# Sensory organ development in the inner ear: molecular and cellular mechanisms

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The molecular mechanisms underlying the specification of sensory organs in the inner ear and the development of hair and supporting cells within these organs are described. The different organs are all derived from a common pro-sensory region, and may be specified by their proximity to the boundaries between compartments – broad domains within the otocyst defined by the asymmetric expression patterns of transcription factors. Activation of Notch may specify the pro-sensory region, and lateral inhibition mediated by Notch signalling influences whether cells of common lineage in a sensory patch differentiate as either hair cells or supporting cells. The transcription factors Math1 and Brn3.1 are required for hair cell differentiation, and supporting cells express negative regulators of neurogenesis, Hes1 and Hes5. Retinoic acid and thyroid hormone influence early aspects and timing of hair cell differentiation, respectively. Development of the hair cell's mechanosensory hair bundle involves interactions between the cytoskeleton, cell-surface adhesion molecules, receptors and associated extracellular matrix.

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There are six distinct sensory organs in the mammalian inner ear: the three cristae of the semicircular canals, the two maculae of the saccule and utricle, and the organ of Corti of the cochlea (Fig. 1). The cristae and the maculae are vestibular organs that respond to angular and linear acceleration, respectively. The organ of Corti is the organ of hearing. These three types of organ differ in their function, and in the fine details of their cellular architecture, but they all conform to the same basic plan. They are relatively simple epithelia composed of two basic cell types, the sensory hair cells and their surrounding, non-sensory supporting cells. These epithelia lie upon a sheet of extracellular matrix, a basal lamina, and also have a prominent extracellular structure, a cupula, an otoconial membrane or a tectorial membrane, associated with their apical surface. The supporting cells sit on the basal lamina, and their lateral membranes surround the hair cells, projecting up to the surface of the epithelium. The hair cells do not contact the basal lamina, and they are isolated from one another by the supporting cells. At the apical surface of the epithelium, the supporting cell processes form tight and adherens junctions with each

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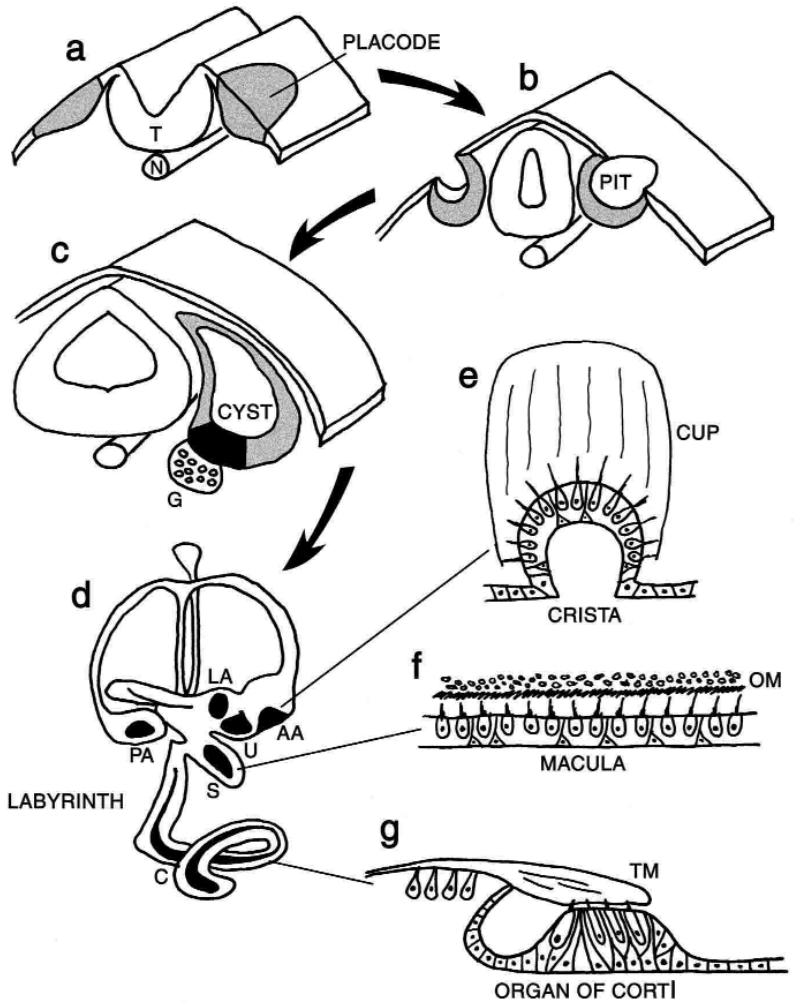


**Fig. 1** Diagram illustrating the main steps in the development of the inner ear (a–d), and the structure of the three types of sensory organ (e–g). The inner ear develops from the otic placode (a) an ectodermal thickening that invaginates to form the otic pit (b) which in turns pinches off from the ectoderm to form the otic vesicle (c). The different sensory organs are derived from a common pro-sensory patch (black) in the ventromedial wall of the otocyst (c). A complex series of morphogenetic events transforms the otic vesicle (c) into the labyrinth (d) containing three cristae (e), two maculae (f), and an organ of Corti (g). Abbreviations: G, VIIIth ganglion; AA, anterior ampulla; LA, lateral ampulla; PA, posterior ampulla;, U, utricle; S, saccule; C, cochlea; CUP, cupula; OM, otoconial membrane; TM, tectorial membrane.

other and with the hair cells. The hair cells have a highly specialized bundle of modified microvilli on their apical surface, a hair bundle, and it is this feature that enables them to detect mechanical stimuli and transduce them into electrical signals.

