A genetic approach to understanding auditory function

Karen P. Steel[1] & Corné J. Kros[2]

Little is known of the molecular basis of normal auditory function. In contrast to the visual or olfactory senses, in which reasonable amounts of sensory tissue can be gathered, the auditory system has proven difficult to access through biochemical routes, mainly because such small amounts of tissue are available for analysis. Key molecules, such as the transduction channel, may be present in only a few tens of copies per sensory hair cell, compounding the difficulty. Moreover, fundamental differences in the mechanism of stimulation and, most importantly, the speed of response of audition compared with other senses means that we have no well-understood models to provide good candidate molecules for investigation. For these reasons, a genetic approach is useful for identifying the key components of auditory transduction, as it makes no assumptions about the nature or expression level of molecules essential for hearing. We review here some of the major advances in our understanding of auditory function resulting from the recent rapid progress in identification of genes involved in deafness.

Deafness genes

Progress in identifying genes involved in deafness has been remarkable over the past few years. At the end of 1996, no non-syndromic deafness genes had been cloned[1]. In the 4 years since then, 19 new genes involved in non-syndromic deafness have been identified, together with an even larger number of genes implicated in syndromic deafness (Table 1). Deafness is a relatively common disorder, with approximately 1 in 800 children born with a serious permanent hearing impairment, and very large proportions of the population suffering progressive hearing loss as they age (60% of people over 70 have a hearing loss of 25 dB or greater[2]). Whereas single-gene defects probably account for over half of the cases of childhood deafness, the nature of the genetic contribution to progressive hearing loss has not yet been clearly defined. So far, 70 loci involved in non-syndromic deafness have been reported (Box 1), and over 400 distinct syndromes including hearing impairment are listed in Online Mendelian Inheritance in Man. Thus, there are many more genes awaiting identification.

Rapid progress has also been made in identifying deafness genes in the mouse. Over 90 different genes have now been identified that affect inner-ear development or function, with many more loci known to be involved in deafness but not yet identified[3] (Box 1). In addition to these, many other genes have been identified that affect the middle ear or central auditory pathways[3]. Relatively few of the identified mouse deafness genes, however, have been shown to be involved in human deafness. Only eight of the mouse deafness genes found provide non-lethal models for non-syndromic deafness in humans: *Myo7a* (ref. 4), *Myo15* (ref. 5), *Pou3f4* (refs. 6,7), *Pou4f3* (ref. 8), *Col11a2* (ref. 9), *Tecta* (ref. 10), *Cdh23* (ref. 11) and *Pds* (ref. 12). Moreover, only around 20 of the mouse deafness genes provide models of the 400-plus human syndromes including deafness (Box 1). The limited overlap in deafness genes identified in mice and humans indicates that there are still many deafness genes that have yet to be found in both species.

Despite the obvious gap in the catalog, we now have identified a significant number of bona fide deafness genes. So, how has the identification of these deafness genes helped our understanding of the molecular basis of auditory function?

Sensory hair cells

Sensory hair cells of the inner ear have precisely organized, finger-like projections called stereocilia, which are arranged in bundles at their upper surface (Figs. 1 and 2). They are packed with actin filaments, forming a core, and are deflected by the vibration of sound. The most likely model for hair-cell function proposes that deflection of the stereocilia pulls on fine links that join adjacent stereocilia at their tips. The tip link acts as a gating spring to open one or more transduction channels, allowing cations to flood into the cell and depolarize it[13–15]. The extremely rapid rate of hair-cell responses (hair cells in some species respond at over 100 kHz) requires that the opening of the transduction channel be direct, because transduction using a second messenger system (as found in olfaction or vision) would be far too slow.

Essential molecules for hair-cell transduction

Several molecules have been identified as having a vital role in hair-cell transduction because they are specifically expressed in or around the stereocilia and mutations in their genes lead to deafness. Three are unconventional myosins: myosins VI, VIIA and XV (refs. 4,5,16). Mutant myosin VI leads to progressive fusion of hair cell stereocilia, mutant myosin XV results in short stereocilia, and mutant myosin VIIA leads to progressive disorganization of the stereocilia bundle[5,17,18]. These specific defects have been discovered through the study of mice carrying the relevant mutations; defects in myosins VIIA and XV are also involved in human deafness[19–22]. The human mutations presumably have effects on hair-cell development similar to those in the mouse. Myosin VI may serve to anchor the apical hair cell membrane covering the

[1]*Medical Research Council Institute of Hearing Research, University of Nottingham, Nottingham, UK.* [2]*School of Biological Sciences, University of Sussex, Falmer, Brighton, UK. Correspondence should be addressed to K.P.S. (e-mail: karen@ihr.mrc.ac.uk).*

stereocilia to the actin-rich intracellular cuticular plate located just below the stereocilia array (Fig. 1), to counter the natural tendency of the lipid membranes to 'zip up'[18], which would lead to fusion of the stereocilia. This suggestion fits well the observation that, compared with other myosins, myosin VI acts in a 'reverse' direction along actin filaments[23]—that is, it would move down the stereocilium. Myosin XV may have a more direct effect on actin filament formation, as abnormal actin aggregates are observed in hair cells of mice with mutant myosin XV (ref. 5).

Single-hair-cell measurements from mice with mutations in the myosin VIIA gene (*Myo7a*) indicate that when the stereocilia are in their resting position (that is, not stimulated by sound), the gating springs seem to be slack and the transduction channels are closed[24]. This contrasts with normal hair cells, which maintain tension on the gating springs, keeping a small proportion of the channels open even at rest. In the *Myo7a* mutants, transducer currents can be measured, reflecting cation flow into the hair cell through open transduction channels, but only when the stereocilia bundles are strongly deflected[24]. This observation suggests that myosin VIIA may have a key role in adjusting the tension of the tip-link/transduction channel complex.

