
Article:
Whitfield, T.T. (2015) Development of the inner ear. Current Opinion in Genetics & Development, 32. 112 - 118.

https://doi.org/10.1016/j.gde.2015.02.006

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Current Opinion in Genetics & Development

Development of the inner ear

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The vertebrate inner ear is a sensory organ of exquisite design and sensitivity. It responds to sound, gravity and movement, serving both auditory (hearing) and vestibular (balance) functions. Almost all cell types of the inner ear, including sensory hair cells, sensory neurons, secretory cells and supporting cells, derive from the otic placode, one of the several ectodermal thickenings that arise around the edge of the anterior neural plate in the early embryo. The developmental patterning mechanisms that underlie formation of the inner ear from the otic placode are varied and complex, involving the reiterative use of familiar signalling pathways, together with roles for transcription factors, transmembrane proteins, and extracellular matrix components. In this review, I have selected highlights that illustrate just a few of the many recent discoveries relating to the development of this fascinating organ system.

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Current Opinion in Genetics & Development 2015, **32**:112–118

This review comes from a themed issue on **Developmental mechanisms, patterning and organogenesis**

Edited by **Deborah J Andrew** and **Deborah Yelon**

http://dx.doi.org/10.1016/j.gde.2015.02.006

Introduction

The mature vertebrate inner ear has a highly ordered and complex architecture, and contains a multitude of different cell types. Understanding the generation of this organ in the embryo requires an analysis of developmental processes at many different levels: the factors that establish otic identity in the early embryo, the dynamics of cell fate decisions, the morphogenetic movements that sculpt the labyrinth, and the expression of cell type-specific proteins that govern the maturation and physiological function of specialist cell types such as the sensory hair cell. The following sections cover some of the recent advances in each of these steps in a range of different model organisms.

Early ear development: otic placode induction and otic vesicle formation

The inner ear develops from pre-placodal region (PPR), a zone of ectoderm running around the anterior border of the neural plate (Figure 1a). It has been known for many years that graded BMP activity contributes to the overall dorso-ventral patterning of the embryo, but it is now clear that substantial modulation of the initial gradient is important for the establishment of different ectodermal fates, in particular to generate the PPR (reviewed in Ref. [1]). Using a reporter line to give a direct visual readout of BMP signalling in the zebrafish embryo, Reichert and colleagues have provided direct confirmation that BMP activity is specifically attenuated in the presumptive PPR at neural plate stages. A strong candidate to mediate this down-regulation is the BMP inhibitor Bambi-b, which is expressed in the PPR under the control of Dlx3b [2•].

The PPR is further segregated according to fate, first into a common otic/epibranchial precursor domain (OEPD), followed by induction of the otic placode itself. These steps remain an area of active research interest, and the identity of new molecular players is adding detail to a model that is now reasonably well established. Otic placode induction requires not only inducing signals from surrounding tissues, but also the expression of appropriate competence factors in the PPR. Transcription factors of the Foxi, Gata, Tfap and Dlx families are important for conferring competence to form otic tissue, while signalling molecules of the Fgf family are critical for providing the inducing signals [3–6]. Within the PPR, otic placode cells must segregate from neighbouring trigeminal, lateral line (if present) and epibranchial fates. In chick and *Xenopus*, mutual repression between Gbx2 and Otx2 controls segregation between otic (Gbx2-positive) and trigeminal (Otx2-positive) progenitors [7•], while in zebrafish, graded levels of Pax transcription factors are important for the segregation of otic and epibranchial fates [8••].

A detailed fate map provides the foundation for interpreting the results of any perturbation of the otic developmental programme. A recent study used the classical technique of homotypic quail-chick grafting to generate a fate map of the chick otic placode at the 10 somite stage, showing that different otic fates arise from distinct dorso-ventral zones in the placode, with little evidence of cell mixing [9]. While it is tempting to speculate that this arrangement reflects the influence of a morphogen gradient distributed across the dorso-ventral axis, such as Wnt signalling, the morphogenetic movements that form the otocyst may bring ventral regions into contact with dorsal

signalling sources at later stages. It will be necessary to integrate gene expression, morphogenetic and fate map data to get a full understanding of the dynamics and control of fate acquisition in the ear.

Following induction, the otic placode undergoes invagination (amniotes) or cavitation (fish) to form the otocyst or otic vesicle. The task of linking the placodally expressed transcription factors to the cellular behaviours that effect these morphogenetic events is just beginning. One approach is to search for transcriptional targets of genes that are expressed in the PPR and otic placode at early stages. For example, a microarray study using an over-expression assay in *Xenopus* has identified nearly 30 genes expressed in the otocyst that are possible Six1 targets [10]. This and similar studies will provide not only a more complete picture of the transcriptional profile of early otic cells, but also new candidate genes for auditory disorders such as Branchio-Oto-Renal syndrome.

