

# Auditory transduction in the mouse

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**Abstract** The sensory hair cells of the mammalian cochlea transduce acoustic stimuli into auditory nerve activity. The biomechanical and molecular details of hair cell mechanotransduction are being acquired at an ever-finer level of resolution. In this review, we discuss how selected mouse mutants and transgenic models have contributed to, and will continue to shape, our understanding of the molecular basis of hair cell mechanotransduction. Functional and structural discoveries made originally in hair cells of nonmammalian vertebrates have been further pursued in the mouse inner ear, where transgenic manipulation can be applied to test molecular mechanisms. Additional insights have been obtained from mice bearing mutations in genes underlying deafness in humans. Taken together, these studies emphasize the elegance of mechanotransduction, enlarge the team of molecular players, and begin to reveal the remarkable adaptations that provide the sensitivity and temporal resolution required for mammalian hearing.

**Keywords** Sensory hair cells · Hair cell mechanotransduction · Mouse mutants and transgenic models

## The auditory periphery

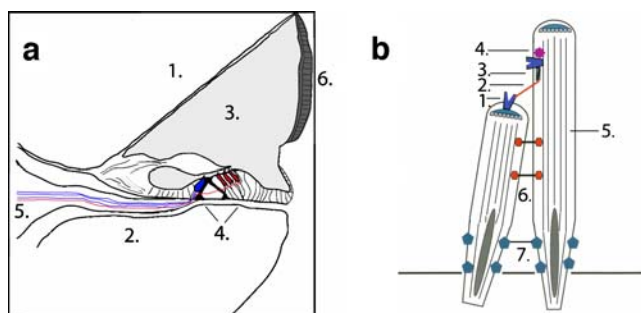
Sound waves propagate through the external and middle ear to cause fluid motion within the inner ear. This fluid-filled cavity within the petrous temporal bone includes both vestibular and auditory sensory epithelia. Mechanical

energy in the acoustic frequency spectrum preferentially vibrates the membranous compartments of the spiraled cochlea, the auditory sensory organ. Much lower-frequency head motions are encoded in the saccule, utricle, and semicircular canals of the vestibular compartment. A cross-section of the cochlea (Fig. 1a) shows its three fluid-filled chambers. The upper and lower (scala vestibuli and scala tympani, respectively) chambers contain perilymph, similar in composition to cerebrospinal fluid. The intervening scala media, bounded by Reissner's membrane and the cuticular surface of the organ of Corti, contains endolymph, a potassium-rich, calcium-poor fluid similar to cytoplasm in ionic composition. A specialized secreting endothelium, the stria vascularis on the lateral wall of the scala media, is responsible for generating the endolymph and the associated endolymphatic potential of  $\sim +80$  mV. Mechanosensory hair cells and supporting cells make up the organ of Corti, with their cuticular surfaces facing the potassium-rich endolymph. Thus, mechanotransduction in the inner ear takes place at an asymmetric ionic boundary, with potassium serving as the charge carrier for mechanically gated ionic currents.

## Mechanotransduction

Sensory hair cells of vertebrates possess a graduated bundle of stereocilia, specialized actin-cored microvilli, whose deflection leads to ionic flux and generation of receptor potentials (Fig. 1b). The receptor potential alters the open probability of voltage-gated calcium channels found at presynaptic active zones ("ribbon" synapses after similar structures found in retinal photoreceptors and bipolar cells), modulating transmitter release onto associated dendrites of afferent neurons. Many details of mechanotransduction were learned originally from excised inner ear preparations

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**Fig. 1** Mechanosensitive hair cells of the mammalian cochlea. **a** Cross-section of the mammalian cochlea showing disposition of perilymphatic [scala vestibuli (1) and scala tympani (2)] and endolymphatic [scala media (3)] chambers. The sensory epithelium (organ of Corti) composed of hair cells (4), supporting cells, and neuronal processes (5), provides the cuticular boundary of the endolymphatic space. Stria vascularis (6) on the lateral wall of the scala media generates endolymph and the endolymphatic potential. **b** Stereocilia atop the hair cells stand in serried rows by height. Deflection toward the taller row opens transducer channels (1). Tip links (2) joining the tops and sides of adjacent cilia may transmit hair motion [through an associated gating spring (3)] to the stretch-sensitive transducer channels thought to reside near the tips of stereocilia. Nonmuscle forms of myosin (4) are thought to tension the transducer apparatus by pulling against the stereocilia's actin core (5). Other forms of myosin and “scaffolding” proteins are found elsewhere in the stereocilium, possibly associated with side-links (6) between stereocilia or ankle links (7) near the insertion into the hair cell's cuticular plate

of nonmammalian vertebrates: viz., the saccule of frogs and the basilar papilla (analogous to the mammalian cochlea) of turtles. As shown to be universally true, deflection of the hair bundle toward its tallest stereocilia (the “positive” direction) causes increased conductance and depolarization, and opposite (“negative”) motion produces decreased conductance and hyperpolarization [38]. Movements perpendicular to this axis cause no change in membrane potential [68]. These observations imply that the transducer apparatus is directional, aligned with the stereociliar “staircase,” and is partially activated at rest, as though a constant tension is produced by an intrinsic mechanical force, and then is modulated by extrinsic stimuli. A possible structural basis is suggested by the “tip links” that attach the top of one stereocilium to the side of the next taller one [62]. It has been proposed that tip links transmit a gating force to mechanosensitive channels in each stereocilium, based in part on the observation that the loss of tip links eliminates mechanosensitivity [7], which returns with the regenerated tip link [94].