These sensory organs of the inner ear are all derived during development from the wall of the otocyst, a hollow, pear-shaped structure that forms, just after neural tube closure, from a thickening of the head ectoderm that lies adjacent to the rhombencephalon and is known as the otic placode. The neurones that innervate the hair cells in each organ of the ear are also derived from the otocyst, by a process of delamination, prior to the formation the sensory organs. Although these neurones become critically dependent on trophic support from the hair cells[1,2], there is little evidence that they influence the development of hair and supporting cells. In this brief review, we will focus specifically on the sequence of molecular and cellular events that leads to the differentiation of hair and supporting cells within these epithelial organs, rather than the process of synaptogenesis. We will describe the origin of the different sensory organs, the lineage relationships of hair and supporting cells, and how the two cell types come to adopt different fates. The roles of retinoic acid and thyroid hormone will be discussed, and recent evidence indicating that a complex interplay between the cytoskeleton, components of the hair cell surface and the associated extracellular matrix controls the process of hair bundle development will be reviewed.

Much of our understanding about the development of the inner ear comes from a number of different systems, and not just from the experiments done with mammalian species. Studies on the developing chick inner ear, and more recently that of the zebrafish, provide considerable information on basic mechanisms and principles that are likely to be applicable to the process of hair and supporting cell development in both mice and humans. For certain aspects, detailed data are only available for the chick and, when necessary, these will be used to illustrate how the sensory patches in the inner ear are formed.

## Generation and specification of sensory organs

Histological studies[3] originally suggested that the different sensory organs of the chick inner ear are all derived from a single patch of cells in the ventromedial wall of the otocyst. Molecular studies have recently revived support for this suggestion, and have revealed that there is a prosensory area in the ventromedial region of the otocyst that can be defined by the expression patterns of *Serrate1*[4], *Lunatic fringe*[4] and BEN[5]. Serrate1 (known as Jagged1 in mammals) is a transmembrane ligand for Notch, a membrane receptor that is involved in many aspects of development[6]. Lunatic fringe is a protein that modulates interaction between Notch and its ligands[7]. BEN is a cell–cell adhesion molecule of the Ig superfamily[8]. Notch is widely expressed throughout the otocyst[9], and Serrate1 may serve to maintain a high level of Notch activation

within the pro-sensory patch, endowing this region with the capacity to form sensory organs, and preventing the premature differentiation of hair cells[10]. The role of Lunatic fringe in the pro-sensory patch is uncertain. *Lfng*$^{-/-}$ mice do not have any inner ear abnormalities[11], and it may only act as a weak inhibitor of Notch signalling[10]. As a cell–cell adhesion molecule, BEN may serve to stop the cells of the pro-sensory patch from mixing with cells in other regions of the otocyst, *i.e.* those that are destined to become non-sensory parts of the ear[5]. Although these studies provide evidence for the existence of a common pro-sensory patch and reveal molecular constituents with potential roles, it is not yet known what signals determine the formation of this region, or from where these signals emanate.

A boundary model has been proposed to account for how the different sensory organs of the inner ear are specified[12]. This model postulates that the different sensory organs of the inner ear form at, or in proximity to, the boundaries between a small number of different compartments. These compartments are defined by the asymmetric expression patterns of a few genes and may, for example, correspond to the ventral and dorsal halves, or the anterior and posterior segments of the otocyst. Genes for transcription factors such as Pax2, Dlx5, Otx1 and Hmx3 are expressed in such broad domains in the otocyst, and data from knockout mice are consistent with the boundary model (see Brigande *et al*[13] and Cantos *et al*[14] for reviews). For example, anterior and posterior cristae along with their associated canals are missing in *Dlx5*$^{-/-}$ mice[15]. Also *Pax2*$^{-/-}$ mice fail to form a cochlear duct[16]. Compartment boundary intersection points may, therefore, define where a sensory organ forms within the ventromedial pro-sensory patch, and whether it becomes a crista, a macula, or a hearing organ.

The expression of *BMP4* in spatially discrete regions within the ventromedial pro-sensory patch marks the first appearance of individual sensory organs[17,18]. BMP4 is a member of the TGF-β family of secreted growth factors and, in the chick otocyst, *BMP4* is expressed in all of the sensory organs as they first emerge[17]. However, BMP4 is not a good marker for every sensory organ in the mouse otocyst[18], and it is only expressed in some of the organs in the zebrafish ear[19]. Although BMP4 could autonomously regulate the development of hair and supporting cells within the organs within which it is expressed, it may actually play a major role in controlling the development of the accessory structures that form adjacent to the sensory patches. For example, the BMP4 antagonist Noggin severely disrupts semicircular canal formation but has comparatively little effect on the development and differentiation of hair cells[20,21]. FGF10 is also expressed by the presumptive sensory epithelia of the mouse otocyst, and it too may operate in a paracrine fashion, signalling through the IIIb isoform of FGFR-2 to control the

development of adjacent non-sensory tissues[22]. A number of other genes are also expressed in sensory patches at the very early stages of their development. These include *BMP5* and *BMP7*[23], *p75NGFR*[24,25], *Msx1*[17], *NT3*[18,24], *bmp2b*[19], and *MsxC*[19,26]. Some of these, like *MsxC*, are only expressed in certain sensory patches, and may specify the type of organ that develops.