The initial identification of myosin VIIA by genetic means[4] has led to a flurry of activity involving other approaches to try to identify its precise role in hearing and deafness, and to identify other components of the transduction apparatus that may be candidates for additional deafness genes. For example, a yeast two-hybrid screen has identified a new transmembrane protein, vezatin, that binds to the FERM (4.1, ezrin, radixin, moesin) domain of the myosin VIIA tail[25]. Vezatin may be associated with the ankle links, a subset of the lateral links that hold the stereocilia together. It has also been suggested that myosin VIIA might interact with harmonin, the recently discovered PDZ

Table 1 • Genes and hearing loss

Some molecules involved in non-syndromic deafness[a]

Molecule	Gene	Locus	Type of protein
connexin 26	*GJB2*	DFNB1, DFNA3	channel component
connexin 31	*GJB3*	DFNA2+recessive form	channel component
connexin 30	*GJB6*	DFNA3	channel component
KCNQ4	*KCNQ4*	DFNA2	channel component
pendrin	*PDS*	DFNB4+Pendred	ion transporter
myosin 7A	*MYO7A*	DFNB2, DFNA11+Usher	motor molecule
myosin 15	*MYO15*	DFNB3	motor molecule
MYH9	*MYH9*	DFNA17	motor molecule
diaphanous	*DIAPH1*	DFNA1	cytoskeletal protein
POU3F4	*POU3F4*	X-linked DFN3	transcription factor
POU4F3	*POU4F3*	DFNA15	transcription factor
α-tectorin	*TECTA*	DFNB21, DFNA8/12	extracellular matrix
coch	*COCH*	DFNA9	extracellular matrix
collagen11α2	*COL11A2*	DFNA13+Osmed	extracellular matrix
otoferlin	*OTOF*	DFNB9	synapse component
DFNA5	*DFNA5*	DFNA5	novel
TMPRSS3	*TMPRSS3*	DFNB10	serine protease
claudin14	*CLDN14*	DFNB29	junction protein
otocadherin	*CDH23*	DFNB12	cadherin

Some molecules involved in syndromic deafness[b]

Molecule	Gene	Structures involved	Syndrome	Type of protein
connexin 32	*GJB1*	peripheral nerves	Charcot-Marie-Tooth	channel component
ATP6B1	*ATP6B1*	renal acidosis	renal tubular acidosis and deafness	ion pump
pendrin	*PDS*	thyroid	Pendred	ion transporter
KvLQT1	*KCNQ1*	heart	Jervell and Lange-Nielsen	channel component
IsK	*KCNE1*	heart	Jervell and Lange-Nielsen	channel component
myosin 7A	*MYO7A*	retina	Usher 1B	motor molecule
EYA1	*EYA1*	kidney, jaw	branchio-oto-renal	transcription factor
PAX3	*PAX3*	pigmentation	Waardenburg type 1	transcription factor
MITF	*MITF*	pigmentation	Waardenburg type 2	transcription factor
SOX10	*SOX10*	pigmentation, gut	Waardenburg type 4	transcription factor
EDNRB	*EDNRB*	pigmentation, gut	Waardenburg type 4	receptor
EDN3	*EDN3*	pigmentation, gut	Waardenburg type 4	ligand
FGFR3	*FGFR3*	skull	craniosynostosis with deafness	receptor
treacle	*TCOF1*	skull and jaw	Treacher Collins	trafficking protein
norrin		eye, brain	Norrie	extracellular matrix
collagens 4	*COL4A3/4/5*	kidney	Alport	extracellular matrix
collagen 2	*COL2A1*	eye, joints, palate	Stickler	extracellular matrix
collagen 11	*COL11A1/2*	skeleton	Osmed, Stickler	extracellular matrix
USH2A	*USH2A*	retina	Usher 2A	extracellular matrix
harmonin	*USH1C*	retina	Usher 1C	PDZ clustering protein
otocadherin	*CDH23*	retina	Usher 1D	cadherin
DDP	*TIMM8A*	muscle	DFN1	mitochondrial protein

[a]DFNA loci are autosomal dominant, DFNB loci are autosomal recessive, and DFN are X-linked. Syndromes are indicated where the molecule is also involved. [b]Other major systems involved in the syndrome are indicated. For further details see Box 1.

