Development 137, 1777-1785 (2010) doi:10.1242/dev.040055

From shared lineage to distinct functions: the development of the inner ear and epibranchial placodes

Raj K. Ladher[1,*], Paul O'Neill[1] and Jo Begbie[2]

Summary

The inner ear and the epibranchial ganglia constitute much of the sensory system in the caudal vertebrate head. The inner ear consists of mechanosensory hair cells, their neurons, and structures necessary for sound and balance sensation. The epibranchial ganglia are knots of neurons that innervate and relay sensory signals from several visceral organs and the taste buds. Their development was once thought to be independent, in line with their independent functions. However, recent studies indicate that both systems arise from a morphologically distinct common precursor domain: the posterior placodal area. This review summarises recent studies into the induction, morphogenesis and innervation of these systems and discusses lineage restriction and cell specification in the context of their common origin.

Key words: Epibranchial, Inner ear, Neurogenesis, Placode, Signalling

Introduction

Cranial placodes, found in all vertebrates, are transient thickenings of ectoderm that contribute extensively to the sensory component of the cephalic peripheral nervous system (see Box 1 and Glossary, Box 2). Individual placodes give rise to characteristic cell types, although the diversity of placodal derivatives varies (Box 1). Some placodes, such as the olfactory, otic and lateral line placodes, can form the receptive cell that responds to a stimulus, as well as the sensory neurons that transmit this information (Box 1). Others, such as the epibranchial and trigeminal placodes, only give rise to sensory neurons. The lens and adenohypophyseal placodes generate no sensory derivatives (Baker and Bronner-Fraser, 2001; Webb and Noden, 1993; Begbie and Graham, 2001b). In this review, we focus on the inner ear (or otic) placode and the epibranchial series of placodes and discuss their origins from a common progenitor domain: the posterior placodal area (PPA) (Fig. 1).

The otic placode forms the complex inner ear structure that detects sound and balance, as well as the neurons that convey this information to the auditory hindbrain. The otic placodes form distinctive paired depressions adjacent to the caudal hindbrain and progressively deepen to form otocysts (see Glossary, Box 2). Transcriptional networks, influenced by extrinsic signals, drive the regional differentiation of the otic placode to generate mechanosensory hair cells, supporting cells and neurons (see Box 1 and Glossary, Box 2). This progressive differentiation results in a remarkable convolution of the simple spherical otocyst into an intricate structure that is dedicated to receiving information on balance, angular velocity and sound. These later morphogenetic events have been well reviewed (Bok et al., 2007; Fritzsch et al., 2006; Torres and Giráldez, 1998) and will not be covered here.

The epibranchial placodes give rise to the geniculate, petrosal and nodose ganglia, which contribute sensory neurons to cranial nerves VII (facial), IX (glossopharyngeal) and X (vagus), in that order (see Box 1 and Glossary, Box 2; Fig. 1). Epibranchial placodes are located ventral to the otic placode, but dorsocaudal to the pharyngeal clefts, which separate each branchial arch (see Glossary, Box 2; Fig. 1) (Graham, 2008). Differentiation occurs by delamination of neuroblast cells from the thickened placode. Delaminated neuroblasts then coalesce to form ganglia that make appropriate connections to their targets. In contrast to the inner ear and lateral line, the epibranchial placodes generally do not generate mechanosensory cells (Baker and Bronner-Fraser, 2001).

It had been thought that the development of otic and epibranchial placodes occur independently of each other. However, recent data suggest that these placodes form from a common *Pax2*-positive precursor domain (Sun et al., 2007). Aquatic vertebrates possess an additional sensory structure, the lateral line, which also forms from this domain. We use the term PPA to describe this common domain (Schlosser and Ahrens, 2004). Alternate names include the otic-epibranchial precursor domain (Freter et al., 2008) and the pre-otic field (Ohyama et al., 2007). In this review, we present an updated model of inner ear and epibranchial induction. By considering the development of both otic and epibranchial placodes, we ask how

Box 1. The cranial placodes

Sense organs and most of the neurons that ferry sensory information from the head are formed from cranial placodes. Of the placodes that make sensory cells and neurons, the olfactory placode forms the epithelium of the nose, which is important in chemosensation, and the first cranial nerve (the olfactory nerve). The otic placode forms the inner ear and is responsible for mechanosensation, detecting sound and balance, and will also give rise to the eighth cranial nerve (the cochleovestibular nerve). In aquatic vertebrates, the lateral line placodes form superficially located mechanosensors that detect water flow. Some placodes only form sensory neurons; the trigeminal placode, which is split into ophthalmic and maxillomandibular placodes, forms the sensory component of the fifth cranial nerve (the trigeminal nerve) that mediates pain, touch and temperature sensation in the skin of the head, eyes and jaw muscles. There are three epibranchial placodes. The geniculate placode forms the sensory component of the seventh cranial nerve (the facial nerve) and innervates most of the taste buds, tonsils and also receives sensory information from the ear lobes. The petrosal placode contributes to the ninth cranial nerve (the glossopharyngeal nerve), which innervates the tongue and also the carotid sinus, which is important for regulating blood pressure, as well as the carotid body, which is important in detecting blood oxygen content, pH and temperature. Finally, the nodose placode contributes to the tenth cranial nerve (the vagus nerve), which conveys sensory information from almost all of the organs in the body.

[1]RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan. [2]Department of Physiology, Anatomy and Genetics, Le Gros Clark Building, South Parks Road, University of Oxford, Oxford OX1 3QX, UK.

*Author for correspondence (raj-ladher@cdb.riken.go.jp)

Box 2. Glossary

Ganglia. A mass of nerve cell bodies.

Interkinetic nuclear migration. The apical-to-basal and then basal-to-apical migration of a cell nucleus during the cell cycle of neuroepithelial cells.

Mechanosensory hair cells. Cells that respond to mechanical stimuli. They have specialised apical structures called stereocilia or microtubule-based kinocilia that are deflected by mechanical stimuli, which cause them to deflect, opening ion channels resulting in changes in potential.

Otocyst. An intermediate stage of inner ear development consisting of an epithelial sphere embedded in the head mesenchyme that forms after the otic placode has invaginated.

Paraxial mesoderm. Mesoderm that lies immediately adjacent to, and either side of, the axial mesoderm.

Peripheral nervous system (PNS). A series of nerves and sensory structures located outside of the central nervous system (CNS).

Pharyngeal arches. Paired segmented structures (also called branchial arches) located either side of the developing pharynx that consist of ectoderm, mesoderm and neural crest cells and that contribute to pharyngeal organs and to the connective, skeletal, neural and vascular tissues of the mammalian head and neck.

Pharyngeal pouches. Endodermal pockets located at the top of the clefts between neighbouring branchial arches.

Utricle and saccule. Sense organs in the vestibular part of the inner ear that respond to changes in linear acceleration, especially those caused by gravity.

cellular diversification is induced, and whether the common lineage of both systems is reflected in the later development of the inner ear and epibranchial ganglia.

The posterior placodal area

The concept of a common precursor domain for the otic and epibranchial placodes, as well as for the lateral line in aquatic vertebrates, was initially based on gene expression patterns, in particular those of *Pax2* (Schlosser and Ahrens, 2004). This idea was also reinforced by vital dye lineage analysis of the lateral edges of *Pax2* expression in chick, which showed that these cells give rise to epibranchial placodes (Streit, 2002). This is also true in mouse, in which descendants of the early *Pax2* expression domain and of *Pax8*-expressing cells (*Pax8* is expressed redundantly in the PPA) give rise to both otic and epibranchial placodes (Bouchard et al., 2004; Ohyama et al., 2006).

In chick, *Pax2* is detectible from Hamburger and Hamilton stage (HH) 8 in an ectodermal domain located rostral to the first somite (Groves and Bronner-Fraser, 2000; Streit, 2002) (Fig. 1A). In mouse and chick, the *Pax2/8*-positive region is morphologically distinct and is apparent as thickened ectoderm that extends laterally to encompass both otic and epibranchial regions (Fig. 1) (Wright and Mansour, 2003). This observation is reminiscent of certain fish species in which the inner ear and lateral line system derive from a common cranial ectodermal thickening: for example, in selachians (Mitrophanow, 1893), salmon (Wilson and Mattocks, 1897) and the bowfin *Amia clava* (Beckwith, 1907). Based on our current understanding of the relationship between the inner ear and lateral line, it is likely that in aquatic vertebrates the otic/lateral line preplacode also includes epibranchial precursors.