The morphogenetic changes that generate the otocyst from the otic placode have been investigated in the chick embryo [11]. Here, invagination to form the otic cup and otocyst involves two phases: an initial basal expansion of placodal cells, followed by their apical constriction. Sai and colleagues used a variety of inhibitory approaches to elucidate a pathway — triggered by activation of the planar cell polarity mediator Celsr1 and involving RhoA, ROCK and myosin-II activation — leading to actin-mediated apical constriction of otic placodal cells, driving the second phase of the invagination process [11]. This model has close similarities with the events leading to neural tube closure. In the fish, both the otic vesicle and the neural tube form via cavitation (from the otic placode and neural keel, respectively), rather than invagination [12,13]. It will be interesting to compare similarities and differences between the molecular mechanisms of invagination and cavitation in the different species.

Neurogenesis: generation of the VIIIth ganglion

The otic vesicle is the source of nearly all the cell types in the inner ear, including the afferent neurons of the VIIIth cranial ganglion, which innervate the auditory and vestibular sensory hair cells. A neurogenic/non-neurogenic fate decision is made very early in the otic developmental programme (reviewed in Ref. [14]). In zebrafish, the *b380* deletion mutant has been informative in revealing — and ruling out — some of the key players in this process [15•]. The *b380* deletion removes the genes *dlx3b*, *dlx4b* and *sox9a*, resulting in an almost complete loss of otic tissue. Nevertheless, *neurod*-expressing otic neuroblasts still form, although are reduced in number. Development of these neuroblasts is dependent on *foxi1* activity: additional knockdown of *foxi1* abolishes expression of neuronal markers in the otic region. Knockdown of *foxi1* or *dlx3b/4b* alone has highlighted their roles in specifying neuronal and sensory competence, respectively, within the otic region [15•]. Notably, however, a population of common neurosensory progenitors (giving rise to both neuroblasts and hair cells) has been identified in the posteromedial part of the zebrafish ear [16•].

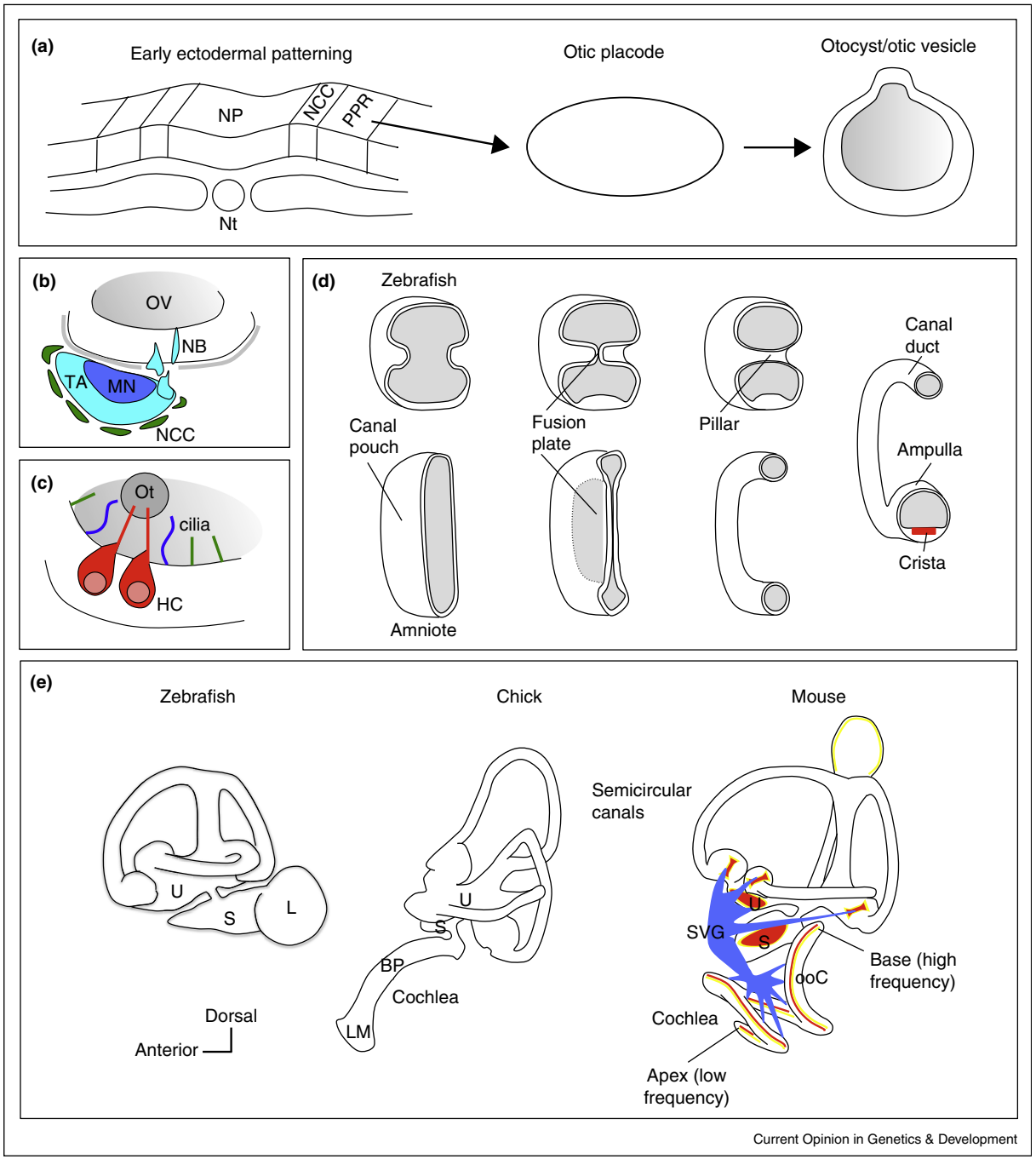
Various signalling pathways are required for otic neurogenesis, in particular Fgf and RA signalling in the zebrafish [17•,18•]. Once specified, neuroblasts leave the zebrafish otic vesicle and enter a transit amplifying population (Figure 1b); Fgf-dependent feedback inhibition from mature neurons in the newly-formed statoacoustic (VIIIth) ganglion is thought to regulate both specification and maturation of neuroblasts, ensuring control over numbers of differentiating neurons [18•]. Neurogenesis in the ear, as in the central nervous system, is also under the control of lateral inhibition mediated by Notch signalling: classical neurogenic phenotypes (an overproduction of neuroblasts) result when Notch signalling is disrupted, as reviewed elsewhere. In the mouse and chick, imaging and ablation studies have revealed the close association between the developing cochleovestibular (VIIIth) ganglion neurons and neural crest-derived glial precursors [19].

