REVIEWS

GENETIC INSIGHTS INTO THE MORPHOGENESIS OF INNER EAR HAIR CELLS

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The mammalian inner ear is a sensory organ that has specialized hair cells that detect sound, as well as orientation and movement of the head. The 'hair' bundle on the apical surface of these cells is a mechanosensitive organelle that consists of precisely organized actin-filled projections known as stereocilia. Alterations in hair-bundle morphogenesis can result in hearing loss, balance defects or both. Positional cloning of genes that underlie hereditary hearing loss, coupled with the characterization of corresponding mouse models, is revealing how hair cells have adapted the molecular mechanisms of intracellular motility and intercellular adhesion for the morphogenesis of their apical surfaces.

STEREOCILIUM
(Pl. stereocilia). A large, rigid, actin-filled microvillus on the apical surface of hair cells in the inner ear.

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The selective advantage of sensitive hearing to monitor the environment and communicate has produced various morphologies of hearing organs in vertebrate species[1,2]. Although the physiological mechanisms that underlie the sensitivity and frequency selectivity of hearing might differ between mammalian and non-mammalian species[2,3], there is a recurrent architectural theme in hearing organs: they all use hair cells to detect mechanical stimuli and convert them into afferent nerve signals that are directed towards the brain[4].

The mammalian inner ear contains approximately 15,000–30,000 neurosensory hair cells. These cells are located inside the temporal bone of the cranium, which makes their post-mortem isolation a daunting task in many mammalian species, including humans. For this reason, much of what is known about the structure and function of hair cells has been derived from studies of non-mammalian species, including frogs, turtles and chickens. However, many of the proteins that are involved in the morphogenesis of hair cells have been identified through the positional cloning of genes that are responsible for different forms of hereditary deafness in humans, or through the analysis of mouse models of hereditary hearing loss[5]. Surprisingly, many intracellular motor and intercellular adhesion molecules have been implicated in hair cell morphogenesis. Only recently has it become possible to incorporate the insights from all of these studies into integrated, testable models of hair cell morphogenesis. Here, we review these models and the genes involved in the 'micro-morphogenesis' of structural elements that are required for the mechanosensitivity of hair cells. These morphogenetic events include changes in actin packing and the contour of the hair cell's mechanosensory organelle (the STEREOCILIUM), adhesion of adjacent stereocilia to form a hair bundle and elongation of stereocilia to form a staircase-shaped bundle. Irreversible hair cell degeneration is a common feature of many genetic and environmental forms of hearing loss, and our evolving knowledge of hair cell morphogenesis could eventually lead to strategies to prevent, reduce or reverse hearing loss caused by hair cell damage.

Development of the inner ear in mammals

The mammalian inner ear consists of the cochlea, a snail-shaped organ that mediates sound transduction, and the vestibular labyrinth, which detects gravitational force, and angular and linear accelerations (FIG. 1a,b). The extraordinary ability of the mammalian cochlea to detect and distinguish sounds over a wide range of frequencies depends on the precise organization of its highly specialized neurosensory epithelium,

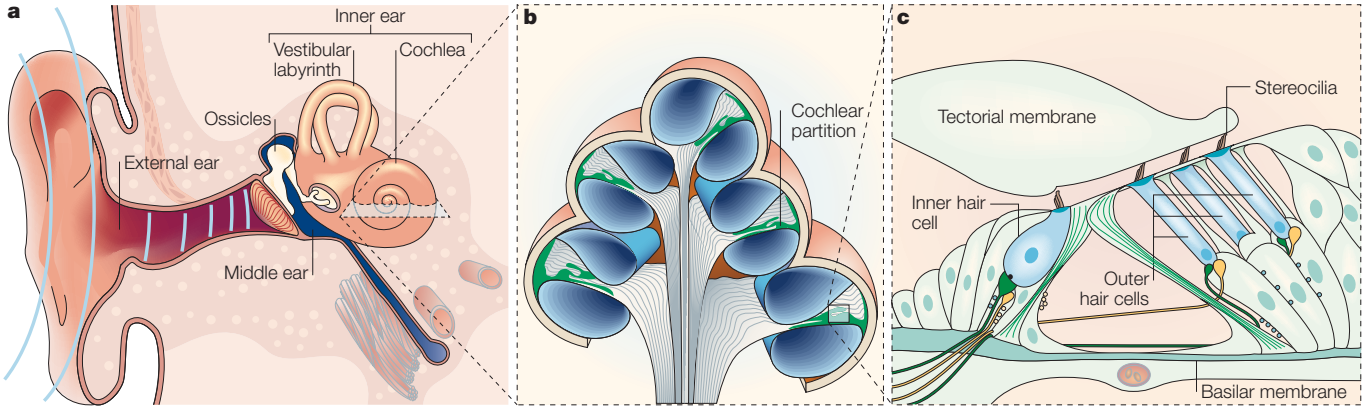

Figure 1 | **Structure and function of the mammalian ear. a** | Detection of environmental sound begins when incoming sound waves reach the external ear. Sound vibrates the chain of ossicles in the middle ear, which transmit it into the inner ear, where mechanical vibrations are converted into electrical nerve impulses. **b** | The fluid-filled hearing end-organ, known as the 'cochlea', is a continuous coiled duct that is separated longitudinally by a cochlear partition. Mechanical stiffness and resonant frequency of a basilar membrane that underlies the cochlear partition gradually decreases from the base of the cochlea to its apex. Sound vibrations of a particular frequency cause the basilar membrane to resonate at a particular location that is determined by its stiffness. **c** | A sensory part of cochlear partition, the organ of Corti, has two types of specialized mechanosensitive cell: inner and outer hair cells, both of which convert mechanical stimuli into variations of intracellular potential. Only inner hair cells seem to transmit information to the brain, whereas outer hair cells receive abundant efferent innervation (yellow) but have few afferent fibres (dark green). Outer hair cells demonstrate a unique ATP-independent voltage-driven motility that might provide positive cycle-by-cycle feedback to amplify sound-induced vibrations of the organ of Corti[3]. Both cochlear amplification and outer hair cell motility are absent in mice that are deficient for prestin[123], a novel voltage-sensing motor protein[124] that is localized exclusively to the lateral plasma membrane of these cells[125]. Hair cells can be lost or damaged by ageing, noise exposure, ototoxic drugs such as aminoglycoside antibiotics, or by the inheritance of mutant alleles of genes that are essential for hearing. For further details, see the online supplementary information S1 (movie).

known as the organ of Corti (FIG. 1c). Given this complexity, it is therefore not surprising that many genes are required for the development of the mammalian inner ear.