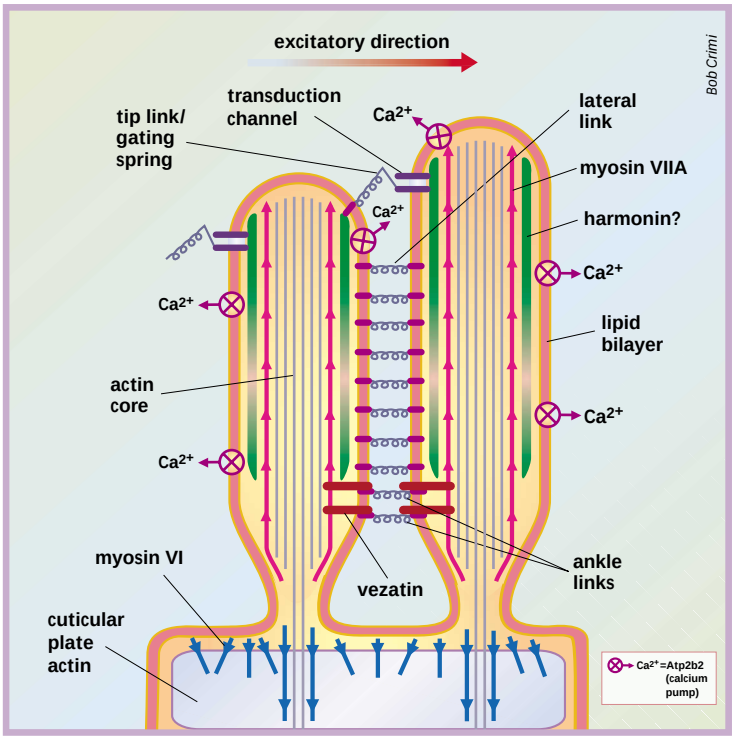

Fig. 1 Schematic illustration of the top, apical surface of a hair cell, showing two of the tens to hundreds of stereocilia that make up the hair bundle and are inserted into the cuticular plate. Movement in the excitatory direction tips the stereocilia over to the right and opens the transduction channels due to increased force on the tip links, which act as gating springs. The stereocilia contain a core of parallel actin filaments oriented so as to enable upward movement (towards the tips of the stereocilia) of myosins, except myosin VI, which moves backwards. Main functional localizations of myosin VIIA and myosin VI are indicated, with the direction of movement/force indicated by arrowheads. Myosin VI, which may hold down the apical cell membrane below the stereocilia, is uniformly abundant throughout the (pale blue) cuticular plate actin (not shown). Vezatin may be involved in tensioning the ankle links by connecting them, by means of myosin VIIA, to the actin core. Harmonin (speculative localization shown, green) or other as yet undiscovered PDZ-domain–containing proteins may similarly mediate tensioning by myosin VIIA of other lateral links and tip links. The Atp2b2 calcium pump is distributed quite uniformly over the stereociliary membrane.

(postsynaptic density protein, disc-large, zo-1)-domain–containing protein present in stereocilia and known to underlie Usher syndrome type 1C (refs. 26,27). By indirectly binding such transmembrane and membrane-associated proteins to the actin core, myosin VIIA might tension the various types of linkages (tip links and lateral links) between the stereocilia.

Mutations in two new cadherin-related genes were reported in the deaf mouse mutants waltzer[11] and Ames waltzer[28] in the last issue of *Nature Genetics*. Intriguingly, both mouse mutants show disorganization of the stereocilia bundle reminiscent of the disorganization seen in *Myo7a* mutants[17] (Fig. 3). Cadherins are often thought to be involved in cell-cell interactions, but disorganized stereocilia in waltzer and Ames waltzer mutants indicate that these cadherins might instead be involved in the lateral links or even the tip links that join adjacent stereocilia. Vezatin or harmonin might be involved in these linkage complexes as well.

Another newly identified and essential cytoskeletal component of stereocilia is espin, an actin-bundling protein reported to be depleted in the deaf mouse mutant jerker, which has a frameshift mutation in the espin gene[29] (*Espn*).

The transducer channel seems to be relatively non-selective for different cations, and it is likely that both potassium and calcium ions flow into the hair cell through the open transducer channel[30,31]. A rapid influx of calcium would have immediate effects on the cytoskeleton of the stereocilia, and so might be expected to be carefully controlled. A calcium pump was shown to be abundant in the cell membrane covering each stereocilium, allowing local control of calcium levels by pumping the ions out of the stereocilium[32]. The finding of mutations in *Atp2b2* in the deaf waddler mouse mutant, associated with a lack of the calcium pump in hair cells of this mutant, provided the molecular identity of the pump[33,34]. The pump is also located at the base of inner hair cells of the cochlea (Fig. 2), where auditory nerve fibers form synapses, and may thus also be involved in local control of calcium at the synapse.

Unique components of hair-cell synaptic activity

Synapses of cochlear hair cells, specifically inner hair cells that receive the bulk of the afferent innervation of the cochlea, have unique demands placed on them by the extremely rapid response time of the hair cells and the requirement for this to be faithfully transmitted to the cochlear nerve. Accurate timing information is necessary to permit a range of functions, such as perception of timing differences between the two ears to allow localization of the source of a sound. Thus, it is not surprising that some components of the synaptic apparatus seem to be specialized. The expression of *Atp2b2* is one example, although it is expressed in the brain as well as in hair cells[33].

The otoferlin gene, *OTOF*, is mutated in some cases of dominantly inherited, progressive deafness in humans[35]. Its protein is located at the base of inner hair cells, next to the synaptic region (Fig. 2). Together with its sequence similarity to other ferlin molecules, these features indicate that it might be involved in synaptic vesicle recycling—although a mouse model is not yet available to test this hypothesis.

In contrast to neurons of the central nervous system, which use N-, P- or Q- type calcium channels, L-type calcium channels are required to couple sound-induced depolarization to neurotransmitter release in auditory hair cells. This was demonstrated using mice lacking a class D L-type calcium channel[36] (D-LTCC, encoded by *Cacna1d*). The null mutants grew and reproduced normally, despite a propensity to cardiac arrhythmia, but were profoundly deaf and their inner hair cells had diminutive voltage-gated calcium currents. L-type calcium channels activate rapidly and inactivate very slowly, thus allowing the precisely timed periodic release of neurotransmitter essential for normal hearing. So far, it is not known whether any forms of human deafness are associated with defects in this ion channel.

