The roles of unconventional myosins in hearing and deafness

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Introduction

Unconventional myosins are crucial for the proper development and function of several sensory systems (for a detailed discussion of unconventional myosins see Chapter 4 in this volume by Kalhammer & Bähler). Specifically, mutations in unconventional myosins have been found to cause deafness and retinal degeneration in humans, and similar pathologies can be found in their animal models. Here, we will concentrate our discussion of myosins on their roles in hearing and balance; in particular, we will examine the function of unconventional myosins in the cells in the inner ear that are responsible for transducing sound and vestibular information into neural code, the hair cells.

There are reasons for studying the role of unconventional myosins in the inner ear beyond the fact that mutations in them can cause human disease. The inner ear is a good model system for assessing the function in vivo of unconventional myosins for several reasons. First, at least five unconventional myosins are expressed in the inner ear, each having a unique expression pattern. Second, the anatomy and basic physiology of the inner ear are well understood, and there are well-defined techniques available to ascertain

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whether the inner ear is functioning normally; thus, there are established experimental paradigms available to analyse the effects of disrupted myosin function. Finally, there are already several mouse models where a mutation in an unconventional myosin is known to affect the normal function of the inner ear. These mouse models can be, and have been, used to ascertain the function of these molecules \textit{in vivo}.

**Hearing and balance**

The inner ear contains the apparatus that is responsible for hearing and balance. These two systems are physically separated in the inner ear into distinct areas. In the mammalian inner ear, sound waves are transduced by specialized hair cells in the cochlea and balance information is transduced by a similar type of specialized cell in the vestibular system (which is subdivided into five distinct areas). Although these two systems transduce very different stimuli and are physically separated, they both rely on the same specialized cell, the hair cell. An overly simplistic view of the anatomy of the sensory epithelium of the inner ear is that it consists of two basic cell types, hair cells and the supporting cells. The supporting cells function, as their name suggests, to support the hair cell. Their main function is to provide the proper structural, physiological and metabolic environment for hair cells. The hair cell contains the molecular components necessary for transducing sound and vestibular information (Figure 1a). The basal region of the hair cell contains the synaptic machinery necessary to synapse with neurons of the VIII cranial nerve. The hair cell’s apical surface is even more specialized than the basal surface; protruding from the apical surface is the structure that detects the stimulus, the hair bundle (Figure 1b). This consists of a highly ordered alignment of stereocilia (stereocilia are filled with actin and are not true cilia), the exact arrangement depending on the species and whether the hair cell is in the cochlea or in the vestibular system. Hair bundles are a highly ordered, stepped arrangement of stereocilia. Individual stereocilia are cross-linked to each other by extracellular extensions called side links. There is a single specialized connection (tip link) at the tip of the stereocilia that is thought to be important in transduction. The stereocilia are anchored in an actin-rich structure that lies just below the apical surface of the hair cell, the cuticular plate. The proper organization and maintenance of the hair bundle is required for normal hearing and balance. As we will see, unconventional myosins play several critical roles in the development and function of the inner ear and, more specifically, the hair cell and its stereociliary bundle.

**Myosin VIIa**

Human myosin VIIa is predicted to be 2215 amino acids in length and its gene (\textit{MYO7A}) contains 48 coding exons [1,2]. The protein contains a terminal head domain, four IQ motifs, a coiled-coil domain and three myosin tail homology
4 (MyTH4) domains. Myosin VIIa is unique in that it is the only unconventional myosin to contain two talin homology domains (talin is a member of the band-4.1 protein superfamily). Talin-like domains are thought to interact with membranes. As with other members of the superfamily that contain a coiled-coil domain, myosin VIIa is thought to form a homodimer [3]. In mammals the protein is expressed in many different adult and developing tissues and appears to be a common component of cilia and microvilli [4]. Mutations in MYO7A can cause either syndromic deafness (deafness linked with other non-inner-ear pathologies) or non-syndromic deafness (pathology confined to the ear). As we will see, different mutations in MYO7A can be correlated with the level of hearing impairment, i.e. some lead to progressive deafness and some lead to a profound congenital deafness. A careful analysis of the mutations causing disorders in humans and mice can help us to better understand the function of myosin VIIa. However, one must be careful when correlating genotype with phenotype because the patient’s genetic background can affect the severity of the phenotype caused by a particular mutation.