Sensory hair cell differentiation and cochlear tonotopy

Sensory hair cells in the ear are the mechanoreceptors that convert sound into electrical energy. They have a spectacular and highly polarised cellular architecture, with a stereociliary bundle on the apical surface and ribbon synapses at the basal surface. The developmental mechanisms that control the specification and differentiation of hair cells are often conserved across the different model systems. Expression of Sox2, for example, marks the prosensory domain in different species, prefiguring the appearance of hair cells (reviewed in Ref. [20]). Fgf signalling is required for the maintenance of *Sox2* expression and normal hair cell development in the developing mouse cochlea [21•,22•]. Interestingly, while complete inhibition of Fgf signalling in the zebrafish resulted in a loss of hair cells, low level inhibition resulted in a significant expansion of the *sox2*-expressing sensory domain, which went on to develop supernumerary hair cells after relief of Fgf inhibition [17•]. Treatment with retinoic acid (RA) gave an identical result [17•]. These and other studies indicate that precise levels of signalling, together with balance and feedback between different signalling pathways, are essential for normal sensory patterning.

As for otic neurogenesis, development of the sensory epithelium is also dependent on Notch signalling. Here, Notch has a dual role: initially, Notch-mediated lateral induction results in specification of the *Sox2*-positive prosensory domain, within which Notch-mediated lateral inhibition selects hair and supporting cell fates (see Refs. [23,24••], and references within). A study combining

Figure 1



Schematic illustrations of the various stages of ear development highlighted in the text (not to scale). See text for details of progress in understanding the developmental mechanisms that pattern each of these steps. **(a)** Formation of the PPR, otic placode and otocyst (otic vesicle) from cranial ectoderm. The otocyst is the source of nearly all cell types of the mature ear (F). **(b)** Otic neurogenesis: neuroblasts are specified from otic vesicle epithelium, but delaminate from it and accumulate beneath the ear in a transit amplifying population (light blue). Neurons (dark blue) differentiate from this population, and innervate sensory hair cells in the overlying otic epithelium. The ganglion develops in close association with neural crest cells (green), which give rise to glia. **(c)** Early otolith formation in the zebrafish otic vesicle. At least three distinct populations of cilia can be distinguished: immotile hair cell kinocilia (red), which tether the otolith at early stages; motile cilia (blue) in the vicinity of the sensory hair cells, which do not bind otolithic material, and shorter immotile cilia (green). **(d)** Schematic comparison of semicircular canal formation in the zebrafish ear (top row) and a generalised amniote ear (bottom row). A single canal is illustrated for clarity. Epithelia adhere at a fusion plate, from which cells are cleared to make the duct. The end result of both events is the same (right hand image), but the fusion plate is much smaller in the zebrafish. **(e)** Comparative sketches of inner ears from adult zebrafish and late stage chick and mouse embryos. Sensory (red), neuronal (blue) and

experimental manipulation in the chick embryo and mathematical modelling has underlined the importance of differential signalling strength driven by different Notch ligands (Jag1 and Dl1). Competition between the two ligands allows for the switch from lateral induction to lateral inhibition, and biases selection of hair cell fate [24••].

A key player in hair cell differentiation is the autoregulatory basic helix-loop-helix factor Atoh1, which induces expression of *Dl1*, and is regulated by both Sox2 and Notch [24••,25,26]. Atoh1 is known to be both necessary and sufficient for hair cell specification and differentiation (see Ref. [27] for review), but recent work has revealed additional roles for Atoh1 at different stages of hair cell development. Conditional knockout of Atoh1 in mice, based on an inducible Cre-lox system, has demonstrated roles for Atoh1 in hair cell survival, stereociliary bundle maturation and hair cell function [28,29]. Other new insights into hair cell integrity and survival include the identification of the actin bundling proteins of the Eps8 family, which show exquisite localisation to stereociliary tips in murine cochlear hair cells, and are required for maturation, maintenance and function of the stereociliary bundle [30,31]. Work in the zebrafish has generated new models of Usher syndrome, demonstrating that ER stress is likely to underlie the hair cell death in this disorder [32].