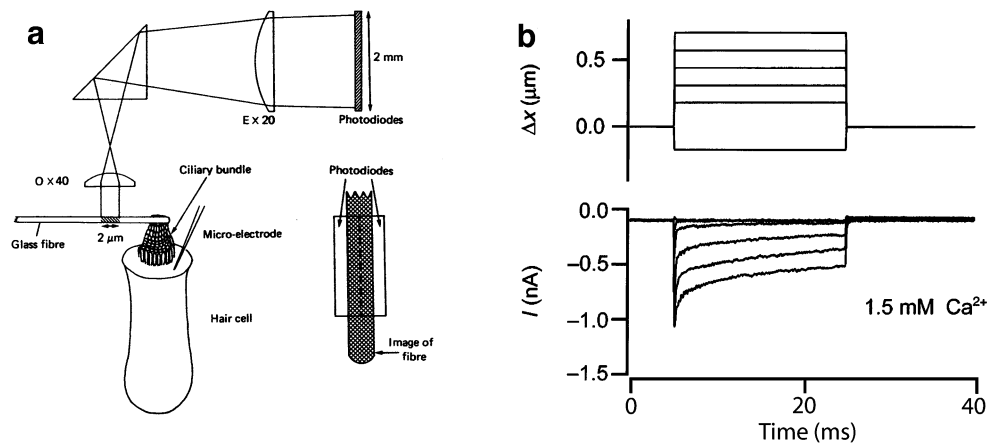
Hair cell mechanotransducer channels are permeable to calcium and other cations, have a relatively large single-channel conductance (100–300 pS), and have a mean open time that varies with bundle displacement [21]. Several aspects of gating require that the channel either contain or be intimately associated with a spring-like element that is extended to open the mechanotransducer channel [36]. The

identity of the gating spring remains unknown, and it could be intrinsic or extrinsic to the channel. However, the gating spring is likely to be distinct from the tip link, whose probable molecular structure lacks the required elasticity [41] (see below).

### Studying hair cell mechanotransduction

Progress in the detailed understanding of hair cell mechanotransduction accelerated with the development of *ex vivo* preparations of inner ear epithelia, principally from cold-blooded vertebrates, especially frogs and turtles. These approaches were subsequently replicated with mammalian tissues. While important insights were obtained with trans-epithelial current measurements [26], whole-cell voltage clamp of individual hair cells with patch electrodes has provided much of our present understanding. Essential technical developments for this work were the controlled application of microscopic force to the hair bundle and optical resolution of resulting movements in the nanometer range (Fig. 2a). To impose submicron movements, a fine glass fiber was attached to a piezo-electric bimorph operating in the cantilever mode [18]. A relatively stiff fiber could then be stepped against the hair bundle to impose a steady deflection. Dynamic measurements could be made using a more flexible fiber to move the bundle because this was then bent by the relative stiffness of the hair bundle. As will be described more fully below, the flexible fibers also provided the surprising observation that the hair bundle imposed its own self-generated motion onto the fiber! The other advantage of the flexible fibers is that, by calibrating their stiffness, the stiffness of the hair bundle could also be calculated. Calibration of the glass fiber probes followed methods originally developed for studies of molluscan cilia [92], and depends on comparison of the experimental fiber to a standard glass fiber of known stiffness [75]. The stiffness of a standard glass fiber was measured optically by deflection with known weights (crystals of p-nitrophenol whose mass was subsequently determined spectrophotometrically in a known volume of solvent) [22]. Finer, more flexible fibers were then pushed against the standard, and the relative bending of the two was used to calculate the stiffness of the more flexible fiber by reciprocity. Similarly then, the relative motion of the flexible fiber and the hair bundle enabled experimental estimation of hair bundle stiffness.