## Lineage and birth of hair and supporting cells

Hair and supporting cells in the mammalian inner ear are born over a brief period of development after the sensory patches have been specified[27,28]. The cells then differentiate and remain mitotically inactive. In the cochlea of the mouse, the cyclin-dependent kinase inhibitor, $p27^{Kip1}$, is expressed by cells of the organ of Corti as soon as they withdraw from the cell cycle[29,30]. The pattern of $p27^{Kip1}$ expression precisely delineates the region of the cochlear duct within which the hair and supporting cells of the organ of Corti will differentiate. The expression of $p27^{Kip1}$ is down-regulated in hair cells as they begin overt cytodifferentiation, but in supporting cells it persists into adulthood[29]. In $p27^{Kip1-/-}$ mice, cell proliferation within the organ of Corti continues, and an excess of hair and supporting cells is found[29,31]. These data indicate $p27^{Kip1}$ negatively regulates cell proliferation in the organ of Corti. However, other molecules must also control this process as many hair and supporting cells do leave the cell cycle and differentiate in the cochleae of $p27^{Kip1}$ knockout mice.

In the hearing organs of both mammals[27] and birds[32], hair and supporting cells at any one place within the structure are born simultaneously, suggesting they may share a common lineage. Retroviral tracing studies in the chick auditory organ have provided firm experimental evidence for this suggestion[33,34]. Furthermore, it has been shown that the potential to become either a hair or a supporting cell is retained by a progenitor cell until it has passed through its final mitotic division, as two-cell clones were found that contained either two supporting cells, two hair cells or both cell types[33].

## The differentiation of hair and supporting cells

The decision to become either a hair cell or a supporting cell most probably involves lateral inhibition[35,36], a process by which cells of common origin adopt different fates. Lateral inhibition is usually mediated by Notch signalling, and a number of studies have now shown that the products of the neurogenic genes of the Notch signalling pathway play a key role in mediating the differentiation of hair and

**Table 1** Expression patterns of transcription factors and components of the Notch signalling pathway observed during sensory organ development in the inner ear, and the effects of experimentally manipulating their expression

| Name of gene or protein | Nature of protein | Expression pattern | Mutant/experimental phenotype | References |
|---|---|---|---|---|
| *Math1* | bHLH transcription factor | Mouse vestibule at E12, cochlea at E13.5; expressed transiently by hair cells and down-regulated by P3 | Complete absence of hair cells in the inner ear of *Math1*$^{-/-}$ mice by P1 | 30, 42, 50, 54 |
| *Brn3.1* | POU domain transcription factor | Mouse vestibule E12.5, cochlea at E14.5; expressed specifically by hair cells | Loss of hair cells by P14 in *Brn3.1*$^{-/-}$ mice | 46–48 |
| *GATA3* | C4 zinc finger transcription factor | Mouse auditory epithelium at E14; down-regulates in hair cells, then in supporting cells | | 49 |
| *Hes1* | bHLH transcription factor | Greater and lesser epithelial ridges of rat (E17.5) and mouse (E18.5) cochlea; supporting cells of rat utricle by E17.5 | Increase in inner hair cells and utricular hair cells in *Hes1*$^{-/-}$ mice | 52, 53 |
| *Hes5* | bHLH transcription factor | Mouse cochlea at E15, restricted to Deiter's cells by E17 | Increased in outer hair cells and macular hair cells in *Hes5*$^{-/-}$ mice | 53, 54 |
| *Notch1* | Transmembrane receptor | Wide-spread in epithelium of otocyst from early stages, becomes restricted to supporting cells as they differentiate | Increase in OHCs in *Notch1*$^{+/-}$ mice. Increased rows of IHCs and OHCs in rat cochlear cultures treated with Notch1 antisense oligonucleotides | 9, 37–40, 42, 43 |
| *Delta1* | Transmembrane ligand for Notch | Mouse vestibular hair cells at E12.5, mouse cochlear hair cells at 14.5. In sensory patches of chick otocyst from E3.5 onwards, and zebrafish otocyst at 14 hpf | Large increase in hair cells numbers in zebrafish dominant negative DeltaA$^{dx2}$ mutants. Retroviral expression of dominant negative Dl1$^{dn}$ in chick down-regulates *Ser1* expression | 9, 10, 38, 39 |
| *Serrate1 (Jagged1)* | Transmembrane ligand for Notch | In all sensory organs of the mouse inner ear by E12.5, and becomes restricted to supporting cells as hair cells differentiate In pro-sensory patch of chick otocyst from E2.5 (stage 19) | Extra IHCs but loss of third row OHCs along with loss of cristae in mice with dominant missense Jag1 mutations. Extra rows of IHCs and OHCs in rat cochlear cultures treated with Jag1 antisense oligonucleotides | 4, 9, 38, 39, 43–45 |
| *Serrate2 (Jagged2)* | Transmembrane ligand for Notch | In hair cells of mouse vestibule at E13.5, and cochlea at E14.5. In rat IHCs at E18, and OHCs at E20. In zebrafish from 18 hpf | Extra rows of IHCs and OHCs in Jag2 null mutant mice | 37, 40, 43 |
| *Lunatic fringe* | Glycosyl transferase that modifies Notch extracellular domain | In sensory organs of mouse inner ear by E11.5, restricted to supporting cells in organ of Corti by E16. In pro-sensory patch of chick otocyst by E2.5 | No obvious phenotype in inner ear of Lfng deficient mice, but the extra rows of IHCs seen in Jag2 null mutant are suppressed on a Lfng null mutant background | 4, 11, 18 |
| *Numb* | Intracellular protein that blocks Notch activation | In all cells of chick sensory patch at E3, restricted to hair cells at E12 | | 10 |