Tonotopy of the auditory system (its ordered arrangement according to frequency sensitivity) is one of the marvels of the inner ear and its central processing pathways. In the cochlea, tonotopy is manifest as gradients of hair cell density, morphology, physiology and gene expression, many of which are established at very early stages of cochlear development (reviewed in Ref. [33]). Recent studies have suggested mechanisms that contribute to the establishment of this tonotopic arrangement. The first of these used conditional approaches in mice to demonstrate a role for Shh signalling from the spiral ganglion in controlling growth of the cochlear duct and timing of hair cell differentiation in the organ of Corti [34•]. In the absence of Shh signalling from the spiral ganglion, hair cell precursors — normally differentiating from base to apex — now underwent premature cell cycle exit, and differentiated precociously in an apical to basal wave. Similar precocious hair cell differentiation in apical regions was observed in mice with a conditional knockout of the Hh transducer Smoothened in the cochlea [35•]. In the chick, Mann and colleagues propose that non-canonical BMP signalling is a key mechanism in establishing the tonotopic organisation of the cochlea [36•]. Using microarray, RNA-seq and qPCR approaches, they demonstrated that *Bmp7* is expressed in an increasing proximal-to-distal gradient in the cochlea, while *Chordin-like1*, a BMP antagonist, is expressed in an increasing distal-to-proximal gradient. Moreover, manipulation of Bmp signalling by over-expression of either Bmp7 or Chordin-like1 abolished gradients of hair cell density and morphology as expected [36•]. A second RNA-seq transcriptome analysis has highlighted the graded expression of genes coding for RA-synthesising or RA-degrading enzymes along the developing cochlea in the chick [37•]. Although further elements of each model remain to be elucidated, dynamic gradients of signalling molecules, established and maintained by cross-regulatory feedback loops, are attractive candidates for the instructive cues that establish tonotopic differences in hair cell morphology and function along the cochlea.

Fluid production in the ear: the endolymph

As the otic vesicle develops, it becomes filled with endolymph, a specialised extracellular fluid with unusual ionic composition that is essential for sensory hair cell function. Disruption to endolymph generation or homeostasis can have profound effects on otic development and physiology. For example, in mice lacking function of the anion exchanger SLC26A4 (Pendrin), an endolymphatic hydrops develops, resulting in both hearing loss and vestibular dysfunction. In an exciting and thorough study, Li and colleagues restored *Slc26a4* expression specifically to the endolymphatic sac in mice otherwise lacking *Slc26a4* function. Although *Slc26a4* is normally expressed in many sites throughout the labyrinth, expression in the endolymphatic sac alone was sufficient to restore normal morphology and function to the entire inner ear [38••]. This promising work paves the way for the design of spatially and temporally restricted therapeutic interventions for human hearing loss caused by mutations in the *SLC26A4* gene.

Formation and tethering of otoliths and otoconia

Normal endolymph composition is also important for the development of otoliths or otoconia in the ear. These are the biomineralised 'ear stones' that sit above vestibular hair cells of the saccule and utricle, enabling the detection of gravity and linear acceleration. In the zebrafish, it is possible to observe the very earliest steps in otolith formation in the live embryo (Figure 1c). Here, otolith precursor particles tether to the tips of the kinocilia of the first hair cells (tether cells) in the ear, in a process defined as otolith seeding. Cells bearing motile cilia are found in close proximity to the tether cells; the motile cilia do not

(Figure 1 Legend Continued) endolymph-regulating (yellow) cells are shown for the mouse ear. *Abbreviations*: A, ampulla; BP, basilar papilla; HC, hair cell; L, lagena; LM, lagenar macula; MN, maturing neurons; NB, neuroblasts; NCC, neural crest cells; NP, neural plate; Nt, notochord; ooC, organ of Corti; Ot, otolith; OV, otic vesicle; PPR, preplacodal region; S, saccule; SVG, spiral and vestibular ganglion; TA, transit amplifying population of neuroblasts; U, utricle.

bind otoliths, but contribute to the accuracy of the seeding process [39•]. Surprisingly, disruption of cilia or ciliary motility results in only mild perturbations of otolith seeding and characteristic otolith defects; in the absence of cilia, otolith precursor particles adhere directly to the apical surfaces of the hair cells [40•,41•]. Disruption of hair cell differentiation, however (through morpholino-mediated knockdown of *atoh1b*), results in a failure of otolith seeding [40•]. Although these results predict the existence of a hair cell-specific otolith precursor-binding factor that becomes localised to the kinociliary tips, the identity of such a factor has so far proved elusive.