The inner ear develops from the otic placode, a thickened region of cranial ECTODERM that invaginates and closes to form an otic vesicle, where early development and differentiation of epithelial and neuronal structures occur[6,7]. The genetic basis of these early morphogenetic events has been reviewed elsewhere[6,7], but morphogenesis of the mechanosensory cells of the inner ear occurs at the later stages of inner ear development. Investigations of these later stages have been greatly facilitated in small rodents including the mouse, in which the inner ear continues to develop through the first three postnatal weeks[8]. In the mouse, formation of sensory hair cells begins when cells of the inner ear sensory epithelium synchronously exit the cell cycle at embryonic day 12.5–13.5 (REF. 9). Some of them subsequently differentiate into hair cells, the highly specialized mechanoreceptors with hair-like projections on their apical surfaces (FIG. 2). These projections, known as stereocilia (FIG. 2b,c), have mechanosensitive ion channels[10,11] of unknown molecular identity and are precisely organized to detect sub-nanometer deflections[12,13] (FIG. 2d). Unlike hair cells in birds and fish, mammalian cochlear hair cells do not regenerate and their loss or damage results in irreversible deafness or hearing impairment. Hair cells of mammalian vestibular organs show some potential for regeneration[14–17], although the mechanism of renewal is unknown.

ECTODERM
Embryonic tissue that is the precursor of the epidermis and the nervous system.

MICROVILLUS
(Pl. microvilli). A thin, cylindrical, membrane-covered projection on the surface of an animal cell that contains a core bundle of actin filaments.

ACTIN FILAMENT
A helical protein filament that is formed by the polymerization of globular actin molecules.

VESTIBULAR AREFLEXIA
An abnormal absent response to artificial caloric (hot or cold) stimulation of the neurosensory organs of balance in the inner ear.

From microvillus to stereocilium

In spite of the misleading name that indicates a relationship with true cilia, hair cell stereocilia are specialized derivatives of actin-based MICROVILLI[18]. The core of both microvilli and stereocilia consists of parallel ACTIN FILAMENTS that are held together by different sets of actin-bundling proteins, such as espin and fimbrin/plastin in hair cell stereocilia, or villin, fimbrin/plastin and 'small espin' in intestinal epithelial microvilli[19]. The filaments are unidirectionally aligned with their barbed end — the site of high-affinity actin polymerization — oriented away from the surface of the cell[20]. Growth of both microvilli and stereocilia therefore occurs by the addition of new actin monomers to their tips[21–23].

When stereocilia emerge from the apical surface of a hair cell, they have a similar appearance to microvilli[24] (FIG. 3A). The loosely packed actin filaments of the precursor microvillus are then replaced by the dense, hexagonally ordered paracrystalline array of actin filaments in a mature stereocilium[25] (FIG. 3B,C). There are at least three models for this transformation (FIG. 3D). The first model posits that some signal might initiate the growth of a new, denser actin core that begins at the tip of the stereocilium (FIG. 3Da). The new core would progressively replace the old actin fibres by a treadmill process, in which actin monomers are depolymerized from the pointed end while new monomers are added to the barbed end, resulting in overall downward movement of the entire array[26]. Alternatively, new actin fibres might be added between existing fibres (FIG. 3Db). In a third model, the hair cell might modify the