Missing molecules

It is clear that although these known essential molecules are highly likely to have critical roles in hair-cell function, the molecular basis of many key steps has yet to be established. Two key molecules that have not yet been identified are the transducer channel and the outer hair cell motor. The latter is known to be abundant in the basolateral membranes of outer hair cells, and responds to hair cell stimulation by changing conformation, thus changing the shape of the hair cell and providing amplification of the vibration of the cochlear partition in a localized region. In the past year, exciting candidates for these two molecules have emerged. A fructose transporter, Glut5, and the newly identified prestin molecule (which has similarity to sulfate transporters) have both been proposed as candidates for the outer hair-cell motor on the basis of physiological features and expression patterns[37,38] (Fig. 2). When expressed in a

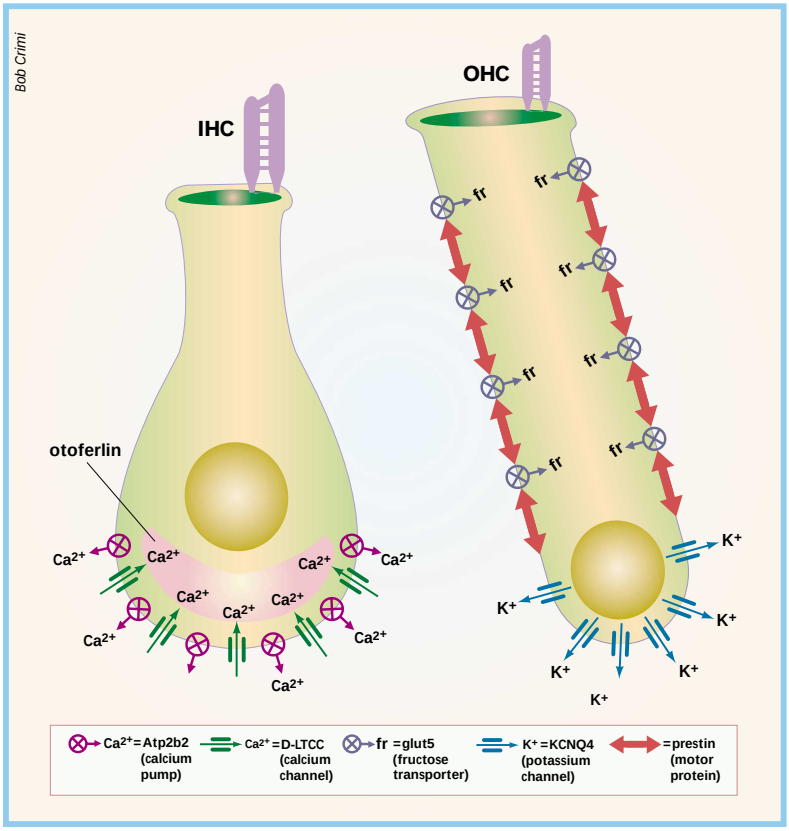

Fig. 2 Schematic illustrations of an inner hair cell (IHC, flask shaped) and an outer hair cell (OHC, cylindrical) showing locations in the basolateral membrane of functionally important gene products. Inner hair cells are true sensory receptors that signal the reception of sound to the brain through afferent auditory nerve synapses. It is thus perhaps not surprising to find hearing defects associated with molecules that may affect the synaptic transmitter release function of these cells: the D-LTCC L-type calcium channel, the Atp2b2 calcium pump and otoferlin. Outer hair cells have a localized motor function within the cochlea with which prestin and possibly Glut5 are thought to be associated. The potassium channel KCNQ4 is likely to be involved in potassium recycling. The different expression pattern of these genes in inner compared with outer hair cells emphasizes their different functions in cochlear responses.

kidney cell line, prestin endows the cells with electro-motile properties much like outer hair cells, an important test to which Glut5 has not yet been put. Moreover, the developmental increase of prestin immunolabeling in the lateral wall of outer hair cells has the same time course as the acquisition of electromotility, whereas Glut5 immunolabeling appears later[39]. Ablation of these two genes in mice will no doubt shed light on their respective roles.

A genetic approach in *Drosophila melanogaster* has led to the identification of a candidate for the transduction channel of vertebrate hair cells. Random mutagenesis by ethyl methanesulfonate (EMS) followed by screening for bristle defects led to the identification of the gene *nompC*, which encodes a product with features in common with the hair-cell transduction channel, including adaptation to sustained stimuli and directional selectivity[40].

Tectorial membrane defects

The tectorial membrane (shown in pale gray in Fig. 4) is an extracellular gel-like matrix delicately positioned above the hair cells, providing a mass against which stereocilia can bend. Mutations in three different genes, encoding α-tectorin (*Tecta*), collagen 11α2 (*Col11a2*) and otogelin (*Otog*), all lead to ultrastructural defects of the tectorial membrane associated with moderate impairment of cochlear function[9,10,41]. The *Tecta*$^{-/-}$ mouse has been used to define precisely the role of the tectorial membrane in cochlear biophysics. As expected, mechanical responses in the cochlea of the mutants show raised thresholds and thus reduced sensitivity, but surprisingly the responses do still show reasonable tuning[10] (enhancement of the response at a particular frequency of sound stimulation compared with responses to other frequencies). Mutations in two of these genes (*COL11A2* and *TECTA*) also lead to human hearing impairment[9,42].

Ionic environment of hair cells

One of the surprising aspects of the genetic approach to understanding auditory function has been the number of deafness genes that appear to affect ionic homeostasis in the cochlear duct. The fluid bathing the upper surface of the hair cell, called endolymph, has a high potassium and low sodium concentration and is maintained at a high positive resting potential of around +100 mV in the mouse. This high resting potential is essential for normal hair cell function, because when it is reduced to zero, the result is deafness[43]. The organ of Corti, including the sensory hair cells and supporting cells, sits on a relatively permeable basilar membrane. Below this is another fluid-filled channel containing perilymph (shown in pink in Fig. 4), high in sodium and low in potassium, much more like a normal extracellular fluid. Within the organ of Corti, the basolateral membranes of inner hair cells are surrounded by support cells, but the body of outer hair cells is exposed to another fluid, cortilymph (shown in yellow in Fig. 4), with a potassium concentration slightly higher than that of perilymph. Potassium ions accumulate in the cortilymph during exposure to loud sounds, and reach even higher concentrations in the extracellular space around inner hair cells[44]. The particular ionic environment of the hair cell is