The second technical requirement was to be able to resolve submicrometer movements with the light microscope. This was achieved by projection from the microscope so that a magnified image of the glass fiber, or the hair bundle, fell across a photodiode pair (Fig. 2a). As the projected image “shadow” moved, difference currents from



**Fig. 2** Measuring mechanotransduction by hair cells. **a** Flexible glass fibers attached to piezo-electric “steppers” are used to produce controlled deflections of the hair bundle. The image of the glass fiber is projected onto a photodiode pair, whose output signals motions in the range of 1–500 nm. **b** Submicron deflections imposed onto the stereociliary bundle of an outer hair cell in the rat cochlea (postnatal

day 8, at 20°C, 1.5 mM calcium) caused inward membrane currents that activated and deactivated rapidly. In addition, the mechanosensitive current showed both fast and slow rates of decay (adaptation) during the sustained deflection (**a** from Crawford and Fettiplace [22], with permission; **b** from Kennedy et al. [42], with permission)

the photodiode pair could be used to indicate displacements in the range of 1–500 nm [22]. Thus, imposed, or intrinsic, motions of the hair bundle could be related to transducer currents measured from the same hair cell under voltage-clamp (Fig. 2b). In all hair cells examined to date, including those of mammals, the resulting membrane currents are inward-going (at negative membrane potentials) for “positive” deflections (toward the tallest stereocilia) and reverse in sign near 0 mV. The transducer currents activate and deactivate very rapidly (within a fraction of 1 ms) and inactivate more slowly during sustained deflections, as will be described further.

### Hair bundle adaptation

A fundamental aspect of mechanotransduction is that it adapts in the presence of a sustained deflection of the hair bundle. Adaptation is calcium-dependent and its time course varies from a submillisecond range in auditory hair cells of turtles [20] and mammals [42] to tens of milliseconds in vestibular hair cells of frogs [26] and mammals [34]. Several lines of evidence support the hypothesis that slower, “vestibular” adaptation is mediated by a nonmuscle form of myosin (myosin 1C) through calcium and ATP-dependent interaction with the stereocilia’s actin core [35]. Rapid, “auditory” adaptation has been postulated to result from direct calcium feedback onto the transduction channel itself, or a closely associated protein, favoring the closed configuration [21]. Recent studies have suggested some role for myosin in rapid adaptation as well [73]. For both fast and slow adaptation, the mechanism involves force feedback to generate active movements of the hair bundle that can be visualized in living cells. Under appropriate experimental conditions (especially

micromolar external calcium, as found in endolymph), these movements can become oscillatory [22, 51], and are related to the acoustic tuning frequency of hair cells in the turtle basilar papilla [64]. Thus, active force-generation by the hair bundle could serve as a source of nonlinear amplification and tuning. In addition to these effects on kinetics, adaptation causes a steady-state shift in the set-point of the stimulus–response curve of the hair bundle [20, 26]. This shift enables the hair cell to preserve its sensitive dynamic range in the face of sustained stimuli.

Thus, studies on mammalian and nonmammalian hair cells have detailed our picture of mechanotransduction. The “gating spring,” and its association to bundle movement through tip links, form the basis of this model. A myosin-based adaptation motor is proposed to tension the transducer channel/gating spring. The pulling strength of the myosin motor falls with a rise in calcium, resetting resting tension. Calcium also provides a much more rapid negative feedback that, perhaps directly, causes mechanotransducer channel closure. A variety of candidates have been nominated as the molecular bases for these functions. However, while myosin 1c has emerged as a likely adaptation motor [35], and cadherin 23 as a potential tip link [70], the identity of other elements remains obscure, in particular, those of the gating spring and the mechanotransducer channel. Direct biochemical isolation and identification seems near impossible for such rare proteins, with perhaps only 100 or so copies of the transducer channel in each of ~10,000 hair cells within the entire cochlea. Thus, molecular genetic strategies in the mouse have played an increasingly visible role in further pursuit of these questions. Numerous naturally occurring “deafness” mutations, as well as the ability to conduct transgenic experiments, make the mouse an important model for studying hair cells

of mammals, including topics ranging from age-related hearing loss [59] to central processing disorders [56].

### Transgenic mice

Genetically modified mice have played a central role in the study of hearing and deafness. As early as the turn of the twentieth century, mice with inherited hearing defects were an important tool for studying the function of the auditory system [50]. As genetic technology advances and we begin to identify individual molecular components of the auditory transducer, mutant mice have become increasingly valuable experimental models. This utility is further enhanced with the advent of cell-specific, inducible transgenesis.

An adaptation of the Cre-LoxP method, in which an extrinsically induced form of Cre-recombinase, such as Cre-ER<sup>T2</sup> that is activated by tamoxifen [27], or tetracycline-inducible Cre [78], is driven by a hair cell-specific promoter, offers promise for specific elimination of target gene function (see review by Gao et al. [29]). For example, hair-cell-specific, inducible Cre activity under the control of the *Math1* gene has been demonstrated [15]. *Math1* is expressed from around embryonic day 13 to postnatal day 7 in mouse hair cells [10, 87], providing a means by which floxed genes can be eliminated within this timeframe, but not thereafter. Additional efforts will involve other candidate “hair cell” genes, such as the POU domain class 4 transcription factor (*Pou4f3*) or the  $\alpha 9$  subunit of the hair cell acetylcholine receptor [29]. The ultimate goal of truly “ear-specific” genetic induction will require control of multiple promoters, such as the “deadbolt/doorlatch” approach, in which limited overlapping expression patterns apply to only the targeted tissue or cell type.