Figure 1. Anatomy of the mammalian hair cell
(a) Entire hair cell. (b) An enlargement of the apical region; note that there are three actin-rich compartments, stereocilia, cuticular plate and circumferential band (actin is represented by dark blue lines).
Non-syndromic deafness

Mutations in MYO7A cause both dominant and recessive forms of non-syndromic deafness. Analysis of the recessive cases of non-syndromic deafness clearly show that one normal allele is sufficient to enable normal function, i.e. 50% or less of the normal protein level of myosin VIIa is enough to allow normal hair-cell physiology [5]. For a mutation in MYO7A to be dominant, the mutant allele must affect the function of the product of the normal allele, and when this has a deleterious effect on myosin VIIa function it is called a dominant negative mutation. Thus far, only one mutation has been identified in MYO7A that causes dominant non-syndromic deafness; DFNA11 [6] (note that non-syndromic deafness loci are given standard names; DFN followed by either A for dominant or B for recessive and then a number). This mutation is an in-frame deletion resulting in loss of three residues within the coiled-coil region thought to be responsible for myosin VIIa homodimer formation. Presumably, the lack of these three residues does not prevent the product of the mutant allele from dimerizing with the product of the normal allele, but the mutation may effect the tertiary structure of the protein, rendering the entire dimer functionless (a dominant negative).

To date, four mutations have been identified in MYO7A that lead to recessive non-syndromic deafness (DFNB2) [3,7]. One of these is particularly interesting because it demonstrates how the analysis of mutations can provide insight into the roles of certain domains within proteins but that it is not always clear why a mutation causes a disease. In one family, a missense mutation (where one amino acid is substituted for another) in the last nucleotide of an exon was shown to cause profound deafness [3]. Mutations in the last nucleotide of an exon are known to affect splicing and in other genes similar mutations can result in the whole exon being spliced out of the transcript. Exon 15 of MYO7A (the exon that would be affected) encodes the actin-binding site, whose absence would severely affect the protein’s function. However, the reason this mutation causes deafness is not completely clear since 40% or more of the transcripts appear to be full length and presumably encode enough myosin VIIa for normal function. The amino acid change, a methionine to an isoleucine, is not believed to affect the function of the protein, despite being close to the actin-binding site. The authors give two reasons for this [3]. First, methionine and isoleucine have similar properties and this residue is not conserved across the myosin family, suggesting that it is not a critical residue. Secondly, analysis of the three-dimensional structure of the actin-binding site in muscle myosin predicts that this residue is not important in actin binding. So how do we explain why individuals homozygous for this mutation are deaf? It may simply be that the mutation causes the exon to be spliced out in the vast majority of transcripts and/or that the prediction that this amino acid is not crucial for myosin VIIa to function is incorrect. However, it is still possible to explain the deafness even if the analysis of the mutation is correct, i.e. that 40% of MYO7A transcripts are correctly spliced and 60% are missing
exon 15. The incorrectly spliced \textit{MYO7A} transcript results in a 46-amino acid in-frame deletion that probably affects the tertiary structure of myosin VIIa. A disruption of the tertiary structure could alter the coiled-coil domain just 220 amino acids downstream from the actin-binding site \cite{3}. As with the dominant mutation discussed above, this abnormal myosin VIIa could be acting in a dominant negative fashion by dimerizing with normal myosin VIIa. Thus for this mutation, it is still unclear what its exact effects are on myosin VIIa function. Furthermore, the analysis of this mutation points out that we still have a lot of questions remaining about all levels of the biology of myosin VIIa.

