Regulation of cell fate in the sensory epithelia of the inner ear

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Abstract | The sensory epithelia of the inner ear contain mechanosensory hair cells and non-sensory supporting cells. Both classes of cell are heterogeneous, with phenotypes varying both between and within epithelia. The specification of individual cells as distinct types of hair cell or supporting cell is regulated through intra- and extracellular signalling pathways that have been poorly understood. However, new methodologies have resulted in significant steps forward in our understanding of the molecular pathways that direct cells towards these cell fates.

Mechanosensory hair cells
The primary transducers of pressure waves in the inner ear. Each is characterized by the presence of a stereociliary bundle on its lumenal surface.

Otocysts/otic vesicles
Bilateral ectodermal invaginations that constitute the primordia of the inner ear.

Organ of Corti
The sensory epithelium of the mammalian cochlea, characterized by the presence of inner and outer hair cells as well as at least four different types of supporting cell.

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In vertebrates, the perceptions of sound and balance are mediated by epithelial sensory patches located in the cochlear and vestibular regions of the inner ear. Each patch is comprised of mechanosensory hair cells and non-sensory supporting cells that surround the hair cells to form an alternating mosaic. Hair cells and supporting cells are derived from a pool of epithelial progenitors initially located in the ventromedial region of the otocyst. During the course of embryonic development, these progenitors become specified as prosensory precursors that then become partitioned to different regions of the ear. Depending on their position, prosensory precursors then give rise to either vestibular or cochlear sensory patches, and cells in those patches ultimately assume final fates as either hair cells or supporting cells. The generation of this diversity requires multiple rounds of genetic and cellular interactions, resulting in the progressive restriction of cells to particular cellular phenotypes.

The inner ear represents a fascinating microcosm for the study of vertebrate development. From a single initial sphere of cells, individual regions of the otocyst must be specified along three anatomical axes, with both sensory and non-sensory structures specified according to their location. Sensory structures must be directed to develop in the correct regions of the ear, and individual cells in those structures must develop as specific types of hair cells or supporting cells. Moreover, hair cells and supporting cells must be arranged in a precise, alternating mosaic that is as important for its function as it is intriguing in its development. In fact, the regularity of the cellular pattern in the mammalian auditory sensory epithelium (the organ of Corti) far exceeds that of nearly all other vertebrate epithelial structures. These unique and varied requirements for the development of a functional inner ear provide us with a rare opportunity to discover and understand not only the nature of the molecular signalling pathways that direct cell fate and patterning, but also how those factors work together in a fairly small number of cells to generate a remarkably diverse, yet precisely patterned structure.

Studies of inner ear development also promise to identify the genes and pathways that are crucial in generating specific types of hair cell and supporting cell. Because the loss of either cell type leads to a progressive and permanent loss of auditory or vestibular function, forced regeneration through gene therapy could represent the best opportunity for the development of regenerative therapies. In this review, recent results identifying specific factors that regulate cell fate choices along the sensory lineage will be reviewed and discussed in light of the existing body of knowledge regarding sensory development and regeneration in the inner ear.

Structure of the inner ear

The inner ear is recognized as a remarkable structure that mediates multiple sensory inputs, including sound, balance and acceleration, using a relatively limited number of sensory cell types (FIG. 1). In fact, the sensory cell for each of these inputs is the hair cell, a modified epithelial cell that utilizes a group of derived microvilli, referred to as stereocilia, to perceive pressure waves induced through either sound or motion. However, significant variations in hair cell morphology exist both between different sensory epithelia, and even within a single epithelium. For example, vestibular epithelia contain Type I and Type II hair cells, which are characterized by differences

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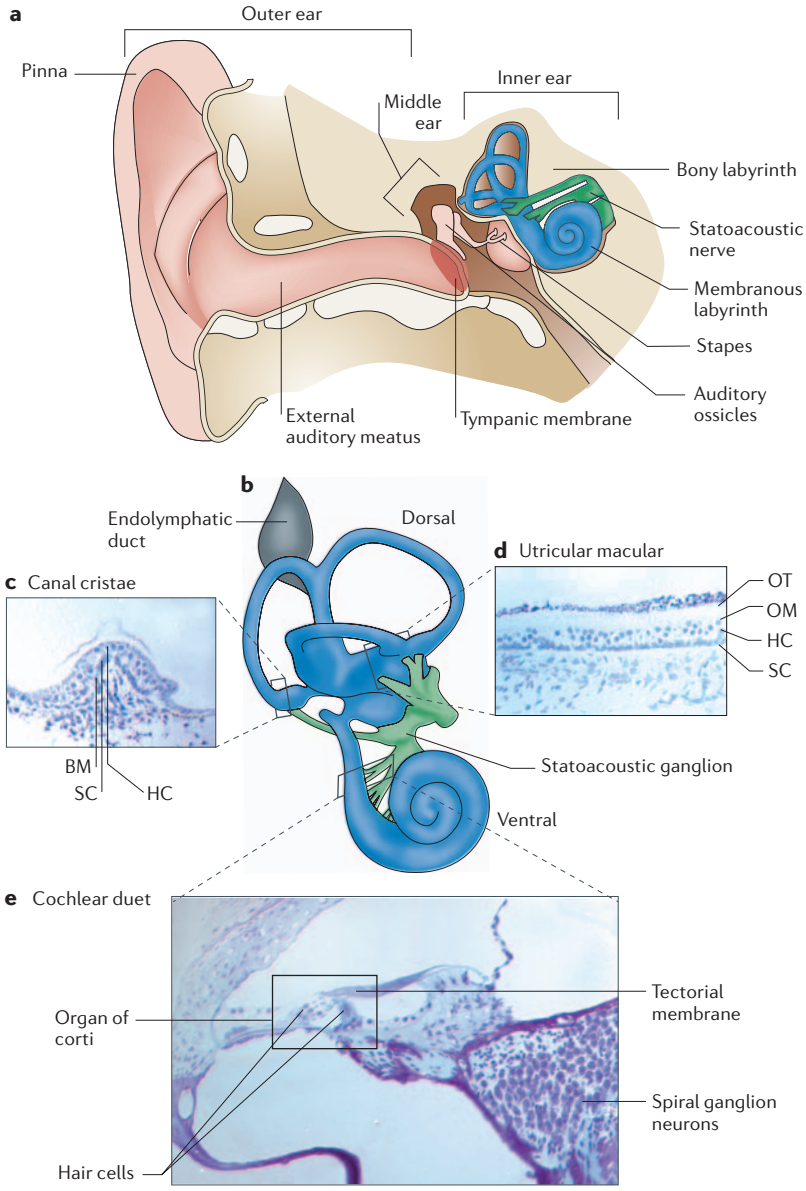

Figure 1 | **Anatomy of the auditory system, membranous labyrinth, and sensory epithelia. a** | Diagrammatic cross-section through the human head illustrating the three (outer, middle and inner) regions of the auditory system. The outer ear is comprised of the pinna and external auditory meatus, and is bounded on its medial side by the tympanic membrane. The middle ear is an air-filled space containing the three auditory ossicles bounded by the tympanic membrane and the round and oval windows. The third ossicle, the stapes, covers the oval window. The inner ear is comprised of the membranous labyrinth (blue), which is surrounded by a bony labyrinth and innervated by the VIIIth (statoacoustic) cranial nerve. **b** | The isolated membranous labyrinth, endolymphatic duct and statoacoustic ganglion. The membranous labyrinth is comprised of a dorsal, vestibular portion that mediates the senses of balance and acceleration, and a ventral, auditory region that mediates the sense of hearing. **c** | Cross-section through one of the canal cristae, illustrating the raised sensory epithelium. A layer of mechanosensory hair cells (HC) is located at the lumenal surface with a layer of non-sensory supporting cells (SC) located adjacent to the basement membrane (BM). **d** | Cross-section through the utricular macula (sensory epithelium). The structure is similar to that of the cristae, with a lumenal layer of hair cells located above basally located supporting cells. In addition, in the utricular and saccular maculae, hair cell stereociliary bundles project into an otoconial membrane (OM) that also contains otoconia (OT). **e** | Cross-section through the cochlear duct, illustrating the sensory epithelium (the organ of Corti; boxed region) and the associated spiral ganglion neurons . As in the utricular macula, the hair cells in the organ of Corti are covered by a gelatinous membrane referred to as the tectorial membrane.

in their morphology, electrophysiology and innervation[1]. Similarly, cochlear epithelia in both birds and mammals contain two distinct hair cell types, inner and outer hair cells in mammals, tall and short hair cells in birds. As is the case for vestibular hair cells, cochlear hair cell types can be distinguished based on their morphology and physiology[2–4]. In addition to hair cells, each sensory patch also contains a variable number of non-sensory cells, collectively known as supporting cells. In many sensory epithelia, the population of supporting cells seems largely homogenous, with no consistent morphological or molecular heterogeneity. However, in the mammalian cochlea, at least four unique types of supporting cell can be identified, indicating that there is diversity in supporting cells as well (FIG. 2).

