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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