\textbf{Syndromic deafness}

\textbf{Usher syndrome type 1B (USH1B)}

Usher syndrome is one of the most common forms of syndromic deafness and is characterized by hearing loss, retinitis pigmentosa and, in some types, vestibular dysfunction. The most severe form of the disease is type 1; USH1 patients have profound congenital deafness, vestibular dysfunction and the onset of retinitis pigmentosa prior to puberty. The disease types are further divided (e.g. USH1A and USH1B) based on genetic-mapping studies which have shown that several genetic loci cause the same type of Usher syndrome. Mutations in \textit{MYO7A} are responsible for one subtype, USH1B \cite{1}.

There are at least 35 different mutations in \textit{MYO7A} that lead to USH1B. The effects of these mutations are spread over the entire length of myosin VIIa, with the majority of them concentrated in the protein’s head region (Table 1). Nonsense, missense, deletion and splice-site mutations have all been identified. Missense mutations that lead to USH1B can be used to help elucidate the importance of particular residues within myosin VIIa. One example is a base substitution (G→A) that causes a glycine residue (2137) to be changed to glutamic acid (a non-conservative substitution). This area of myosin VIIa shows homology to the membrane-binding domain of members of the band-4.1 protein superfamily and, therefore, is thought to be involved in myosin VIIa interactions with membranes. Thus this mutation may significantly affect the function of myosin VIIa by preventing it from being properly targeted within the cell. Furthermore, it shows that this residue is critical for proper function of myosin VIIa.

\textbf{Atypical Usher syndrome}

Patients whose clinical features do not allow them to be classified as having one of the three main forms of Usher syndrome are classified as having atypical Usher syndrome. In two siblings diagnosed with atypical Usher syndrome, the underlying cause was found to be a mutation in \textit{MYO7A} \cite{8}. Unlike the profound congenital deafness and the early onset of retinitis pigmentosa associated with USH1B, a progressive hearing loss began during infancy and retinitis pigmentosa was not diagnosed until after puberty. Both
Table 1. Known mutations in MYO7A

<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of mutation</th>
<th>Location in myosin VIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFNA11</td>
<td>Deletion</td>
<td>2658–2666del(IF) (CC)*</td>
</tr>
<tr>
<td>DFNB2</td>
<td>Insertion</td>
<td>Vally19insT (FS 28 aa)</td>
</tr>
<tr>
<td></td>
<td>Missense</td>
<td>Arg244→Pro</td>
</tr>
<tr>
<td>USH1B</td>
<td>Deletion</td>
<td>724delC (IQ)</td>
</tr>
<tr>
<td></td>
<td>Missense</td>
<td>Gly25→Arg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ala826→Thr (IQ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gly214→Gin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg140→Gln</td>
</tr>
<tr>
<td></td>
<td>Arg212→His</td>
<td>Arg1602→Gln (Talin)*</td>
</tr>
<tr>
<td></td>
<td>Arg212→Cys</td>
<td>Arg1743→Trp</td>
</tr>
<tr>
<td></td>
<td>Arg302→His</td>
<td>Gly2137→Glu (Talin)*</td>
</tr>
<tr>
<td></td>
<td>Ala397→Asp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gly450→Glu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pro503→Leu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg756→Tyr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cys31→Stop</td>
<td>Arg1861→Stop</td>
</tr>
<tr>
<td></td>
<td>Arg150→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gln234→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glu314→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyr333→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cys628→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg634→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg669→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ile668→Stop</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS5+1g→a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS13–8c→g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS18+1g→a</td>
<td></td>
</tr>
<tr>
<td>Atypical USH</td>
<td>Missense</td>
<td>Leu651→Pro</td>
</tr>
<tr>
<td></td>
<td>Arg1602→Gln (Talin)*</td>
<td></td>
</tr>
</tbody>
</table>