Abbreviations: IHC, inner hair cell; OHC, outer hair cell; E, embryonic day; P, postnatal day; hpf, hours post-fertilisation.

supporting cells in the inner ear[9,10,37–45]. The expression patterns of these genes in the ear, and the phenotypes of the different mutants that have been examined, are summarised in Table 1. In addition, a number of transcription factors have been shown to be involved in the process of hair and supporting-cell differentiation[46–54], some of which may directly interact with the Notch signalling pathway. These are also listed in Table 1. The expression patterns of both the neurogenic genes and the transcription factors, the consequences of experimentally manipulating their expression, are now described and discussed in detail.

## *Math1*

*Math1*, a mouse homologue of the *Drosophila* proneural gene *atonal*, encodes a basic, helix-loop-helix (bHLH) transcription factor. It is expressed in the primordium of the organ of Corti after the cells in this region have withdrawn from the cell cycle and have begun to express $p27^{Kip1}$, but before hair cells have started to express myosin VIIa, a marker of overt differentiation[30]. Math1 is initially expressed by thin bands of cells that span the entire thickness of the epithelium, and expression becomes restricted to hair cells located at the lumenal surface of the epithelium as they differentiate[30,54]. These thin bands of Math1-expressing cells that initially span the thickness of the epithelium may be bi-potential progenitor cells[54]. Alternatively, they may be vertical stacks of hair cells that subsequently undergo movement within the thickness of the epithelium and spread out along the longitudinal axis of the cochlea as it elongates[30].

Hair cells are absent from the inner ears of $Math1^{-/-}$ mice by birth[50], and the ectopic expression of Math1 in the non-sensory cells of the greater epithelial ridge that lie adjacent to the organ of Corti results in the formation of supernumerary hair cells[51]. These results indicate that Math1 is both 'necessary and sufficient' for hair cell differentiation[30]. Early markers of overt hair cell differentiation, myosin VI and calretinin, are never expressed during inner ear development in $Math1^{-/-}$ mice[50]. The sensory epithelia are thinner and non-stratified, but have an overlying extracellular matrix suggesting the supporting cells may have differentiated. An apparent overcrowding of supporting-cell nuclei and a failure to observe apoptosis in the sensory epithelia of $Math1^{-/-}$ mice was originally interpreted as indicating that a fate switch had occurred, leading to an overproduction of supporting cells[50]. However, apoptotic cells have recently been reported in the organ of Corti of $Math1^{-/-}$ mice, so the hair cells may be produced, fail to express any known markers of differentiation, and then die[30]. Sensory epithelia, albeit eventually devoid of hair cells, do form in $Math1^{-/-}$ mice, so it is unlikely that *Math1* is

acting as a true proneural gene like its invertebrate homologue *atonal*[50]. A proneural gene required for sensory organ specification in the inner ear has, therefore, yet to be discovered.

### *Delta1, Jagged2 and Notch1*

*Delta1* and *Jagged2* (known as *Serrate2* in chick) are expressed by hair cells in the mouse cochlea approximately 1 day after the onset of Math1 expression[38,40]. In *Jagged2* null mutant mice, there is an increase in the linear density of inner and outer hair cells in the cochlea, with a nearly complete duplication of the normal, single row of inner hair cells, and many stretches where there are four instead of three rows of outer hair cells[40]. Delta mutants have not been studied in the mouse, but a dominant negative allele of the zebrafish *deltaA* gene, *deltaA*$^{dx2}$, results in a 5–6-fold increase in hair cell numbers in the inner ear, and a loss of most of the supporting cells[41]. Notch expression is initially widely distributed throughout the inner ear epithelium and the sensory patches, and becomes restricted to the supporting-cell layer as the hair cells differentiate[9,39,40,42,43]. *Notch1*$^{-/-}$ mice are early embryonic lethals, but in heterozygotes with presumably reduced levels of Notch1, a significant increase is observed in the numbers of regions along the cochlea where there are four instead of three rows of outer hair cells[11]. Treating rat cochlear cultures with antisense Notch oligonucleotides causes the production of extra rows of both inner and outer hair cells[43]. The expression of *Delta1* and *Jagged2* in hair cells, and the overproduction of hair cells seen in corresponding mutants and with antisense oligonucleotides to *Notch1*, all indicate that hair cells use Notch signalling to inhibit laterally their neighbouring cells and thereby prevent them from adopting the same fate.