Induction of the PPA

The PPA is induced from non-neural ectoderm located adjacent to the still open neural plate at the caudal hindbrain level during mid-neurula stages. The PPA overlies the lateral and later paraxial mesoderm (see Glossary, Box 2), although, as the neural tube closes, the topographic relationships between ectoderm and mesoderm, as well as with the endoderm, change so that the more lateral reaches of the PPA are brought into closer apposition with the paraxial mesoderm. Interactions with these tissues regulate the formation of the PPA, as well as the specification of its derivatives: the inner ear and epibranchial ganglia. In the paragraphs below, we describe the tissues that regulate PPA induction and the molecules that mediate this process (Fig. 2).

Tissues that regulate PPA induction

Classic experimental embryological studies suggest that the source of PPA-inducing signals is the mesoderm (for a review, see Groves, 2005). These studies used the presence of an otocyst as a marker for the inner ear, and did not look at the effect on the epibranchial ganglia. However, modern molecular techniques also support a role for the mesoderm in PPA induction. Zebrafish embryos mutant in *no tail* (*ntl*) and *one-eye pinhead* (*oep*) do not form cranial mesoderm. These mutants have delayed or absent expression of the early PPA marker *pax8*. By contrast, mutants that lack only axial mesoderm form a relatively normal PPA (Mendonsa and Riley, 1999). Studies in chick indicate that this inductive region localises to the paraxial mesoderm beneath the PPA, as its ablation causes the downregulation of the PPA marker *Pax2*; other regions of the paraxial mesoderm do not rescue the ablation (Kil et al., 2005). This study also showed that transplanting sub-otic mesoderm to ectopic locations induces ectopic *Pax2*, further implicating this mesoderm in the induction of the PPA, at least in chick (Kil et al., 2005). Grafting experiments have shown that the region from the first somite to the level of rhombomere 2/3 has PPA-inducing activity (Groves and Bronner-Fraser, 2000). This corresponds closely to the actual location of the PPA in chick. Although these experiments suggest that only the mesoderm provides the PPA-inductive signal, other findings argue against this view.

Recombination experiments between ectodermal tissue and different regions of mesoderm suggest that the posterior cephalic paraxial mesoderm is sufficient for PPA induction, but only when neural precursors are also included (Ladher et al., 2000). Indeed, experiments in which the chick hindbrain is grafted to ectopic locations also suggest that hindbrain-derived signalling can induce ectopic inner ear vesicles, although as Groves points out "the absence of clear markers to distinguish host from donor" demands care in the interpretation of these findings (Groves, 2005; Kuratani and Eichele, 1993; Sechrist et al., 1994). It is now clear that both mesoderm and hindbrain are important for the induction of the inner ear proper, but the necessity of the hindbrain in inducing the progenitor region has neither been clearly shown nor can it be completely discounted. Conclusive evidence might prove tricky to obtain, as several key inducing molecules are expressed in the mesoderm and later in the hindbrain, suggesting redundancy in both the identity and the source of the inducing signal.

Signals in PPA induction

There is considerable evidence that members of the fibroblast growth factor (FGF) family contribute to PPA induction. As shown in Fig. 3A, FGF ligand expression during PPA induction is dynamic, with expression detected in cranial mesoderm, ectoderm and endoderm at different stages during early head development (Schimmang, 2007). Furthermore, many of the same FGFs are expressed at multiple sites around the PPA and thus there is not only redundancy between different FGF molecules, but also some degree

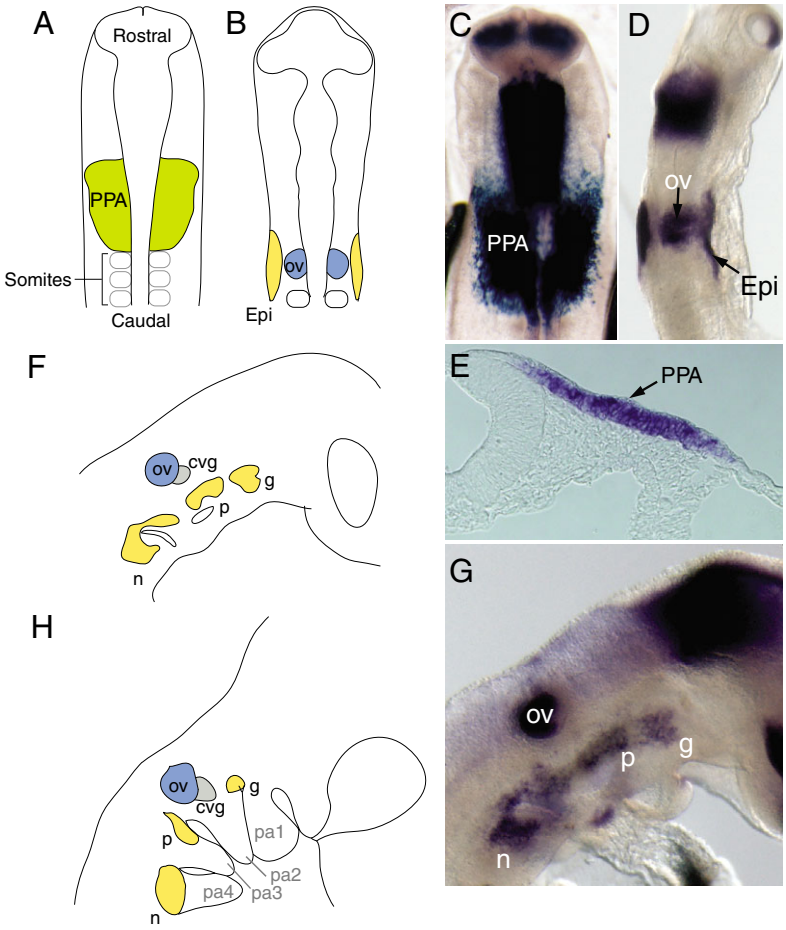

Fig. 1. The posterior placodal area is the precursor of the otic and epibranchial placodes. (**A**) Schematic of the posterior placodal area (PPA, green) at Hamburger and Hamilton stage (HH) 8 of chick development. (**B**) Schematic, based on D, showing specification of otic (blue) and epibranchial (yellow) territories. (**C**) *Pax2* expression in a HH8 chick embryo shows the extent of the PPA. (**D**) Lateral view of chick *Pax2* epibranchial expression at HH12, showing the specification of otic and epibranchial domains. (**E**) Transverse section through C showing that the entire mediolateral extent of the *Pax2* expression domain is thickened at HH8. (**F**) Schematic, based on G, showing the resolution of the epibranchial domain into individual neurogenic foci in a chick embryo at HH14. At this stage, the first neuroblasts delaminate from the otocyst to form the CVG (grey). Rostral is to the upper right. (**G**) Lateral view of HH14 chick embryo, showing *Pax2* epibranchial expression and epibranchial resolution. (**H**) Schematic, based on G, of the final positions of the otic (blue) and epibranchial (yellow) placodes. Rostral is to the upper right. Images courtesy of Dr Yuko Muta, RIKEN CDB. Epi, epibranchial domain; g, geniculate placode; p, petrosal placode; n, nodose placode; ov, otic vesicle; cvg, cochleovestibular ganglion; pa, pharyngeal arch.

of redundancy between FGFs acting from different sites of expression. Moreover, and somewhat confusingly, there is some variation between species as to the exact identity and organisation of the FGF ligands involved (Fig. 3B).

FGF-mediated PPA induction has been well reviewed recently (Fig. 2) (Schimmang, 2007). Briefly, the localised mesodermal expression of an FGF (*Fgf3* and *Fgf19* in chick, *Fgf3* and *Fgf10* in mouse, and *fgf3* and *fgf8* in zebrafish) is important for the induction of the PPA in overlying non-neural ectoderm (Leger and Brand, 2002; Liu et al., 2003; Mahmood et al., 1995; Maroon et al., 2002; Phillips et al., 2001; Vendrell et al., 2000; Ladher et al., 2005; Zelarayan et al., 2007; Wright and Mansour, 2003; Freter et al., 2008; Dominguez-Frutos et al., 2009; Nechiporuk et al., 2007). The exact details do vary between species (Fig. 2, Fig. 3B). However, this somewhat complex picture of FGF interactions can be simplified by thinking of PPA induction as being mediated by the ligands Fgf3 and Fgf8, and, in terrestrial vertebrates, by an additional FGF, such as Fgf10 or Fgf19. The fact that this additional FGF has different identities in birds and mammals suggests that its role might have arisen recently, and independently, during evolution. However, it is not clear what the role of this additional FGF ligand is.