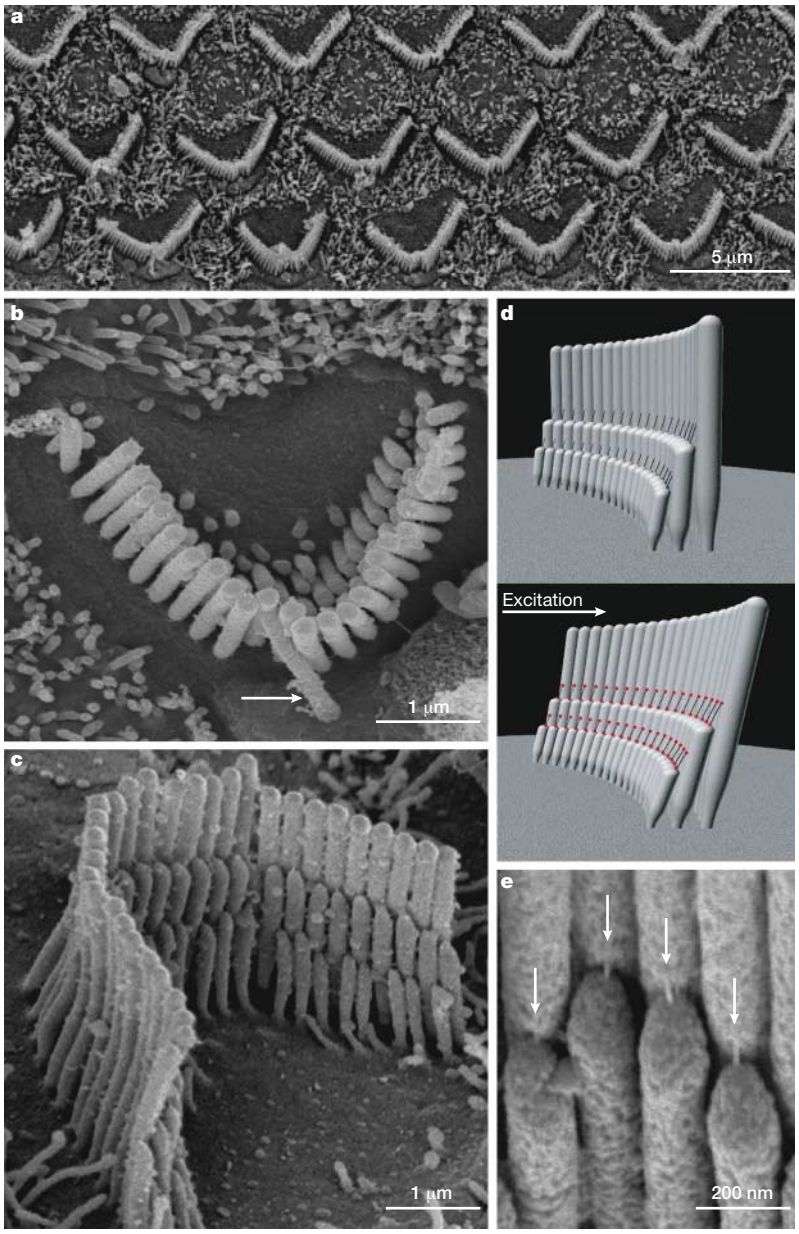

Figure 2 | **The hair bundle of mammalian auditory sensory cells. a** | Scanning electron microscopy (SEM) image of three rows of outer hair cells that is viewed from the top of the organ of Corti and that shows unidirectional orientation of V/W-shaped hair bundles. **b** | Close-up view of one of these almost-mature hair bundles that consists of three rows of individual stereocilia and a true cilium, the kinocilium (white arrow), which is always positioned at the vertex of the bundle. Images in panels **a** and **b** were obtained from the middle cochlear turn of a wild-type mouse at postnatal day 7. **c** | Side view of a mature outer hair cell bundle that shows the precise staircase organization of stereocilia rows. **d** | Sound-induced, nanometer-scale deflections of the hair bundle[13] open mechanically gated ion channels (shown in red)[10,11]. These channels are thought to be located at the ends of tip-links[126]. **e** | The tip-link[92] (indicated by white arrows) is a tiny filament that connects neighbouring stereocilia. Images in panels **c** and **e** were obtained from the middle cochlear turn of an adult guinea pig.

FILOPODIUM
(Pl. filopodia). A thin, spike-like protrusion with an actin filament core that is generated on the leading edge of a motile animal cell.

actin-bundling components by adding or removing certain proteins, which would allow denser packing of actin filaments. According to this last model, existing filaments migrate to the centre of the stereocilium and new filaments are added at the periphery (FIG. 3Dc). Certainly, some combination of these models is also possible. In the stereocilia of chicken hair cells, developmental changes of actin filaments seem to follow the third model (FIG. 3C,Dc), although there are no data that compare the rates of growing new filaments at the periphery and treadmilling of the existing filaments at the centre. An obvious molecular candidate for mediating the transition from loose to dense packing of actin filaments in chick hair cell stereocilia is espin, the expression of which coincides with stages when stereocilia become thicker[27]. In *Drosophila melanogaster*, the replacement of Forked by Fascin controls the proper formation of the actin core in the sensory bristles[28]. Whether a similar developmental mechanism is functioning in mammalian hair cells is not known, but two lines of evidence support this possibility. First, L-plastin, a homologue of the actin-bundling protein fimbrin, is transiently present during mammalian stereocilia bundle formation, but not in mature bundles[29]. Second, espin is an important structural element of the hair bundle of mammalian hair cells, and a recessive mutation of the gene (*Espn*) that encodes espin in the deaf jerker mouse results in failure to accumulate detectable amounts of this protein in the hair bundle, which leads to shortening, loss of mechanical stiffness and eventual disintegration of stereocilia[30]. In humans, recessive mutations of *ESPN* at the *DFNB36* locus cause profound prelingual hearing loss and peripheral VESTIBULAR AREFLEXIA[31].

Stereocilia and microvilli compared. In various mammalian and non-mammalian cells, microvilli are dynamic structures that continuously form and disassemble with an average lifetime of approximately 12 minutes[32]. By contrast, stereocilia of auditory hair cells retain their mature shape throughout the life of the organism. Nevertheless, the actin core of a stereocilium seems to be renewed continuously through an actin treadmill mechanism[22]. This renewal process is at least two orders of magnitude slower than the cytoskeletal renewal in typical actin bundle-based structures such as FILOPODIA[33] or intestinal epithelial microvilli[21,23]. The extraordinary stability of a stereocilium was attributed long ago to the remarkable paracrystalline structure of its actin core[20,25]. Recent structural studies indicate that the assembly of similar actin–fimbrin arrays *in vitro* proceeds as a whole crystal unit and requires strong coupling between actin polymerization, fimbrin binding, cross-bridge formation and conformational changes of actin[34]. Therefore, the marked differences in the stability of stereocilia compared with microvilli might result from differences in actin crosslinking and/or the predominant isoform of actin. In chick hair cells, the β-isoform of actin seems to be targeted selectively to stereocilia, where it is crosslinked by fimbrin/plastin, which has a higher binding affinity for β-actin than for γ-actin[35]. γ-actin, which differs from β-actin in only four amino acids at the amino terminus, is found throughout the hair cell, including microvilli[35], as well as throughout many other cell types, and could be considered as a housekeeping gene product. Nevertheless, there are several dominant missense alleles of *ACTG1* that encode γ-actin that result in non-syndromic,

SENSORINEURAL HEARING LOSS Hearing loss that results from abnormalities of the inner ear or auditory neural pathways.

progressive, SENSORINEURAL HEARING LOSS in humans[36–38], indicating a unique requirement for γ-actin, perhaps in hair cell stereocilia.

Shaping the stereocilium from tip to taper

A stereocilium differs from a microvillus not only in length and thickness, but also in its prominent taper at the junction with the cell body (FIG. 3C). The taper forms when a stereocilium grows in width, which represents a distinct developmental step in the morphogenesis of chick hair cells[24], but a more gradual process in hamster hair cells[39]. Ultrastructural studies show that most of the actin fibres terminate at the taper, and only the central core fibres project into the body of the cell[20] (FIG. 3C), predisposing the stereocilium to bend at the taper and not at a higher position[40]. To maintain this morphology, tight control of actin polymerization and depolymerization is required at both locations: the tip and the taper.

The processes that control actin polymerization at the tips are generally assumed to be similar in stereocilia and microvilli[18]. Several proteins have been implicated in the actin cytoskeleton dynamics of stereocilia. A mutation in a human homologue of the *D. melanogaster* gene *diaphanous* is linked to autosomal dominant, non-syndromic sensorineural progressive hearing loss at the *DFNA1* locus[41]. Diaphanous belongs to the formin family of proteins, which accelerate actin nucleation while interacting with the barbed end of actin filament[42]. Although the localization of diaphanous in the inner ear is unknown, its involvement in actin polymerization in the stereocilia was proposed[41]. The tips of stereocilia might also contain an actin-binding protein, kaptin, which is recruited to the leading edge of lamellipodia of motile fibroblasts[43]. Another actin-binding protein, radixin, was detected at the taper of the hair cell stereocilium in the chick, frog, mouse and zebrafish[44]. Proteins of the ezrin/radixin/moesin (ERM) family crosslink actin filaments to plasma membranes and are involved in organizing the cortical cytoskeleton, especially in the formation of microvilli[45]. So far, there are no reports of hair-bundle abnormalities associated with mutations of genes that encode ERM proteins. However, these proteins might interact with unconventional myosins VIIa or XVa, which have FERM (band 4.1, ezrin, radixin, moesin) binding domains[46], are specifically expressed in hair cells[47,48] and have mutant allele products that are known to disrupt formation of the hair bundle[49,50] (see below).

