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Sculpting the labyrinth: Morphogenesis of the developing inner ear

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ABSTRACT

The vertebrate inner ear is a precision sensory organ, acting as both a microphone to receive sound and an accelerometer to detect gravity and motion. It consists of a series of interlinked, fluid-filled chambers containing patches of sensory epithelia, each with a specialised function. The ear contains many different differentiated cell types with distinct morphologies, from the flask-shaped hair cells found in thickened sensory epithelium, to the thin squamous cells that contribute to non-sensory structures, such as the semicircular canal ducts. Nearly all cell types of the inner ear, including the afferent neurons that innervate it, are derived from the otic placode, a region of cranial ectoderm that develops adjacent to the embryonic hindbrain. As the ear develops, the otic epithelia grow, fold, fuse and rearrange to form the complex three-dimensional shape of the membranous labyrinth. Much of our current understanding of the processes of inner ear morphogenesis comes from genetic and pharmacological manipulations of the developing ear in mouse, chicken and zebrafish embryos. These traditional approaches are now being supplemented with exciting new techniques—including force measurements and light-sheet microscopy—that are helping to elucidate the mechanisms that generate this intricate organ system.

Contents

1. Introduction

In any study of organogenesis, it is important to gain an appreciation not only of the genetic control of patterning but also of the morphogenetic events that give rise to the three-dimensional form of the mature organ system. Understanding the coupling of signalling pathways and transcription factor network activity to the cell behaviours and physical forces that effect these morphogenetic events is thus one of the major challenges in the field. The development of new technologies, particularly in live imaging, is now opening up new possibilities for tackling these challenges. For the inner ear, such studies have clinical relevance: congenital hearing loss can also be accompanied by some form of morphological

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anomaly, such as Mondini dysplasia (incomplete partition or coiling of the cochlea). Aplasia of the semicircular canals or whole labyrinth can also cause severe disruption of vestibular function.

In this review, we consider a selection of the morphological rearrangements that take place as the inner ear develops. We focus on four topics: formation of the otic placode and vesicle; neurogenesis and generation of the VIIIth ganglion; segregation of sensory epithelia; and formation of the semicircular canal ducts. We have omitted discussion of a number of other important processes, including formation of the endolymphatic duct and sac, establishment of the precise cytoarchitecture of the mammalian organ of Corti, the role of surrounding tissues (including hindbrain and periotic mesenchyme), and the morphogenesis of ancillary structures, each of which would justify a separate review in its own right. We end with a perspective on the new methodologies that are pushing the boundaries of our understanding of how patterning is coupled to morphogenesis in the developing inner ear.

2. Segregation of the otic placode from the pre-placodal region (PPR)

2.1. Cell movements

The otic placode together with other cranial placodes (adenohypophyseal, olfactory, lens, trigeminal, epibranchial, otic and lateral line) and the neural crest give rise to the elements of the cranial peripheral nervous system. The cranial placodes do not develop directly as individual entities from the ectoderm but emerge from the common pre-placodal region (PPR), a horseshoe-shaped subdomain of the ectoderm adjacent and lateral to the neural plate and neural crest [1–5]. The PPR expresses a combination of transcription factors of the Six1/2, Six4/5, Dach, Eya, Dlx, Gata, and Foxi families that confer its identity and competence for specific placode-inducing signals [6–14]. The latter, by acting upon the PPR precursors, drive the splitting of the PPR and emergence of individual placodal fates [15–17]. The segregation of the PPR into placodes is progressive. At the level of the hindbrain for example, prior to the appearance of the otic placode, a large *Pax2/8*-expressing domain encompasses the precursors of future epibranchial and otic placodes (also lateral line precursors in anamniotes). This domain has been coined the otic-epibranchial precursor domain (OEPD) to highlight the close developmental relationship between these placodes [9,10,18–20]. The inductive events involved in the development of the OEPD and otic placode are reviewed elsewhere in this issue [21]; we focus here on the morphogenetic movements leading to the segregation of the large PPR into discrete placodes within the cranial ectoderm.