### *Serrate1/Jagged1*

*Serrate1/Jagged1* is expressed throughout the pro-sensory patch initially[4,9,38], but becomes progressively restricted to the supporting cells as they differentiate[38,39]. The expression of Serrate1/Jagged1 by supporting cells may not appear to be consistent with the idea that hair cells inhibit their neighbours. However, there is evidence from the chick that hair cells express Numb, a protein that blocks Notch signalling[10]. These hair cells would, therefore, be deaf to signals delivered by Serrate1 from adjacent supporting cells. Furthermore, there is also evidence from the chick inner ear that the expression of *Serrate 1* in supporting cells is positively regulated by Notch signalling via lateral induction[10]. This would serve to increase the level of Notch activation amongst the

supporting cells, and ensure that they do not differentiate as hair cells. The overproduction of hair cells in late embryonic rat cochlear cultures caused by Jagged1 antisense oligonucleotides[43] is consistent with this, as reduced Notch activation should lead to an excess of hair cells. However, in mouse mutants[44,45], Jagged1 mutations that are assumed to be dominant negative lead to the complete loss of some sensory organs (cristae) and only perturb hair cell patterning in others (cochlea). This could be because Serrate1/Jagged1 has an early role in specifying sensory

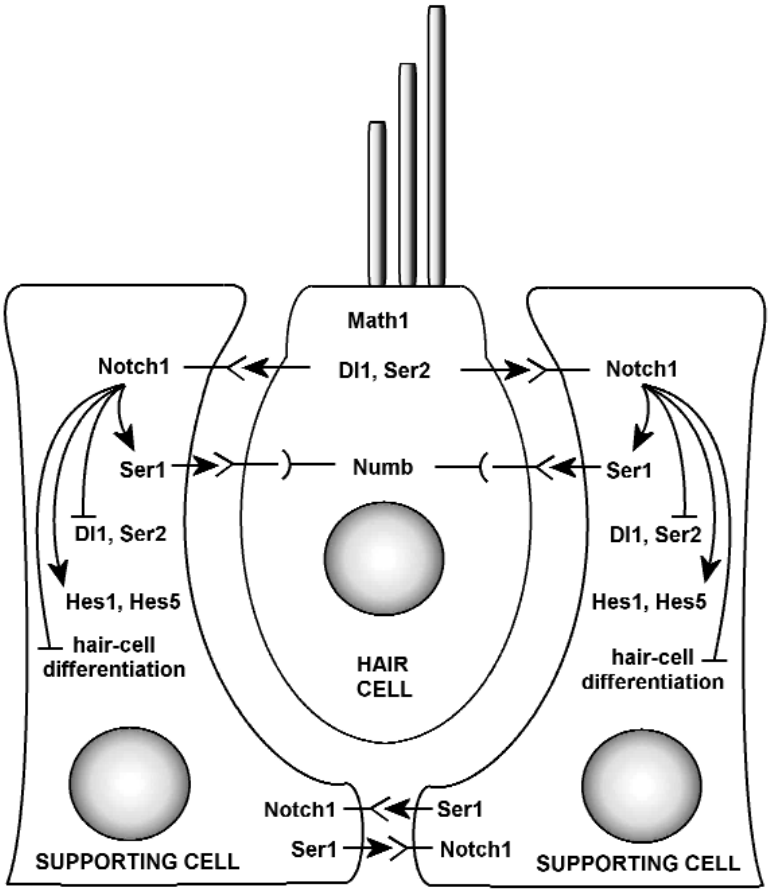


**Fig. 2** Diagram illustrating the regulatory interactions occurring between components of the Notch signalling pathway that lead to the differentiation of hair and supporting cells in the sensory organs of the inner ear. A single hair cell is shown that is surrounded by two supporting cells. Activation of Notch1 stimulates Serrate1/Jagged1 (Ser1) production, represses expression of Delta1 (Dl1) and Serrate2/Jagged2 (Ser2), positively regulates expression of Hes1/5 and inhibits hair cell differentiation. Hair cells express Delta1 (Dl1), Serrate2/Jagged2 (Ser2) and Numb. Numb prevents Serrate1/Jagged1 (Ser1) expressed by supporting cells from activating Notch. A high level of Notch activation leads to cells adopting the secondary fate, *i.e.* they become supporting cells. Cells with low levels of Notch activation adopt the primary, default state and become hair cells. Modified from Eddison *et al*[10] with permission from the author.

patches and/or preventing their premature differentiation, and a later role in regulating the development of hair and supporting cells within the patch[44]. Whilst this may partially explain why the mutations, which are effective throughout development, and antisense treatment, which has only been applied at relatively late developmental stages, have different effects, it does not explain why the different organs in the ears of *Jagged1* mutant mice respond differently to the same mutation. However, the loss of cristae may occur secondarily to the truncation of the canals, which in turn may be due to diminished BMP4 signalling from the sensory patch resulting from reduced Notch activation.

The expression patterns of the neurogenic genes described above and the phenotypes of the different mutants that have been examined are generally in accordance with the theory that hair cells laterally inhibit their neighbours and force them to become supporting cells. However, the situation is clearly more complex than originally envisioned[35,36]. Although a stochastic fluctuation in Notch signalling in a field of cells initially expressing uniform levels of Notch and Delta can theoretically lead, as a result of negative feedback, to the production of regular mosaics of hair and supporting cells[55,56], Delta1 is not uniformly expressed[38], there are two other Notch ligands operating in the ear, and the signalling pathway can be modulated at many levels, making the simple scenario, although attractive, less likely. Furthermore, an active re-arrangement of hair and supporting cells relative to one another may contribute to the formation of a precise cellular mosaic, and refine the imperfect patterns that are initially observed during the early stages of development[57], indicating that lateral inhibition with feedback is not the only mechanism contributing to pattern formation in the sensory epithelia. The expression patterns for the neurogenic genes and the experimental findings described above are largely consistent with a system of regulatory interactions (see Fig. 2) recently proposed by Lewis and colleagues[10].