In its most basic state, a sensory patch is comprised of a population of several thousand or more hair cells arranged in a circular or oblong patch. However, there are examples of morphological specializations, such as the elongated auditory sensory epithelia found in some reptiles and all birds and mammals[5], with the most striking example of these specializations being the highly derived sensory epithelium of the mammalian organ of Corti (FIG. 2).

Morphological development of the inner ear

The inner ear begins development as a bilateral thickening of the surface ectoderm, referred to as the otic placode, in the region located adjacent to the developing hindbrain[6]. Transplantation studies conducted in amphibians and birds have indicated that a large region of head ectoderm can develop as otic placode if it is placed in the appropriate location, showing that inductive signals that arise from the region of the otic placode are sufficient to induce otic identity[7–9]. Recent results addressing the intrinsic and extrinsic signalling pathways involved in otic induction have been the subject of several reviews[10–13], and therefore will not be discussed here. Soon after induction, the developing placodes descend beneath the surface ectoderm to form first the otic cups and ultimately the otic vesicles.

Following the formation of the otic vesicles, a series of morphological changes occur (FIG. 3). Neuroblasts that will give rise to the statoacoustic ganglion (SAG) begin to delaminate from the ventral region of the otocyst[14–16]. At the same time or soon after, a narrow extension originates in the dorsomedial region of the otocyst and extends towards the brain to form the endolymphatic duct and sac[17,18]. Similarly, the ventral region of the otocyst extends to form the cochlear duct (FIG. 3). As development continues, individual regions in the developing labyrinth become specified to develop as the prosensory patches that will contain mechanosensory hair cells and non-sensory supporting cells. These regions can be identified by an increased thickness of the epithelium and the presence of developing SAG neurites[19,20]. A total of six sensory regions are present in the mammalian inner ear: the three cristae associated with the semi-circular canals, the utricular and saccular maculae, and the organ of Corti.

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In each prosensory region, hair cells are the first identifiable differentiated cell types[21–23]. Typically, the first hair cells develop in a specific region of the epithelium, such as the centre of the utricular epithelium or the mid-basal region of the cochlear duct[24,25]. As development continues, additional hair cells begin to appear in a stereotypical wave of differentiation. Supporting cells develop in a similar pattern to hair cells, although with a delay that depends on the species and region of the inner ear.

Specification of prosensory patches

The first step in the development of the sensory epithelia is the specification of a prosensory anlage/anlagen in the otocyst. At present it is not clear whether all of the prosensory regions are derived from a single posterior–ventral anlage that rapidly becomes subdivided, or if two anlagen, one anterior and one posterior, are specified independently. Regardless, the development of the anlage/anlagen has been examined using a number of different markers including bone morphogenetic protein 4 (*Bmp4*), lunatic fringe (*Lfng*), jagged/serrate 1 (*Jag1*), *Islet1*, prospero-related homeobox 1 (*Prox1*), and fibroblast growth factor 16 (*FGF16*), all of which are expressed in patterns that are consistent, to some extent, with the early development of the prosensory anlage/anlagen[26–32]. For example, in the chicken, *BMP4* expression is initially observed in a somewhat broad and diffuse pattern along the posterior ventral edge of the developing otic cup[30,33,34]. However, as the cup closes, *BMP4* expression resolves to a single posterior ventral spot and an anterior ventral stripe. Subsequently, *BMP4* expression can be localized to each of the developing prosensory patches. *Bmp4* similarly defines the prosensory patches for the three developing cristae in the developing mouse embryo, but surprisingly is not expressed in the prosensory patches that will give rise to the utricular or saccular maculae or the organ of Corti[29].

BMP4 in prosensory formation. Based on its pattern of expression, it has been suggested that BMP4 has a role in the specification of prosensory patches. However, modulation of BMP4 signalling in developing chick embryos through the ectopic expression of noggin, a BMP4 inhibitor, produced equivocal results in terms of a direct role for BMP4 on prosensory patch formation[35,36]. Although sensory patches were affected when located near a source of noggin, the most common change was in cellular patterning rather than in the size of the sensory patch. As the presence of noggin results in significant morphological changes in the overall structure of the inner ear, the basis for these defects was not clear. More recently, two separate studies have addressed the role of BMP4 in hair cell formation *in vitro* using chick otocyst cultures. Surprisingly, despite using similar protocols and reagents, the two studies obtained opposing results, with one concluding that BMP4 promotes hair cell formation[37], while the other indicated an inhibitory role for BMP4 (REF. 31). The basis for these dichotomous results are unclear, but might be related to differences in the concentrations of BMP4 and the duration of the culture period used in the two studies. Li *et al.*[37] observed a significant increase in hair cell formation in otocysts after 7 days in the presence of 3–5 ng ml^{-1} of BMP4 but also found a downward trend in hair cell number at concentrations between 10 and 20 ng ml^{-1}. By contrast, Pujades *et al.*[31] observed a decrease in the expression of the early hair cell marker *CATH1* (chicken atonal homologue) after only 18 hours in the presence of 50 ng ml^{-1} of BMP4. Perhaps more intriguing, despite using very similar concentrations of noggin (0.75 mg ml^{-1} versus 1.0 mg ml^{-1}), the two studies reached opposite conclusions about the effects of inhibition of BMP4, with one finding that hair cell number was decreased[37] whereas the other found an increase[31]. Again, it seems possible that the differences in the durations of the experiments could account

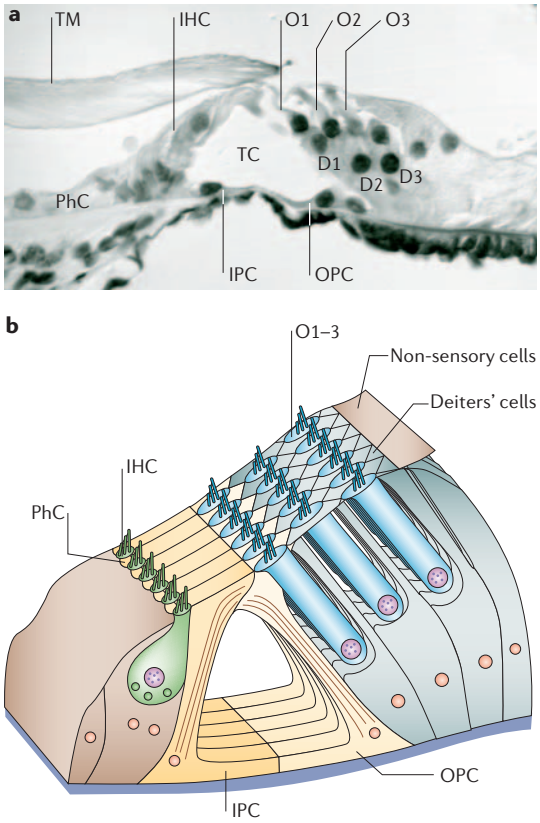

Figure 2 | **The organ of Corti. a** | Cross-section through the organ of Corti in an adult mouse. A single inner hair cell (IHC) and three outer hair cells (O1–3) are separated by the tunnel of Corti (TC). The tunnel is formed by two cell types, the inner pillar cells (IPC) and the outer pillar cells (OPC), each of which extends a projection to the lumenal surface. Inner hair cells are separated from one another by inner phalangeal supporting cells (PhC), whereas outer hair cells are separated by Deiters' supporting cells (D1–3). The tectorial membrane (TM) overlies the sensory epithelium and normally contacts the stereocilia of the outer hair cells (the TM in this section has retracted away from the outer hair cells as a result of fixation). **b** | Three-dimensional depiction of the organ of Corti illustrating the organization of hair cells and supporting cells into rows. A single row of IHCs and PhCs is located adjacent to single rows of IPCs and OPCs. Three rows of outer hair cells and Deiters' cells are located next to the row of OPCs.