*These amino acid changes are discussed in the text.
†Mutation may also result in exon 15 being spliced out of the transcript.
There are 35 different mutations in MYO7A that lead to deafness. These deafnesses can be syndromic (USH1B and atypical Usher syndrome) or non-syndromic (DFNA11 and DFNB2).
The domains affected by mutations in the tail are noted in parentheses following the mutation information. The substitution shown in bold is associated with two clinically distinct forms of (contd.)
siblings are compound heterozygotes for mutations in MYO7A. The mutation in one allele leads to a non-conservative leucine-to-proline substitution at residue 651. Position 651 is a highly conserved residue within the motor domain and is thought to be important in the overall structural integrity of the head domain [9]. Thus this mutation is thought to affect severely the function of the protein. The second allele carries a mutation resulting in another amino acid substitution, at residue 1602, in which a glutamine replaces an arginine. Some patients homozygous for this mutation have the severe USH1B [10]. Thus we have a problem in interpreting the basis of the deafness in the compound heterozygote siblings with atypical Usher syndrome. If the transcript from the first allele is non-functional and homozygosity for the second mutant allele produces the more severe USH1B, why do these patients not also acquire the more severe form of Usher syndrome? Liu et al. [8] suggest that the genetic background of these families may be a contributing factor to the severity of the disease. The importance of this extends beyond the clinical manifestation of the disease as it suggests that other proteins may affect and/or be affected by myosin VIIa, or that the function of myosin VIIa can be compensated for by other proteins. Identifying these other proteins will be important in understanding the function of myosin VIIa in vivo and, potentially, in treating diseases caused by abnormal myosin-VIIa function.

**Mouse models**

Myosin VIIa was identified as the gene involved in deafness at the shaker1 (sh1) locus in mice (Myo7a) [11]. The sh1 mutation arose spontaneously and was first described by Lord and Gates in 1929 [12]. Since then, a further nine sh1 alleles have been found, and a total of seven mutations have been identified.

<table>
<thead>
<tr>
<th>Table 1 (contd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usher syndrome. The mutations are given in the standard nomenclature for human gene mutations [28]; this information can be found on the worldwide web (<a href="http://interscience.wiley.com/pages/1059%E2%80%937794/nomenclature.html">http://interscience.wiley.com/pages/1059–7794/nomenclature.html</a>). The number prior to a deletion (del) is the number of the nucleotide deleted and a letter after it shows which type of nucleotide has been deleted (A, T, G or C). The type of deletion is given in parentheses after the nucleotide affected; FS, frame shift (if a number follows, there is a stop codon that number of amino acids, or aa, downstream); IF, in-frame deletion. The number before an insertion (ins) is the number of the affected amino acid and after 'ins' is information describing the exact nature of the insertion. For nonsense and missense mutations, the first three letters are the amino acid affected, followed by its position in the protein, then the amino acid that results from the mutation (or Stop). In the case of intronic mutations, the number directly after IVS (intervening sequence) is the intron affected and the characters following IVS describe where the mutation is in the intron with respect to the splice-acceptor site. Regions of the protein affected by mutations are indicated by: IQ, IQ domain; CC, coiled-coil domain; Talin, talin homology domain. Data are from [1,3,6–8,10,16].</td>
</tr>
</tbody>
</table>
The sh1 mouse lines are characterized by hearing loss and vestibular defects. To date, none of the Myo7a alleles examined has been shown to lead to any signs of retinal degeneration [5]. The sh1 mice are a useful model for inner-ear abnormalities in humans that are caused by myosin-VIIa dysfunction and are also useful for determining the role of myosin VIIa in vivo.

Experimental analysis of the shaker1 mouse has provided great insight into the biological function of myosin VIIa in the inner ear and into the pathology caused by its disruption. However, before we address the possible biological roles of myosin VIIa in hearing and balance, it is important that we understand which cells in the auditory system express myosin VIIa and its intracellular location. We can use an understanding of the location of myosin VIIa within a cell in conjunction with phenotypic analysis of the sh1 mice to better understand the biological function of myosin VIIa.