All four FGF receptors are expressed in the putative PPA ectoderm. Mutant mouse studies suggest that *Fgfr3* and *Fgfr4* do not play a role in PPA induction. Mutants for *Fgfr1* and *Fgfr2* die before the PPA forms (Deng et al., 1994; Yamaguchi et al., 1994; Xu et al., 1998). Thus, tissue-specific knockouts of these receptors will be necessary to determine the role that they play in PPA induction. In the absence of the genetic ablation of FGF receptors, pharmacological inhibition provides valuable information. For example, inhibiting FGF signalling with SU5402 prior to the 4-somite stage in chick prevents *Pax2* expression and placodal thickening (Martin and Groves, 2006). Thus, any signalling transduction intermediates that are active at this stage could mediate PPA induction. FGF signals through a number of pathways to trigger, for example, ERK/MAP kinase phosphorylation, the phosphorylation of phospholipase C gamma (Plcγ), or the activation of phosphoinositol 3-kinase. Genes responsive to ERK/MAP kinase signalling are expressed in the PPA at around these stages (Lunn et al., 2007). This suggests that the activation of ERK might mediate PPA induction. Genetically ablating and pharmacologically inhibiting the different FGF signalling intermediates should provide an unequivocal answer to this question.

Diversification of the PPA

The progenitors of the otic and epibranchial placodes form from a common ectodermal territory induced by FGF signalling. In this section, we discuss the mechanisms by which the PPA segregates into distinct domains.

Several Wnt family members are expressed in the hindbrain adjacent to the PPA and have been shown to be crucial for the formation of the inner ear (Ladher et al., 2000; Ohyama et al., 2006; Park and Saint-Jeannet, 2008). Wnt signalling functions after FGF-mediated PPA induction, as modulating Wnt activity in chick does not affect the initial induction of the PPA (Freter et al., 2008). Furthermore, selective inactivation of β-catenin (a transducer of Wnt signalling) in mouse PPA ectoderm leads to a loss of otic cells

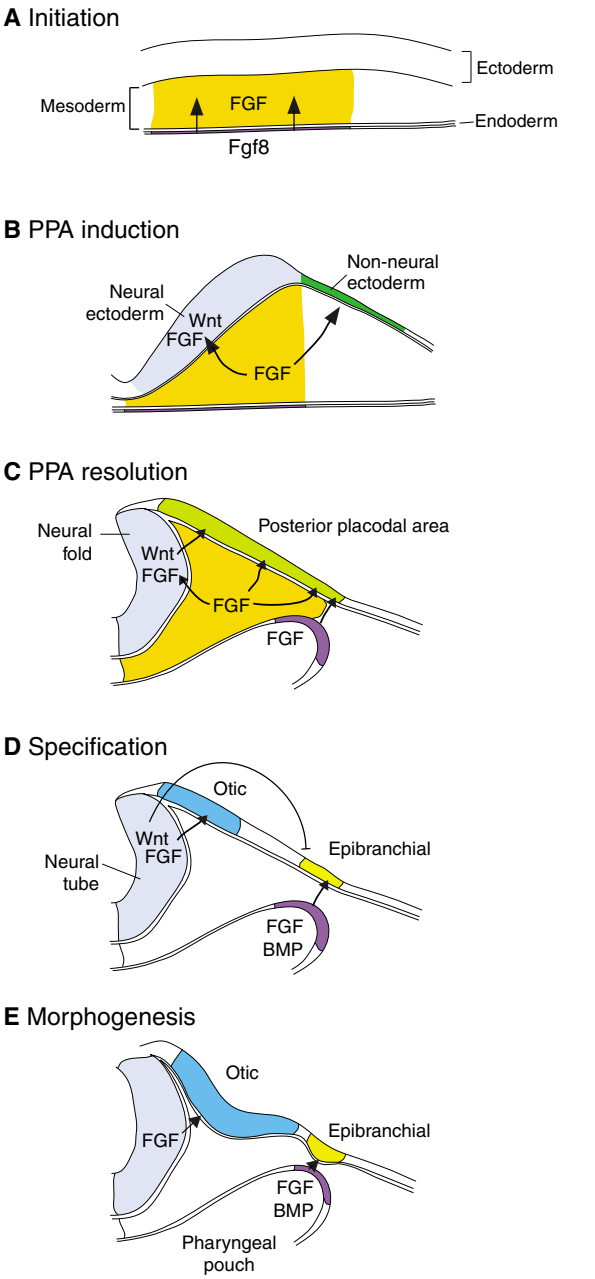

Fig. 2. Model of the cellular and molecular interactions involved in PPA induction and in otic and epibranchial placode specification. A scheme for PPA induction and inner ear and epibranchial development synthesised from mouse, chick and zebrafish embryonic data. Dorsal is uppermost. (**A**) Initiation occurs during early neurula stages in the hindbrain when Fgf8 secreted by endoderm induces FGF expression in overlying mesoderm. (**B**) During mid-neurula stages, mesodermal FGF acts on overlying non-neural ectoderm to induce the PPA and acts on neural ectoderm to induce FGF and *Wnt8a* expression. (**C**) The PPA domain expands and is sharpened by FGF emanating from neural ectoderm, mesoderm and endoderm. (**D**) During late neurula stages, mesodermal FGF expression is attenuated. This allows neural Wnt8a and endodermal FGF to act. Wnt8a acts positively to regulate otic fate, while negatively influencing epibranchial fate; FGF specifies epibranchial fate. (**E**) In early pharyngula stages, neural FGF expression acts on the basal side of the newly induced otic placode to induce its invagination. Epibranchial placodes are resolved into individual foci through the action of BMP and FGF.

(Ohyama et al., 2006). Importantly, analysis of TCF/LEF transgenic reporter mice indicates that Wnt signalling acts only on medial regions of the *Pax2*-positive PPA (Ohyama et al., 2006). Similarly, in chick, only the medial portion of the PPA is affected following the electroporation of the secreted Wnt antagonist dickkopf 1 (Freter et al., 2008). Conversely, forced activation of β-catenin in the PPA results in an enlarged otic placode in mouse and in repression of epibranchial placode fate in both chicken and mouse (Freter et al., 2008; Ohyama et al., 2006). These data imply that secreted Wnt from the hindbrain is important in determining lineage choice within the PPA, promoting otic fate in the medially located ectoderm, while suppressing epibranchial fate laterally.

Wnt8a is expressed in the caudal part of the hindbrain adjacent to the PPA, and it is likely to be the Wnt family member that is responsible for otic specification (Ladher et al., 2000; Ohyama et al., 2006; Park and Saint-Jeannet, 2008). Interestingly, in chick, *Wnt8a* is induced by mesodermal Fgf19 (Ladher et al., 2000), indicating a dual role for FGF signalling in inducing the PPA and in the control of its later segregation. Curiously, otic specification requires transient downregulation of *Fgf3* and *Fgf19*; if expression is sustained, the inner ear is unable to differentiate (Freter et al., 2008). Thus, FGF can be thought of as a checkpoint to prevent Wnt signals from precociously committing PPA cells to an otic fate. The mechanism by which this control is mediated remains to be elucidated.

Similar to otic specification from the PPA by signals from the adjacent hindbrain, epibranchial specification also depends on signals from its neighbouring tissue: the pharyngeal endoderm. During embryogenesis, the lateral regions of the PPA are brought into close proximity with the forming pharyngeal pouches (see Glossary, Box 2) (Landacre, 1912). In zebrafish embryos, there is a close correlation between endodermal pouch formation and the start of epibranchial neurogenesis (Holzschuh et al., 2005). In zebrafish endoderm-deficient *casanova* (*sox32*) mutants, the epibranchial ganglia fail to form, although other placode-derived cranial ganglia remain intact (Holzschuh et al., 2005; Nechiporuk et al., 2007). Similarly, tissue recombination experiments in chick have demonstrated that the apposition of pharyngeal endoderm with cranial ectoderm promotes epibranchial neurogenesis (Begbie et al., 1999).