Another unconventional myosin, myosin VI, is also involved in stereocilium formation. Myosin VI is a 'backward-stepping' actin-based motor that moves towards the pointed (minus) end of actin filaments[51]. In humans, dominant and recessive mutations of *MYO6* (which encodes myosin VI) can cause hearing loss[52–54]. In mammalian hair cells, myosin VI has not been observed in stereocilia, but instead, is localized at the base of the hair bundle[47], within the cuticular plate that is thought to provide mechanical stability to the apex of the hair cell. The Snell's waltzer (*sv*) mouse is deaf and has no detectable myosin VI protein in any tissues[55]. Stereocilia of the *Myo6*$^{sv/sv}$ mouse are fused at their bases, indicating that myosin VI is required to moor the apical plasma membrane to the base of stereocilia and/or anchor stereocilia rootlets[56,57]. Ultrastructural studies show numerous links between the apical plasma membrane and the actin network of the cuticular plate[58], which might correspond to macromolecular complexes that contain myosin VI. In addition to this potential anchoring function, the 'backward' movement

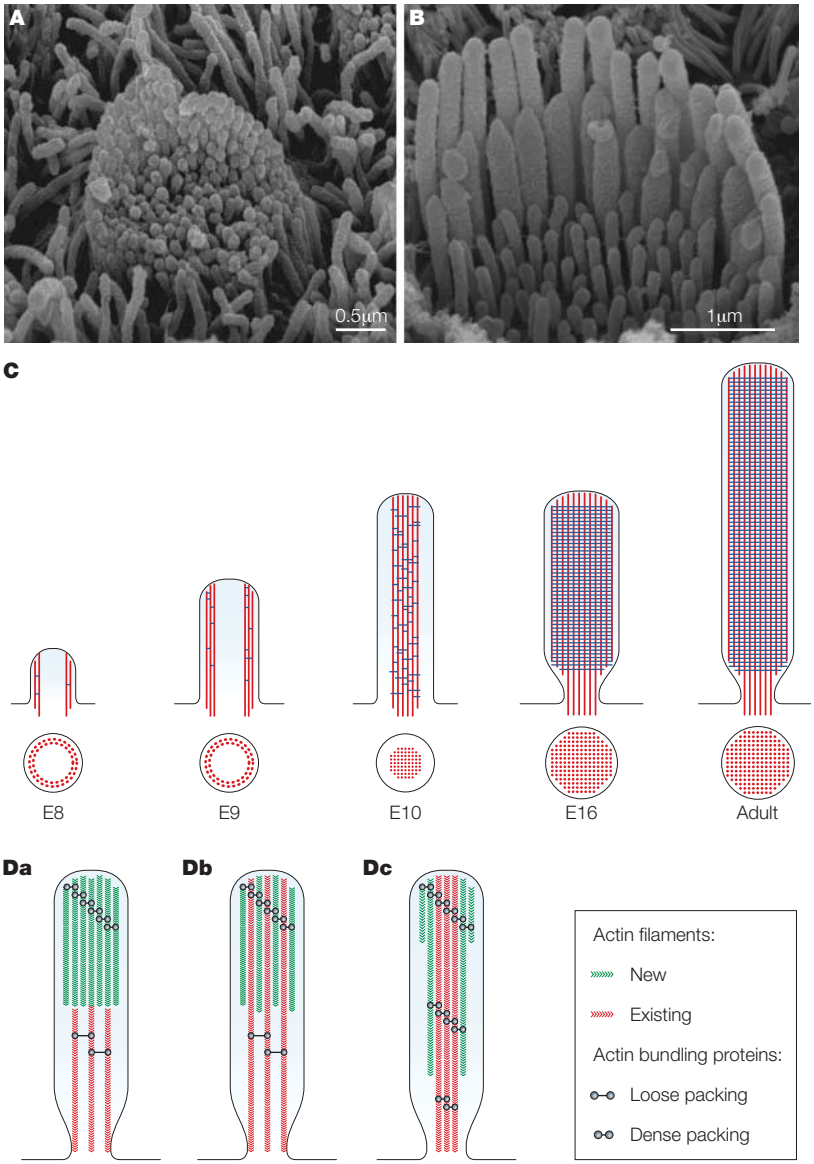

Figure 3 | **Maturation of hair cell stereocilia. A** | Scanning electron microscopy (SEM) image of the immature hair bundle of a mouse inner hair cell (apical turn of the cochlea, neonatal mouse). The nascent stereocilia are similar in appearance to the neighbouring microvilli. **B** | SEM image of mouse inner hair cell stereocilia during the final stage of stereocilia elongation (middle turn, postnatal day 7). **C** | Reorganization of the actin core during stereocilium maturation in chicks at various embryonic stages of development (indicated below the drawings). Actin filaments (red) migrate to the centre and form a hexagonal paracrystal (bottom row). Crosslinking of actin filaments (blue) increases with maturation and acquires transverse periodicity (upper row). Drawing is based on data reported by Tilney and DeRosier[24]. **D** | Three models of the actin-core transformation from the microvillus to the stereocilium. **a** | Replacement of the whole actin-core structure by treadmilling. **b** | Growth of new actin filaments between existing ones. **c** | Migration of existing actin filaments to the centre of the core and the addition of new filaments to the periphery. For further details, see the main text.