Otic placode
Bilateral thickening of the surface ectoderm located adjacent to the developing hindbrain. With continued development, placodes invaginate to form the otocyst.

Otic cup
Bilateral depressions of the otic placodes that form as a transitional phase between the otic placode and the otocyst.

Statoacoustic ganglion
(SAG). Also known as cranial nerve XIII. The cell bodies of the afferent nerves that innervate both the vestibular and auditory regions of the inner ear. All neurons in the ganglion are derived from cells that delaminate from the otocyst.

Cristae
The inner ear sensory epithelia associated with the three semicircular canals.

Utricular/saccular maculae
Inner ear sensory epithelia associated with the utricle, a part of the vestibular system that mediates the perception of balance.

Anlage/anlagen
Describes a region in the otocyst that has become specified to develop as a prosensory patch but in which no morphological indications of prosensory identity have yet become obvious.

Deiters' cells
A specialized type of supporting cell within the organ of Corti that surrounds the outer hair cells.

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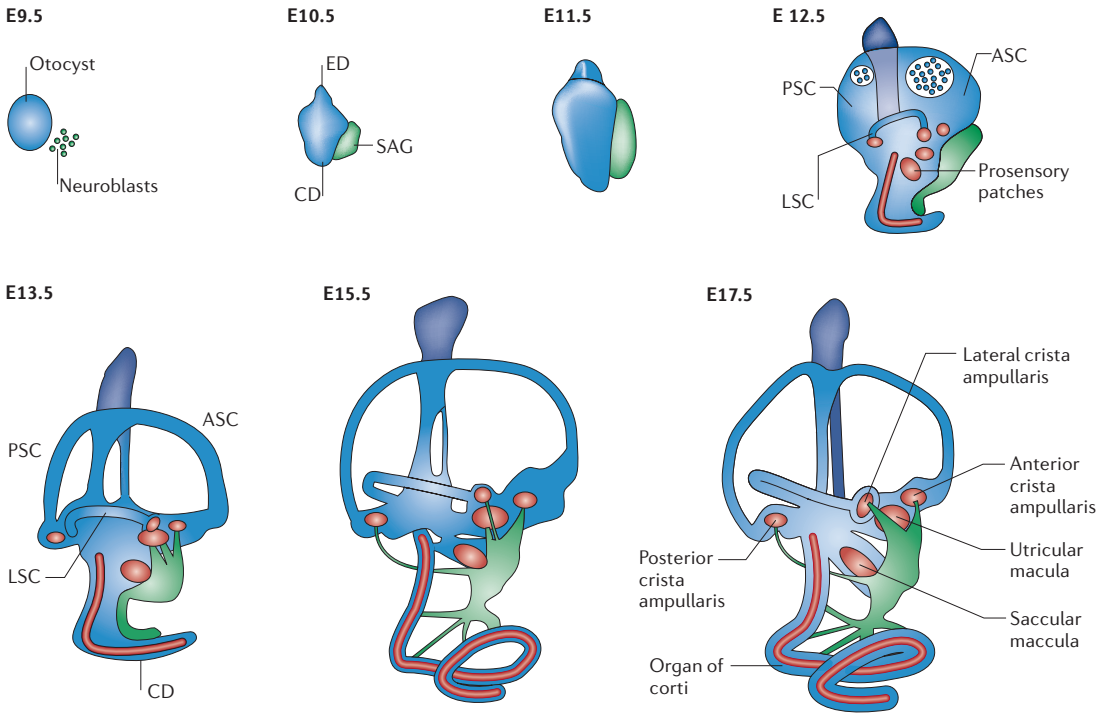

Figure 3 | **Development of the inner ear in the mouse.** All the structures in the inner ear develop from an otic placode that invaginates to form the spherical otocyst by E9.5. Soon after closure of the otic pit to form the otocyst, neuroblasts delaminate from its ventral region. These neuroblasts will coalesce adjacent to the developing inner ear and begin to form the statoacoustic ganglion (SAG). By E10.5, the otocyst begins to change shape as a result of the formation of dorsal and ventral protrusions that will ultimately develop as the endolymphatic duct (ED) and the cochlear duct (CD). By E12.5, the developing semicircular canals, anterior (ASC), posterior (PSC) and lateral (LSC) can be identified. Each canal forms as a flattened outgrowth from the otocyst. The central portion of each outgrowth ultimately undergoes a period of cell resorption (speckled regions) to form the mature canal phenotype[124]. In addition, the positions of the developing sensory patches can be identified, and the developing cochlear duct begins to form a spiral. At E13.5, the cochlear duct has completed approximately three-quarters of one turn and the neurons in the developing SAG have extended dendrites that will form contacts with developing hair cells in all of the sensory epithelia. Between E15.5 and E17.5, different regions of the inner ear continue to grow and expand, including the cochlea, which reaches its mature length of approximately one and three-quarter turns, and the semicircular canals. Modified, with permission, from REF. 29 © (1998) Society for Neuroscience.

for the differing conclusions. Therefore, although the expression of *Bmp4* in developing prosensory patches seems most consistent with a role in their specification, as results from my laboratory suggest (K. Puligilla and M.W.K., unpublished observations), the results from the two *in vitro* studies, along with the observation that *Bmp4* is not expressed in half of the sensory epithelia in the mouse inner ear, suggest that the role of BMP4 is probably more complex than a straightforward role in prosensory specification.

Notch signalling is required for prosensory patch formation. Two additional genes that are expressed in patterns that are largely consistent with a role in the specification of prosensory patches are *Jag1* and *Lfng*, both of which are components of the Notch signalling pathway, with JAG1 acting as a ligand for Notch, while LFNG modulates the activity of some Notch ligands[38,39]. Although both genes are initially expressed in more diffuse patterns in the optic cup, each ultimately resolves to the developing prosensory regions[16,29,30,34]. However, an important exception is the expression of *Jag1* in the mammalian cochlea which abuts with, rather than overlaps, the prosensory domain until well past the developmental stages at which prosensory specification takes place[40,41]. Consistent with a role for Notch signalling in prosensory specification, Notch 1 is expressed throughout the otocyst, beginning at the pre-placodal stage in fish, chicks and mice, and its expression is maintained throughout inner ear development[13,16,42,43]. In *Drosophila*, the expression of Notch, delta (a Notch ligand) and fringe (a homologue of LFNG) regulates boundary formation such that cells that express all three molecules are refractory to delta-mediated Notch activation, while Notch can be activated in adjacent cells that do not express delta or fringe[44–47]. Therefore, it seems reasonable to predict that Notch signalling could have a similar role in specifying the boundaries of the prosensory patches, with Notch being activated in cells on the non-sensory side of the sensory/non-sensory border (FIG. 4).

However, the existing experimental data, although still incomplete, suggests a more varied role for Notch signalling in the specification of prosensory patches. The most obvious testable hypothesis would be that loss of Notch signalling should lead to an increase in

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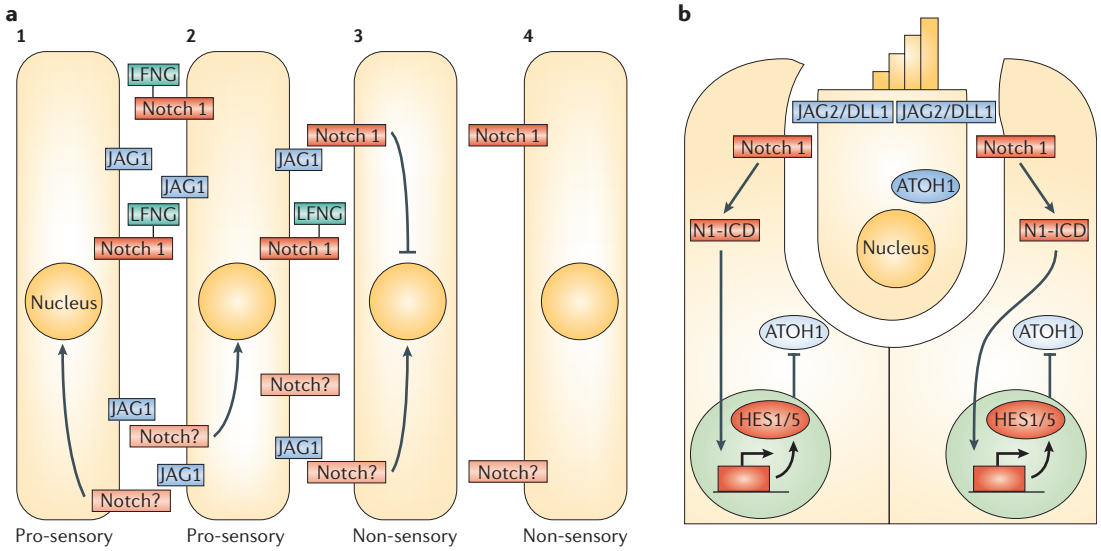