The pharyngeal endoderm expresses both FGF and bone morphogenetic protein (BMP) signals and both have been implicated in the formation of epibranchial placodes. Inhibiting BMP signalling in chick and zebrafish endoderm inhibits epibranchial neurogenesis (Begbie et al., 1999; Holzschuh et al., 2005). There is also strong evidence from zebrafish that FGF signalling from the endoderm is necessary for the specification and neurogenesis of epibranchial placodes (Nechiporuk et al., 2005; Nechiporuk et al., 2007; Nikaido et al., 2007). The expression patterns of FGF and BMP ligands are suggestive of a possible hierarchy of signalling in epibranchial specification. Using *Sox3* and *Pax2* expression to distinguish epibranchial precursors, two stages in epibranchial specification become apparent (Ishii et al., 2001). Epibranchial precursors are initially specified from the PPA as a contiguous stripe, lateral to the otic placode (Fig. 1D). This stripe

then resolves into the individual placodes. A number of FGF ligands are expressed in a contiguous stripe within the endoderm (Abelló et al., 2010), whereas the expression of *Bmp7* is restricted to the pharyngeal pouches (Begbie et al., 1999). This implies that the sequential action of endoderm-derived FGF ligands and *Bmp7* operate to establish the individual epibranchial placodes. Support for BMP signalling being involved in inducing the foci of epibranchial neurogenesis comes from the ectopic expression of a constitutively active BMP receptor in chick, which induces ectopic neurogenesis in a broader swathe of lateral ectoderm in the head (Tripathi et al., 2009). These spatially restricted inductive cues enable epibranchial neurogenesis to be localised correctly, allowing the appropriate association of an epibranchial ganglion with each pharyngeal arch (see Fig. 1H). This correlation is important, as the number of pharyngeal arches can vary between species, and where it does the number of epibranchial ganglia varies accordingly (O'Neill et al., 2007).

Otic and epibranchial placode morphogenesis

The end result of the signals and tissue interactions described so far is the formation of thickened placodes, which are pseudostratified epithelia that are poised to differentiate. In this section, we compare and contrast the morphological changes that accompany the differentiation of otic and epibranchial derivatives.

The epithelium of the inner ear placode deforms, buckling so that it forms a progressively deepening invagination, which eventually pinches off to form an enclosed sphere inside the head, called an otocyst (see Glossary, Box 2; Fig. 2) (Meier, 1978a; Meier, 1978b). Surface area measurements in chicken embryos show that initially, between HH10 and HH11, the basal side of the inner placode expands without a significant change in the area of the apical surface. Shortly afterwards, from HH12, the apical surface area dramatically decreases (Alvarez and Navascués, 1990). Thus, two types of cell shape change can be inferred: an initial basal expansion, followed by apical constriction. Basal expansion correlates with the generation of polarity in one component of the cytoskeleton: actin filaments. Prior to morphogenesis, actin filaments can be detected both apically and basally in the inner ear placode; however, when invagination begins, actin filaments are depleted basally and enriched apically (Sai and Ladher, 2008). FGF signalling plays an important role in this process. Chick embryos treated with the FGF inhibitor SU5402 after the inner ear has segregated from the PPA (at HH10), show impaired inner ear invagination and do not apically enrich actin filaments (Sai and Ladher, 2008). In contrast to the induction of the PPA, which seems to involve ERK activation, actin polarisation in response to FGF signalling is mediated by the activation of a second branch of the signal transduction pathway – the phosphorylation of Plcγ – and ends with the activation of the regulatory subunit of the motor protein, myosin II. At these initial stages, phosphorylated myosin light chain, a read-out for active myosin II, and actin filaments are reciprocally localised. This suggests that myosin II might exhibit a non-canonical activity in actin depletion during basal expansion (Sai and Ladher, 2008). During the second phase of inner ear invagination, active myosin II is translocated to the apical side of the inner ear epithelium, and it is likely that together with actin filaments, the actin-myosin complex mediates apical constriction (Sai and Ladher, 2008). Once closed, the otocyst undergoes further complex morphogenetic movements as it gives rise to a wide range of highly patterned derivatives, including the specialised epithelial structures of the vestibular canals and cochlea, as well as the sensory hair cells and sensory neurons required for function. The development of the epithelial structures is beyond the scope of this review and has been reviewed recently (Bok et al., 2007). Instead, we focus on the development of the sensory and neurogenic regions of the otic and epibranchial placodes.

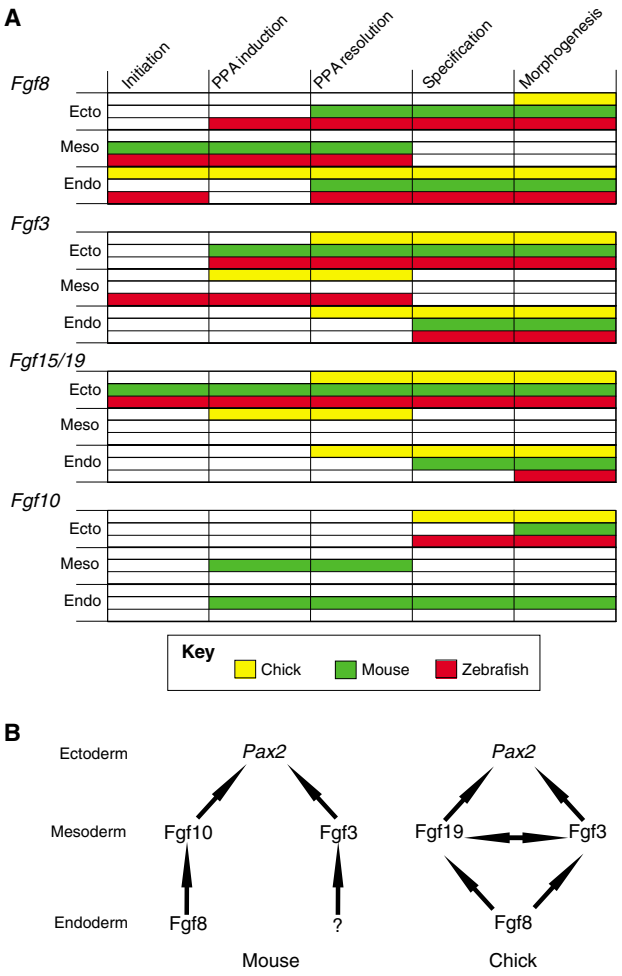

Fig. 3. Expression and action of FGF ligands during PPA induction and diversification. (**A**) The expression of *Fgf8*, *Fgf3*, *Fgf15/19* and *Fgf10* in chick (yellow), mouse (green) and zebrafish (red) shown in all three germ layers at different stages during the initiation, induction and diversification of the PPA. The staging follows the scheme introduced in Fig. 2. Ecto, ectoderm; Meso, mesoderm; Endo, endoderm. (**B**) The differing hierarchical relationships between mouse and chick FGF ligands involved in PPA initiation and induction. The question mark indicates an as yet uncharacterised upstream factor controlling localised *Fgf3* expression in the mouse.

Compared with the inner ear, the morphogenesis of the epibranchial placodes is less complex, as they do not invaginate but remain on the surface of the embryo. GFP labelling has shown that the epibranchial placodal epithelium is pseudostratified. This resembles the germinal neuroepithelium of the CNS as found, for example, in the ventricular zone. Similar to the ventricular zone, cell division within the epibranchial placodes occurs mainly on the apical surface, with the nascent neuroblasts migrating basally as they commit to their neuronal fate. This process is termed interkinetic nuclear migration (see Glossary, Box 2). In contrast to the CNS, epibranchial neuroblasts keep migrating basally and exit from the basal surface of the placode through breaches in the basal lamina (Graham et al., 2007). Delamination of neuroblasts is the main morphogenetic event in the epibranchial placodes, but this is not an epithelial-to-mesenchymal

transition (EMT), in contrast to what might be expected by analogy with other delaminating cell types, such as the neural crest. In fact, strictly speaking, the epibranchial placodal cells do not actually assume a mesenchymal morphology as they migrate away from the epithelium. Consistent with this is the finding that the genes involved in typical EMT processes are not expressed during epibranchial delamination (Graham et al., 2007). When an epibranchial neuroblast leaves the placodal epithelium it migrates internally and, guided by neural crest streams, joins its siblings that were born in the same placode. Following migration, epibranchial neuroblasts condense in a process known as gangliogenesis.

The inner ear also gives rise to neurons, and, like the epibranchial placodes, inner ear neuroblasts delaminate from the epithelium and coalesce to form ganglia. The cellular features of this delamination have not been determined in as much detail as for the epibranchial placodes. Instead, studies have focused on the patterning of inner ear neurons, particularly the mechanisms that distinguish the neuronal field from the regions of the inner that will make sensory hair cells. Molecular analysis has shown that the inner ear placode is regionally patterned into neural versus non-neural portions at early placode stages (Abelló et al., 2007; Raft et al., 2007). The mechanosensory and neuronal lineages of the inner ear appear to be generated in different regions of the inner ear placode, with only a small region of overlap in the utricle and saccule sensory organs (see Glossary, Box 2) (Bell et al., 2008; Satoh and Fekete, 2005). The sensory component of the inner ear derives from a thickened region in the ventral and medial regions of the otocyst (Knowlton, 1967), whereas the neurogenic portion of the placode becomes localised to a thickening in the rostromedial portion of the otic vesicle, close to the developing geniculate placode of the epibranchial series (Fekete and Wu, 2002). Thus, there is very little evidence of a common, immediate progenitor for sensory hair cells and neurons in the inner ear.