### Mutant mouse models for mechanotransduction

Independent of direct molecular genetic manipulation, naturally occurring deafness mutations in mice provide a rich experimental resource. We have selected a number of genes in this review to provide an overview both of

mechanotransduction and of the techniques being used to solve the problems associated with identifying the transducer channel and associated proteins. These include the transient receptor potential (TRP) channels, which have been shown to be important in mechanotransduction in other sensory systems (see articles by Kernan and Chalfie this issue), the genes implicated in Usher syndrome, which are thought to relate to the complex of accessory proteins at the hair cell stereociliary tips, and other nonconventional myosin molecules present in hair cell stereocilia.

### Usher syndrome

Usher syndrome type I (USH1) manifests as sensorineural deafness associated with blindness due to retinitis pigmentosa, accounting for over 50% of hereditary deafness and blindness in humans. Of the six recognized loci for USH1 mutations, five genes have been identified encoding the proteins Myosin 7a, Cadherin 23, Protocadherin 15, Harmonin, and Sans (Ush1g) (see Table 1). Mice with defects in these five proteins have been characterized and, despite normal retinal phenotypes, exhibit severe hearing impairments. Here we discuss how investigations using these mouse models have advanced our understanding of the complex interaction of proteins within hair cell stereocilia involved in shaping mechanotransduction.

### Myosin 7a

Defects in the *MYO7A* gene underlie syndromic (associated with other symptoms) and nonsyndromic recessive deafness in humans [30, 49, 83, 85]. *Shaker 1* mice, homozygous for *myo7a* mutations, exhibit cochlear and vestibular dysfunction [50]. A number of mutations in *myo7a* have been characterized with a variable range of phenotypic severity [53]. Kros et al. [44] utilized two of the shaker mutants to examine the function of myosin 7a in cochlear hair cells. *Myo7a*<sup>6J</sup> mice expressed low but detectable levels of (presumably) defective myosin 7a, while *Myo7a*<sup>4626SB</sup> mice expressed extremely low levels of myosin 7a and displayed

**Table 1** Summary of the genes and protein products discussed in this review

Gene	Gene product	Mouse model
<i>Myo7a</i>	Myosin 7a	Shaker1
<i>Cdh23</i>	Cadherin 23	Waltzer
<i>Pcdh15</i>	Protocadherin 15	Ames waltzer
<i>Ush1c</i>	Harmonin	Deaf circler and deaf circler 2 Jackson
<i>Ush1g</i> (Sans)	<i>Ush1g</i> (Sans)	Jackson shaker
<i>Trpa1</i>	TRPA1	TRPA1 knockout
<i>Mcoln3</i>	TRPML3	Varitint waddler
<i>Trpv4</i>	TRPV4	TRPV4 knockout

a more severe phenotype. To study effects on mechano-transduction, stereociliary bundles were deflected by a fluid jet (an alternative to the glass fiber method described above) and the resulting ionic currents recorded in voltage-clamp from cochlear hair cells of control mice and mice homozygous for mutations in myosin7a.

Transducer currents from both wild-type and heterozygous mice replicated those identified in earlier recordings from mammalian [45] and lower vertebrate [6, 20] hair cells. However, the deflection needed to generate a threshold current in the *myo7a* mutants was far greater than for wild type. Bundle movements of at least 150 nm were required to activate any transducer current in the mutants; a near saturating displacement in the wild type and heterozygote hair cells. These results demonstrated preserved functionality of the transducer channels in *Myo7a* mutants; however, the shifted operating range suggested a role for myosin 7a in adaptation. Indeed, adaptation was faster and more complete in mutant homozygotes, and *Myo7a* mutants did not display the transient rebound current following inhibitory bundle deflections characteristic of the adaptive process. These observations, and the knowledge that myosin 7a is expressed throughout the length of the stereocilia [33], led to a proposed refinement of the adaptation motor model. That is that myosin 7a connects the stereociliar membrane to the actin core, thereby contributing to anchoring and tensioning of membrane-bound elements such as the transducer channel. The effect of myosin 7a is to help maintain transducer channel and gating spring tension. With dysfunctional myosin 7a, the gating spring would be slack at rest, resulting in a low open probability of the transducer channel.