Figure 4 | **Proposed roles for Notch signalling in the development of sensory epithelia. a** | Proposed interactions that specify the boundaries between prosensory patches and non-sensory epithelium. Prior to the specification of prosensory patches, all epithelial cells in the otocyst express Notch 1 (REF. 13) and, it is proposed, a second, unidentified Notch (Notch?). Cells that will develop in a prosensory patch upregulate the expression of the Notch 1 ligands jagged (*Jag1*) and lunatic fringe (*Lfng*)[6,29,32]. LFNG inhibits binding of JAG1 to Notch 1 by selective glycosylation[39,40,43,96], but does not affect the ability of Notch? to bind JAG1. In cells 1 and 2, activation of Notch? is instructive for development of a prosensory cell (black arrow) and the presence of LFNG inhibits activation of Notch 1. Cell 3 is directly adjacent to the prosensory domain and does not express *Jag1* or *Lfng*. JAG1 (expressed by cell 2) binds to Notch 1 and Notch? on cell 3, activating both the instructive and inhibitory pathways. The inhibitory pathway is sufficient to block the effects of the instructive pathway, and cell 3 develops as a non-sensory cell. In cell 4, although both Notch 1 and Notch? are expressed, there is no activation of these pathways in the absence of a ligand and this cell also develops as a non-sensory cell. **b** | Once a prosensory patch has been specified, interactions between individual cells in the patch determine which cells will develop as hair cells or supporting cells. Developing hair cells express *Jag2* and delta 1 (*Dll1*)[49,95,96]. Both ligands bind to Notch 1 in adjacent cells, leading to the generation of Notch intracellular domain (ICD) fragments[88,99]. This upregulates the expression of two inhibitory basic helix–loop–helix proteins, hairy and enhancer of split 1 (HES1) and HES5 (REFS 85,94,97,98), which block the effects of prosensory genes such as *Atoh1*, leading to inhibition of the hair cell fate. Inhibited cells subsequently develop as supporting cells. Recent results have indicated that expression of *Notch 1-ICD* (N1-ICD; for example, by viral transfection) in non-sensory regions of the ear can lead to the formation of ectopic sensory patches[55]. These results seem to be contradictory to the interactions proposed above. However, functional compensation of one Notch ICD for another has been demonstrated[125], suggesting that the effects of Notch 1-ICD in the specification of prosensory patches could be a nonspecific response to the presence of any Notch ICD within the cell, and that an as yet unidentified Notch acts to specify prosensory identity during inner ear development.

the number or size of prosensory patches. And, in fact, complete inhibition of Notch signalling in the zebrafish otocyst as a result of a mutation in *mind bomb* (*mib*), a ubiquitin ligase that is essential for Notch function[48], results in an increase in the size of the initial sensory patch[43,49]. Similarly, deletion of one of the Notch ligands, deltaA, also results in a larger initial sensory patch in zebrafish[50].

Notch 1 has also been selectively inactivated in mice using a *FoxG1–Cre* driver that results in its excision throughout the otocyst beginning around embryonic day (E) 9.0. Analysis of the inner ears of these mice was restricted to the cochlea. Nevertheless, the results seem to be consistent with the result from *mib* zebrafish, in that there was an increase in the size of the sensory epithelium[51]. However, it is not clear whether this increased size occurred as a result of an increase in the size of the prosensory patch or from defects in lateral inhibition at the single cell level (which will be discussed below). In addition, no ectopic prosensory patches were observed. Therefore, whereas the results obtained in zebrafish are consistent with a role for Notch signalling in the placement of sensory/non-sensory boundaries, the data from inactivation of *Notch 1* are less conclusive. However, there are four mammalian Notch genes[52], suggesting that functional redundancy or compensation could also have a role.

In contrast to the results described above, several more recent studies have suggested a different role for Notch signalling during prosensory patch formation. As discussed, *Jag1* is the only known Notch ligand that is expressed in developing prosensory patches in the early otocyst. Therefore, if JAG1 acts as a ligand for Notch 1 in the otocyst, then deletion of *Jag1* should result in a phenotype similar to that observed when *Notch 1* is deleted. Complete deletion of *Jag1* results in early embryonic lethality[53]. However, conditional deletion using the *FoxG1–Cre* line to delete *Jag1* in the otocyst resulted in a contrasting phenotype to that of deleted *Notch 1*. Rather than having an overproduction of hair

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Box 1 | **Prosensory progenitors and SAG neuroblasts – a clonal relationship?**

Recently, it has been suggested that the neuroblasts that give rise to the neurons of the statoacoustic ganglion (SAG) and the progenitors that give rise to hair cells and supporting cells might derive from a common precursor, and therefore hair cells and the sensory neurons that innervate them might be clonally related[108–112]. A number of observations support this hypothesis. Spatial profiling of gene expression in the otocyst indicates that both neuroblasts and sensory progenitors develop in the same ventromedial region[16,62,113]. In addition, some factors, such as lunatic fringe (*Lfng*) and *Islet1* are expressed, at least at some point during development, in cells that will develop as either neuroblasts or hair cell progenitors[26,27,29,34]. Moreover, there is evidence suggesting that *Atoh1* is expressed in some auditory and vestibular ganglion neurons, and there is expression of neurogenin 1 (*Ngn1*) derivatives in a limited number of hair cells (S. Raft, A. Groves and N. Segil, personal communication). Finally, the loss of hair cells in *Atoh1* mutants is accompanied by ectopic neurogenesis in parts of the vestibular system that normally generate sensory epithelia (Raft, Groves, N. Segil, personal communication). These last findings are particularly relevant because the deletion of individual proneural genes in the developing CNS results in an increase in glial cells as a result of a shift in the cell fate bias of a common progenitor.

Although these indirect data support a clonal relationship between hair cells and sensory neurons, it is important to consider that a direct test of this hypothesis by lineage tracing with dye injection or retrovirus has never been conducted in the auditory system of any mammal because of the inaccessibility of the otocyst. By contrast, lineage tracing has been conducted in the more accessible chicken otocyst. In the most relevant analysis, 11 samples with clonally related cells were identified in different regions of the ear[114], of which only three contained clonally related cells in the auditory or vestibular ganglion, or both, and in one of the sensory patches. Eight clones were restricted to the auditory ganglion or spanned the auditory and vestibular ganglions with no contribution to any of the sensory patches. The authors concluded that although ganglion cells and mechanosensory hair cells can arise from a common progenitor, this lineal relationship tends to be the exception rather than the rule. Therefore, although further study is required, at present it seems that while a single cell in the otocyst can generate daughter cells that develop as either neuroblasts or sensory progenitors, the number of these cells is fairly small and they do not represent the most common type of progenitor in the otocyst.

cells, *Jag1* mutant ears have a significant decrease in the size of all sensory patches, with some of the vestibular sensory regions decreased almost to non-existence[40,54]. Similar phenotypes have been reported in two mutant mouse lines generated by treatment with the alkylating agent ethylnitrosourea (ENU) and carrying missense mutations in the second extracellular epidermal growth factor repeat of *Jag1*, a region that is important for interactions with Notch[55,56]. In addition, overexpression of an activated form of chicken Notch 1, *cNotch 1-ICD* (Notch-intracellular domain), in the non-sensory regions of the chick otocyst leads to the formation of ectopic sensory patches[57] (FIG. 4).