As an aside, the developing zebrafish ear is a really beautiful system in which to study cilia. All cells of the early otic epithelium are monociliated, and at least three different ciliary types (hair cell kinocilia, motile cilia and immotile short cilia) are present from early stages, and can be visualised easily in the live embryo [40•] (Figure 1c). Moreover, different otic hair cell kinociliary subtypes have different genetic requirements [42], and the kinocilium plays an unexpected role in the development of mechanosensitivity in zebrafish hair cells [43•]. The possibilities for live imaging coupled with transgenic and mutant analysis make this an area ripe for further study.

Semicircular canal morphogenesis

The three semicircular canals of the ear sense rotational movements (angular accelerations) of the head. The generation of these canals — involving the topological conversion of the otic vesicle into a labyrinth of interconnected ducts and chambers — is a fascinating problem for the developmental biologist (Figure 1d and e). Work in the zebrafish has identified an adhesion class G protein-coupled receptor, Gpr126 that is required for the early fusion step in canal formation [44•]. Possible transcriptional targets for the Gpr126 signalling pathway in the ear include genes coding for various extracellular matrix (ECM) components: dynamic and spatially restricted expression of several ECM genes accompanies outgrowth of the epithelial projections that form the canal system. In the *gpr126* mutant, expression of *versican* and other ECM genes persists at abnormally high levels in the ear [44•]. It remains to be tested whether down-regulation of ECM genes is a prerequisite for the fusion events that ensure normal development of the canal ducts.

Fgf, RA and Wnt signalling also play an important role in the development of the vestibular system. In the zebrafish, Fgf promotes, whereas RA restricts, the otic expression of *otx1b* [17•], which has a conserved role in formation of the horizontal semicircular canal. In mice, disruption of the RA-synthesising enzyme gene *Raldh3* results in both morphological and functional deficits of the entire vestibular system [45]. Another study in mice has demonstrated the importance of Wnt/β-catenin signalling in sculpting the semicircular canal ducts via regulation of Netrin1-mediated cell resorption at the canal fusion plate [46].

Sensory hair cell regeneration

The quest to understand the regenerative capacity of hair cells in some organisms and the inability to regenerate hair cells in others is still a major research endeavour and one that is of enormous clinical significance. Recent studies demonstrate that species-specific differences in hair cell regenerative capacity correlate with the degree of thickening and stability of F-actin bands at junctions between supporting cells, which may explain the inability of mammals to replace damaged hair cells [47,48]. Nevertheless, various approaches may be able to overcome this problem. Following on from promising work using Notch inhibitors in embryonic or neonatal systems, two studies have shown that localised treatment with inhibitors of Notch signalling can rescue both outer hair cell number (via direct transdifferentiation of supporting cells) and some limited hearing function in the noise-damaged mature mammalian cochlea [49,50]. Indeed, the importance of Notch signalling in the regenerative process has been underlined by a comprehensive transcriptome analysis of the regenerating chick utricle [51]. This and related studies in the zebrafish [52] provide a rich source of candidate genes and pathways to target in the mammalian system.

Conclusions

Over the past two years, there have been many exceptional new insights into the developmental mechanisms that pattern the inner ear. The diversity of studies relating to this single sensory organ meant that it was a real challenge to decide what to include for this compilation. Inevitably, I have had to leave out discussion of many interesting findings, including studies on the development of hair cell apico-basal and planar polarity, physiological function and neuronal circuitry, together with new studies on the evolutionary developmental biology of the ear. Progress in developmental studies has also underpinned a large body of work aiming to restore hearing using cell-based therapies (see, for example, Ref. [53•]). One particularly exciting report demonstrates recapitulation of the entire otic developmental programme — from murine embryonic stem cells to vesicular organoids containing functional hair cells and sensory neurons — *in vitro* [54••]. This illustrates the sophisticated level of understanding that we now have for the developmental mechanisms underlying inner ear organogenesis, and holds promise for the design of improved and personalised therapies for human hearing loss. Nevertheless, many areas remain to be explored, and these are likely to yield new discoveries well into the future.

Acknowledgements

I am grateful to Sarah Baxendale and Matthew Holley for discussion and constructive comments on the manuscript. Work in the Whitfield lab is funded by the BBSRC (BB/J003050).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Groves AK, LaBonne C: **Setting appropriate boundaries: fate, patterning and competence at the neural plate border**. *Dev Biol* 2014, **389**:2-12.

2. • Reichert S, Randall RA, Hill CS: **A BMP regulatory network controls ectodermal cell fate decisions at the neural plate border**. *Development* 2013, **140**:4435-4444.
Analysis of the feedback loops that regulate BMP signalling in embryonic ectoderm, distinguishing neural crest from preplacodal ectoderm, using a transgenic reporter to visualise the different domains of BMP activity.

3. Bhat N, Kwon HJ, Riley BB: **A gene network that coordinates preplacodal competence and neural crest specification in zebrafish**. *Dev Biol* 2013, **373**:107-117.

4. Padanad MS, Bhat N, Guo B, Riley BB: **Conditions that influence the response to Fgf during otic placode induction**. *Dev Biol* 2012, **364**:1-10.

5. Khatri SB, Edlund RK, Groves AK: **Foxi3 is necessary for the induction of the chick otic placode in response to FGF signaling**. *Dev Biol* 2014, **391**:158-169.