### Cadherin 23

In addition to hearing loss, the *waltzer* mouse model for Usher 1D exhibits erratic circling and head tossing behavior associated with vestibular defects. Stereocilia of *waltzer* hair cells are disorganized [25]. In 2001, the gene product of the *waltzer* locus was identified as cadherin 23, also known as otocadherin [13, 14, 25, 81, 86]. Cadherin 23 comprises a large transmembrane protein with an extracellular domain containing 27 cadherin motifs [25]. The extracellular domains of two homophilically interacting cadherin 23 molecules are predicted to span a distance as great as 200 nm [70]. As functional cadherin 23 is necessary for hair bundle integrity and the formation of cadherin dimers is known to be calcium-dependent [91], the extracellular domain of cadherin 23 emerged as a promising tip link candidate. Antibodies targeted against the cytoplasmic domain of cadherin 23 labeled throughout the stereocilia of developing mice (up to P5) and in the stereociliary tips of

mature mice. This immunolabeling was not seen in *waltzer* (cadherin mutant) mice. Immunogold labeling was apparent at both ends of the tip links in electron micrographs. When tip links were disrupted by  $\text{La}^{3+}$  or with the calcium chelators ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) or 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) [7, 70], this removed the cadherin immunolabeling, which reappeared given sufficient time for tip links to reform. Cadherin 23 can bind to myosin 1c when expressed in human embryonic kidney (HEK) cells, and cadherin 23 has a similar sensitivity to proteolytic digestion as does the hair cell's tip link. Finally, mutations in cadherin 23 are associated with a loss of tip links in zebrafish hair cells [71], and disrupts myo1c binding to the stereocilia of mouse hair cells [61].

However, Boeda et al. [12] had previously reported that cadherin 23 was only expressed in developing hair bundles. In light of the study published by Siemens et al., discussed above, they undertook a further immunological study of cadherin 23 expression in developing mouse and rat cochleae using antibodies raised against epitopes in both the intra- and extracellular domains of cadherin 23 [55]. These antibodies only detected cadherin 23 in developing hair bundles where, at the electron microscopic level, it appeared to be associated with links running between the rows of stereocilia. From P13, just before the auditory system had reached functional maturity, cadherin 23 could no longer be detected in hair bundles. Michel et al. also examined the immunoreactivity following treatment with calcium chelators and  $\text{La}^{3+}$ . They demonstrated that, in contrast to the results of Siemens et al., neither BAPTA nor  $\text{La}^{3+}$  affected the immunolabeling. They also showed that subtilisin treatment abolished cadherin 23 labeling in the stereociliary bundle. This led Michel et al. to conclude that cadherin 23 is a component of the transient lateral links in the developing hair bundle, which have properties distinct from those of the tip links.

Further complicating the issue is the fact that a polymorphism in the exon-encoding part of the extracellular domain of cadherin 23 has been connected to age-related hearing loss in humans [58]. If the extracellular cadherin motifs of cadherin 23 are components of the tip link, why should this mutation not affect the tip links of affected individuals from birth? If, on the other hand, cadherin 23 is an important component of the transient lateral links of hair bundles and vital for the development of the functional hair bundle, how do the bundles develop in afflicted patients and function until age-related hearing loss sets in?

### Protocadherin 15

Shortly after the discovery of cadherin 23 and its involvement in cochlear function, the gene product of the

*Ames waltzer* mouse was identified as a novel protocadherin, protocadherin 15 [4]. Protocadherin 15 was also confirmed to be one of the genes mutated in Usher syndrome type 1 [3, 5]. Subsequent analysis of the peptide sequence of the tip link antigen, a distinct antigen associated with tip and kinociliary links, identified this protein as protocadherin 15 [2]. Ahmed et al. reported the presence of multiple protocadherin 15 transcripts expressed in mouse inner ear using cDNA cloning and sequence analysis and demonstrated labeling compatible with that of the tip link complex in an immunohistochemical study targeting some of these protocadherin 15 isoforms.

Cooperativity between myosin 7a and protocadherin 15 was illustrated by Senften et al. [66]. The expression patterns of protocadherin 15 were examined immunohistochemically in myosin 7a mutant mice, while myosin 7a expression patterns were investigated in protocadherin 15 mutant mice. In both cases, the expression of one protein was perturbed by mutation of the other. Furthermore, binding studies demonstrated biochemical interactions between protocadherin 15 and myosin 7a. Senften et al. suggest that protocadherin 15 interacts with myosin 7a to regulate the developmental formation of the hair bundle. Protocadherin 15 is unlikely to form the tip link itself, as its extracellular domain is thought to be too short [72].

### Harmonin

This gene mutated in Usher 1C patients was independently identified by two groups in 2000 [11, 80]. Verpy et al. also identified the protein encoded by this gene using a cDNA library from mouse inner ear, demonstrated its presence in cochlear hair cells, and named it harmonin. Verpey et al. [80] described eight alternative exons from mouse *Ush1c* cDNA, five of which were previously uncharacterized. Eight different transcripts of harmonin, falling into three subclasses, were shown to be present in the inner ear. All of the subclasses contained two PDZ domains and a coiled coil domain. Two of the subclasses contained a third PDZ domain, one of which contained an additional coiled coil domain and a proline–serine–threonine-rich (PST) domain. PDZ, coiled coil, and proline-rich PST domains are important protein–protein interaction sites [39, 67, 88, 93]. In keeping with this composition, harmonin is suggested to be a key scaffolding protein in the stereociliary complex.