There are instances in which non-otic regions of the PPA can also generate hair cells, the best example being the lateral line system in fish. Some clear examples can also be found in terrestrial vertebrates. In birds, for example, the first epibranchial placode (geniculate) generates the paratympanic organ (PTO), which is a small baroreceptor located in the middle ear that is thought to act as an altimeter during flight (D'Amico-Martel and Noden, 1983; von Bartheld, 1990; von Bartheld, 1994; Nesser and von Bartheld, 2002). Importantly, it contains mechanosensory hair cells similar to those of the inner ear (Giannessi and Ruffoli, 1996), and is innervated by neurons of the geniculate ganglion (von Bartheld, 1990). Clonal relationships between some PTO hair cells and geniculate neurons have also been demonstrated (Satoh and Fekete, 2005). This might suggest that the ability to form sensory hair cells in terrestrial vertebrates is actually more widespread within the PPA, and that the geniculate placode is simply using this latent sensory potential to generate the PTO.

Neuronal differentiation

Many similarities are emerging between the molecular mechanisms that control lineage specification and neurogenesis in the placodes of the PPA and the neuroepithelium of the CNS. Neurogenesis is prefigured by expression of the HMG-domain SoxB1 transcription factor family, of which *Sox3* is the earliest marker of epibranchial and otic neurons to be expressed (Abu-Elmagd et al., 2001). As described above, *Sox3* is initially expressed in a fairly broad domain within the PPA and then becomes restricted to the neurogenic regions as they are specified (Ishii et al., 2001). This expression is controlled by FGF signalling, although this control is independent of the signals that induce the PPA (Abelló et al., 2010). Sox3 is necessary for epibranchial neurogenesis in the chick and zebrafish (Tripathi et al., 2009; Dee et al., 2008). However, cranial nerves still form in mice lacking *Sox3*, suggesting possible redundancy with the other SoxB1 family member *Sox2* (Rizzoti and Lovell-Badge, 2007). Overexpressing Sox3 in epibranchial placodal cells inhibits migration and neurogenesis (Abu-Elmagd et al., 2001). This suggests that similar to the developing neuroepithelium of the CNS (Bylund et al., 2003), epibranchial placodal cells must downregulate Sox3 before progressing into a more differentiated state.

The upregulation of proneural basic helix-loop-helix (bHLH) genes, such as atonal-related family members or achaete-schute-like genes, is considered to be the start of neurogenic differentiation (Guillemot, 2007; Powell and Jarman, 2008). The expression of proneural genes initiates a cascade of gene expression that commits the progenitor to a neuronal phenotype and to subtype specificity. The expression of proneural genes upregulates that of 'pan-neural' genes, as well as the expression of the neurogenic gene *Delta*, which, together with its receptor Notch, is responsible for lateral inhibition in the CNS. *Delta* is expressed in a punctuate pattern in otic and epibranchial placodes, indicating that lateral inhibition controls neurogenesis in these tissues. Thus, although the expression patterns of proneural bHLH genes may be broad, only a subset of these cells will generate neuronal, or even mechanosensory, cells.

How is sensory neuronal subtype specificity encoded in the tissues arising from the PPA? It has been suggested that the proneural bHLH family members are responsible. For example, in mouse neurogenin 1 (*Ngn1*; *Neurog1*) mutants, the cochleovestibular neurons are lost (Ma et al., 1998), and *Ngn2* mutants do not form epibranchial neurons (Fode et al., 1998). However, in chick, the expression of *Ngn1* and *Ngn2* is reversed relative to the mouse, whereas zebrafish has lost neurogenin 2 (Andermann et al., 2002; Begbie et al., 2002). These and other findings suggests that the two neurogenin genes are interchangeable and most likely have a general role in sensory development (Furlong and Graham, 2005) rather than being involved in conferring neuronal specificity. Instead, sensory subtype specificity is the product of distinct neuronal homeodomain proteins that act in parallel pathways. Cochleovestibular neurons express the POU homeodomain transcription factor *Brn3a* (*Pou4f1*), whereas the epibranchial neurons express the paired homeodomain transcription factor *Phox2a* (Begbie et al., 2002). Further subtype segregation of the cochlear and vestibular neuronal phenotype might also rely on GATA transcription factors in the mouse (Lawoko-Kerali et al., 2004) and chick (Jones and Warchol, 2009). Epibranchial neurons derived from specific placodes also carry out distinct sensory functions; however, the specification of these subpopulations is not clear.

Molecular analysis has revealed similarities between mechanosensory inner ear hair cell progenitors and the neurogenic cells of both the inner ear and epibranchial placodes. This might not be so surprising as the sensory hair cells are considered to be derived neuronal populations (Manley and Ladher, 2008). Mechanosensory cells develop from sensory patches, which are thickened regions of epithelium that are developmentally regulated by FGF and BMP signalling and are maintained by Notch signalling through one of its receptors, jagged 1 (Daudet et al., 2007; Kelley, 2006). This establishes a domain expressing a SoxB1 family member, in this case *Sox2*, which predicts the competence to form sensory cells (Kiernan et al., 2005). *Sox2* expression is necessary for the expression of *Atoh1*, the bHLH gene that is considered the starting point for mechanosensory differentiation. However, the continued expression of *Sox2* antagonises *Atoh1* (Dabdoub et al., 2008). It is

thought that this is one way by which precocious differentiation is controlled. In the mouse, *Ngn1* and *Atoh1* expression, in the neuronal and mechanosensory lineages, respectively, is mutually exclusive (Raft et al., 2007), consistent with the idea that these cell types do not share a clonal relationship in the inner ear (Bell et al., 2008; Satoh and Fekete, 2005).

Development of ganglia and connections

Following their delamination, the neuroblasts of both the inner ear and epibranchial placodes migrate and coalesce to form ganglia. The neurons within these ganglia send out axonal projections that connect with their peripheral and central targets to form functional circuits (Fig. 4).

Several studies have shown that the cranial neural crest plays a pivotal role in cranial sensory gangliogenesis and in the organisation of central connections (Fig. 4A-C). Neural crest ablation in chick embryos results in misplaced epibranchial ganglia with aberrant projections that fail to connect to the hindbrain. These defects are also evident in the trigeminal and cochleovestibular ganglia (CVG), demonstrating a broader requirement for the neural crest in organising all of the cranial sensory ganglia and their central innervation (Begbie and Graham, 2001a). Molecular perturbations affecting neural crest migration provide further insight; disrupting neuropilin/semaphorin signalling in both mouse and chick results in misplaced ganglia and aberrant projections (Osborne et al., 2005; Schwarz et al., 2008). Similarly, mouse mutations in *Erbb4*, an EGF receptor family member that recognises the axon guidance molecule neuregulin 1, show misplaced ganglia as a result of the disruption of normal neural crest cell migration pathways (Golding et al., 2004).

Although it is not known how neural crest cells guide the organisation of epibranchial ganglia and CVG, studies in the trigeminal ganglion have demonstrated that ganglion condensation is dependent on interactions between neural crest and placodal cells, and are mediated by the axonal regulator Slit and its receptor Robo (Shiau et al., 2008). Generally, Robo-Slit interactions are considered to be repulsive, and thus it is possible that the neural crest acts to corral the placodal cells into a ganglion. *Robo* and *Slit* are also expressed in developing epibranchial ganglia and CVG, so it will be interesting to determine whether these same molecules also facilitate ganglion formation in these systems.

The peripheral projections of the CVG have been studied in much greater depth than the central projections (for a review, see Fekete and Campero, 2007). Lineage analysis in mouse has revealed a degree of autonomy in the peripheral and central projections of the CVG that depends on neuronal phenotype (Koundakjian et al., 2007). Yet the projections also depend on their targets for guidance and survival, with many of the typical axon guidance molecules involved in targeting as well as in trophic support, with brain-derived neurotrophic factor (Bdnf), in particular, implicated in survival (Fritzsch et al., 2005). Similarly, recombination experiments carried out in axolotl have shown that projections from epibranchial placode-derived neurons depend on their peripheral target tissue, in this case taste-bud-bearing endoderm, for guidance and support (Gross et al., 2003). Thus, like the CVG, targeting of both the peripheral and central projections of the epibranchial ganglia depend on neuronal origin and phenotype (Harlow and Barlow, 2007). Finally, recent data have shown that the peripheral projections of the geniculate ganglion to the tongue also guide efferent innervation from the hindbrain, as well as controlling the formation of the parasympathetic ganglia (Coppola et al., 2010).