6. Yao D, Zhao F, Wu Y, Wang J, Dong W, Zhao J, Zhu Z, Liu D: **Dissecting the differentiation process of the preplacodal ectoderm in zebrafish**. *Dev Dyn* 2014, **243**:1338-1351.

7. • Steventon B, Mayor R, Streit A: **Mutual repression between Gbx2 and Otx2 in sensory placodes reveals a general mechanism for ectodermal patterning**. *Dev Biol* 2012, **367**:55-65.
Misexpression, knockdown and cell sorting experiments reveal mechanisms that distinguish and sort otic cells from neighbouring placodal fates.

8. •• McCarroll MN, Lewis ZR, Culbertson MD, Martin BL, Kimelman D, Nechiporuk AV: **Graded levels of Pax2a and Pax8 regulate cell differentiation during sensory placode formation**. *Development* 2012:2740-2750.
Lineage tracing, ablation, misexpression and knockdown experiments demonstrate the importance of Pax levels in controlling the otic/epibranchial fate decision: high levels favour otic fate.

9. Sánchez-Guardado LÓ, Puelles L, Hidalgo-Sánchez M: **Fate map of the chicken otic placode**. *Development* 2014, **141**:2302-2312.

10. Yan B, Neilson KM, Ranganathan R, Maynard T, Streit A, Moody SA: **Microarray identification of novel genes downstream of Six1, a critical factor in cranial placode, somite and kidney development**. *Dev Dyn* 2015, **244**:181-210.

11. Sai X, Yonemura S, Ladher RK: **Junctionally restricted RhoA activity is necessary for apical constriction during phase 2 inner ear placode invagination**. *Dev Biol* 2014, **394**:206-216.

12. Tay HG, Schulze SK, Compagnon J, Foley FC, Heisenberg CP, Yost HJ, Abdelilah-Seyfried S, Amack JD: **Lethal giant larvae 2 regulates development of the ciliated organ Kupffer's vesicle**. *Development* 2013, **140**:1550-1559.

13. Zigman M, Laumann-Lipp N, Titus T, Postlethwait J, Moens CB: **Hoxb1b controls oriented cell division, cell shape and microtubule dynamics in neural tube morphogenesis**. *Development* 2014, **141**:639-649.

14. Maier EC, Saxena A, Alsina B, Bronner ME, Whitfield TT: **Sensational placodes: neurogenesis in the otic and olfactory systems**. *Dev Biol* 2014, **389**:50-67.

15. • Hans S, Irmscher A, Brand M: **Zebrafish Foxi1 provides a neuronal ground state during inner ear induction preceding the Dlx3b/4b-regulated sensory lineage**. *Development* 2013, **140**:1936-1945.
Demonstration that neuronal specification occurs very early in the zebrafish, during induction of the otic/epibranchial precursor domain, and is dependent on Foxi1 activity.

16. • Sapède D, Dyballa S, Pujades C: **Cell lineage analysis reveals three different progenitor pools for neurosensory elements in the otic vesicle**. *J Neurosci* 2012, **32**:16424-16434.
Lineage tracing and loss-of-function experiments reveal that sensory hair cell and neuronal lineages segregate early in anterior regions of the zebrafish otic vesicle, but common progenitor cells for both lineages exist in posterior regions.

17. • Maier EC, Whitfield TT: **RA and FGF signalling are required in the zebrafish otic vesicle to pattern and maintain ventral otic identities**. *PLoS Genet* 2014, **10**:e1004858.
A combination of gain-of-function and loss-of-function approaches demonstrate that RA and Fgf continue to pattern the ear at otic vesicle stages to establish sensory, neurogenic and non-neural domains in ventral otic epithelium.

18. • Vemaraju S, Kantarci H, Padanad MS, Riley BB: **A spatial and temporal gradient of Fgf differentially regulates distinct stages of neural development in the zebrafish inner ear**. *PLoS Genet* 2012, **8**:e1003068.
Conditional approaches demonstrating roles for Fgf signalling at multiple stages of otic neurogenesis in the zebrafish. The authors identify Fg5, expressed by mature neurons, as a candidate for regulating neuronal differentiation from the transit amplifying population.

19. Sandell LL, Butler Tjaden NE, Barlow AJ, Trainor PA: **Cochleovestibular nerve development is integrated with migratory neural crest cells**. *Dev Biol* 2014, **385**:200-210.

20. Neves J, Vachkov I, Giraldez F: **Sox2 regulation of hair cell development: incoherence makes sense**. *Hear Res* 2013, **297**:20-29.

21. • Ono K, Kita T, Sato S, O'Neill P, Mak SS, Paschaki M, Ito M, Gotoh N, Kawakami K, Sasai Y *et al.*: **FGFR1-Frs2/3 signalling maintains sensory progenitors during inner ear hair cell formation**. *PLoS Genet* 2014, **10**:e1004118.
This study elaborates on the mechanism of Fgf signalling, through FGFR1 and the maintenance of Sox2 expression, in the developing mammalian cochlea and utricle.