Harmonin has been shown to interact with myosin 7a [12], cadherin 23 [12, 69], protocadherin 15 [1, 63], Sans, and itself [1]. The ability of harmonin to form complexes with all of the identified Usher 1 proteins implies a central scaffolding role for this protein. Mice with mutated copies of the gene encoding harmonin (*Ush1c*) were profoundly deaf and displayed vestibular defects. Morphological

analysis revealed pathologically disorganized splayed stereocilia on cochlear and vestibular hair cells [40].

Recently, an additional role for harmonin in hair cell stereocilia was proposed by Yan et al. [90]. They demonstrated that harmonin can bind a novel isoform of GTPase regulator DOCK4, which is a potent activator of one of the Rho family of GTPases (Rac) [89]. Immunolabeling experiments revealed this isoform of DOCK 4 to be present in hair cell stereocilia. As Rho GTPases coordinate assembly of actin cytoskeleton and provide coupling between the cytoskeleton and signal transduction, the authors suggest that a harmonin-activated pathway could regulate actin cytoskeletal organization in stereocilia. Thus, harmonin could serve as an organizing link between the macromolecular complex of proteins mediating mechanotransduction and the dynamic actin core of the stereocilia.

### Sans (Ush1g)

Sans, the protein most recently identified with the Usher complex, is also a scaffolding protein. The gene encoding Sans was shown to be mutated in Usher syndrome 1G patients and Jackson shaker mutant mice [43, 84]. In *Jackson shaker* mice, hair bundles are disrupted and mice display cochlear and vestibular defects. In addition to identifying Sans as the mutated protein in Usher syndrome 1G, Weil et al. [84] demonstrated that Sans associates with harmonin. Adato et al. [1] also probed the possible interactions of Sans with all of the known Usher proteins and demonstrated that, in addition to harmonin, Sans can bind to itself and myosin 7a, tying it into the complex of usher proteins.

### TRP channels in auditory transduction

The identity of the transducer channel has proven to be a tantalizingly difficult question to address. The miniscule amount of protein present in the cochlea defeats current molecular biological approaches, demanding insightful experimental approaches to probe the molecular composition of the channel. A few promising candidates have been advanced, but none has claimed permanent title. Central to these investigations has been the need for genetic manipulations in mice to address the ultimate question of whether the candidate protein actually functions as the mechanotransducer channel. In the late 1980s, interest in amiloride-sensitive chloride channels [epithelial sodium channel (ENaC)] as the transducer channel arose. However, the ENaC knockout mouse generated a decade later exhibited no symptoms of abnormal cochlear or vestibular function and displayed

transducer currents indistinguishable from those of wild-type hair cells [65]. More recently, channels from the TRP family have come to light as promising transducer channel candidates, as recently reviewed [16]. Here, we summarize the involvement of TRP channels in mammalian auditory transduction.

### TRPA1

The involvement of TRP channels in mechanosensitivity is widely acknowledged (for reviews in nonmammalian systems, see articles by Kernan and Chalfie, this issue). Corey et al. [17] screened for the presence of all 33 members of the TRP family in the mammalian inner ear using *in situ* hybridization. The probes for TRPA1 labeled hair cells of the cochlear and vestibular systems, prompting a closer look at this subtype and its function in the inner ear. In addition to the *in situ* hybridization, immunofluorescent labeling suggested that TRPA1 was present in hair cell stereocilia. Exposure to  $\text{La}^{3+}$  and calcium chelators, agents known to disrupt tip links [7, 70], resulted in a loss of TRPA1 immunoreactivity in saccular hair cells. These data provided some evidence that TRPA1 could be present in hair cells in a manner compatible with that of the transducer channel. To gain functional data to corroborate these localization studies, small interfering RNAs (siRNAs) were used to inhibit the translation of TRPA1 in hair cells. In hair cells infected with siRNAs, transducer currents were reduced in amplitude, though not eliminated. Adaptation and the resting position of the activation curve for the transducer current remained unchanged. Collectively, this evidence led the authors to conclude that TRPA1 played some role in mechanotransduction in mammalian hair cells. In complementary studies, Nagata et al. [57] reported immunolabeling for TRPA1 in hair cell stereocilia using another antibody targeted to a different region of TRPA1. This study also reported similarities between the conductance properties of TRPA1 expressed in HEK cells and those of native hair cell transducer channels. Despite this strong circumstantial evidence, a TRPA1 knockout mouse had no hearing defects, and transducer currents were normal in vestibular hair cells [46]. On balance then, TRPA1 may play some role in maintaining mechanotransduction, but either it is not essential or another molecule can be upregulated to restore complete functionality.

### TRPML3 and TRPV4

Defects in two other TRP channels have been implicated in hearing loss in mouse mutants. *Varitint waddler* mice have

mutations in the gene encoding TRPML3. These mice exhibit deafness and circling behavior attributable to degeneration of stereocilia and disruption of cellular trafficking [24]. Although TRPML3 antibody labeled hair cell stereocilia faintly, the highest levels of expression were associated with cytoplasmic organelles [24]. Consistent with the progressive degeneration of hair cells in these mutants, it is likely that TRPML3 is involved in organelle trafficking and that a dysfunctional TRPML3 protein inhibits hair cell development and function.