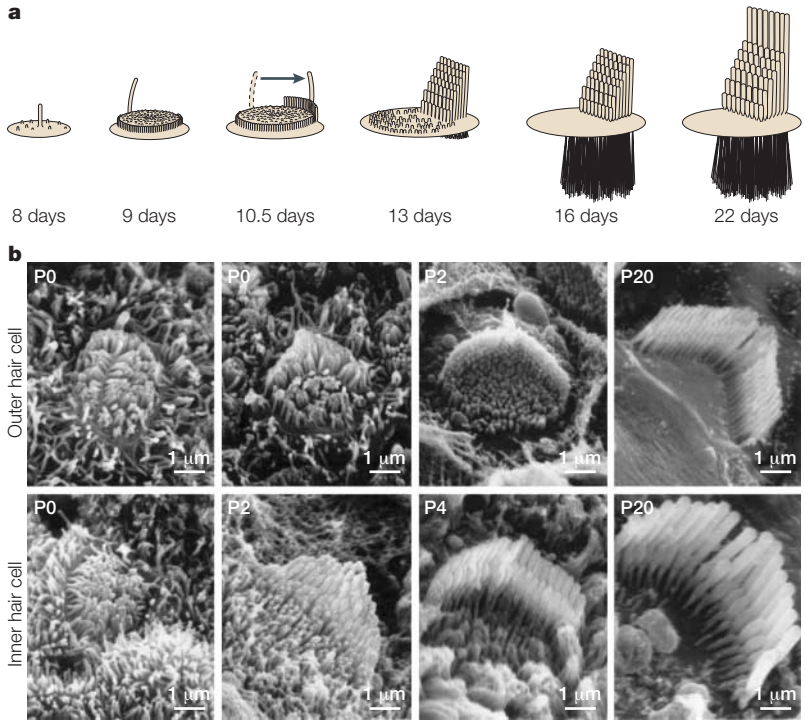

Figure 4 | **Stages of bundle formation in auditory hair cells.** Panel **a** | chick hair cells. Panel **b** | hamster hair cells. In both species, the hair bundle develops from precursor microvilli through the programmed and differential elongation of nascent stereocilia to pre-determined lengths and through the retraction of redundant microvilli at the end of the maturation process. For further details, see the main text. Panel **a** adapted with permission from REF. 127 © (1992) Annual Reviews. Embryonic ages are shown below the illustrations of the hair bundle. Panel **b** reproduced with permission from REF. 39 © (1994) Wiley. Postnatal ages are indicated on the scanning electron microscopy images. All micrographs were obtained from the middle turn of the cochlea.

of myosin VI along the actin filaments might be essential for removing molecular components that are released by actin treadmilling at the taper of the stereocilium.

Arrangement of stereocilia in the hair bundle

In the mammalian cochlea, stereocilia are arranged into precise V- or W-shaped arrays (FIG. 2a). It is thought that these configurations are stabilized by stereocilia rootlets that project into a densely organized cortical cytoskeleton, the cuticular plate, at the apex of the hair cell. The cuticular plate consists of a gel-like network of actin filaments[59] that are held together by actin-binding proteins, such as α-actinin, fodrin and fimbrin[60,61]. In the early stages of stereocilia formation, actin filaments of the precursor microvilli are simply projected into the cortical cytoskeleton of the hair cell (FIG. 3C). When stereocilia grow in diameter, their rootlets elongate and interconnect with perpendicular actin fibres to form the cuticular plate[24]. The formation and maintenance of the distinct actin arrangements of the cuticular plate and the stereocilia might involve selective sorting of different actin-binding proteins to these structures[62]. When normal stereocilia morphogenesis is disrupted, abnormal sorting of actin-bundling proteins could account for the formation of an aberrant actin bundle structure, referred to as a 'cytocaud', which emerges from the cuticular plate and projects into the cell[63,64]. However, the orientation and overall arrangement of the bundles are generally intact in these conditions, indicating the presence of a mechanism that stabilizes the overall orientation of the cuticular plate and hair bundle as an integrated complex. This mechanism might involve myosin VIIa, which, anchored by vezatin to a cadherin–catenins complex, could link the cortical cytoskeleton to the adhesion junctions between hair cells and neighbouring supporting cells[65]. Consistent with this hypothesis, mutations in myosin VIIa and a cadherin-related protein, cadherin 23, result in loss of the V/W configuration and disorientation of the bundle, respectively[49,66].

Orientation of the stereocilia bundles. Stereocilia bundles are directionally sensitive[67] and their precise orientation (FIG. 2a) is necessary for normal auditory perception[68]. Deflections towards the tallest stereocilia increase the MECHANOTRANSDUCTION current, whereas deflections in the opposite direction decrease the current[67] (FIG. 2d). In most mammalian and non-mammalian species, a specialized extracellular matrix, the tectorial membrane, overlies the auditory neuroepithelium and deflects hair bundles in response to sound (FIG. 1c). In the chick, growth and concomitant radial movement of the tectorial membrane was proposed to orient hair bundles by a traction mechanism[69]. However, in mammals, the orientation of cochlear hair cell bundles becomes evident as soon as microvilli emerge, well before the tectorial membrane is formed[70]. Furthermore, targeted deletion of α-tectorin, one of three key glycoproteins that constitute the tectorial membrane, seems to cause complete detachment of the tectorial membrane from the mouse organ of Corti[71,72], but does not affect the orientation or structure of the hair cell bundles[72]. The signalling processes that control hair-bundle orientation might be similar to those that control PLANAR-CELL POLARITY in the eye of *D. melanogaster*. Indeed, Flamingo, a member of the cadherin superfamily of adhesion proteins, the transmembrane protein Van Gogh and the secreted molecule Wingless, have been implicated in the regulation of planar-cell polarity in flies[73,74]. The mammalian homologues of each of these molecules is also involved in the polarization of cochlear hair cell stereocilia bundles[75–77].

Programmed elongation of stereocilia

The evolutionary conservation of a precise arrangement of stereocilia rows in a staircase-like pattern (FIG. 2c) indicates that this unique organization is required for mechanotransduction[2]. Formation of the staircase pattern in chick hair cells has been described as a four-step process[78]. First, numerous precursor microvilli emerge around a single primary cilium (kinocilium) and grow in unison with neighbouring microvilli (FIG. 4a). Second, precursor microvilli stop elongating and increase their width, while the kinocilium migrates to the edge of the apical cell surface, an event that determines the eventual orientation of the bundle. Third, nascent stereocilia elongate sequentially to form a staircase-like bundle.

MECHANOTRANSDUCTION
Conversion of a mechanical stimulus, such as sound, into an electrochemical signal.

PLANAR-CELL POLARITY
The polarized organization of cells in the plane of an epithelium.