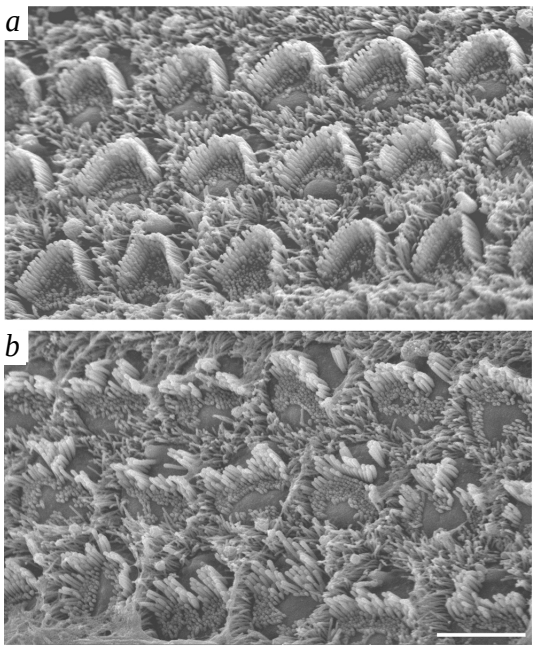

Fig. 3 Scanning electron micrographs of the upper surface of outer hair cells, showing the stereocilia bundles. ***a***, Control at four days old, showing three rows of outer hair cells with neat V-shaped arrays of stereocilia. ***b***, A waltzer mutant (v^{Alb}/v^{Alb}) showing three rows of outer hair cells with disorganized stereocilia. The v^{Alb} mutation is described in ref. 11. Scale bar, 5 μm. Figure provided by R. Holme.

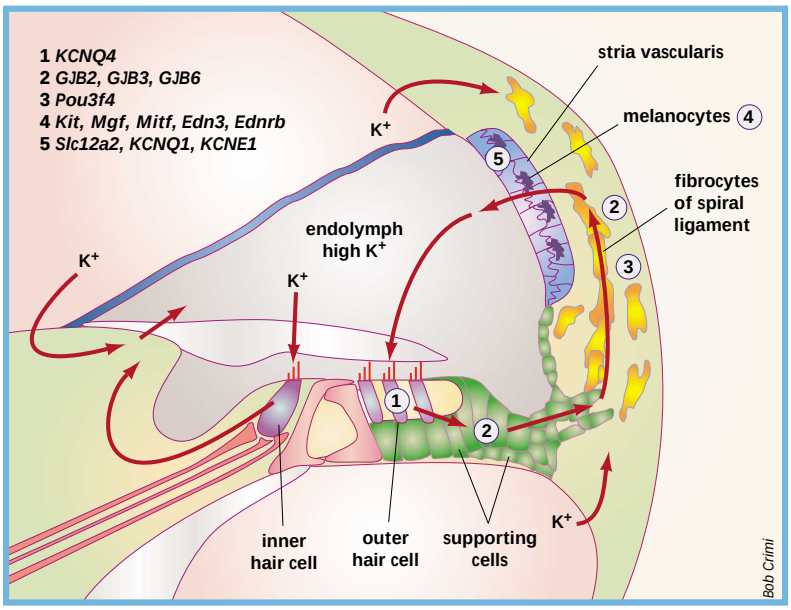

Fig. 4 Illustration of cochlear duct showing the proposed lateral (right) and medial (left) K^+ ion recycling pathways from the sensory hair cells within the organ of Corti back to the endolymph[48]. During sound stimulation, K^+ floods into the hair cells and must be removed. The lateral route for recycling involves K^+ transport through supporting cells of the organ of Corti and fibrocytes of the lateral wall through gap junctions to the stria vascularis, where it is pumped out into the endolymph. The medial route involves passage through supporting cells to the left of the inner hair cells via gap junctions to the interdental cells on the lumenal surface, which may pump the K^+ back into the endolymph (small red arrow on left). Excess K^+ in the perilymph above and below the endolymph-filled cochlear duct may be taken up for recycling by either route, as shown by arrows.

clearly important to its function, because mutations affecting endolymph lead to deafness.

It has been known for some time that the potassium pumped into the endolymph is not immediately derived from the rich blood supply of the stria vascularis[45,46] (the structure on the lateral wall of the duct responsible for generating the endolymph; shown in blue in Fig. 4). Instead, it has been proposed that the potassium ions may be recycled within the cochlear duct[47,48] (Fig. 4). After leaving the hair cells, the potassium is thought to be taken up by the supporting cells of the organ of Corti, and returned to the stria vascularis for pumping back into the endolymph. There are several possible routes for potassium recycling, including a lateral route through an extensive network of gap junctions linking supporting cells to the fibrocytes of the spiral ligament and back to the stria vascularis[47], and routes through the perilymph to the spiral ligament either above or below the endolymph compartment and hence to the stria[49]. Another route is a medial recycling pathway not involving the stria vascularis[48]. In this proposed pathway, excess potassium is returned to the endolymph by means of medial supporting cells, spiral limbus fibrocytes and interdental cells, all coupled by gap junctions, and pumped out into endolymph by Na-K-ATPase pumps expressed in interdental cells[47–49].

Molecules involved in homeostasis

Mutations have been reported to affect several components of these recycling routes, providing strong support for the model as well as identifying some of the molecules involved.