TRPV4 knockout mice have delayed-onset hearing loss and increased susceptibility to noise damage [76]. However, this TRP channel clearly is not associated with the transduction apparatus in the sensory hair cells. Rather, TRPV4 is expressed in the stria vascularis [48], the epithelium involved in generating endolymph and the endocochlear potential across the hair cells—the driving force for the transducer current. It is not surprising that interruption of endolymphatic function would have deleterious effects on cochlear function, illustrating just one of the many alternative ways a given class of proteins can influence hearing.

### Gating spring properties—ankyrin vs cadherin

An additional route by which to explore the nature of the transducer channel is through the gating spring. Analysis of channel gating and the architecture of the spring further our understanding of the intrinsic properties necessary for a protein to act as the gating spring. It is unknown if the gating spring is part of the mechanotransducer channel itself or a distinct protein in series with it and the tip link. In keeping with the hypothesis that the transducer channel may be a TRP, the suitability of ankyrin repeats, extracellular domains of TRP channels, for the demanding role of the gating spring has been considered. The functionally defined transducer channel gating spring is an elastic element with a maximal extension of 125–250 nm to accommodate the largest bundle deflections, and a relaxation time in the order of 10  $\mu\text{s}$  necessary for following high-frequency acoustic stimuli [19, 35, 37]. In the first atomic force microscopic experiments to fully resolve the refolding force of a protein domain, Lee et al. [47] directly measured properties of ankyrin repeats. They demonstrate that ankyrin repeats can act as linear and fully reversible springs and, furthermore, exert a force during refolding [47]. This goes some way towards substantiating modeling predictions [72] that the properties of ankyrin repeats are better suited to those of the gating spring, a tidy solution if a candidate TRP is found to be the transducer channel. Of the TRP channels, only TRPA1 and TRPN1 have enough ankyrin repeats to form a molecular spring [72].

It is thought to be unlikely that the tip link itself is the gating spring for the mechanotransducer channel. Kacher et al. [41] analyzed the structure of the tip link using rapid-freeze, deep-etch electron microscopy. The resulting high-resolution images showed the tip link to be a helically coiled pair of protofilaments. The coiled double filament is anchored by two points at its uppermost end on the taller stereocilium and by three points to the tip of the next lower stereocilium. It is argued that the stiffness of such a coiled structure renders it incompatible with the elastic gating spring. Sotomayor et al. [72] performed molecular dynamic simulations with crystallographic structures of ankyrin and cadherin repeats to examine their mechanical properties. Their models suggest that cadherin repeats require huge forces to unfold and extend, with a slow time course of refolding. This is in agreement with the notion that cadherin repeats could form the tip link but not the elastic gating spring.

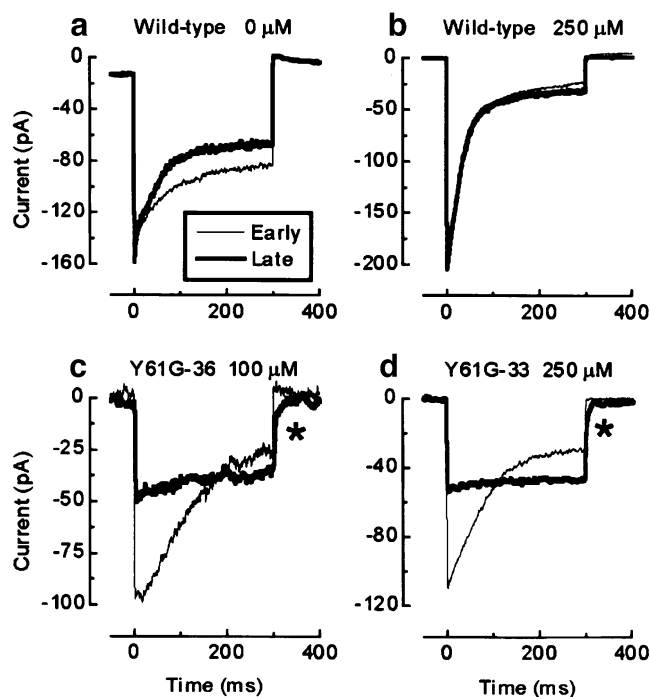
### Slow adaptation and nonconventional myosins

#### Myosin 1c

Although existing studies into the function of myosin 1c in stereocilia were carried out on vestibular hair cells, they merit discussion here, as conserved mechanisms may exist between vestibular and cochlear hair cells. On the other hand, differences between the two cell types could be enlightening if they provide examples of the ways in which hair cells with similar overall structure are specialized to carry out different functions. In addition, as described next, transgenic mice have been put to innovative use in studies of myosin 1c.