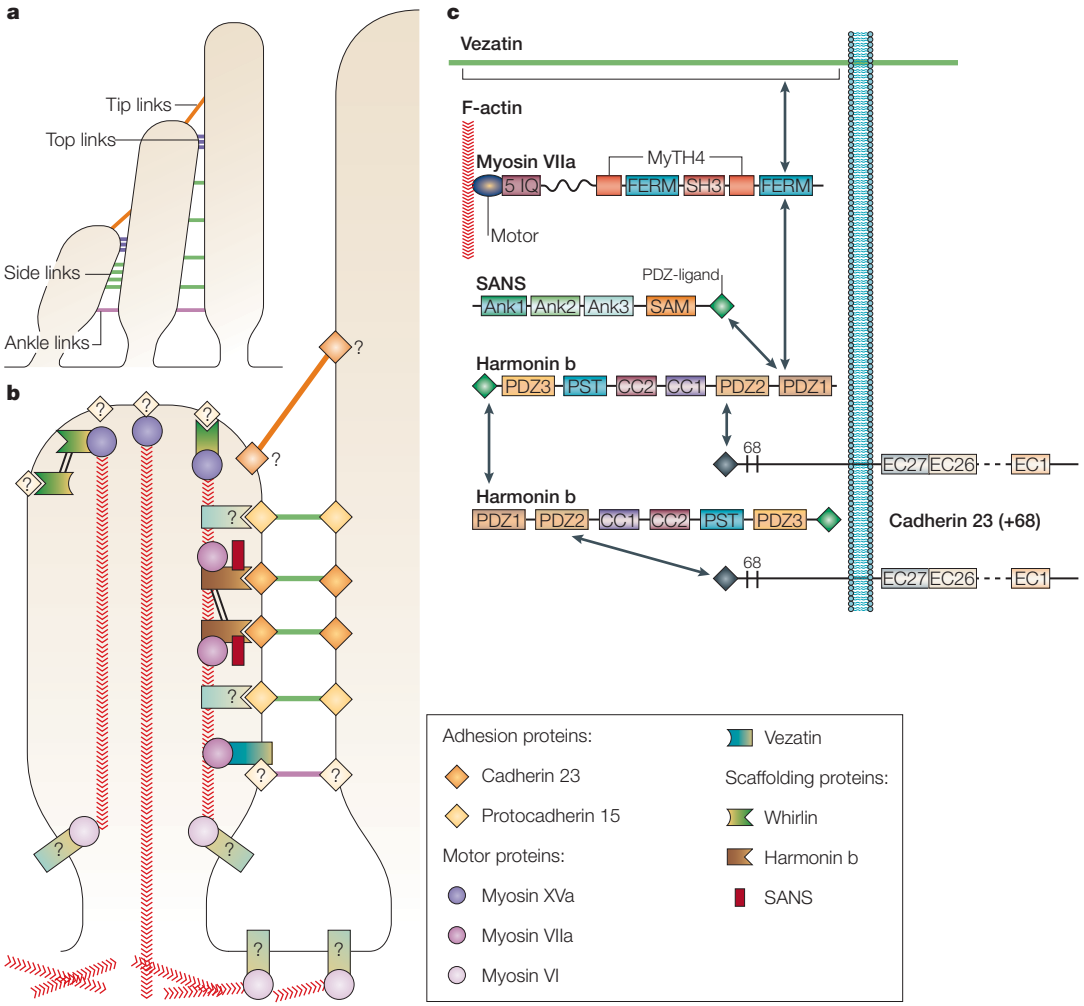

Figure 5 | **Adhesion in the hair bundle. a** | Four known types of link between adjacent stereocilia[86]. **b** | Schematized illustration of proteins that constitute adhesion complexes on the plasma membrane of stereocilia. Experimentally demonstrated interactions between myosin VIIa, harmonin, cadherin 23, SANS and vezatin are shown, as well as the interaction of myosin XVa with whirlin (I.A.B. *et al.*, unpublished observations). **c** | Interacting domains among the proteins that are essential for stereocilia micromorphogenesis and function. These proteins were identified through positional cloning of genes that underlie type 1 Usher syndrome. Ank, ankyrin repeat domain that is thought to be involved in protein–protein interactions; CC, coiled coil domain that mediates dimerization; EC, cadherin extracellular repeat; FERM, domain that is also known as the talin homology domain, which is thought to be important for linking cytoskeletal proteins to the membrane; IQ, a motif that serves as a binding site for myosin light chains; Motor, a domain that mediates actin binding, ATP binding and hydrolysis, and force generation; MyTH4, a domain of unknown function in some myosin and kinesin tails; PDZ, a domain that mediates interactions with other proteins that contain a PDZ ligand sequence and that is thought to be important for targeting signalling molecules to sub-membranous sites; PST, a proline-, serine- and threonine-rich region; SAM, a sterile α-motif, a domain that is found in many signalling proteins and that is thought to be involved in protein–protein interactions; SH3, a Src homology-3 domain that is involved in protein–protein interactions.

Finally, excess microvilli on the apical cell surface are resorbed (FIG. 4a). Morphogenesis of the hair bundle in mammalian auditory hair cells generally follows the same principles[39,79], although the stages are less distinct (FIG. 4b). Moreover, the kinocilium of mammalian auditory hair cells (FIG. 2b) degenerates shortly after formation of the bundle, indicating that it is not required to maintain bundle structure and function. However, its persistence throughout the lifetime of other types of hair cell with the capability to regenerate (mammalian vestibular hair cells, and hair cells in frogs and chicks) indicates that it might indeed be important for bundle morphogenesis.

Mutations in several genes are known to cause hearing loss by disrupting bundle morphogenesis (FIG. 5b; TABLE 1). Mutations of *MYO15A*, which encodes unconventional myosin XVa, cause non-syndromic sensorineural deafness in humans (*DFNB3*) as well as deafness and vestibular disorders in the shaker-2 mouse[50,80,81]. Hair cell stereocilia in homozygous shaker-2 mice are present and properly positioned, but are much shorter than wild-type stereocilia[50]. In the shaker-2 mouse, all stereocilia within a bundle are approximately the same length and there is no staircase organization of the mature hair bundle. Myosin XVa is discretely located at the tip of every stereocilium in