**Expression and localization of myosin VIIa**

In the adult vertebrate inner ear, myosin VIIa is expressed exclusively in the hair cells [13,14] and, during development, is expressed extremely early, prior to stereocilia formation [15]. Antibodies raised against myosin VIIa localize the protein at the light- and electron-microscopic levels within vertebrate hair cells, diffusely spread throughout the cytoplasm but concentrated in several regions, such as the cuticular plate, the pericuticular necklace and within the stereocilia of the hair bundles (Figure 2) [13,14]. Interestingly, the distribution of myosin VIIa within the stereocilia is not the same in all species [13]. In the bullfrog, myosin VIIa is concentrated in a band towards the base of the stereocilia, whereas in mammals myosin VIIa appears to be distributed throughout the stereocilia. This discrepancy in myosin VIIa localization in the stereocilia corresponds to a difference in the distribution of side links: in bullfrogs, side links are concentrated towards the basal end of the stereocilium, whereas in mammals they are distributed evenly along the length of the stereocilium. This interesting correlation implies a role for myosin VIIa in side-link formation and/or maintenance. The localization of myosin VIIa in the cuticular plate, the structure within the hair cell that anchors the stereocilia, suggests that myosin VIIa may be involved in anchoring the stereocilia. Thus the localization of myosin VIIa within the hair cell suggests that it is involved in maintaining the structural integrity of the hair bundle. This hypothesis can be tested by analysing the hair cells of mice with mutations in myosin VIIa.

**Lessons from shaker1 mice**

The 10 different Myo7a alleles identified are all recessive mutations and the exact mutations are known for seven alleles (Table 2). These different mutations affect to some degree the amount of myosin VIIa expressed [5] and its function (Table 2). The 10 different alleles provide the researcher with tools with which to investigate the physiological function of myosin VIIa (for a more complete discussion of myosin VIIa alleles, refer to Mburu et al. [16]). Numerous studies...
<table>
<thead>
<tr>
<th>Allele</th>
<th>Mutation</th>
<th>Protein domain</th>
<th>Protein level</th>
<th>Cochlear physiology</th>
<th>Hair-cell development</th>
</tr>
</thead>
<tbody>
<tr>
<td>sh1</td>
<td>Missense, Arg502 → Pro</td>
<td>Head</td>
<td>0.93</td>
<td>Some responses</td>
<td>Normal</td>
</tr>
<tr>
<td>6J</td>
<td>Missense, Arg241 → Pro</td>
<td>Head</td>
<td>0.21</td>
<td>Very few responses</td>
<td>Abnormal</td>
</tr>
<tr>
<td>26SB</td>
<td>Missense, Phe1800 → Ile</td>
<td>Tail</td>
<td>0.46*</td>
<td>No activity</td>
<td>Abnormal</td>
</tr>
<tr>
<td>816SB</td>
<td>Intrinsic, del aa 646–655</td>
<td>Head</td>
<td>0.063</td>
<td>No activity</td>
<td>Abnormal</td>
</tr>
<tr>
<td>44945B</td>
<td>Intrinsic, Stop</td>
<td>Head</td>
<td>0.0089</td>
<td>Very few responses</td>
<td>Abnormal</td>
</tr>
<tr>
<td>46265B</td>
<td>Nonsense, Gln720 → Stop</td>
<td>Head</td>
<td>0.0072</td>
<td>No activity</td>
<td>Abnormal</td>
</tr>
<tr>
<td>33365B</td>
<td>Nonsense, Cys2182 → Stop</td>
<td>Tail</td>
<td>0.13</td>
<td>No activity</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