Fate mapping of pre-placodal precursors in chick indicates that otic precursors are interspersed with future neural tissue, neural crest and other placodal cells until the four-somite stage; extensive cell movements have been observed to accompany placode development, enabling the segregation of the different cell types [22]. At early stages (stage 5–6 in chick), otic precursors were found over a large territory of the PPR, at the level of rhombomeres 2–7 of the hindbrain, but were then progressively restricted to form the otic placode at the level of rhombomeres 5–6. Convergence of lateral cells to medial positions was the most dramatic cell movement, accompanied by splitting and cell mixing between groups of cells. In zebrafish, live imaging of cells expressing GFP driven by the *pax2a* promoter within the OEPD showed that most GFP-positive cells converge from anterior, posterior and lateral positions to form the otic placode, but more anterior and posterior GFP-positive cells also contribute to epibranchial ganglia [23]. Analysis of Pax2a protein expression, together with heat-shock-induced mis-expression and morpholino-based gene knockdown, demonstrated that cells with high levels of Pax2a protein have a tendency to contribute to the otic placode, while lower levels of Pax2a bias precursors to the epibranchial placodes [20]. Exactly how the levels of Pax2a can influence the sorting and/or convergence of pre-placodal precursors needs to be investigated further, but probably involves changes in cell adhesivity. Interestingly, a morpholino-based study suggests that directed cell movements and convergence of pre-otic precursors to form the zebrafish otic placode relies partly on the function of the extracellular matrix receptor Integrin-α5 [23].

Similar cellular movements leading to the segregation of intermingled anterior PPR precursors into the anterior cranial placodes (olfactory, lens, adenohypophyseal, trigeminal) have also been described [3,24–26]. The extent of the directional cell migration differs between species; in *Xenopus* and zebrafish, cell movements are restricted to small areas and no large-scale cell sorting is detected [17,20]. Differences could be related to the species or to the periods in which movements have been analysed, which are limited prior to placode coalescence. When cells of the OEPD display small-scale movements, they contribute to distinct otic regions depending on their initial anteroposterior location in the OEPD, the most anterior cells being preferentially allocated to the anterior neurogenic domain and statoacoustic ganglion (SAG) [20] and not to the posterior domain of the inner ear. In conclusion, while migratory movements have recently been followed in real time, the underlying molecules involved in the chemotaxis, sorting and collective movements are little known. Moreover, it is still debated whether PPR cells are already lineage-restricted before their sorting out or whether random movements favour their position along the anteroposterior axis, followed by the reception of distinct signals that direct cells to specific placodal fates (see also [27]).

2.2. Placode formation

2.2.1. Coalescence into a placode

How do placodes appear as a cluster of cells after the segregation of PPR cells? Compared with other placodes, morphogenetic events of otic placode formation have received scant attention, but studies on other placodes hint at generic mechanisms. Whitlock and Westerfield have shown that before the final appearance of the olfactory placode, olfactory precursors extend over a long and thin territory that progressively converges to a shorter and wider domain along the anteroposterior axis and mediolateral axis respectively [24]. A similar event takes place during the coalescence or convergence of the otic placode. Alvarez and Navascués found long cytokinetic bridges during this process, and have proposed a link between cellular displacements after mitosis and placode formation [28]. In line with this, recent imaging of convergence movements during chick gastrulation identified mitosis as a driver for epithelial rearrangements [29]. This highlights the need to re-evaluate, with modern imaging techniques, the contribution of cell division orientation and shapes to otic placodal morphogenesis.

Other tissues also impact on placodal development. In particular, coalescence and positioning of the zebrafish olfactory and epibranchial placodes are linked to migration of the adjacent neural crest [30,31]. The latter influences the timing of establishment of a basal lamina surrounding the olfactory placode and the neural tube that segregates both populations and favours condensation of the olfactory placode. During formation of the epibranchial placodes in chick, coordinated cell migration between placodal and neural crest cells results from a “chase and run” cellular behaviour, in which neural crest cells chase placodal cells and then placode cells run away as they are contacted [32]. The classical chemokine system Sdf1-Cxcr4, known to underlie various migratory events (for example, migration of germ cells and lateral line primordia) is involved in the coordinated placode-neural crest migratory behaviour [32]. Epibranchial placodal cells express the ligand *Sdf1* and remain in