22. • Huh SH, Jones J, Warchol ME, Ornitz DM: **Differentiation of the lateral compartment of the cochlea requires a temporally restricted FGF20 signal**. *PLoS Biol* 2012, **10**:e1001231.
Identification of FGF20 as a key signal for outer hair cell development in the murine cochlea.

23. Pan W, Jin Y, Chen J, Rottier RJ, Steel KP, Kiernan AE: **Ectopic expression of activated notch or SOX2 reveals similar and unique roles in the development of the sensory cell progenitors in the mammalian inner ear**. *J Neurosci* 2013, **33**:16146-16157.

24. •• Petrovic J, Formosa-Jordan P, Luna-Escalante JC, Abelló G, Ibañes M, Neves J, Giraldez F: **Ligand-dependent Notch signaling strength orchestrates lateral induction and lateral inhibition in the developing inner ear**. *Development* 2014, **141**:2313-2324.
Differential signalling strength and competition between two different Notch ligands regulate the switch between lateral induction and lateral inhibition in the chick inner ear.

25. Neves J, Uchikawa M, Bigas A, Giraldez F: **The prosensory function of Sox2 in the chicken inner ear relies on the direct regulation of Atoh1**. *PLOS ONE* 2012, **7**:e30871.

26. Ahmed M, Wong EY, Sun J, Xu J, Wang F, Xu PX: **Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating Atoh1 expression in cooperation with Sox2**. *Dev Cell* 2012, **22**:377-390.

27. Cai T, Groves AK: **The role of atonal factors in mechanosensory cell specification and function**. *Mol Neurobiol* 2014. (in press).

28. Cai T, Seymour ML, Zhang H, Pereira FA, Groves AK: **Conditional deletion of Atoh1 reveals distinct critical periods for survival and function of hair cells in the organ of Corti**. *J Neurosci* 2013, **33**:10110-10122.

29. Chonko KT, Jahan I, Stone J, Wright MC, Fujiyama T, Hoshino M, Fritzsch B, Maricich SM: **Atoh1 directs hair cell differentiation and survival in the late embryonic mouse inner ear**. *Dev Biol* 2013, **381**:401-410.

30. Zampini V, Rüttiger L, Johnson SL, Franz C, Furness DN, Waldhaus J, Xiong H, Hackney CM, Holley MC, Offenhauser N *et al.*: **Eps8 regulates hair bundle length and functional maturation of mammalian auditory hair cells**. *PLoS Biol* 2011, **9**:e1001048.

31. Furness DN, Johnson SL, Manor U, Rüttiger L, Tocchetti A, Offenhauser N, Olt J, Goodyear RJ, Vijayakumar S, Dai Y *et al.*: **Progressive hearing loss and gradual deterioration of sensory hair bundles in the ears of mice lacking the actin-binding protein Eps8L2**. *Proc Natl Acad Sci U S A* 2013, **110**:13898-13903.

32. Blanco-Sánchez B, Clément A, Fierro J Jr, Washbourne P, Westerfield M: **Complexes of Usher proteins preassemble at the endoplasmic reticulum and are required for trafficking and ER homeostasis**. *Dis Mod Mech* 2014, **7**:547-559.

33. Mann ZF, Kelley MW: **Development of tonotopy in the auditory periphery**. *Hear Res* 2011, **276**:2-15.

34. • Bok J, Zenczak C, Hwang CH, Wu DK: **Auditory ganglion source of Sonic hedgehog regulates timing of cell cycle exit and differentiation of mammalian cochlear hair cells**. *Proc Natl Acad Sci U S A* 2013, **110**:13869-13874.
See annotation below Ref. [35•].

35. • Tateya T, Imayoshi I, Tateya I, Hamaguchi K, Torii H, Ito J, Kageyama R: **Hedgehog signaling regulates prosensory cell properties during the basal-to-apical wave of hair cell differentiation in the mammalian cochlea**. *Development* 2013, **140**:3848-3857.
These two papers [34•,35•] demonstrate that Hh signalling regulates the graded timing of hair cell differentiation along the murine cochlea.

36. • Mann ZF, Thiede BR, Chang W, Shin JB, May-Simera HL, Lovett M, Corwin JT, Kelley MW: **A gradient of Bmp7 specifies the tonotopic axis in the developing inner ear**. *Nat Commun* 2014, **5**:3839.
See annotation below Ref. [37•].

37. • Thiede BR, Mann ZF, Chang W, Ku YC, Son YK, Lovett M, Kelley MW, Corwin JT: **Retinoic acid signalling regulates the development of tonotopically patterned hair cells in the chicken cochlea**. *Nat Commun* 2014, **5**:3840.
These two papers [36•,37•] propose that gradients of BMP and RA signalling contribute to the establishment of tonotopic patterning along the murine cochlea.

38. •• Li X, Sanneman JD, Harbidge DG, Zhou F, Ito T, Nelson R, Picard N, Chambrey R, Eladari D, Miesner T *et al.*: **SLC26A4 targeted to the endolymphatic sac rescues hearing and balance in Slc26a4 mutant mice**. *PLoS Genet* 2013, **9**:e1003641.
Restoration of Pendrin (SLC26A4) expression to the endolymphatic sac of Slc26a4 mutant mice rescues inner ear development and function.