Note that for all alleles studied to date, the animals eventually become profoundly deaf. Alleles 7J, 8J and 9J have only recently been identified and the mutations are unknown (?); these mice are deaf and have vestibular abnormalities. Protein levels were determined from kidney and testes and are expressed as a proportion of the wild-type level (taken from [5]). Myo7a<sup>26SB</sup> is the only allele that gave different results for the protein levels in the two tissues, the value for the testes is shown (*) and the kidney is 0.18, suggesting that there is a differential tissue response to the mutation in Myo7a. Cochlear physiology and hair-cell development are from [16]. Deletion of amino acids is indicated by del aa.
over the last 70 years have analysed the effect of mutations in myosin VIIa on the function of the inner ear with most of the studies concentrating on the original shaker1 allele, Myo7ash1 [12]. The Myo7ash1 mutation is a missense mutation within the part of the gene encoding a poorly conserved region of the myosin head domain [11] and so may have only subtle effects on the function of the head [16]. Myo7ash1 mutants do produce myosin VIIa; in fact, in some tissues it reaches nearly normal levels [5]. Even so, these animals do eventually become deaf and the hair cells in the cochlea degenerate [17].

In a detailed study, electron microscopic and electrophysiological techniques were used to analyse the effect that Myo7ash1 has on the structure and function of the developing hair cells of the cochlea [18]. Ultrastructurally, hair-cell development appears to be approximately normal for the first few weeks after birth, with the exception that only two rows of stereocilia are in the hair bundle instead of the normal three. Not surprisingly, young Myo7ash1 mutants do have some electrophysiological function remaining, although the physiology of the hair cells is not normal. The analysis of the Myo7ash1 mice suggests

Figure 2. Localization of unconventional myosins in the mammalian hair cell
(a) There are at least five different unconventional myosins expressed in the inner ear (indicated by Roman numerals). The location of myosin XV is not known. Myosin V is located in the postsynaptic terminals of the neurons that synapse with the hair cells. (b) Myosins Iβ, VI and VIIa are all expressed within the hair cell but have unique, though overlapping, expression patterns within the apical portion of the hair cell.
that myosin VIIa is necessary for maintaining hair-cell viability (they eventually degenerate); in particular, it plays a role in stereocilia development [18].

Self et al. [18] also analysed another allele, *Myo7a<sup>816SB</sup>* , with more severe effects. These mutants have very low protein levels [5] and any residual protein is thought to be non-functional [16]. The mice have an extensive disruption of hair-cell development in the cochlea, characterized by severely disorganized hair bundles. Furthermore, the *Myo7a<sup>816SB</sup>* mutants have no electrophysiological response to sound. Thus the absence of myosin VIIa leads to a severe disruption of hair-bundle development and hair-cell function.

**Potential roles for myosin VIIa**

By correlating the intracellular localization of myosin VIIa and the phenotypes of the mouse mutants we can begin to elucidate myosin VIIa function *in vivo*. Both its localization near side links and within the cuticular plate suggest that it is involved in maintaining the structural integrity of the hair bundle and that when myosin VIIa is mutated the hair bundle is severely disorganized. Thus myosin VIIa’s function in the hair cell appears to be to maintain the proper position of the stereocilia.

**Myosin VI**

Mutations in the mouse myosin VI gene (*Myo6*) are responsible for deafness and vestibular dysfunction in the Snell’s waltzer mouse [19]. To date, no mutations in the human gene for myosin VI (*MYO6*) are known to cause deafness; however, several deafness loci map near the *MYO6* locus [20]. Myosin VI is predicted to be 1266 amino acids in length. Its motor domain contains one ATP-binding domain and one actin-binding domain, and its tail has one IQ motif and a coiled-coil region. Two Snell’s waltzer alleles have been identified; both are recessive alleles and both lead to hair-cell degeneration. One mutant line, *sesv*, is the result of an inversion that has disrupted the function of at least one other gene; in fact, the mutant is named after the other locus that is disrupted—the short-ear locus. The inversion does not disrupt the coding sequence of *Myo6*, but does affect regulatory regions of the gene; *sesv* mutants have normal-size RNA transcripts but myosin-VI protein levels are only about 15% of those in controls [19]. The second mutation, *sv*, is a deletion that includes 130 bp of coding sequence, resulting in a termination codon truncating the protein just after the head region. There is no detectable myosin VI and this deletion is effectively a null mutation.