### *Hes1 and Hes5*

*Hes1* and *Hes5*, mammalian homologues of the *Drosophila hairy* and *enhancer of Split* genes, are expressed in patterns that are both complementary and overlapping in the developing cochlea and utricle of the mammal[52,53]. The products of these genes act as negative regulators of neurogenesis in vertebrates[58]. Expression of *Hes5* is first observed in the supporting cells of the organ of Corti after the expression of *Math1* has begun in differentiating hair cells, and probably slightly after the onset of expression of Delta1 and Jagged2[54]. The expression of Hes5 may be positively regulated by Notch activation as it is much reduced in

the supporting cells (Deiter's cells) of *Jag2*$^{-/-}$ mice[54]. Additional inner hair cells are observed in *Hes1*$^{-/-}$ mutant mice, and additional outer hair cells are observed in *Hes5*$^{-/-}$ mutants[52,53], generally consistent with the expression patterns of these genes and their proposed role as negative regulators of hair cell differentiation. Hes1 may directly antagonize the activity of Math1, as co-transfecting cells in cochlear cultures with Hes1 and Math1, blocks the effects of ectopic Math1 expression[52], reducing the production of supernumerary hair cells.

### *Brn3.1 and GATA3*

Brn3.1 (also called Brn3c) is a POU domain transcription factor that is specifically expressed by hair cells within the adult mouse inner ear[46,47]. Brn3.1 is expressed by postmitotic hair cells at approximately the same time as Jagged2 and Delta1, 1 day before the hair cell markers myosin VI and myosin VIIa[48]. In *Brn3.1*$^{-/-}$ mice, hair cells are generated and express myosin VI, myosin VIIa and calretinin, but they never develop sensory hair bundles and are lost from the inner ear by P14[46,47]. The ectopic overexpression of Brn3.1 does not lead to the production of hair cells[51], indicating Brn3.1 is only required for the later aspects of hair cell differentiation. GATA3 is another transcription factor that may be involved in cell differentiation within the sensory patches. All epithelial cells in the dorsal wall of the cochlear duct express GATA3, and its expression selectively decreases in both hair and supporting cells as they differentiate, indicating it may act as a negative regulator of hair and supporting-cell differentiation[49].

## Roles of thyroid hormone and retinoic acid in hair cell differentiation

Nuclear receptors for thyroid hormone and retinoic acid are expressed in the developing sensory epithelia of the inner ear and their respective ligands are known to play roles in hair cell development (see Raz and Kelley[59] for recent review). Retinoic acid is produced by the embryonic, but not adult, organ of Corti, and treating cochlear cultures from early, but not late, embryonic stages of development with retinoic acid leads to the production of extra rows of inner and outer hair cells[60]. The RARα/RXRα heterodimer is the most likely hair cell receptor for retinoic acid, and application of the RARα antagonist Ro-41-5253 to cochlear cultures leads to a reduction in the number of hair cells that eventually develop[61]. However Ro-41-5253 does not block the initial differentiation of hair cells, as judged by the expression of Brn3.1 and

myosin VI[61], so it has been suggested that retinoic acid induces certain early aspects of hair cell differentiation rather than determining the size of the pro-sensory cell population[61].

The three functional thyroid hormone receptors, TRα1, TRβ1 and TRβ2, and the non-ligand binding TRα2 are expressed in the sensory epithelia of the inner ear from early stages, with the expression of TRβ1 and TRβ2 being restricted to the cochlear epithelium[62]. Chemically induced hypothyroidism results in a delay in the maturation of most elements in the organ of Corti, and the same effect is observed in transgenic mice that lack all known thyroid hormone receptors[63]. TRβ is essential for the development of hearing[64] and in *Thrb*$^{-/-}$ mice there is a delay in the onset of the fast potassium conductance ($I_{K,f}$) in inner hair cells, but the onset of outer hair cell motility is unaffected[65]. Although $I_{K,f}$ is eventually expressed by inner hair cells in *Thrb*$^{-/-}$ mice, hearing does not recover, possibly due to a permanent impairment of the tectorial membrane[63]. In *Thra*$^{-/-}$/*Thrb*$^{-/-}$ compound null mutant mice both the onset of $I_{K,f}$ and outer hair cell motility is delayed[63], and a recent study has shown that the gene encoding prestin, the outer hair cell motor[66], is regulated by thyroid hormone[67].

## Development of the sensory hair bundle

The sensory hair bundle, a precisely determined array of actin-filled stereocilia located on the hair cell's apical surface, is a distinguishing characteristic of the hair cell and is essential for the process of mechanotransduction. The development of the hair bundle is a complex process and, for the auditory organ of the avian inner ear, the morphological details have been described thoroughly (*see* Tilney and Tilney[68] for review). For the mammal, this process has been best characterized in the mouse vestibular system[69,70], and the cochlea of the hamster[71] and the rat[72]. For the mouse cochlea, information is somewhat limited.