The results discussed above seem contradictory, and additional experiments are clearly required. However, with the exception of the results observed in *mib* zebrafish, a reasonable hypothesis would be that interactions between JAG1 and a Notch protein other than Notch 1 have a role in the specification of prosensory patches. As prosensory patches are present in otocysts that lack Notch 1 from E9 onwards[51], it seems unlikely that this receptor mediates prosensory patch formation. However, functional redundancy or compensation by another Notch protein at the level of prosensory patch specification could also account for the observed results. The ability of cNotch 1-ICD to induce ectopic sensory patches would suggest that the effects of Notch signalling on prosensory specification are non-specific, and that the placement of patches is regulated at the level of *Jag1* expression. As activation of Notch 1 is not required for the specification of prosensory patches, but activation of Notch 1 at later stages in sensory patch development is known to inhibit cell commitment (as will be described below), a further implication of this hypothesis would be that LFNG might prevent JAG1-dependent activation of Notch 1 through glycosylation-dependent inhibition of binding of JAG1 to Notch 1, while allowing or facilitating JAG1-dependent activation of another Notch protein. One of the most informative experiments at this point would be to completely inactivate the Notch signalling pathway at the level of the common effector RBP-J (recombining binding protein suppressor of hairless)[58]. If the Notch signalling pathway does have a role in prosensory patch specification, then deletion of RBP-J should lead to the complete loss of prosensory patches in the ear.

Tbx1 regulates prosensory versus neural identity. The neuroblasts that will delaminate from the otocyst to develop as the SAG arise from a region of the otocyst that closely abuts or overlaps with the prosensory anlage (BOX 1). Recently, the transcription factor T-box 1 (*Tbx1*)[59–61] has been implicated in the specification of neural versus sensory domains. *Tbx1* is initially expressed in a posterior–ventral region of the otocyst that correlates with the location of the first expression of *Bmp4* (REF. 62). In *Tbx1* mutant mice, expression of *Bmp4* is reduced, sensory epithelia fail to form and there is an increase in the size of the SAG[62]. In addition, ectopic expression of *Tbx1* throughout the otocyst results in larger and ectopic sensory epithelia[63]. Finally, DiGeorge syndrome, in which sensorineural hearing loss occurs, results from mutations in *TBX1* (REF. 64). These results clearly demonstrate a role for TBX1 in the specification of inner ear sensory epithelia; however, it is not clear whether it acts directly to induce the formation of the prosensory anlage or indirectly through its effects on the anterior–posterior patterning of the otocyst. In fact, several markers of anterior–posterior identity are altered in *Tbx1* mutants[62], indicating a role in otocyst axial patterning. Axial patterning markers are also altered in mice from the bacterial artificial chromosome

T-box transcription factors
A family of transcription factors characterized by similarity in the DNA binding domain (the T domain).

DiGeorge syndrome
Also known as velocardiofacial syndrome. A genetic disorder caused by mutations in *TBX1*. Affected individuals can have malformations of the heart, face, limbs and auditory system.

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(BAC) transgenic line 316.23, in which *Tbx1* is broadly expressed throughout the otocyst, indicating that the changes in size of the sensory epithelia could be a result of axial respecification. Therefore, it would be appropriate to directly test the ability of TBX1 to induce prosensory patches, possibly by using a retroviral approach similar to the one used to examine the effects of expression of *cNotch 1-ICD*[57].

Sox2 is required for the development of sensory epithelia. Recently, two ENU-generated mouse mutants were identified in which the expression of the high mobility group (HMG)-box transcription factor *Sox2* in the inner ear was either significantly reduced (*Ysb*) or completely eliminated (*Lcc*)[65,66]. Both *Ysb* and *Lcc* mice are deaf, exhibit circling behaviour[65] and have profound defects in inner ear development[66]. Formation of the sensory epithelia was shown to be significantly disrupted in both mutants, with a direct correlation between the number of hair cells and the level of *Sox2* expression. Analysis of the expression of late markers for sensory epithelia indicated either a significant reduction (*Ysb*) or complete loss of expression (*Lcc*). By contrast, *Sox2* expression is lost in *Jag1* conditional mutants[56], suggesting that *Sox2* acts after the early specification of prosensory patches. However, in chickens, *SOX2* is initially expressed throughout the otic placode[67], in a pattern that is broader and precedes *JAG1*.

The specific role of SOX2 in inner ear development is unclear. It has been shown to be necessary for the transition from a proliferating neuroblast to a postmitotic precursor in the developing CNS[68,69], and its loss in the inner ear results in a disruption in the expression of at least one cell cycle regulator, *p27^{kip1}* (REF. 66). However, appropriate assays have not been carried out to determine if the loss of SOX2 leads to an increase in cellular proliferation or in the size of the population of progenitor cells in the inner ear.

Auditory versus vestibular specification

Although all the prosensory patches in the inner ear derive from one or two anlage/anlagen, each patch develops with a unique morphology and contains unique cell types. In particular, hair cells located in auditory sensory epithelia are morphologically and physiologically distinct from hair cells in vestibular epithelia. The factors that generate this diversity are probably dependent, to some extent, on global patterning signals that act to specify the inner ear along its three dimensional axes. However, as disruption to axial patterning genes often leads to gross morphological changes in the overall structure of the inner ear[10,11], it has been difficult to analyse specific effects of these genes on sensory patch identity.

Recently, forced activation of the canonical WNT signalling pathway by retrovirally-mediated overexpression of either a constitutively active form of β-catenin (caβ-cat, a molecule that modulates transcriptional activity in response to canonical WNT signalling) or WNT3A in the developing chicken inner ear indicated a role for this pathway in the specification of sensory patch identity and, to a lesser extent, in the specification of prosensory patches[70]. When caβ-cat was expressed in regions in the developing auditory sensory patch, hair cells and supporting cells in that region developed characteristics that were consistent with a vestibular phenotype. Similar results were observed in response to overexpression of WNT3A, showing that this effect is mediated through the WNT pathway. Expression of caβ-cat in regions outside of the auditory sensory patch induced the formation of ectopic sensory patches. However, although caβ-cat was overexpressed in many different regions of the inner ear, ectopic sensory patches only occurred when caβ-cat overexpression was localized to regions near the developing auditory patch. Moreover, although ectopically induced sensory patches usually had a vestibular phenotype, some developed with an auditory phenotype, suggesting that activation of the WNT pathway might not directly specify vestibular fate.

Although the results discussed above represent the first experimental evidence of a factor that has a role in the determination of auditory versus vestibular identity, the specific role of the WNT signalling pathway remains unclear. As part of the same study, the authors demonstrated that *WNT4* is expressed exclusively in the auditory portion of the chicken ear but that the highest levels are actually located adjacent to two vestibular sensory patches, the saccular macula and the lagenar macula. Based on this pattern of expression, they suggested that high levels of WNT4 might specify vestibular identity; however, the lack of expression in the vestibular region of the ear would seem to contradict this hypothesis. Nonetheless, the results of overexpression of caβ-cat or WNT3A are compelling. At this point, most of the main regulators in the WNT pathway have been identified[71,72]. Therefore, a thorough screening of those factors would probably provide insights into the specific mechanisms that control auditory versus vestibular identity.

Regulation of cell cycle exit

A key step in the development of any differentiated structure is the transition from proliferating blast cells to non-proliferating progenitor cells. As discussed above, SOX2 has a role in this transition within the developing neural tube, but the possibility of a similar role in the inner ear has not been examined. Moreover, *Sox2* expression is initiated at the early otocyst stage, well before the terminal mitoses of any sensory cells, suggesting that other factors must also be involved.