Holt et al. [35] employed transgenic mice with a mutation in myosin 1c to examine the slow component of adaptation in vestibular hair cells [31, 33, 82]. The alteration of myosin 1c (tyrosine 61 to glycine; hence, *Y61G* mice) rendered it susceptible to inhibition by modified  $N^6$ ADP analogs. Thus, in these mice, myosin 1c remained functional *until* the modified  $N^6$ ADP analogs were applied. Prior to drug application, transducer currents in *Y61G* mice were comparable with those of wild-type mice. However, when the  $N^6$ ADP analog was introduced into a hair cell through the recording pipette, adaptation of the transducer current was eliminated. As it took time for the  $N^6$ ADP analog to diffuse into the cell from the pipette, it was possible to record control transducer currents in the same cells from which currents were recorded in the presence of the  $N^6$ ADP analog.

Figure 3 shows representative transducer currents in response to a 0.6- $\mu$ m hair bundle deflection prior to and following the diffusion of the  $N^6$ ADP analog into the cell [35]. Wild-type hair cells showed pronounced slow adap-



**Fig. 3** Mechanosensory adaptation depends on functional myosin 1c. Mechanosensitive currents activated in mouse utricular hair cells by a 0.6- $\mu$ m positive deflection of the hair bundle. **a** In wild-type hair cells, transducer currents adapt with 40–50 ms time constant at room temperature. **b** Wild-type transducer currents are unchanged in the presence of a modified form of ADP that interrupts mutant but not wild-type myosin. **c** Application of NMB-ADP gradually removes adaptation in mice with a sensitizing mutation to myosin 1c. **d** Similar effects obtained in another myosin 1c mutant mouse line (from Holt et al. [35], with permission)

tation that was unchanged in the presence of  $N^6$ ADP. However, in hair cells from mice containing the *Y61G* form of myosin, adaptation was gradually lost in the presence of the modified ADP. This appeared to be a specific effect on adaptation because transducer currents in the *Y61G* hair cells were initially identical to those of wild-type cells. More recently, vestibular transducer channel adaptation was studied using mice in which *Y61G* myosin entirely replaced the wild-type form by homologous recombination [73]. Here, both slower and faster adaptation was substantially diminished when the “blocking” ADP analog was introduced into the cell, suggesting that myosin 1c may contribute to both processes. It remains to be determined whether similar effects can be shown for the order-of-magnitude-faster adaptation in cochlear hair cells [42].

The presence of other myosin isoforms has been reported in stereocilia and the cuticular plate, in addition to myosin 7a and myosin 1c, to date myosin 15a (see below), myosin 6 [33], and myosin IIa [54] have been identified. Understanding the interactions between these different myosin molecules and their significance in transducer channel adaptation requires further investigation. Transgenic or mutant mice for these myosins will help

elucidate the interactions with other stereociliary proteins and the transducer channel complex.

### Myosin 15a and whirlin

*Shaker-2* mice that possess mutated forms of myosin 15a exhibit profound hearing and balance disorders [28]. Stereocilia of *shaker-2* mice are stunted but arranged in a regular fashion. Myosin 15a interacts with the PDZ-domain-containing-protein whirlin, and the interaction of these proteins during development is vital for formation of a functional hair bundle [8, 9, 23, 52]. However, transducer currents in mutant mice resembled those of wild type [74], suggesting that, although myosin 15a is essential for hair bundle formation, it does not affect mechanotransduction and is not involved in adaptation of the transducer current.

### Summary

Sensory hair cells of the inner ear convert mechanical energy into graded receptor potentials through the gating of displacement-sensitive ion channels in the ciliary (hair) bundles. Mechanotransduction is regulated dynamically to maintain optimum sensitivity and speed. We are beginning to gain insights into how this regulation may occur and which proteins may be involved. Certainly, many different proteins and structures will interact to set the mechanical properties of the hair bundle. These interactions will provide linkages to previously unknown participants. Equally, however, these linkages will limit the functional interpretation possible for single-protein modification. Nonetheless, transgenic mice promise opportunities for future research into the process of mechanotransduction in the auditory system. The technology to time the knockout of a gene of interest in a cell-specific manner offers opportunities for determining the functional interactions among the proteins that facilitate mechanotransduction in the stereocilia. For example, TRPML3 could be knocked out in a controlled, hair-cell-specific manner after the onset of hearing. Mechanotransduction could then be studied with less impact on cellular trafficking mediated by TRPML3 during hair cell development.

Questions remaining open at present concern the identity of the transducer channel and its gating element, the structure of the tip link and the gating spring, and the role of different myosin isoforms in slow adaptation. Transgenic technology will undoubtedly contribute to deciphering the molecular mystery of auditory transduction. When the enigmatic transducer channel is identified, we will be better positioned to investigate intrinsic properties of the channel, how these properties control mechanotransduction, and to what extent they are affected by external factors.

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