Although there are some inter-species and inter-organ differences, the general features of the process of hair bundle development can be described as follows. First, small stereocilia sprout up over the entire apical surface of the hair cell, clustering around a centrally located kinocilium to form a short bundle of uniform height. Second, the kinocilium migrates to one side of the cell and the stereocilia nearest the kinocilium begin to elongate and generate the staircase pattern in which adjacent rows of stereocilia become arranged in increasing height with the tallest row lying next to the now eccentrically placed kinocilium. The position of the kinocilium, therefore, defines the planar polarity of the hair cell and its bundle. Third, rootlets project down from the stereocilia, anchoring them into the cuticular plate, and the excess

supernumerary stereocilia that have not been incorporated into the ranked rows of the bundle are re-absorbed. Finally, the stereocilia within the bundle grow to their final width and height, and the bundle achieves its mature shape and form. In the chick auditory organ, increases in the width and height of stereocilia occur over different time periods[68], whereas in mammals both processes, thickening and lengthening, occur concurrently[71]. Also, differences in bundle height in the avian hearing organ may be governed by regulating the period over which the growth of stereocilia occurs at a constant rate[68], whereas in the mammalian cochlea they may be generated by differences in growth rate[71].

Surprisingly little is known about the molecular basis of hair bundle development in any species. Clearly, it will involve molecules and pathways that direct the assembly of the actin cytoskeleton, like the Rho-GTPases along with their upstream effectors and their downstream targets, and two recent reviews have suggested how this may be accomplished[73,74]. However, thus far, studies on mouse mutants and human deafness genes have provided the greatest insight into the molecular processes that may be involved in hair bundle development.

Mice with mutations in the genes that underlie deaf-blindness in three of the genetic forms of the human Usher type I syndrome, USHIB, USHID and USHIF, exhibit defects in hair-bundle development. These genes encode the unconventional myosin, myosin VIIa (*shaker-1* mouse[75]), and two cell adhesion molecules, cadherin 23 (*waltzer* mouse[76]) and protocadherin 15 (*Ames waltzer* mouse[77]). Bundles with ranked rows of stereocilia form in all three mouse mutants, but by the early stages of postnatal development, they show varying degrees of disruption and are often fragmented into several smaller units[75–77], suggesting there may be defects in the mechanisms of inter-stereociliary adhesion. The *USHIC* gene encodes a PDZ domain protein that is present in stereocilia and could act as an interface between the cytoskeleton and the plasma membrane[78], although it is not yet known how mutations in this gene affect hair bundle development. Interactions between the actin cytoskeleton, unconventional myosins, PDZ domain proteins and cell-surface adhesion molecules may, therefore, play a pivotal role in the development of hair bundle integrity. Mutations in two other unconventional myosins, cause non-syndromic human hereditary deafness and cause defects in hair bundle development in the corresponding mouse mutants. Defects in myosin VI (*Snell's waltzer* mouse[79]) lead to the fusion of stereocilia and the formation of giant bundles in the early postnatal period[80]. Myosin VI is unusual as it is a minus-end directed actin motor[81], unlike most other myosins that move towards the plus, or barbed, ends of actin filaments. It is not found in stereocilia and it has been proposed[80] that it tethers the apical membrane around the base of each stereocilium to the rootlet or the cuticular plate,

thereby stabilising the stereocilium. Defects in myosin XV (*shaker-2* mouse[82]) lead to the production of hair bundles that are abnormally short, although of normal shape and with height-ranked rows of stereocilia. Abnormally long actin filament bundles, cytocauds, are found in these hair cells that project many microns from the base of the cell[83], suggesting a mis-regulated deployment of a limited supply of actin monomers may account for the reduction in bundle height observed.

Integrins are cell surface receptors for extracellular matrix molecules and a recent study[84] has shown that the α8 integrin subunit specifically localizes to the apical pole of developing utricular hair cells along with focal adhesion kinase (FAK), and that the extracellular matrix molecules fibronectin and collagen type IV are associated with the apical surface of the developing epithelium. Transgenic inactivation of the α8 integrin subunit leads to the malformation of a subset of utricular hair bundles, a loss of FAK from the apical pole of the hair cell and a disappearance of fibronectin from the apical epithelial surface. The growth of sensory hair bundles may, therefore, involve reciprocal interactions between hair bundle receptors and matrix molecules associated with the apical surface of the sensory epithelia.

## Conclusions and key points for clinicians

The evidence reviewed suggests the following sequence of molecular events occurs during the differentiation of sensory organs in the inner ear (Fig. 3). The different sensory organs all originate from a single common patch in the otocyst that can be defined by the expression of *Serrate1*, *Lunatic fringe*, and BEN. Notch is present throughout the otocyst, and is maintained in an activated state within the pro-sensory patch by Serrate1. Notch activation can also laterally induce Serrate1/Jagged1, re-inforcing the expression of Serrate1/Jagged1 in cells with activated Notch. Notch activation may make the patch sensory-competent, and prevent the premature differentiation of hair cells. Discrete regions within this pro-sensory patch then become specified to form different types of organs, cristae, maculae or a cochlea, possibly by virtue of their position relative to compartment boundaries within the inner ear. Hair and supporting cells share a common lineage, and express $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, as soon as they withdraw from the cell cycle. Once postmitotic, the hair cells express Math1, followed by Delta1, Jagged2/Serrate2 and Brn3.1 At some stage during this process, possibly during the final round of cell division, the presumptive hair cells acquire Numb, thereby rendering them deaf to Jagged1/Serrate1 signalling from the supporting cells. This decreases Notch activation in the presumptive hair cells, further promoting their