KCNQ4 encodes a potassium channel and is mutated in dominant, progressive hearing loss[50]. Within the cochlea, immunolabeling of the channel protein is found predominantly in outer hair cells, and may serve as the first step in recycling by allowing potassium to flow out of the cells through their basolateral membranes, into the cortilymph. Its physiological properties when expressed in *Xenopus laevis* oocytes support this suggestion[50]. Subsequent experiments[51] established that KCNQ4 is a component of the ion channel that underlies the potassium current $I_{K,n}$. This current is characteristic of outer-hair cells[52] and is activated at the cell's normal resting potential, thus allowing potassium ions flowing in through the transduction channels to exit the cell. Most of the reported mutations act in a dominant-negative manner *in vitro* in *X. laevis* oocytes, but at least one mutation seems to exert its effect through haploinsufficiency[53]. KCNQ4 immunolabeling has also been seen in auditory brainstem nuclei[54] and mRNA expression is seen in some inner-hair cells[55]. Still, there is no evidence for the presence of $I_{K,n}$ in inner-hair cells[51], and no animal model is yet available to unravel the mechanism leading to hearing impairment consequent to KCNQ4 mutation.

At least three connexin genes, *GJB2*, *GJB3* and *GJB6*, are involved in human genetic deafness[56–58], and the proteins they encode are located in regions of the cochlear duct that are rich in gap junctions, suggesting that all three connexins are essential components of the gap junctions. Mice carrying mutant versions of some of these connexins are being developed to test this suggestion. As the potassium reaches the stria, it is believed to be pumped into the marginal cells on the surface of the stria by a Na-K-ATPase, aided by a K-Na-Cl cotransporter[59]. This cotransporter has now been identified as the product of *Slc12a2*, by the finding of its mutation in the shaker-with-syndactylism mouse mutant and by two independent mouse mutants in which the gene is deleted, all of which fail to secrete endolymph[60–62]. Potassium then flows into the endolymph through a channel at the lumenal surface of marginal cells, and the two components of this channel have been identified by a genetic approach: mutations in either *KCNQ1* (a member of the same family of potas-

Box 1 • Web sites dedicated to the genetics of hearing impairment in mice and humans

The Hereditary Hearing Loss Homepage (Van Camp, G. & Smith, R.J.H.) includes current listings of identified genes and loci involved in non-syndromic deafness with references and convenient markers for linkage analysis. Details of many types of syndromic deafness are also included, plus links to other relevant sites.
http://www.uia.ac.be/dnalab/hhh

Mouse mutants with hearing or balance defects (Steel, K.P.). This table lists many of the mouse mutants with known or suspected auditory system defects, arranged by type of defect and including chromosomal localizations and underlying genes where known, plus key references.
http://www.ihr.mrc.ac.uk/hereditary/mousemutants.htm

Homepage of Hereditary Hearing Impairment in Mice (Zheng, Q.Y., Johnson, K.R. & Erway, L.C.). A table of mouse mutants with hearing impairment organized by chromosomal location, together with tables giving corresponding human forms of deafness.
http://www.jax.org/research/hhim/

sium channel subunits as *KCNQ4*) or *KCNE1* (the gene encoding the auxiliary subunit minK or IsK) cause Jervell and Lange-Nielsen syndrome in humans, and mutation of *Kcne1* in the mouse leads to failure of endolymph secretion[63–65]. Reports of mutations in these genes, plus the pathology observed in the mutant mice where available, all support the model for potassium recycling.

Two other molecules have been implicated in endolymph homeostasis after mutations were found to be associated with human deafness. These were interesting in that the genes are expressed in discrete patches of epithelial cells lining the endolymphatic compartment. *ATP6B1* mutations were found in people with renal tubular acidosis and deafness; the gene is expressed in interdental cells and is thought to be involved in pH control of endolymph[66]. The pendrin gene (*PDS*) is mutated in Pendred syndrome and some cases of non-syndromic deafness[67,68], and is expressed in the endolymphatic duct and sac, but also in epithelial cells just below the stria vascularis on the lateral wall of the cochlear duct[69]. Pendrin acts as an iodide/chloride transporter *in vitro*, and its location indicates it may be involved in endolymph homeostasis. A mouse mutation of *Atp6b1* has not yet been described, but the *Pds*$^{-/-}$ mouse shows very early dilation of the endolymphatic compartments and otoconial defects, both supportive of a role for pendrin in endolymph homeostasis[12].

An ideal approach

The genetic approach is proving to be a powerful tool in unraveling the molecular basis of cochlear function. Progress in the past few years in identifying deafness genes has been remarkable. Knowing some of the molecules involved can lead directly to others, and the use of the yeast two-hybrid system to discover molecules interacting with myosin VIIA is a good example of this. As we knew all along, however, moving from identification of a gene to understanding its function is usually a slow process. We have summarized some of the major advances in understanding cochlear physiology, but we have not considered many genes now known to be essential for auditory system development because of their involvement in genetic deafness. We have also ignored the growing evidence that single genes can be involved in late-onset hearing loss in humans, especially in dominantly inherited deafness, which suggests that lifelong maintenance of the cochlea is critical. The large number of deaf mouse mutants with no obvious human homologue, and human deafness genes localized or identified with no equivalent mouse model available, indicates that we still have much to learn about deafness from a genetic approach.

Acknowledgments

Supported by the MRC, Defeating Deafness and EC contract QLG2-CT-1999-00988.

Received 22 November; accepted 20 December 2000.

