of microtubule ends. Therefore, the pericuticular necklace may represent a release point for vesicles carrying cargo such as myosins in transit between the microtubule arrays in the cell body and the actin arrays in either the cuticular plate or stereocilium. Both myosin-VI and myosin-VIIa are implicated in membrane trafficking, so, perhaps, it is these actin-based movements that are truly essential for inner-ear function.

Other Myosins as Human Deafness Genes?

An autosomal dominant deafness locus (*DFNA11*) has recently been assigned to 11q13, in the region of *USH1B* and *DFNB2* (Tamagawa et al. 1996), and *MYO7A* has been suggested as a candidate for this gene. On the basis of the presence of a coiled-coil motif within its tail domain (fig. 1), myosin-VIIa has been predicted to be found within the cell as a dimer (Hasson et al. 1995; Weil et al. 1996). A dominant mutation could therefore be created if the mutated myosin-VIIa protein was stable and capable of forming heterodimers with one wild-type and one mutant copy of the protein. These heterodimers could then act in a dominant-negative fashion, and, indeed, some carriers of Usher disease exhibit progressive deafness. Therefore, *DFNA11* may well result from expression of a stable but nonfunctional myosin-VIIa enzyme.

Currently, three other unconventional myosins are candidates for human deafness genes. Both human myosin-VI (*MYO6*) and myosin-Iβ (*MYO1C*) have recently been cloned (Avraham et al. 1997; Crozet et al. 1997). *MYO6,* which maps to chromosome 6, does not map to a previously identified human deafness gene. *MYO1C,* however, maps to 17p13 and, as such, is a possible candidate for the recessive deafness gene, *DFNB3*. The third candidate is the gene for human myosin-ID (*MYO1F*), which is also expressed in the inner ear (Crozet et al. 1997). *MYO1F* maps to 19p13.2-19p13.3 (Hasson et al. 1996; Crozet et al. 1997), a region that potentially overlaps with *DFNB15,* a recently identified autosomal recessive locus for nonsyndromic hearing loss (Van Camp et al. 1997). As more human unconventional myosins are cloned and mapped, undoubtedly more candidates for deafness genes will arise.

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