**Expression and localization**

In the ear, myosin VI expression is, in some respects, similar to the expression of myosin VIIa; both are expressed during the earliest stages of hair-cell differentiation [15] and in the adult mammalian hair cell [13]. Furthermore, myosin VI, like VIIa, is concentrated in the cuticular plate and in the
pericuticular necklace [13]. However, in contrast with myosin VIIa, myosin VI was not present in the stereocilia of any vertebrate examined [13].

Lessons from Snell’s waltzer mice

The phenotype of the sv mutant mouse is similar to the Myo7ash1 mouse in that there is a severe disorganization of the stereocilia; however, the exact nature of the disorganization is quite different to that of the Myo7ash1 mouse. The stereocilia of the sv mutants become fused together, resulting in either a single stereocilium or a few large stereocilia per hair cell [20a]. By the time hair cells would normally be morphologically mature, many hair cells in Snell’s waltzer mutants are dead or dying and the sensory epithelium of the cochlea eventually degenerates.

Potential roles for myosin VI

The expression pattern of myosin VI in hair cells is similar to that of myosin VIIa and the phenotype of myosin VI-deficient hair cells suggests that myosin VI is necessary for the proper development of the stereocilia. T. Self et al. [20a] suggest that myosin VI directly or indirectly anchors the apical membrane between the stereocilia, preventing the surface tension (resulting from the stereocilia protrusions) from ‘zipping up’ the stereocilia into one large stereocilium, the exact phenotype of the sv mouse. This idea is supported by experiments on Drosophila syncytial blastoderms where the injection of antibodies against myosin VI prevented the furrow canals (inward extensions of the membrane) from forming completely [21]. Although the furrow canals are in the opposite direction to the stereocilia, they still must overcome the surface tension created as they are drawn further and further into the cell. Myosin VI function may be to provide the motor that allows some cellular extensions to form normally.

Myosin XV

Myosin XV is the most recent member of the myosin superfamily to be identified in mammals. It was identified as the gene responsible for a non-syndromic recessive deafness (DFNB3) [22] and for the shaker2 (sh2) mouse phenotype [23]. The myosin XV gene in humans (MYO15) has 50 exons and its longest open reading frame found to date is 4757 bp. Structurally, the head domain of myosin XV appears to contain one ATP-binding site and two putative actin-binding sites. The tail region has two IQ motifs, one MyTH4 domain, and one talin-like domain towards the C-terminus. Three different mutations in MYO15 have been identified that lead to deafness in humans [22]. One is a nonsense mutation in exon 39 that is predicted to result in a truncated protein lacking the talin-like domain. The two other mutations identified by Wang et al. [22] are both single-base transversions causing amino acid substitution within the MyTH4 domain. In fact, the affected amino acids are
separated by only two residues. This suggests that the MyTH4 domain may be an important domain for auditory function and analysis of these mutants may shed light on the function of this domain.

**Expression and localization**
Unfortunately, as MYO15 has only just been cloned, there are no antibodies available to establish where in the hair cell the protein is. In fact, it is not known whether myosin XV is expressed in the hair cell at all, although it is extremely likely that it is. It will be important to determine the precise localization of myosin XV in order to establish its function in the inner ear.

**Lessons from shaker2 mice**
The sh2 mouse is profoundly deaf and has vestibular abnormalities. The stereocilia of sh2 hair cells are arranged normally but are abnormally short, and the actin cytoskeleton within the cell body is malformed [23]. sh2 mice have large ectopic actin bundles that stretch from the cuticular plate to the base of the hair cell. This abnormal actin structure suggests that myosin XV is necessary for normal actin/cytoskeleton organization. The mutation in Myo15 in the sh2 mouse lies within one of the putative actin-binding domains; it is a single-base transition that produces a cysteine-to-tyrosine (non-conservative) substitution at codon 674. The cysteine at this position is conserved in the vast majority of myosin heads. Thus this residue may be instrumental in actin binding.