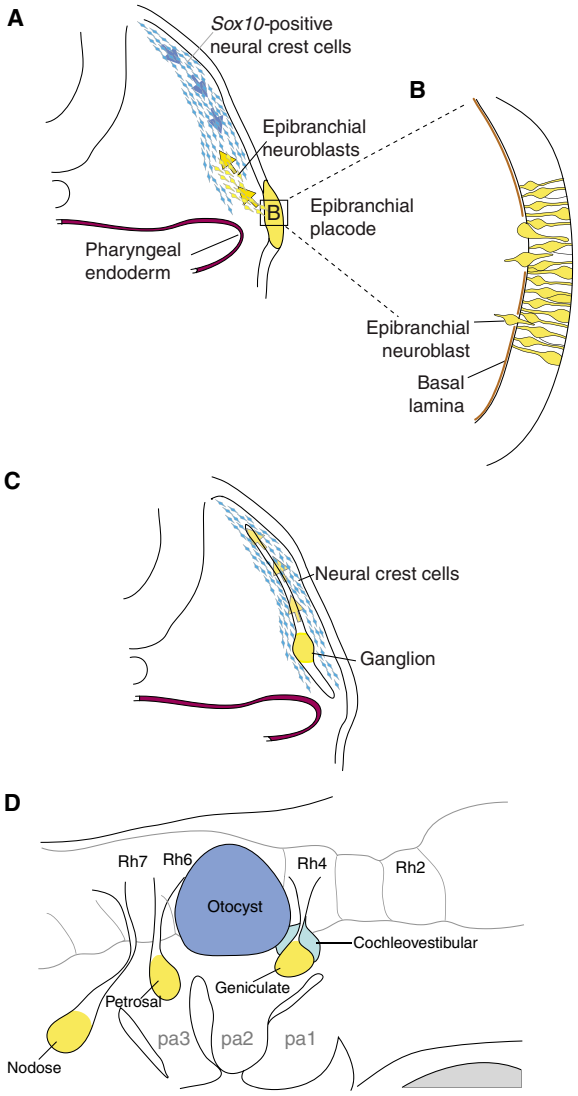

Fig. 4. Epibranchial neuroblast delamination and migration. (**A**) Transverse section of a chick embryo at HH15 at the level of the pharyngeal pouches. Dorsal is uppermost. Endodermal signals induce neurogenesis in epibranchial placodes, promoting delamination and gangliogenesis. (**B**) Magnified view of the boxed area in A. Neuroblasts leave the placodal epithelium through breaches in the basal lamina during interkinetic nuclear migration. (**C**) A transverse section through the pharyngeal region of the HH16 chick embryo. Neural crest streams guide ganglionic axons to the hindbrain. (**D**) Final pattern of ganglia and connections to the hindbrain in a lateral view of the pharyngeal arch region of an HH18 chick embryo. pa, pharyngeal arch; Rh, rhombomere.

Conclusions

Work in recent years has established a more complete understanding of how a naïve ectodermal cell can adopt an inner ear or epibranchial fate. The finding that the inner ear and epibranchial ganglia share a common progenitor domain adds clarity to our understanding of the process of induction. However, the concept of the PPA also raises some questions. Perhaps the most pressing question concerns what the PPA actually represents. One view is that it is simply a product of the topographic restriction of cranial ectoderm, and is thus a step in a hierarchically ordered pathway of commitment employed to ensure that correct cell types are generated in the correct place.

Another view is that the PPA is an ancient mechanosensory placode, and that epibranchial placodes represent a derived, non-mechanosensory state (Baker et al., 2008). The existence of a geniculate-derived sense organ, the PTO, might suggest that this is the case, and that regions of the PPA have a latent mechanosensory potential. Studies that investigate the ability of different regions of ectoderm to form PTO hair cells will resolve this issue and will provide further insight into the ability of cranial ectoderm to generate mechanosensory cells.

FGF signalling plays a central role in PPA induction and diversification. What are the molecular mechanisms responsible for these transformations? Both genetic and cellular processes are enacted to achieve the PPA phenotype. Fundamental to PPA identity is the ability to generate mechanosensory hair cells and sensory neurons. How FGF signalling can establish this competence remains the subject of active research. The otic and epibranchial placodes, as well as the lateral line in fish, emerge from this region and, despite their common lineage, they adopt distinct phenotypes to mediate distinct functions, and here the utilisation of the Wnt signalling pathway appears to be important. Again, the downstream mechanisms and genetic networks that act as determinants of, and gatekeepers to, these two primary cellular phenotypes of the PPA are not well characterised. The elucidation of these mechanisms and their integration with the competence factors that are induced by FGF signalling would place within reach the tantalising prospect of establishing a blueprint for the development of sensory neurons and mechanosensory hair cells.

Acknowledgements

We thank Suzi Mansour and Yuko Muta for sharing unpublished data and Gerhard Schlosser, Alain Ghysen and laboratory members for discussion and criticism. Research in our laboratories is supported by an Intramural grant from RIKEN Center for Developmental Biology (R.K.L., P.O.), JSPS (R.K.L., P.O.), RIKEN FPR (P.O.), BBSRC (J.B.) and John Fell funding from OUP (J.B.).

Competing interests statement

The authors declare no competing financial interests.

References

Abelló, G., Khatri, S., Giráldez, F. and Alsina, B. (2007). Early regionalization of the otic placode and its regulation by the Notch signaling pathway. *Mech. Dev.* **124**, 631-645.

Abelló, G., Khatri, S., Radosevic, M., Scotting, P. J., Giraldez, F. and Alsina, B. (2010). Independent regulation of Sox3 and Lmx1b by FGF and BMP signaling influences the neurogenic and non-neurogenic domains in the chick otic placode. *Dev. Biol.* **339**, 166-178.

Abu-Elmagd, M., Ishii, Y., Cheung, M., Rex, M., Le Rouëdec, D. and Scotting, P. (2001). cSox3 expression and neurogenesis in the epibranchial placodes. *Dev. Biol.* **237**, 258-269.

Alvarez, I. S. and Navascués, J. (1990). Shaping, invagination, and closure of the chick embryo otic vesicle: scanning electron microscopic and quantitative study. *Anat. Rec.* **228**, 315-326.

Andermann, P., Ungos, J. and Raible, D. W. (2002). Neurogenin1 defines zebrafish cranial sensory ganglia precursors. *Dev. Biol.* **251**, 45-58.

Baker, C. V. and Bronner-Fraser, M. (2001). Vertebrate cranial placodes I. Embryonic induction. *Dev. Biol.* **232**, 1-61.

Baker, C. V., O'Neill, P. and McCole, R. B. (2008). Lateral line, otic and epibranchial placodes: developmental and evolutionary links? *J. Exp. Zool. B Mol. Dev. Evol.* **310**, 370-383.

Beckwith, C. J. (1907). The early development of the lateral line system of Amia calva. *Biol. Bull.* **14**, 23-35.

Begbie, J. and Graham, A. (2001a). Integration between the epibranchial placodes and the hindbrain. *Science* **294**, 595-598.

Begbie, J. and Graham, A. (2001b). The ectodermal placodes: a dysfunctional family. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 1655-1660.

Begbie, J., Brunet, J. F., Rubenstein, J. L. and Graham, A. (1999). Induction of the epibranchial placodes. *Development* **126**, 895-902.

Begbie, J., Ballivet, M. and Graham, A. (2002). Early steps in the production of sensory neurons by the neurogenic placodes. *Mol. Cell. Neurosci.* **21**, 502-511.

Bell, D., Streit, A., Gorospe, I., Varela-Nieto, I., Alsina, B. and Giraldez, F. (2008). Spatial and temporal segregation of auditory and vestibular neurons in the otic placode. *Dev. Biol.* **322**, 109-120.

Bok, J., Chang, W. and Wu, D. K. (2007). Patterning and morphogenesis of the vertebrate inner ear. *Int. J. Dev. Biol.* **51**, 521-533.

Bouchard, M., Souabni, A. and Busslinger, M. (2004). Tissue-specific expression of cre recombinase from the Pax8 locus. *Genesis* **38**, 105-109.

Bylund, M., Andersson, E., Novitch, B. G. and Muhr, J. (2003). Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat. Neurosci.* **6**, 1162-1168.