Retinoblastoma 1 regulates cell cycle exit in sensory patches. Ultimately, exit from the cell cycle is regulated by a limited number of factors including pocket proteins such as retinoblastoma 1 (RB1)[73] and cyclin kinase inhibitors (CKIs), which include members of the INK4 and Cip/Kip families[74]. In most differentiating tissues there is a direct correlation between terminal mitosis and differentiation such that

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the first cells to exit the cell cycle are also the first cells to differentiate. Consistent with this observation, recent studies of the expression of *Rb1* in developing vestibular sensory patches indicate that it is expressed in a pattern that correlates with terminal mitosis[24] and cellular differentiation[75]. Moreover, as would be expected, deletion of *Rb1* in the inner ear results in delayed cell cycle exit, an overproduction of hair cells during the embryonic period and ectopic cellular proliferation in postnatal animals[75,76]. Similar defects in cell cycle exit and hair cell production were also observed in the developing auditory (cochlear) sensory patch; however, regulation of cell cycle control is considerably more complex in the organ of Corti.

p27^{kip1} regulates a unique pattern of cell cycle exit in the mammalian cochlea. As discussed above, in vestibular sensory patches the pattern of *Rb1* expression foreshadows terminal mitosis and cellular differentiation. However, in the mouse auditory sensory patch, *Rb1* expression is not initiated until E15.5 (REF. 75), even though terminal mitosis occurs between E12.5 and E14.5 (REF. 24). In addition, *Rb1* expression and cellular differentiation occur in a wave that begins in the base of the cochlear spiral and extends towards the apex[75]. By contrast, terminal mitosis occurs in a gradient that begins in the apex and extends towards the base[24]. This surprising reverse gradient of terminal mitosis is mediated by the onset of an apical-to-basal gradient of expression of the CKI *p27^{kip1}* in the cochlea beginning on E12.5 (REF. 77). *p27^{kip1}* is only weakly expressed in vestibular sensory patches, suggesting that it has an additional or unique role in the development of the organ of Corti. Deletion of *p27^{kip1}* results in a brief extension of cellular proliferation[78,79], an inversion in the apical-to-basal gradient of terminal mitosis (N. Segil, personal communication) and the generation of supernumerary hair cells and supporting cells[78,79]. Although it has not been specifically demonstrated, in the absence of *p27^{kip1}* the pattern of terminal mitoses seems to follow the pattern of *Rb1* expression. As development continues, *p27^{kip1}* and *Rb1* become segregated such that *Rb1* is expressed in hair cells whereas *p27^{kip1}* is restricted to supporting cells. The expression of *p27^{kip1}* is dependent on both JAG1 and SOX2 (REFS 54,56,66); however, a similar role for these proteins in the regulation of the expression of *Rb1* has not been determined. The *p27^{kip1}* mutant phenotype and the unique pattern and timing of expression of *p27^{kip1}* in the cochlea strongly suggests that the reverse gradient of cell cycle exit has an important role in limiting progenitor number during the development of the organ of Corti. However, the mechanisms governing this pattern of expression remain a mystery.

Specification of hair cells

Once developing prosensory progenitor cells have become postmitotic, the next step in the development of the sensory epithelia is the specification of a subset of cells in each patch to develop as hair cells. Developing prosensory patches are assumed to be comprised of progenitor cells that are uncommitted with regards to whether they will develop as hair cells or supporting cells. The data supporting this assumption derive from three sources. First, retroviral lineage tracing in the developing chicken auditory system identified the existence of two cell clones in which one cell had developed as a hair cell while the other had developed as a supporting cell[80,81]. Second, ablation of individual developing hair cells in the embryonic cochlea leads to a change in the fate of one of the surrounding progenitor cells, such that this cell now develops as a replacement hair cell[82]. Similar results have been obtained from studies of hair cell ablations in lateral line neuromasts in the Axolotl salamander[83]. Finally, extensive studies of cell fates have used a hair cell regeneration model in the adult chicken basilar papilla. Supporting cells act as the source for regenerated hair cells in this paradigm, with some supporting cells directly converting into hair cells through a process referred to as transdifferentiation, while other supporting cells re-enter the cell cycle to generate daughter cells that then become committed to develop as either hair cells or supporting cells[84,85].

Atoh1 specifies hair cells. The factors that specify cells to develop as either hair cells or supporting cells are still poorly understood, and most probably remain to be discovered. However, recent work has identified the basic helix–loop–helix (bHLH) transcription factor *Atoh1* (*Math1*) as a key regulator of hair cell development. *Atoh1* is initially expressed in all inner ear sensory epithelia at developmental stages coincident with, or soon after, terminal mitosis[41,86,87]. Deletion of *Atoh1* leads to a complete loss of hair cells, whereas overexpression of *Atoh1* in the embryonic cochlea is sufficient to induce cochlear progenitor cells to develop as hair cells[86,88]. Similarly, overexpression of *Atoh1* in the postnatal saccular macula induces hair cell formation, probably through the transdifferentiation of supporting cells[89]. Surprisingly, ectopic expression of *Atoh1* is sufficient to induce hair cell formation in non-sensory cells located near the organ of Corti in embryonic, postnatal and adult inner ears[89–91]. In embryonic or early postnatal tissue, *Atoh1*-induced ectopic cells seem comparable to endogenous hair cells in terms of the expression of hair-cell specific markers and the development of a stereociliary bundle[89,90]. However, the degree of differentiation in these ectopic hair cells seems to be affected by the age of the animal, such that ectopic hair cells in adult ears often appear dysmorphic[91]. Although a thorough examination of the molecular, morphological and physiological development of *Atoh1*-induced hair cells is clearly required, the existing data suggest that expression of *Atoh1* alone is sufficient to specify cells in prosensory patches, and in some cases outside of these patches, as hair cells. It should be noted that a second bHLH, hairy and enhancer of split 6 (*Hes6*), is expressed in a similar temporal–spatial pattern to *Atoh1*; however, neither deletion nor overexpression of *Hes6* has any obvious effect on hair cell development[92].

Neuromast
A small patch of hair cell sensory epithelium located in the lateral line organs of fish and amphibians.

Basilar papilla
The auditory organ in birds. The basilar papilla is elongated like the mammalian cochlea but is straight rather than coiled.

Transdifferentiation
The direct conversion of a cell from one differentiated cell type to another. In the inner ear this refers to the direct conversion of supporting cells into hair cells.

Basic helix-loop-helix (bHLH) transcription factors
A family of transcription factors that are characterized by a conserved basic domain that mediates DNA binding and a conserved helix–loop–helix domain that mediates dimerization.

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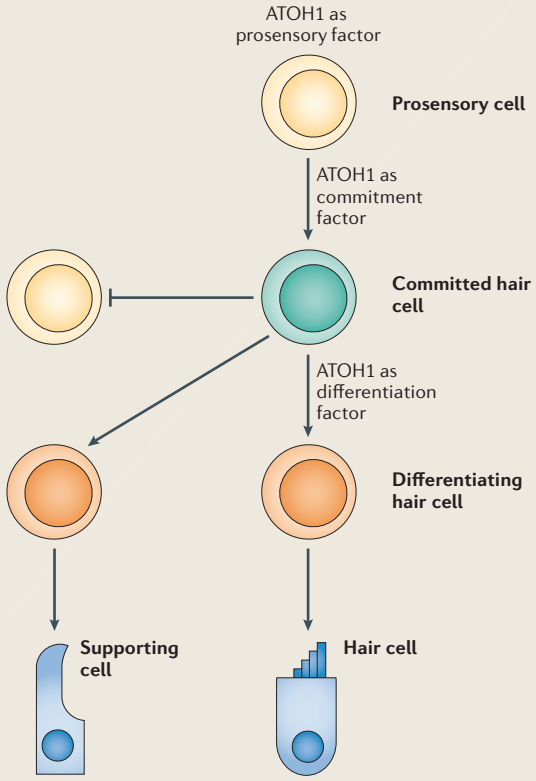
Box 2 | **ATOH1 — Prosensory factor, hair cell commitment factor or hair cell differentiation factor?**

Although ATOH1 has been shown to be necessary and sufficient for hair cell formation[86,89–91], the specific role of this gene remains in dispute. Based on patterns of expression, homology with similar genes in *Drosophila* and results of gain- and loss-of-function experiments, studies have suggested that ATOH1 might act as a prosensory factor, a hair cell commitment factor or a hair cell differentiation factor[41,87,90,93]. These different roles are illustrated in the figure.



If ATOH1 has a prosensory role in the inner ear, then it would be predicted to be broadly expressed in progenitors of both hair cells and supporting cells. Following the initial specification of prosensory domains, *Atoh1* expression might be downregulated entirely or maintained in a subset of cells, such as hair cells. Deletion of *Atoh1* would result in the loss of both cell types and of most precursor markers, and at least transient expression of *Atoh1* would be required for cells to develop as either cell type.

Alternatively, if ATOH1 acts as a commitment factor, and if it is assumed that many cells are initially committed to develop as hair cells, with a subset of these cells ultimately directed away from the hair cell fate through activation of the Notch pathway, then *Atoh1* would still be broadly expressed in progenitors of both hair cells and supporting cells, yet its expression would only be required for hair cell formation. Deletion of *Atoh1* could still lead to the loss of supporting cells indirectly, as a result of the loss of hair cells. Disruption of Notch-mediated lateral inhibition of hair cell development between already committed hair cells and their neighbours would lead to an increase in the number of cells that become committed to develop as hair cells and an increase in the number of cells that maintain *Atoh1* expression.