1. Petit, C. Genes responsible for human hereditary deafness: symphony of a thousand. *Nature Genet.* **14**, 385–391 (1996).
2. Davis, A.C. *Hearing in Adults* (Whurr, London, 1995).
3. Steel, K.P., Erven, A. & Kiernan, A.E. Mice as models for human hereditary deafness. in *Genetics and Auditory Disorders* (eds. Keats, B., Fay, R. & Popper, A.N.) (Springer, New York, in press).
4. Gibson, F. *et al.* A type VII myosin encoded by the mouse deafness gene *shaker-1*. *Nature* **374**, 62–64 (1995).
5. Probst, F.J. *et al.* Correction of deafness in *shaker-2* mice by an unconventional myosin in a BAC transgene. *Science* **280**, 1444–1447 (1998).
6. Minowa, O. *et al.* Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. *Science* **285**, 1408–1411 (1999).
7. Phippard, D., Lu, L., Lee, D., Saunders, J.C. & Crenshaw, E.B. III Targeted mutagenesis of the POU-domain gene *Brn4/Pou3f4* causes developmental defects in the inner ear. *J. Neurosci.* **19**, 5980–5989 (1999).
8. Erkman, L., McEvilly, R.J., Luo, L. & Ryan, A.K. Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. *Nature* **381**, 603–606 (1996).
9. McGuirt, W.T. *et al.* Mutations in *COL11A2* cause non-syndromic hearing loss (DFNA13). *Nature Genet.* **23**, 413–419 (1999).
10. Legan, P.K. *et al.* A targeted deletion in α-tectorin reveals that the tectorial membrane is required for the gain and timing of cochlear feedback. *Neuron* **28**, 273–285 (2000).
11. Di Palma, F. *et al.* Mutations in *Cdh23*, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. *Nature Genet.* **27**, 103–107 (2001).
12. Everett, L.A. *et al.* Targeted disruption of mouse *Pds* provides insight about the inner-ear defects encountered in Pendred syndrome. *Hum. Mol. Genet.* **10**, 153–161 (2001).
13. Corey, D.P. & Hudspeth, A.J. Kinetics of the receptor current in bullfrog saccular hair cells. *J. Neurosci.* **3**, 962–976 (1983).
14. Howard, J. & Hudspeth, A.J. Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. *Neuron* **1**, 189–199 (1988).
15. Holt, J.R. & Corey, D.P. Two mechanisms for transducer adaptation in vertebrate hair cells. *Proc. Natl. Acad. Sci. USA* **97**, 11730–11735 (2000).
16. Avraham, K.B. *et al.* The mouse *Snell's waltzer* deafness gene encodes an unconventional myosin required for structural integrity of inner hair cells. *Nature Genet.* **11**, 369–375 (1995).
17. Self, T. *et al.* Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development* **125**, 557–566 (1998).
18. Self, T. *et al.* Role of myosin VI in the development of cochlear hair cells. *Dev. Biol.* **214**, 331–341 (1999).
19. Weil, D. *et al.* Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* **374**, 60–61 (1995).
20. Weil, D. *et al.* The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nature Genet.* **16**, 191–193 (1997).
21. Liu, X.-Z. *et al.* Mutations in the myosin VIIA gene causing non-syndromic recessive deafness. *Nature Genet.* **16**, 188–190 (1997).
22. Wang, A. *et al.* Association of unconventional myosin *MYO15* mutations with human nonsyndromic deafness DFNB3. *Science* **280**, 1447–1451 (1998).
23. Wells, A.L. *et al.* Myosin VI is an actin-based motor that moves backwards. *Nature* **401**, 505–508 (1999).
24. Richardson, G.P. *et al.* A missense mutation in myosin VIIA prevents aminoglycoside accumulation in early postnatal cochlear hair cells. *Ann. NY Acad. Sci.* **884**, 110–124 (1999).
25. Küssel-Andermann, P. *et al.* Vezatin, a novel transmembrane protein, bridges myosin VIIA to the cadherin-catenins complex. *EMBO J.* **19**, 6020–6029 (2000).
26. Verpy, E. *et al.* A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nature Genet.* **26**, 51–55 (2000).
27. Bitner-Glindzicz, M. *et al.* A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. *Nature Genet.* **26**, 56–60 (2000).
28. Alagramam, K.N. *et al.* The mouse Ames waltzer hearing-loss mutant is caused by mutation of *Pcdh15*, a novel protocadherin gene. *Nature Genet.* **27**, 99–102 (2001).
29. Zheng, L. *et al.* The deaf jerker mouse has a mutation in the gene encoding the espin actin-bundling proteins of hair cell stereocilia and lacks espins. *Cell* **102**, 377–385 (2000).
30. Ohmori, H. Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. *J. Physiol.* **359**, 189–217 (1985).
31. Ricci, A.J. & Fettiplace, R. Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. *J. Physiol.* **506**, 159–173 (1998).
32. Yamoah, E.N. *et al.* Plasma membrane Ca^{2+}-ATPase extrudes Ca^{2+} from hair cell stereocilia. *J. Neurosci.* **18**, 610–624 (1998).
33. Street, V.A., McKee-Johnson, J.W., Fonseca, R.C., Tempel, B.L. & Noben-Trauth, K. Mutations in a plasma membrane Ca^{2+}-ATPase gene cause deafness in deafwaddler mice. *Nature Genet.* **19**, 390–394 (1998).
34. Kozel, P.J. *et al.* Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2. *J. Biol. Chem.* **273**, 18693–18696 (1998).
35. Yasunaga, S. *et al.* A mutation in *OTOF*, encoding otoferlin, a FER-1-like protein, causes DFNB9, a non-syndromic form of deafness. *Nature Genet.* **21**, 363–369 (1999).
36. Platzer, J. *et al.* Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca^{2+} channels. *Cell* **102**, 89–97 (2000).
37. Géléoc, G.S.G., Casalotti, S.O., Forge, A. & Ashmore, J.F. A sugar transporter as a candidate for the outer hair cell motor. *Nature Neurosci.* **2**, 713–719 (1999).
38. Zheng, J. *et al.* Prestin is the motor protein of cochlear outer hair cells. *Nature* **405**, 149–155 (2000).
39. Belyantseva, I.A., Adler, H.J., Curi, R., Frolenkov, G.I. & Kachar, B. Expression and localisation of prestin and the sugar transporter GLUT-5 during development of electromotility in cochlear outer hair cells. *J. Neurosci.* **20**, RC116 (2000).
40. Walker, R.G., Willingham, A.T. & Zuker, C.S. A *Drosophila* mechanosensory transduction channel. *Science* **287**, 2229–2234 (2000).
41. Simmler, M.C. *et al.* Targeted disruption of *Otog* results in deafness and severe imbalance. *Nature Genet.* **24**, 139–143 (2000).
42. Verhoeven, K. *et al.* Mutations in the human α-tectorin gene cause autosomal dominant non-syndromic hearing impairment. *Nature Genet.* **19**, 60–62 (1998).
43. Steel, K.P., Barkway, C. & Bock, G.R. Strial dysfunction in mice with cochleosaccular abnormalities. *Hear. Res.* **27**, 11–26 (1987).
44. Johnstone, B.M., Patuzzi, R., Syka, J. & Sykova, E. Stimulus-related potassium changes in the organ of Corti of guinea-pig. *J. Physiol.* **408**, 77–92 (1989).
45. Konishi, T., Harick, P.E. & Walsh, P.J. Ion transport in guinea pig cochlea. I. Potassium and sodium transport. *Acta Otolaryngol.* **86**, 22–34 (1978).
46. Wada, J., Kambayashi, J., Marcus, D.C. & Thalmann, R. Vascular perfusion of the cochlea: effect of potassium-free and rubidium-substituted media. *Arch. Otorhinolaryngol.* **225**, 79–81 (1979).
47. Kikuchi, T., Kimura, R.S., Paul, D.L. & Adams, J.C. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. *Anat. Embryol.* **191**, 101–118 (1995).
48. Spicer, S.S. & Schulte, B.A. Evidence for a medial K^+ recycling pathway from inner hair cells. *Hear. Res.* **118**, 1–12 (1998).