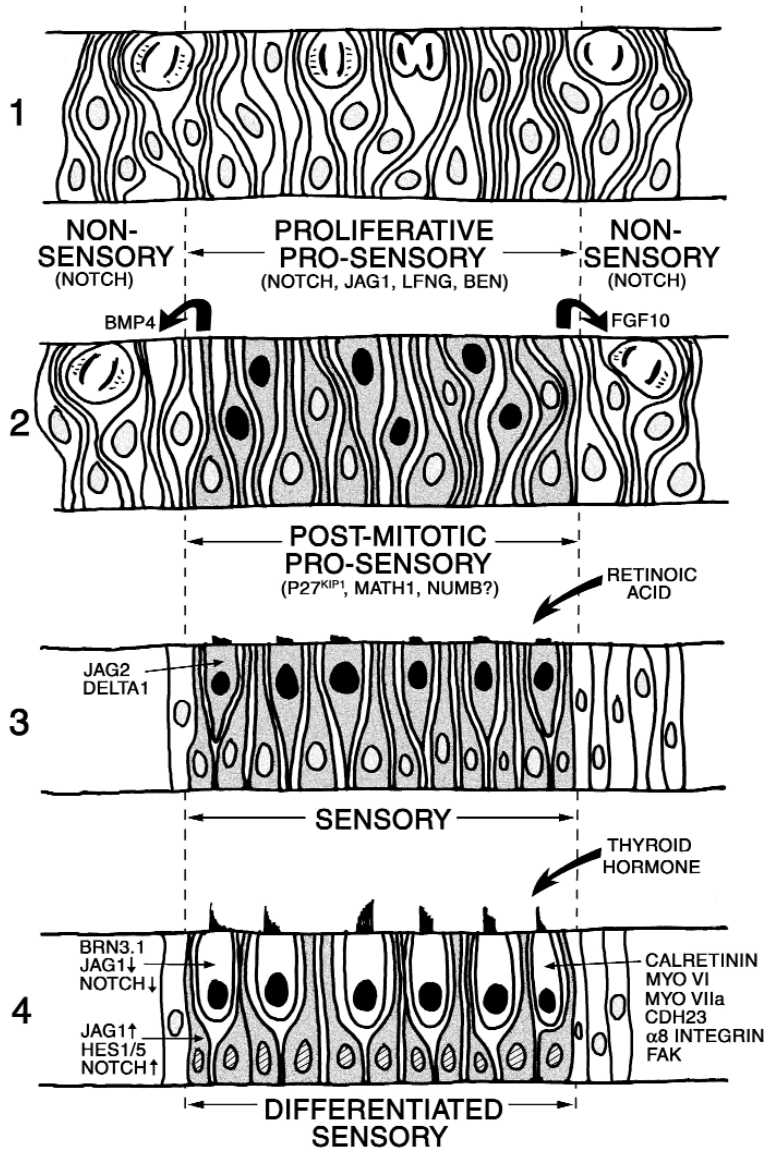


**Fig. 3** Diagram summarizing the key steps in the production of a sensory organ. (**1**) A proliferative pro-sensory region is defined by the expression Jagged1/Serrate1 (JAG1), Lunatic fringe (LFNG) and BEN. Notch1 (NOTCH) is expressed throughout the epithelial wall of the otocyst. (**2**) Cells within a future sensory organ withdraw from the cell cycle and express $p27^{Kip1}$ (grey cytoplasm). Hair cell progenitors may inherit Numb at this stage, via asymmetric division. Shortly after, a subset of cells begins to express Math1 (black nuclei). (**3**) Math1 expressing cells express Jagged2/Serrate2 (JAG2) and Delta1 as they differentiate as hair cells, forcing their neighbours to become supporting cells. (**4**) Hair cells down-regulate Notch1 and Jagged1/Serrate1, and express Brn3.1 followed by a number of hair cell markers including myosin VI, myosin VIIa and calretinin. Notch 1, Jagged1/Serrate1 and Lunatic fringe become restricted to the supporting cells, and these cells begin to express Hes1/5 (hatched nuclei).

differentiation along the hair cell pathway. Delta1 and Jagged2/Serrate2 signalling from hair cells increases the levels of Notch activation in supporting cells, preventing them from expressing Delta1 and Jagged2/Serrate2, and inhibiting hair cell differentiation, probably by positively regulating the expression of Hes1/5. Levels of Notch activation, therefore, determine whether a cell becomes a hair cell (low levels) or a supporting cell (high levels); intermediate levels may maintain cells as pro-sensory progenitors. Retinoic acid may control an early event in the differentiation of hair cells, but probably does not determine the size of the pro-sensory cell population. Brn3.1 is required for the later stages of hair cell differentiation, including the appearance of a sensory hair bundle, the full and complete development of which may involve interactions between the cytoskeleton, cell adhesion molecules, cell-surface receptors and surface associated extracellular matrix. Finally, thyroid hormone controls the timing of certain aspects of hair cell differentiation, including the expression of ion channels and, in cochlear outer hair cells, the motor protein, prestin.

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