However, if ATOH1 acts only as a differentiation factor for cells that are already committed to develop as hair cells, then it would only be expressed in committed hair cells. In addition, although hair cells would be missing in *Atoh1*-null mutants, supporting cells would still be present and forced expression of *Atoh1* would not be sufficient to induce hair cell formation. Disruption of lateral inhibition would not necessarily alter the number of cells that develop as hair cells.

Depending on the method of analysis, the existing data on *Atoh1* expression are consistent with each of these hypotheses[41,87,90,110]. Functionally, virtually all unique markers of hair cell formation are lost in *Atoh1* mutants[86], although it has been suggested that a small number of hair cells might begin to form in the extreme apex[93,110]. Similarly, virtually all markers of supporting cell formation in the organ of Corti are also lost[90], even though analysis of vestibular supporting cells indicated that they are present[86]. Disruption of the Notch pathway does lead to an increase in hair cell numbers[50,51,54,57,97,99,100], and an increase in the number of *Atoh1*-positive (in mammals)[87,115] or *zath1*-positive (in zebrafish) hair cells[116]. Finally, markers of prosensory patch formation, such as *Sox2*, are still expressed in *Atoh1* mutants[66] and *Atoh1* expression is not required for supporting cell formation, as ectopic hair cells successfully induce *Atoh1*-negative cells to develop as supporting cells[90]. These data are most consistent with ATOH1 acting as a commitment factor for hair cells, but it will not be possible to draw this conclusion without cell lineage data showing the expression of *Atoh1* in precursors of both hair cells and supporting cells.

The observation that ectopic expression of *Atoh1* outside of prosensory patches can induce hair cell formation suggests that the idea that prosensory patches are uniquely competent to develop as hair cells and supporting cells might not be entirely correct. A second result that also seems to refute this hypothesis is the recent demonstration that *Atoh1*-induced ectopic hair cells can induce neighbouring cells to develop as ectopic supporting cells[90]. Strikingly, the formation of ectopic supporting cells was solely dependent on proximity to an ectopic hair cell. However, the induction of ectopic supporting cells has only been examined in embryonic tissue, and therefore it is not known whether these cells can also be induced in the adult ear. Overall, these results suggest that competence to develop as hair cells or supporting cells could extend beyond the normal boundaries of the sensory patches. This observation suggests that sensory cell formation might be actively inhibited outside of the sensory patches. However, most of the work on ectopic hair cell and support cell formation has been carried out in Kolliker's organ (also called the greater epithelial ridge), a transient population of epithelial cells located adjacent to the developing organ of Corti during embryogenesis. Further experiments are clearly required to determine whether sensory cell competence extends into other non-sensory regions of the inner ear.

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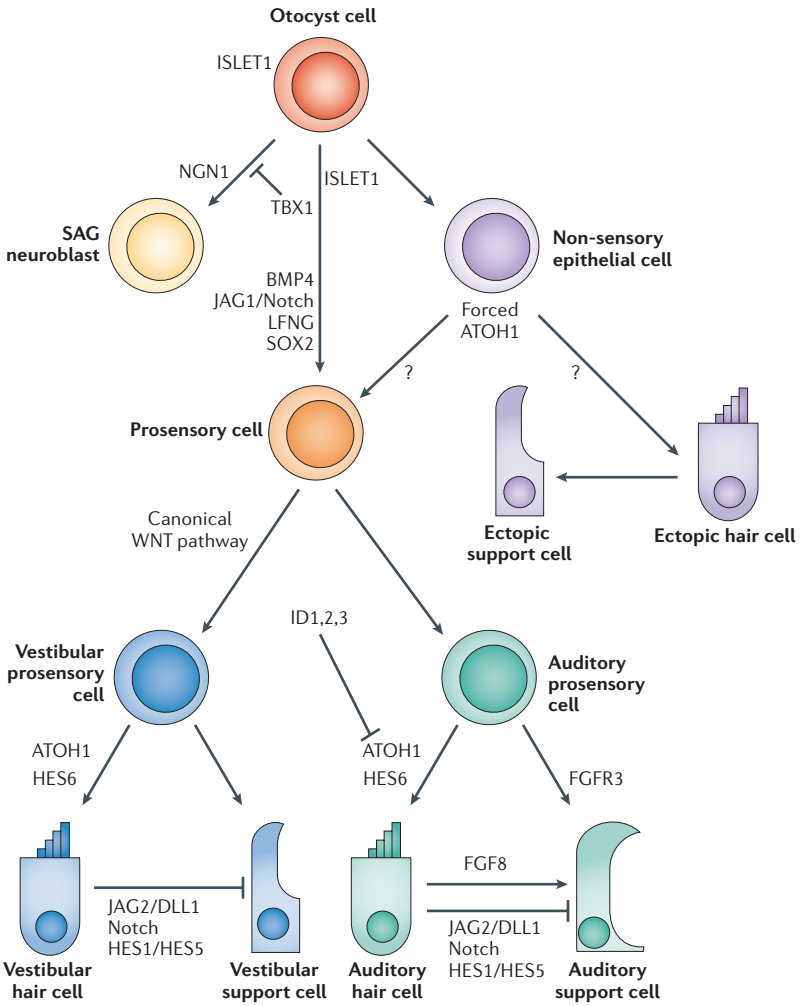

Figure 5 | **Factors that direct sensory cell fates in the inner ear.** Cells that will develop as either prosensory cells or statoacoustic ganglion (SAG) neuroblasts initially express *Islet1*, although its functional role has not yet been demonstrated[26,27]. *Ngn1* is expressed exclusively in SAG neuroblasts and is required for their formation. Next, developing prosensory cells express a number of genes, including *Tbx1*, *Bmp4*, *Jag1*, *Lfng* and *Sox2*, that have been shown to have functional roles in prosensory specification[16,29,31,32,35,37,52–54,60,64,96,126]. These cells are then specified to develop as either vestibular or auditory prosensory cells. The relative position of the prosensory patches in the otocyst and existing spatial identity almost certainly have a role in this process; however, at present, only the canonical WNT pathway, which directs prosensory cells towards a vestibular fate[68], has been shown to directly influence this decision. Regardless of auditory or vestibular identity, the first cell types to develop in prosensory patches are hair cells. Hair cell development is dependent on the expression of *Atoh1* (REF. 84). *Hes6* is expressed in developing hair cells soon after *Atoh1*, but a functional role for this gene has not been demonstrated[90]. The timing and pattern of *Atoh1* expression is regulated, at least in part, through the expression of *Id1*, *Id2* and *Id3* prior to hair cell development[86]. The number of cells that develop as hair cells is regulated through activation of Notch 1 signalling (FIG. 4). In addition, in mammalian auditory prosensory patches, hair cells generate inductive signals that recruit and specify supporting cells. A subset of prosensory progenitors in the cochlea express *Fgfr3* (REF. 101,102) whereas developing inner hair cells express *Fgf8* (REF. 105). Binding of FGF8 to FGFR3 leads to the development of a particular supporting cell type, the pillar cell[102,103]. Finally, recent data has demonstrated that at least some cells thought to be in the non-sensory lineage have the potential to develop as hair cells (as a result of forced expression of *Atoh1*)[87–89] or as supporting cells (as a result of proximity to an ectopic hair cell)[88]. It is unclear whether forced expression of *Atoh1* directs non-sensory cells to enter the prosensory lineage at an undetermined point, or initiates a unique or limited hair cell/supporting cell developmental programme.