Coppola, E., Rallu, M., Richard, J., Dufour, S., Riethmacher, D., Guillemot, F., Goridis, C. and Brunet, J.-F. (2010). Epibranchial ganglia orchestrate the development of the cranial neurogenic crest. *Proc. Natl. Acad. Sci. USA* **107**, 2066-2071.

Dabdoub, A., Puligilla, C., Jones, J. M., Fritzsch, B., Cheah, K. S., Pevny, L. H. and Kelley, M. W. (2008). Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. *Proc. Natl. Acad. Sci. USA* **105**, 18396-18401.

D'Amico-Martel, A. and Noden, D. M. (1983). Contributions of placodal and neural crest cells to avian cranial peripheral ganglia. *Am. J. Anat.* **166**, 445-468.

Daudet, N., Ariza-McNaughton, L. and Lewis, J. (2007). Notch signalling is needed to maintain, but not to initiate, the formation of prosensory patches in the chick inner ear. *Development* **134**, 2369-2378.

Dee, C. T., Hirst, C. S., Shih, Y. H., Tripathi, V. B., Patient, R. K. and Scotting, P. J. (2008). Sox3 regulates both neural fate and differentiation in the zebrafish ectoderm. *Dev. Biol.* **320**, 289-301.

Deng, C. X., Wynshaw-Boris, A., Shen, M. M., Daugherty, C., Ornitz, D. M. and Leder, P. (1994). Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev.* **8**, 3045-3057.

Dominguez-Frutos, E., Vendrell, V., Alvarez, Y., Zelarayan, L. C., Lopez-Hernandez, I., Ros, M. and Schimmang, T. (2009). Tissue-specific requirements for FGF8 during early inner ear development. *Mech. Dev.* **126**, 873-881.

Fekete, D. M. and Wu, D. K. (2002). Revisiting cell fate specification in the inner ear. *Curr. Opin. Neurobiol.* **12**, 35-42.

Fekete, D. and Campero, A. (2007). Axon guidance in the inner ear. *Int. J. Dev. Biol.* **51**, 549-556.

Fode, C., Gradwohl, G., Morin, X., Dierich, A., LeMeur, M., Goridis, C. and Guillemot, F. (1998). The bHLH protein NEUROGENIN 2 is a determination factor for epibranchial placode? Derived sensory neurons. *Neuron* **20**, 483-494.

Freter, S., Muta, Y., Mak, S. S., Rinkwitz, S. and Ladher, R. K. (2008). Progressive restriction of otic fate: the role of FGF and Wnt in resolving inner ear potential. *Development* **135**, 3415-3424.

Fritzsch, B., Pauley, S., Matei, V., Katz, D. M., Xiang, M. and Tessarollo, L. (2005). Mutant mice reveal the molecular and cellular basis for specific sensory connections to inner ear epithelia and primary nuclei of the brain. *Hear. Res.* **206**, 52-63.

Fritzsch, B., Pauley, S. and Beisel, K. W. (2006). Cells, molecules and morphogenesis: the making of the vertebrate ear. *Brain Res.* **1091**, 151-171.

Furlong, R. F. and Graham, A. (2005). Vertebrate neurogenin evolution: long-term maintenance of redundant duplicates. *Dev. Genes Evol.* **215**, 639-644.

Giannessi, F. and Ruffoli, R. (1996). The ultrastructure of the sensory hairs of the paratympanic organ receptor cells in chicken. *Anat. Embryol. (Berl.)* **193**, 569-575.

Golding, J. P., Sobieszczuk, D., Dixon, M., Coles, E., Christiansen, J., Wilkinson, D. and Gassmann, M. (2004). Roles of erbB4, rhombomere-specific, and rhombomere-independent cues in maintaining neural crest-free zones in the embryonic head. *Dev. Biol.* **266**, 361-372.

Graham, A. (2008). Deconstructing the pharyngeal metamere. *J. Exp. Zool. B Mol. Dev. Evol.* **310**, 336-344.

Graham, A., Blentic, A., Duque, S. and Begbie, J. (2007). Delamination of cells from neurogenic placodes does not involve an epithelial-to-mesenchymal transition. *Development* **134**, 4141-4145.

Gross, J. B., Gottlieb, A. A. and Barlow, L. A. (2003). Gustatory neurons derived from epibranchial placodes are attracted to, and trophically supported by, taste bud-bearing endoderm in vitro. *Dev. Biol.* **264**, 467-481.

Groves, A. K. (2005). The induction of the otic placode. In *Development of the Inner Ear* (ed. M. W. Kelley, D. K. Wu, A. N. Popper and R. R. Fay), pp. 10-42. New York: Springer.

Groves, A. K. and Bronner-Fraser, M. (2000). Competence, specification and commitment in otic placode induction. *Development* **127**, 3489-3499.

Guillemot, F. (2007). Spatial and temporal specification of neural fates by transcription factor codes. *Development* **134**, 3771-3780.

Harlow, D. and Barlow, L. (2007). Embryonic origin of gustatory cranial sensory neurons. *Dev. Biol.* **310**, 317-328.

Holzschuh, J., Wada, N., Wada, C., Schaffer, A., Javidan, Y., Tallafuü, A., Bally-Cuif, L. and Schilling, T. F. (2005). Requirements for endoderm and BMP signaling in sensory neurogenesis in zebrafish. *Development* **132**, 3731-3742.