Table 1 | **Genes required for morphogenesis of the hair cell bundle**

Biological role	Gene product	Gene symbol (human)	Location (human)	Human locus	Mouse model (gene symbol)	Refs
Cytoskeleton formation	γ-actin	*ACTG1*	17q25	DFNA20, A26	N/A	36–38
	diaphanous 1	*HDIA1*	5q31	DFNA1	N/A	41,42
	espin	*ESPN*	1p36	DFNB36	Jerker (*je*)	27,30,31
Adhesion	cadherin 23	*CDH23*	10q21-q22	DFNB12, USH1D	Waltzer (*v*)	66,100,101,112,113
	protocadherin 15	*PCDH15*	10q21	DFNB23, USH1F	Ames waltzer (*av*)	107,111,128
Intracellular motors	myosin VI	*MYO6*	6q13	DFNA22, B37	Snell's waltzer (*sv*)	52–56
	myosin VIIa	*MYO7A*	11q12.3	DFNB2, A11, USH1B	Shaker-1 (*sh1*)	49,97,129
	myosin XVa	*MYO15A*	17p11.2	DFNB3	Shaker-2 (*sh2*)	50,81
Scaffolding proteins	harmonin	*USH1C*	11p15.1	DFNB18, USH1C	Deaf circler (*dfcr*)	98,99,108,109
	SANS	*SANS*	17q24-q25	USH1G	Jackson circler (*js*)	104
	whirlin	*WRHN*	9q32-q34	DFNB31	Whirler (*wi*)	85

DFNAx, autosomal dominant deafness type '*x*'; *DFNBx*, autosomal recessive deafness type '*x*'; N/A, not available; USHx, Usher syndrome type 'x'.

wild-type auditory and vestibular hair cells, where it appears just before the staircase emerges, which indicates that it is required for the elongation and formation of the stereocilia-bundle staircase[48].

In a hair bundle, longer stereocilia have more myosin XVa at their tips compared with the adjacent row of shorter stereocilia[48,82]. Similarly, the rate of actin renewal[82] and the amount of espin[23] are proportional to the length of the stereocilium. Overexpression of espin in CL4 porcine kidney epithelial cells initiates the formation of abnormally long microvilli[23], whereas overexpression of myosin XVa in wild-type mouse hair cells or in COS-7 cells does not induce additional growth of stereocilia or filopodia, respectively[48]. One interpretation of these data is that myosin XVa participates in the development of the staircase morphology of the hair bundle by an increased delivery of essential structural or regulatory elements to the tips of the taller stereocilia[48,83].

Localization of myosin XVa to the extreme tips of stereocilia raises the possibility that it is tethered there by integral membrane proteins[48]. Although the proteins that interact with myosin XVa are not known, it has two predicted FERM domains that could mediate interactions with ERM proteins. Perhaps more interestingly, myosin XVa has a PDZ ligand sequence that could interact with PDZ domain-containing proteins to coordinate a macromolecular complex at the tips of stereocilia. PDZ scaffold proteins serve as organizing centres of macromolecular functional complexes[84]. One such PDZ protein, whirlin, has recently been described[85]. Recessive mutations in *WHRN* and *Whrn* (which encode whirlin) cause deafness in humans (DFNB31) and in whirler mice, respectively. Stereocilia of whirler mice are abnormally short and arrayed in a near-normal configuration on the apical hair cell surface[85]. The overall inner ear phenotype of whirler mice is strikingly similar to that of shaker-2 mice, raising the possibility that whirlin and myosin XVa interact physically within a macromolecular complex that is responsible for programmed stereocilia elongation[83]. Whirlin also has a C-terminal PDZ ligand sequence that could interact with one of the PDZ domains of another whirlin protein to organize their multimerization into a higher-order structure.

CALCIUM CHELATORS
Substances that reversibly bind calcium, usually with high affinity, to remove free calcium ions from a solution.

Role of stereocilia links in morphogenesis

Types of link and their distribution. Ultrastructural studies have revealed four different types of link between stereocilia that provide cohesiveness to the stereocilia bundle and might mediate signalling events during morphogenesis (FIG. 5a). From the top to the base of the stereocilia, these links are designated: tip-links, top-links, shaft connections or side-links, and ankle-links, which are located at the beginning of the stereocilia taper[86]. There are also links that connect the kinocilium to the adjacent stereocilia of the tallest row[87]. All of these link types can be distinguished by their differential sensitivities to treatment with CALCIUM CHELATORS and a specific enzyme, subtilisin[88]. After treating the bundle with the calcium chelator BAPTA, tip-links break and multiple linkages sprout from the tips of the stereocilia over the next 12 hours[89]. Inappropriate links subsequently disappear, leaving only regenerated tip-links at 24 hours after treatment. Similar link dynamics were described for developing bundles[39,90]. The molecular identities of stereocilia links are under investigation, but their appearance early in development indicates that they might be important for the morphogenesis of hair bundles. Ankle-links progressively disappear within the first postnatal month in mouse hair cells but persist throughout life in chick hair cells[65]. Tip-links are present in mature hair cells (FIG. 2e) and are believed to be crucial inmechanotransduction[91,92]. Side-links are also present in adult hair cells[93] but do not seem to be a principal factor holding stereocilia in the developing bundle. Hair cells of transgenic mice with different mutations in protein tyrosine phosphatase receptor Q (Ptprq) do not have side links but form transient, normal-appearing bundles that subsequently degenerate shortly after birth[94].

Usher syndrome and hair-bundle morphology. Intercellular adhesion is a key signalling process that initiates cytoskeleton rearrangement during morphogenesis[95,96]. The genetic investigation of type 1 Usher syndrome (USH1) in humans and the characterization of corresponding mouse models has revealed that hair cells might use analogous adhesion mechanisms to control the morphogenesis of the hair bundle. Usher syndrome

is an autosomal recessive disorder that is characterized by sensorineural hearing loss and progressive loss of vision owing to RETINITIS PIGMENTOSA. So far, 5 genes that underlie USH1 have been cloned: myosin VIIa (*MYO7A*)[97], harmonin (*USH1C*)[98,99], cadherin 23 (*CDH23*)[66,100,101], protocadherin 15 (*PCDH15*)[102,103] and *SANS*[104]. There are corresponding mutant mouse models and, in some cases, several mutant alleles for each gene: shaker-1 (*Myo7a*^{sh1}), deaf circler (*Ush1c*^{dfcr}), waltzer (*Cdh23*^{v}), Ames waltzer (*Pcdh15*^{av}) and Jackson shaker (*Sans*^{js}). These mutant phenotypes are all characterized by deafness, vestibular dysfunction and disorganized, splayed stereocilia in homozygous mice[49,66,105–107]. On the basis of similar mutant phenotypes, as well as *in vitro* protein-interaction studies, it was suggested that the protein products of genes that are involved in USH1 might form a macromolecular complex (FIG. 5c) that provides cohesiveness to the stereocilia bundles[104,108,109]. Two of these gene products, harmonin and SANS, represent PDZ domain-containing proteins. Harmonin has been shown to interact with myosin VIIa and cadherin 23[108,109], which has a PDZ ligand sequence at its C-terminus. SANS has also been implicated in an interaction with harmonin[104], and, therefore, SANS and harmonin might act as cytoplasmic scaffold organizers of proteins that are involved in USH1.