**Potential roles for myosin XV**
As MYO15 has only recently been cloned and there is little known about myosin XV, it may be premature to speculate on myosin XV’s role in the ear. However, based on the ultrastructure of the sh2 hair cells, it appears that myosin XV functions, in some way, to organize the actin cytoskeleton in hair cells. It will be important to determine if myosin XV’s temporal and spatial expression patterns correlate directly with this hypothesis.

**Other myosins involved in hearing**
There are several other unconventional myosins expressed in the vertebrate inner ear and they may play important roles in inner-ear physiology; however, to date no inner-ear abnormalities in either humans or mice have been identified that are the result of mutations in their genes. Myosin X is expressed within the inner ear, but its localization is unknown [24]. Myosin V is expressed by the neurons of the VIII cranial nerve and is localized in the nerve terminals that synapse on to hair cells. There are several mouse mutants with mutations in myosin V but none of them have been reported to display any inner-ear abnormalities, suggesting that myosin V may not be critical for hearing and balance. At least four different type-I myosins are expressed in the
mammalian inner ear [24,25]. In vertebrates, myosin 1β is expressed in the cuticular plate and stereocilia of hair cells. Myosin 1β in the stereocilia is of particular interest because it is clustered in and around the area where the tip links are anchored (Figure 1b) [13,26,27]. Tip links are extracellular attachments between the tip of a stereocilium to the side of its taller neighbour. The tension of the tip links is thought to be important in the mechanical adaptation of the hair cells and the motor molecule responsible for this is hypothesized to reside in the tip-link anchor of the taller stereocilia. Thus myosin 1β’s localization in the tip-link anchoring plaque makes it an ideal candidate to be the motor molecule, or at least one of the motor molecules, responsible for adaptation (Figure 2). To test this hypothesis it will be necessary to disrupt myosin-1β expression within the hair cell. As there is an ever-growing number of unconventional myosins, it is likely that even more myosin isoforms will be found to play important roles in the inner ear and its hair cells.

**Perspectives**

There is still much to do before we understand the role of myosins in cochlear function. In particular, the difficulty in expressing the unconventional myosin molecules *in vitro* has hampered research into the biochemistry and biophysics of these important motor molecules. The role of myosin 1β may be critical to hair-cell function, located as it is at the heart of the mechano-electrical transduction mechanism, but as yet no mutations have been reported that might allow a critical investigation of the role of this molecule. Although this review has focused upon the role of myosins in deafness, myosin VIIa at least also has a role in the maintenance of retinal function, shown by the retinitis pigmentosa of Usher syndrome. As this is progressive, there seems to be a reasonable prospect of stopping its progression if we only understood the molecular basis of the disease. Progressive hearing loss is extremely common in the human population, and the involvement of mild mutations of the myosin genes described here have yet to be uncovered.

**Summary**

- The proper expression and function of several unconventional myosins are necessary for inner-ear function. Mutations in MYO7A and MYO15 cause deafness in humans, and mice. Whereas mutations in Myo6 cause inner-ear abnormalities in mice, as yet no human deafness has been found to the result of mutations in MYO6.
- In the mammalian inner ear there are at least nine different unconventional myosin isoforms expressed. Myosin 1β, VI, VIIa and probably XV are all expressed within a single cell in the inner ear, the hair cell.
• The myosin isozymes expressed in the hair cell all have unique domains of expression and in some areas, such as the pericuticular necklace, several domains overlap. This suggests that these myosins all have unique functions and that all are individually targeted within the hair cell.

• The mouse is proving to be a useful model organism for studying both human deafnesses and elucidating the normal functions of unconventional myosins in vivo.

The authors would like to thank Dr. Amy Kiernan for her helpful discussions and critical reading of the manuscript. The work was supported by the MRC, Defeating Deafness and European Commission contracts BMH4-CT96-1324 and BMH4-CT97-2715.

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