The results described above demonstrate the importance of *Atoh1* in hair cell development. However, whether *Atoh1* acts as a prosensory gene, a hair cell competence gene or a differentiation factor remains to be determined (BOX 2). Studies using *Atoh1* knock-in reporter mice or *in situ* hybridization have suggested that *Atoh1* is initially expressed at E13 in a fairly broad and diffuse pattern that extends from the lumenal surface of the epithelium to the basement membrane[86,90]. The authors of these studies concluded that *Atoh1* is initially expressed in progenitors that develop either as hair cells or supporting cells and is subsequently downregulated in cells that develop as supporting cells. By contrast, an *Atoh1* transgenic reporter mouse line and immunohistochemistry have indicated that *Atoh1* expression does not begin until E14 and is restricted to developing hair cells at that time[41]. Based on these results, it was concluded that *Atoh1* is only expressed in cells that have already become committed to develop as hair cells. Finally, a recent study used PCR amplification to demonstrate that transcripts for *Atoh1* are present in the otocyst at E11.5 (REF. 93). However, as vestibular sensory patches begin to develop before the cochlear patch, it is possible that the *Atoh1* transcripts present at E11.5 are restricted to vestibular sensory patches. The reasons for the observed differences in the timing and extent of *Atoh1* expression, and the resulting differences in the suggested role of ATOH1, are not entirely clear. The delay between detection of promoter activity and mRNA versus protein could be a result of a delay in translation or limited antibody sensitivity, which might only detect ATOH1 in cells with high levels of expression. For the transgenic *Atoh1* reporter, the delay seems to be a result of the fact that the transgenic construct does not include all of the *Atoh1* promoter elements, and in particular lacks the promoter regions that regulate initial expression of *Atoh1* (REF. 94). Lineage tracing using an *Atoh1-Cre* knock-in mouse could be used to detect these differences. Such an experiment has been conducted using an *Atoh1-Cre* transgenic line[93], but as this construct also lacks the full complement of *Atoh1* promoter elements, the results have the same limitations as the reporter line discussed above. Even so, the results of these lineage experiments indicated the expression of *Atoh1* in some types of supporting cells, consistent with the idea that the initial pattern of *Atoh1* expression is not limited to hair cells.

Ids regulate Atoh1 activity and hair cell formation. Coordination of cell cycle exit and cellular differentiation has a key role in cell and tissue patterning. In some systems, the activity of bHLH genes such as *Atoh1* must be actively inhibited to prevent premature differentiation. One family of factors that has a role in this inhibition is the *Id* (inhibitors of differentiation) family[95]. These are HLH molecules that inhibit bHLH activity through competition for a common dimer partner, E47 (REF. 95). Prior to hair cell formation, three *Id* genes — *Id1*, *Id2* and *Id3* — are broadly expressed throughout the floor of the cochlear duct including the domain of *Atoh1* expression[88]. As development continues, *Id* expression is specifically downregulated in developing hair cells,

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Box 3 | **Implications for hair cell regeneration**

As our understanding of the development of inner ear sensory epithelia improves, one of the most exciting potential applications of this knowledge is in the area of hair cell regeneration. One of the more puzzling aspects of inner ear biology is the apparent loss of the ability to regenerate hair cells in the mammalian lineage. Although hair cell regeneration from surrounding supporting cells occurs robustly and repeatedly in all other vertebrate classes, hair cell loss in mammals typically results in a permanent deficit[85,117,118]. Considering the highly ordered nature of the organ of Corti, it might be assumed that the complexity of this structure precludes any regenerative events, but hair cell regeneration also fails to occur at any meaningful level in mammalian vestibular epithelia. Therefore, the loss of regenerative ability in mammalian inner ears presumably reflects a fundamental change in the ability of supporting cells to mount a regenerative response. In non-mammalian vertebrates, the supporting cell response to hair cell loss includes two events; one or more rounds of cellular proliferation to generate new cells in the epithelium followed by the differentiation of some of those cells into regenerated hair cells[84,117]. However, recent results have demonstrated that some supporting cells can directly differentiate into hair cells through a process called transdifferentiation[119–121].

These results suggest that the ability to re-enter the cell cycle and/or to switch fates from a supporting cell to a hair cell have been lost in mammals[122]. Therefore, as the specific molecular factors that regulate cell cycle and cell fate in the inner ear are identified, it seems likely that potential targets for regenerative therapies will be revealed. In fact, in a recent study, ATOH1 was overexpressed in the ears of deafened guinea pigs using a virally-mediated delivery system[123]. Although the results were variable, many of the animals demonstrated some recovery of auditory function, suggesting that ATOH1 can be used to force the generation of new hair cells in a mature mammalian inner ear.

suggesting that loss of *Id* function relieves an inhibition of ATOH1 activity in those cells. Consistent with this hypothesis, prolonged expression of *Id3* in sensory progenitors inhibits hair cell formation, suggesting that downregulation of the *Id* family is a key step in hair cell development[88].

Hair cells versus supporting cells

Sensory progenitor cells can develop as either hair cells or supporting cells. As discussed above, ablation studies indicate that removal of a hair cell changes the fate of a surrounding cell from a supporting to a hair cell[82]. This response suggests that hair cells generate inhibitory signals that prevent neighbouring cells from developing as hair cells. This type of interaction is consistent with the effects of Notch-mediated lateral inhibition. Consistent with this hypothesis, two Notch ligands, *Jag2* and delta 1 (*Dll1*) are rapidly upregulated in a subset of *Atoh1*-positive cells[96–98]. The expression of these ligands leads to activation of Notch 1 and the increased transcription of two Notch target genes, *Hes1* and *Hes5* in cells that will develop as supporting cells[87,90,99–101]. Deletion of any of the genes in this pathway leads to an overproduction of hair cells[51,97,99,100], strongly suggesting that Notch signalling has a role in diverting progenitor cells from the hair cell fate (FIG. 4). The mechanism of this diversion has been examined using cells in Kolliker's organ. First, co-transfection of Kolliker's organ cells with *Atoh1* and *Hes1* was sufficient to inhibit hair cell formation, suggesting that *Atoh1* transcription is a target of HES1 in the ear[100]. Second, transient activation of ATOH1 in patches of Kolliker's organ cells leads to activation of the Notch signalling pathway within those cells and to the inhibition of ATOH1 and hair cell fate in a subset of those cells[90].

Supporting cell development

Supporting cell development is dependent on inductive signals generated by hair cells[90]. To a large extent, the molecular nature of those signals is unknown. However, there is evidence for the involvement of the FGF signalling pathway in the development of one type of supporting cell, the pillar cells, in the organ of Corti. *Fgfr3*, one of four tyrosine-kinase receptors in the fibroblast growth factor signalling pathway, is expressed in a population of cells in the cochlear prosensory patch beginning on E15.5 (REFS 102–104). The expression domain of *Fgfr3* includes cells that will develop as pillar cells, outer hair cells and Deiters' cells, and the medial boundary of this domain is located directly adjacent to the developing inner hair cells. Deletion or inhibition of FGFR3 signalling leads to inhibition of the differentiation of pillar cells[104,105]. By contrast, deletion of *Sprouty2*, an FGFR antagonist that is expressed in a pattern similar to *Fgfr3*, results in an overproduction of pillar cells[106]. Finally, FGF8, an FGF with a high binding affinity for FGFR3, is expressed exclusively in developing inner hair cells[107]. Based on these results, it seems likely that an inductive interaction between FGF8 and FGFR3 regulates the position and number of pillar cells in the organ of Corti.

Summary and future directions

The sensory epithelia of the inner ear are comprised of two basic cell types, yet diversity in hair cell and supporting cell form and function exists both between vestibular and auditory sensory epithelia and within the individual epithelia themselves. The specification of individual sensory epithelia and of the unique cell types in those epithelia is regulated through a sequential series of restrictive events (FIG. 5), including specification of prosensory patches and sorting of cells in these patches into hair cells and supporting cells using a conserved developmental paradigm that includes a primary cell fate (hair cell) and a secondary cell fate (supporting cell).

Although our understanding of the basic developmental pathway that directs cells in the otocyst to be specified as hair cells has reached a reasonable level, we know considerably less about the factors that mediate the specific identities of those cells. Activation of the canonical WNT signalling pathway has been shown to change sensory epithelia from auditory to vestibular, but it is not clear how this signalling pathway regulates regional identity in the context of normal development. Similarly, the downstream signalling pathways that generate a vestibular versus an auditory hair cell are completely unknown. The answers to these questions have significant implications for both our understanding of the formation of this system and for the development of regenerative strategies (BOX 3) that might aid with hearing and balance disorders. Although there are still many questions to be answered, the pace of discovery in the auditory field has quickened noticeably in the last 10 years, and significant advances can be expected in the near future.

Inhibitors of differentiation and DNA binding
(IDs). A family of helix–loop–helix proteins. IDs are related to basic helix–loop–helix molecules but lack the basic domain required for DNA binding. As a result, their primary role is to compete with bHLHs for a common dimer partner.

Pillar cells
Specialized types of supporting cell found only in the organ of Corti in the mammalian cochlea. Inner and outer pillar cells combine to form the tunnel of Corti, a fluid-filled space between the inner and outer hair cells.

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