Cadherins in the hair bundle. Cadherin 23 and protocadherin 15 are members of the cadherin superfamily of integral membrane proteins that are responsible for intercellular adhesion as well as signalling[96,110]. Homophilic interaction of these proteins might form links that interconnect stereocilia within a bundle. Protocadherin 15 appears in the stereocilia of developing mammalian hair cells and persists in adult hair cells along the length of stereocilia, indicating that it might be important for the long-term maintenance of lateral connections (sidelinks) between stereocilia[111]. Cadherin 23 is located at the tips of the bundle in frog and zebrafish hair cells and has been proposed as an essential component of tip-links[112,113]. During development in mammals, cadherin 23 and harmonin transiently appear together in stereocilia, and have been reported to diminish to undetectable amounts in adult stereocilia[108]. However, a recent description of cadherin 23 persisting in the tips of adult mammalian stereocilia[112] indicates that its contribution to the maintenance of mature stereocilia links remains to be resolved.

At least some hair-bundle adhesion complexes seem to be linked by unconventional myosin VIIa to the actin core at sites of links between stereocilia (FIG. 5b,c). At the lateral surface, myosin VIIa probably links USH1 macromolecular complexes (harmonin–cadherin 23–SANS) to the actin filaments during hair-bundle maturation[104,108,109]. At the base of the stereocilium, myosin VIIa interacts with a novel transmembrane protein, vezatin, and could comprise part of an adhesion complex that is associated with ankle-links[65]. Ankle-links might determine the precise positioning and concomitant growth of individual stereocilia within a bundle[114]. The unique location and putative composition of ankle-links are consistent with an additional role: an adhesion complex that regulates actin–myosin-based trafficking through the taper of a stereocilium. Although there is no direct evidence to support this model, a barrier at the junction of the taper and the main body of the stereocilium could maintain the segregation of the molecular determinants of these distinct structural domains.

RETINITIS PIGMENTOSA
An aetiologically heterogeneous disorder that is characterized by progressive loss of vision and retinal photoreceptor degeneration.

Mechanotransduction and morphogenesis

Mature mechanotransduction currents can be detected in mammalian vestibular (mouse utricle) hair cells by embryonic day 16 or 17 (REF. 115), in cochlear hair cells earlier than postnatal day 0 (REF. 116) and in chick auditory hair cells by embryonic day 12 (REF. 117). At all of these developmental stages, the morphological maturation of the hair bundles is not fully complete. This temporal relationship raises the possibility that mechanotransduction currents are required to complete the morphogenesis of the hair bundle, and that some or all of the mechanotransduction apparatus is assembled at the tips of stereocilia before the final elongation of stereocilia occurs. Before these observations, Tilney and co-workers speculated that growth of the tallest row of stereocilia increases tension on the tip-links and opens transduction channels in the next row of stereocilia to initiate their growth and generate a staircase morphology[114]. Extending this hypothesis further, a threshold level of transduction current activity might determine whether a stereocilium is incorporated into the bundle or resorbed as a microvillus, providing a potential explanation for the conserved number of rows of stereocilia in mammalian auditory hair cells. Calcium that enters the cell through mechanotransduction channels might destabilize calcium-sensitive crosslinks between actin filaments in the microvilli that are adjacent to nascent stereocilia. By contrast, stereocilia would be unaffected because their actin core is crosslinked by calcium-insensitive espin[30].

Missing pieces of hair-bundle morphogenesis

The genetic investigation of hearing loss has revealed various genes and steps that are required for hair-bundle morphogenesis. The remaining pathways might be determined by characterizing additional mouse mutants, such as the recessive pirouette (*pi*) and dominant Tasmanian devil mutants that have thin stereocilia, which are abnormally short in *pi*[118] and abnormally long in Tasmanian devil mice[119]. Tailchaser (*Tlc*) is a dominant mutation in which stereocilia fail to form the normal V-shaped configuration on outer hair cells[120]. Similarly, Headbanger is a dominant mutation that disrupts development of the V-shaped configuration, but only in the apex of the cochlea, whereas the inner hair cell bundles in the apex fuse postnatally to form giant stereocilia, and the stereocilia of one of the vestibular end organs (the utricle) are abnormally long and thin[121]. The last three mutants were generated and identified in a large-scale ethylnitrosourea (ENU) mutagenesis screen for deafness and vestibular phenotypes[122], which has been a valuable adjunct to the finite supply

of spontaneous mouse mutants. Finally, human families that segregate deafness continue to be a fruitful resource to identify genes that are required for hearing and hair-bundle morphogenesis.

Conclusions

Several developmental themes have emerged from the identification of genes that are required for the morphogenesis of the hair cell bundle. Many of these genes encode intracellular motors, adhesion proteins or scaffolding proteins that interact with them (TABLE 1). Hair cells have therefore adapted and integrated mechanisms of cell-to-cell adhesion and intracellular motility to generate their precisely organized hair bundles. However, it is clear that there are still crucial genes and pathways that remain to be determined, indicating that the genetic investigation of hair-bundle morphogenesis is incomplete. It is prudent to continue positional cloning of genes that underlie hearing or balance disorders in humans and mice, especially genes that are expressed at low levels that elude detection by other screening methods. As some mutations might escape detection in forward-genetic screens owing to embryonic or early postnatal lethal phenotypes, reverse-genetic approaches, including conditional knockouts, are needed to fill in the molecular gaps in hair cell morphogenesis. Candidates might initially be identified through protein-interaction screens, proteomic analyses, genomic approaches or a combination of these methodologies. This integrated approach should lead to a comprehensive understanding of hair-bundle morphogenesis and insights into the pathogenesis of hearing loss and balance disorders, as well as strategies for their prevention and treatment.

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Competing interests statement

The authors declare that they have no competing financial interests.

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