49. Schulte, B.A. & Steel, K.P. Expression of α and β subunit isoforms of Na,K-ATPase in the mouse inner ear and changes with mutations at the *W^v* or *Sl^d* loci. *Hear. Res.* **78**, 65–76 (1994).
50. Kubisch, C. *et al.* KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell* **96**, 437–446 (1999).
51. Marcotti, W. & Kros, C.J. Developmental expression of the potassium current $I_{K,n}$ contributes to maturation of mouse outer hair cells. *J. Physiol.* **520**, 653–660 (1999).
52. Housley, G.D. & Ashmore, J.F. Ionic currents of outer hair cells isolated from the guinea pig cochlea. *J. Physiol.* **448**, 73–98 (1992).
53. Jentsch, T.J. Neuronal KCNQ potassium channels: physiology and role in disease. *Nature Rev. Neurosci.* **1**, 21–30 (2000).
54. Kharkovets, T. *et al.* KCNQ4, a K^+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. *Proc. Natl. Acad. Sci. USA* **97**, 4333–4338 (2000).
55. Beisel, K.W., Nelson, N.C., Delimont, D.C. & Fritzsch, B. Longitudinal gradients of KCNQ4 expression in spiral ganglion and cochlear hair cells correlate with progressive hearing loss in DFNA2. *Brain Res. Mol. Brain Res.* **82**, 137–149 (2000).
56. Kelsell, D.P. *et al.* Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* **387**, 80–83 (1997).
57. Xia, J. *et al.* Mutations in the gene encoding gap junction protein β-3 associated with autosomal dominant hearing impairment. *Nature Genet.* **21**, 363–369 (1998).
58. Grifa, A. *et al.* Mutations in *GJB6* cause nonsyndromic autosomal dominant deafness at DFNA3 locus. *Nature Genet.* **23**, 16–18 (1999).
59. Wangemann, P. Comparison of ion transport mechanisms between vestibular dark cells and strial marginal cells. *Hear. Res.* **90**, 149–157 (1995).
60. Dixon, M.J. *et al.* Mutation of the Na-K-Cl co-transporter gene *Slc12a2* results in deafness in mice. *Hum. Mol. Genet.* **8**, 1579–1584 (1999).
61. Delpire, E., Lu, J., England, R. & Thorne, T. Deafness and imbalance associated with inactivation of the secretory Na-K-2Cl co-transporter. *Nature Genet.* **22**, 192–195 (1999).
62. Flagella, M. *et al.* Mice lacking the basolateral Na-K-2Cl cotransporter have impaired epithelial chloride secretion and are profoundly deaf. *J. Biol. Chem.* **274**, 26946–26955 (1999).
63. Vetter, D.E. *et al.* Inner ear defects induced by null mutation of the *isk* gene. *Neuron* **17**, 1251–1264 (1996).
64. Tyson, J. *et al.* IsK and KvLQT1: Mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome. *Hum. Mol. Genet.* **6**, 2179–2185 (1997).
65. Letts, V.A. *et al.* A new spontaneous mouse mutation in the *Kcne1* gene. *Mamm. Genome* **11**, 831–835 (2000).
66. Karet, F.E. *et al.* Mutations in the gene encoding B1 subunit of H^+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nature Genet.* **21**, 84–90 (1999).
67. Everett, L.A. *et al.* Pendred syndrome is caused by mutations in a putative sulphate transporter (PDS). *Nature Genet.* **17**, 411–422 (1997).
68. Li, X.C. *et al.* A mutation in PDS causes non-syndromic recessive deafness. *Nature Genet.* **18**, 215–217 (1998).
69. Everett, L.A., Morsli, H., Wu, D.K. & Green, E.D. Expression pattern of the mouse ortholog of the Pendred's syndrome gene (*Pds*) suggests a key role for pendrin in the inner ear. *Proc. Natl. Acad. Sci. USA* **96**, 9727–9732 (1999).