39. • Wu D, Freund JB, Fraser SE, Vermot J: **Mechanistic basis of otolith formation during teleost inner ear development**. *Dev Cell* 2011, **20**:271-278.
Measurements and modelling of hydrodynamic flow in the zebrafish otic vesicle caused by beating cilia, and its contribution to otolith morphogenesis.

40. • Stooke-Vaughan GA, Huang P, Hammond KL, Schier AF, Whitfield TT: **The role of hair cells, cilia and ciliary motility in otolith formation in the zebrafish otic vesicle**. *Development* 2012, **139**:1777-1787.
High resolution imaging of ciliary motility in the zebrafish otic vesicle. Analysis of different loss-of-function phenotypes demonstrates the importance of cilia and hair cells (tether cells) for otolith formation.

41. • Yu X, Lau D, Ng CP, Roy S: **Cilia-driven fluid flow as an epigenetic cue for otolith biomineralization on sensory hair cells of the inner ear**. *Development* 2011, **138**:487-494.
Analysis of the role of cilia and ciliary motility in zebrafish otolith formation; demonstration that the immotile hair cell kinocilium is dependent on the expression of Foxj1b, otherwise required for the genesis of motile cilia.

42. Zhao C, Omori Y, Brodowska K, Kovach P, Malicki J: **Kinesin-2 family in vertebrate ciliogenesis**. *Proc Natl Acad Sci U S A* 2012, **109**:2388-2393.

43. • Kindt KS, Finch G, Nicolson T: **Kinocilia mediate mechanosensitivity in developing zebrafish hair cells**. *Dev Cell* 2012, **23**:329-341.
Initial mechanosensitive function in the developing zebrafish lateral line hair cell is mediated by the kinocilium.

44. • Geng FS, Abbas L, Baxendale S, Holdsworth CJ, Swanson AG, Slanchev K, Hammerschmidt M, Topczewski J, Whitfield TT: **Semicircular canal morphogenesis in the zebrafish inner ear requires the function of gpr126 (lauscher), an adhesion G protein-coupled receptor gene**. *Development* 2013, **140**:4362-4374.
Identification of an adhesion class GPCR required for correct growth and fusion of the projections of epithelium that form the semicircular canals in the zebrafish ear.

45. Romand R, Krezel W, Beraneck M, Cammas L, Fraulob V, Messaddeq N, Kessler P, Hashino E, Dollé P: **Retinoic acid deficiency impairs the vestibular function**. *J Neurosci* 2013, **33**:5856-5866.

46. Rakowiecki S, Epstein DJ: **Divergent roles for Wnt/β-catenin signaling in epithelial maintenance and breakdown during semicircular canal formation**. *Development* 2013, **140**:1730-1739.

47. Burns JC, Collado MS, Oliver ER, Corwin JT: **Specializations of intercellular junctions are associated with the presence and absence of hair cell regeneration in ears from six vertebrate classes**. *J Comp Neurol* 2013, **521**:1430-1448.

48. Burns JC, Corwin JT: **Responses to cell loss become restricted as the supporting cells in mammalian vestibular organs grow thick junctional actin bands that develop high stability**. *J Neurosci* 2014, **34**:1998-2011.

49. Mizutari K, Fujioka M, Hosoya M, Bramhall N, Okano HJ, Okano H, Edge AS: **Notch inhibition induces cochlear hair cell regeneration and recovery of hearing after acoustic trauma**. *Neuron* 2013, **77**:58-69.

50. Tona Y, Hamaguchi K, Ishikawa M, Miyoshi T, Yamamoto N, Yamahara K, Ito J, Nakagawa T: **Therapeutic potential of a gamma-secretase inhibitor for hearing restoration in a guinea pig model with noise-induced hearing loss**. *BMC Neurosci* 2014, **15**:66.

51. Ku YC, Renaud NA, Veile RA, Helms C, Voelker CC, Warchol ME, Lovett M: **The transcriptome of utricle hair cell regeneration in the avian inner ear**. *J Neurosci* 2014, **34**:3523-3535.

52. Liang J, Wang D, Renaud G, Wolfsberg TG, Wilson AF, Burgess SM: **The stat3/socs3a pathway is a key regulator of hair cell regeneration in zebrafish**. *J Neurosci* 2012, **32**:10662-10673.

53. • Chen W, Jongkamonwiwat N, Abbas L, Eshtan SJ, Johnson SL, Kuhn S, Milo M, Thurlow JK, Andrews PW, Marcotti W *et al.*: **Restoration of auditory evoked responses by human ES-cell-derived otic progenitors**. *Nature* 2012, **490**:278-282.
Production of otic progenitors from human embryonic stem cells, and transplantion of progenitors into deafened gerbils, resulting in restoration of hearing function.

54. •• Koehler KR, Mikosz AM, Molosh AI, Patel D, Hashino E: **Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture**. *Nature* 2013, **500**:217-221.
Sequential treatment of murine embryonic stem cells with signalling factors, in three-dimensional culture, to generate organoids containing functional hair cells, comparable to immature vestibular epithelia.