Ishii, Y., Abu-Elmagd, M. and Scotting, P. (2001). Sox3 expression defines a common primordium for the epibranchial placodes in chick. *Dev. Biol.* **236**, 344-353.
Jones, J. M. and Warchol, M. E. (2009). Expression of the Gata3 transcription factor in the acoustic ganglion of the developing avian inner ear. *J. Comp. Neurol.* **516**, 507-518.
Kelley, M. W. (2006). Regulation of cell fate in the sensory epithelia of the inner ear. *Nat. Rev. Neurosci.* **7**, 837-849.
Kiernan, A. E., Pelling, A. L., Leung, K. K., Tang, A. S., Bell, D. M., Tease, C., Lovell-Badge, R., Steel, K. P. and Cheah, K. S. (2005). Sox2 is required for sensory organ development in the mammalian inner ear. *Nature* **434**, 1031-1035.
Kil, S. H., Streit, A., Brown, S. T., Agrawal, N., Collazo, A., Zile, M. H. and Groves, A. K. (2005). Distinct roles for hindbrain and paraxial mesoderm in the induction and patterning of the inner ear revealed by a study of vitamin-A-deficient quail. *Dev. Biol.* **285**, 252-271.
Knowlton, V. Y. (1967). Correlation of the development of membranous and bony labyrinths, acoustic ganglia, nerves, and brain centers of chick embryos. *J. Morphol.* **121**, 179-208.
Koundakjian, E. J., Appler, J. L. and Goodrich, L. V. (2007). Auditory neurons make stereotyped wiring decisions before maturation of their targets. *J. Neurosci.* **27**, 14078-14088.
Kuratani, S. C. and Eichele, G. (1993). Rhombomere transplantation repatterns the segmental organization of cranial nerves and reveals cell-autonomous expression of a homeodomain protein. *Development* **117**, 105-117.
Ladher, R. K., Anakwe, K. U., Gurney, A. L., Schoenwolf, G. C. and Francis-West, P. H. (2000). Identification of synergistic signals initiating inner ear development. *Science* **290**, 1965-1967.
Ladher, R., Wright, T. J., Moon, A. M., Mansour, S. L. and Schoenwolf, G. C. (2005). FGF8 initiates inner ear induction in chick and mouse. *Genes Dev.* **19**, 603-613.
Landacre, F. L. (1912). The epibranchial placodes of Lepidosteus osseus and their relation to the cerebral ganglia. *J. Comp. Neurol.* **22**, 1-69.
Lawoko-Kerali, G., Rivolta, M. N., Lawlor, P., Cacciabue-Rivolta, D. I., Langton-Hewer, C., van Doorninck, J. H. and Holley, M. C. (2004). GATA3 and NeuroD distinguish auditory and vestibular neurons during development of the mammalian inner ear. *Mech. Dev.* **121**, 287-299.
Leger, S. and Brand, M. (2002). Fgf8 and Fgf3 are required for zebrafish ear placode induction, maintenance and inner ear patterning. *Mech. Dev.* **119**, 91-108.
Liu, D., Chu, H., Maves, L., Yan, Y. L., Morcos, P. A., Postlethwait, J. H. and Westerfield, M. (2003). Fgf3 and Fgf8 dependent and independent transcription factors are required for otic placode specification. *Development* **130**, 2213-2224.
Lunn, J. S., Fishwick, K. J., Halley, P. A. and Storey, K. G. (2007). A spatial and temporal map of FGF/Erk1/2 activity and response repertoires in the early chick embryo. *Dev. Biol.* **302**, 536-552.
Ma, Q., Chen, Z., del Barco Barrantes, I., de la Pompa, J. L. and Anderson, D. J. (1998). neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron* **20**, 469-482.
Mahmood, R., Kiefer, P., Guthrie, S., Dickson, C. and Mason, I. (1995). Multiple roles for FGF-3 during cranial neural development in the chicken. *Development* **121**, 1399-1410.
Manley, G. A. and Ladher, R. (2008). Phylogeny and evolution of ciliated mechanoreceptors. In *The Senses: A Comprehensive Reference; Audition*, vol. 3 (ed. P. Dallos and D. Oertel), pp. 1-34. San Diego: Academic Press.
Maroon, H., Walshe, J., Mahmood, R., Kiefer, P., Dickson, C. and Mason, I. (2002). Fgf3 and Fgf8 are required together for formation of the otic placode and vesicle. *Development* **129**, 2099-2108.
Martin, K. and Groves, A. K. (2006). Competence of cranial ectoderm to respond to Fgf signaling suggests a two-step model of otic placode induction. *Development* **133**, 877-887.
Meier, S. (1978a). Development of the embryonic chick otic placode. I. Light microscopic analysis. *Anat. Rec.* **191**, 447-458.
Meier, S. (1978b). Development of the embryonic chick otic placode. II. Electron microscopic analysis. *Anat. Rec.* **191**, 459-477.
Mendonsa, E. S. and Riley, B. B. (1999). Genetic analysis of tissue interactions required for otic placode induction in the zebrafish. *Dev. Biol.* **206**, 100-112.
Mitrophanow, P. (1893). Étude embryogénique sur les Selachiens. *Archives de Zool. Exp.* **3**, 160-220.
Nechiporuk, A., Linbo, T. and Raible, D. W. (2005). Endoderm-derived Fgf3 is necessary and sufficient for inducing neurogenesis in the epibranchial placodes in zebrafish. *Development* **132**, 3717-3730.
Nechiporuk, A., Linbo, T., Poss, K. D. and Raible, D. W. (2007). Specification of epibranchial placodes in zebrafish. *Development* **134**, 611-623.
Neeser, J. A. and von Bartheld, C. S. (2002). Comparative anatomy of the paratympanic organ (vitali organ) in the middle ear of birds and non-avian vertebrates: focus on alligators, parakeets and armadillos. *Brain Behav. Evol.* **60**, 65-79.
Nikaido, M., Doi, K., Shimizu, T., Hibi, M., Kikuchi, Y. and Yamasu, K. (2007). Initial specification of the epibranchial placode in zebrafish embryos depends on the fibroblast growth factor signal. *Dev. Dyn.* **236**, 564-571.
Ohyama, T., Mohamed, O. A., Taketo, M. M., Dufort, D. and Groves, A. K. (2006). Wnt signals mediate a fate decision between otic placode and epidermis. *Development* **133**, 865-875.
Ohyama, T., Groves, A. K. and Martin, K. (2007). The first steps towards hearing: mechanisms of otic placode induction. *Int. J. Dev. Biol.* **51**, 463-472.
O'Neill, P., McCole, R. B. and Baker, C. V. (2007). A molecular analysis of neurogenic placode and cranial sensory ganglion development in the shark, Scyliorhinus canicula. *Dev. Biol.* **304**, 156-181.
Osborne, N. J., Begbie, J., Chilton, J. K., Schmidt, H. and Eickholt, B. J. (2005). Semaphorin/neuropilin signaling influences the positioning of migratory neural crest cells within the hindbrain region of the chick. *Dev. Dyn.* **232**, 939-949.
Park, B. Y. and Saint-Jeannet, J. P. (2008). Hindbrain-derived Wnt and Fgf signals cooperate to specify the otic placode in Xenopus. *Dev. Biol.* **324**, 108-121.
Phillips, B. T., Bolding, K. and Riley, B. B. (2001). Zebrafish fgf3 and fgf8 encode redundant functions required for otic placode induction. *Dev. Biol.* **235**, 351-365.
Powell, L. M. and Jarman, A. P. (2008). Context dependence of proneural bHLH proteins. *Curr. Opin. Genet. Dev.* **18**, 411-417.
Raft, S., Koundakjian, E. J., Quinones, H., Jayasena, C. S., Goodrich, L. V., Johnson, J. E., Segil, N. and Groves, A. K. (2007). Cross-regulation of Ngn1 and Math1 coordinates the production of neurons and sensory hair cells during inner ear development. *Development* **134**, 4405-4415.
Rizzoti, K. and Lovell-Badge, R. (2007). SOX3 activity during pharyngeal segmentation is required for craniofacial morphogenesis. *Development* **134**, 3437-3448.
Sai, X. and Ladher, R. K. (2008). FGF signaling regulates cytoskeletal remodeling during epithelial morphogenesis. *Curr. Biol.* **18**, 976-981.
Satoh, T. and Fekete, D. M. (2005). Clonal analysis of the relationships between mechanosensory cells and the neurons that innervate them in the chicken ear. *Development* **132**, 1687-1697.
Schimmang, T. (2007). Expression and functions of FGF ligands during early otic development. *Int. J. Dev. Biol.* **51**, 473-481.
Schlosser, G. and Ahrens, K. (2004). Molecular anatomy of placode development in Xenopus laevis. *Dev. Biol.* **271**, 439-466.
Schwarz, Q., Vieira, J. M., Howard, B., Eickholt, B. J. and Ruhrberg, C. (2008). Neuropilin 1 and 2 control cranial gangliogenesis and axon guidance through neural crest cells. *Development* **135**, 1605-1613.
Sechrist, J., Scherson, T. and Bronner-Fraser, M. (1994). Rhombomere rotation reveals that multiple mechanisms contribute to the segmental pattern of hindbrain neural crest migration. *Development* **120**, 1777-1790.
Shiau, C. E., Lwigale, P. Y., Das, R. M., Wilson, S. A. and Bronner-Fraser, M. (2008). Robo2-Slit1 dependent cell-cell interactions mediate assembly of the trigeminal ganglion. *Nat. Neurosci.* **11**, 269-276.
Streit, A. (2002). Extensive cell movements accompany formation of the otic placode. *Dev. Biol.* **249**, 237-254.
Sun, S. K., Dee, C. T., Tripathi, V. B., Rengifo, A., Hirst, C. S. and Scotting, P. J. (2007). Epibranchial and otic placodes are induced by a common Fgf signal, but their subsequent development is independent. *Dev. Biol.* **303**, 675-686.
Torres, M. and Giráldez, F. (1998). The development of the vertebrate inner ear. *Mech. Dev.* **71**, 5-21.
Tripathi, V. B., Ishii, Y., Abu-Elmagd, M. M. and Scotting, P. J. (2009). The surface ectoderm of the chick embryo exhibits dynamic variation in its response to neurogenic signals. *Int. J. Dev. Biol.* **53**, 1023-1033.
Vendrell, V., Carnicero, E., Giraldez, F., Alonso, M. T. and Schimmang, T. (2000). Induction of inner ear fate by FGF3. *Development* **127**, 2011-2019.
von Bartheld, C. S. (1990). Development and innervation of the paratympanic organ (Vitali organ) in chick embryos. *Brain Behav. Evol.* **35**, 1-15.
von Bartheld, C. S. (1994). Functional morphology of the paratympanic organ in the middle ear of birds. *Brain Behav. Evol.* **44**, 61-73.
Webb, J. and Noden, D. (1993). Ectodermal placodes: contributions to the development of the vertebrate head. *Am. Zool.* **33**, 434-447.
Wilson, H. V. and Mattocks, J. E. (1897). The lateral sensory analage in the salmon. *Anat. Anz.* **13**, 658-660.
Wright, T. J. and Mansour, S. L. (2003). Fgf3 and Fgf10 are required for mouse otic placode induction. *Development* **130**, 3379-3390.
Xu, X., Weinstein, M., Li, C., Naski, M., Cohen, R. I., Ornitz, D. M., Leder, P. and Deng, C. (1998). Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. *Development* **120**, 753-765.
Yamaguchi, T. P., Harpal, K., Henkemeyer, M. and Rossant, J. (1994). fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* **8**, 3032-3044.
Zelarayan, L. C., Vendrell, V., Alvarez, Y., Dominguez-Frutos, E., Theil, T., Alonso, M. T., Maconochie, M. and Schimmang, T. (2007). Differential requirements for FGF3, FGF8 and FGF10 during inner ear development. *Dev. Biol.* **308**, 379-391.