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Review

Changing shape and shaping change: Inducing the inner ear

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ABSTRACT

The inner ear arises from non-neural ectoderm as a result of instructions sent by surrounding tissues. These interactions progressively restrict the potential of the ectoderm, resulting in the formation of the otic placode, a disk of thickened ectoderm that will give rise to all of the inner ear derivatives and its neurons. While otic placode is a surface structure, the inner ear is internalised, embedded within the cranial mesenchyme. Here, the cellular and molecular interactions that restrict the lineage of non-neural ectoderm in its transition to otic placode are reviewed, and how these interactions impinge on the coordination of otic placodal cell shape that drive the dramatic morphogenesis of the placode, as it becomes the otocyst.

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1. Introduction

Organogenesis is an emergent process, with the increase in complexity controlled by sequential molecular and cellular interactions. Understanding how complexity is generated by a small number of possible signalling events remains one of the central challenges in developmental biology. The early development of the inner ear provides a way to understand the fundamental strategies used by molecular and cellular interactions to instruct cellular diversity and how they can encode emergent behaviour. The inner ear forms from a disk of surface-located thickened non-neural ectoderm known as the otic placode [1–5]. From a simple disk of epithelium, appropriately positioned within the embryo, the inner ear rudiment invaginates to become an enclosed sphere in the head of the embryo and then undergoes precise tissue-scale remodelling to form both the vestibular and auditory structures.

The otic placode forms in response to interactions from neighbouring tissues acting on competent ectoderm [6,7]. These interactions are relayed by secreted proteins of the fibroblast growth factor (FGF) family and the Wnt/wingless family [8,9]. Both elicit transcriptional and cellular responses that prime the otic placode for differentiation and for morphogenesis [10]. These initial morphological responses to induction are thickening and then buckling of the otic ectoderm. As development progresses, the otic placode undergoes further dramatic shape changes such that the superficially located structure is internalised, and forms a spherical cyst embedded within the mesenchyme of the head. In this review, I describe the interactions that lead to induction of the inner ear, and how these signals can control both genetic and morphogenetic responses.

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2. Otic inducing signals act on the pre-placodal region

Before describing the tissues and signals that induce the otic placode, it is worth introducing the tissue that these signals act upon. The otic placode is induced from a region of the neural/non-neural border known as the pre-placodal region (PPR). The events that establish the neural/non-neural ectoderm boundary, and hence the PPR are some of the first patterning events to occur in the embryo, and have been detailed elsewhere [5,11]. The neural plate border comprises cells that can form not only the neural plate and non-neural ectoderm, but also the neural crest and PPR.

Lineage-labelling experiments performed in fish, amphibians and chick suggest that placodes exclusively arise from the neural plate border region [12–19]. Furthermore, embryological manipulations point to the PPR as being an intermediate developmental state during the progressive lineage restriction of the ectoderm to form sense organs. Explant studies in which different axial levels of PPR ectoderm have been isolated and placed into culture have suggested that all PPR ectoderm, regardless of its axial level, will form lens placode unless acted upon by further signals [20]. This may suggest that PPR cells are multipotent placode progenitors that in the absence of further instruction, form lens. Support for this idea, comes from rotations of the PPR. These suggest that early anterior PPR, that should form olfactory or lens placodes, can now adopt an inner ear identity when transplanted posteriorly. Similarly caudal, inner ear forming PPR can now adopt a lens or olfactory fate if transplanted anteriorly [21–24]. Perhaps the strongest evidence for the PPR as an area of competence with the ability to respond to specific placode inducing signals comes from experiments in which the ability of different regions of ectoderm to respond to fibroblast growth factor signalling was tested [25]. As discussed later, FGF (fibroblast growth factor) signalling is both sufficient and necessary for the induction of the inner ear in competent ectoderm. Martin et al. found that while ectoderm from the PPR could be induced to form inner ear in response to FGF signalling, other non-PPR ectoderm could not. Only after non-PPR ectoderm was transplanted into the PPR region for 8 h, could this ectoderm now respond to FGF2. This supports the idea of the PPR being an actively specified region, where signalling confers the ectoderm with the competence to respond to the induction of at least one particular type of sensory placode, the inner ear. These experiments are consistent with embryological manipulations that have looked at the regulation of genes that can be considered a molecular signature for the PPR [26–28]. These show that signalling factors from the underlying mesoderm and endoderm are necessary to induce PPR genes.

The genes that are used as the molecular signature for the PPR include members of a class of forkhead box-containing DNA binding proteins called the *FoxI* genes, the *Six* genes (which are homologous to the *Drosophila* gene *sine oculis*), *Six1-4* and *Eya* (homologues of the *Drosophila eyeless* gene) factors, accessory proteins that interact with DNA-binding factors (*Eya1* and *2*) [5,11,29–31]. These genes are expressed in a band of non-neural ectoderm circumscribing the rostral neural plate. Thus, gene expression does provide support to the idea that the PPR is a uniform region of competence that can respond to the inductive cues that specify all rostral or caudally located placodes. However, investigations into the control of expression of one PPR gene, *Six1*, suggest that this view is overly simplistic [31,32]. Enhancers are evolutionarily conserved stretches of non-coding DNA found within a particular gene, which control its expression. Mouse *Six1* has a number of enhancers that drive expression in distinct regions of the mouse embryo, and that have been defined based on their conservation amongst different species. However despite the pan-PPR expression of *Six1*, an individual enhancer that directs expression in this domain has not, as yet, been identified [32]. Of course, one interpretation could be simply that an evolutionarily conserved pan-PPR enhancer cannot be found and is elsewhere in the *Six1* gene. However, it is worth noting that a separate rostral PPR enhancer was identified as a region of homology present in other species. This may imply that rather than evolution setting aside the whole PPR as a region that can give rise to sensory organs, the PPR may be a composite of territories of competence that respond to specific placode inducing cues.

Careful analysis of the fate of *Six1* expressing PPR tissue in Xenopus has shown that all placodes do arise from this region, although there is a caveat: There is a late caudal addition to the Six1 expression domain that contains precursors to posterior epibranchial placodes as well as posterior lateral line [14]. The implication is that perhaps these placode are recent evolutionary innovations in *Xenopus*, but must still form from the PPR. This supports the idea that all sensory placodes must go through an initial PPR state. While the exact nature of the PPR state is still unclear, the hypothesis that PPR genes are involved in priming the genetic response of this ectoderm to subsequent placode inducing cues [33] is particularly attractive.

3. Otic induction is a progressive process

The induction of the inner ear can be more properly considered as a gradual restriction of ectodermal lineage. The formation of the PPR is one of these steps, and subsequent steps take the PPR ectoderm through a series of fate choices to form the precursor to the inner ear. The importance of signalling in inducing the inner ear has been established by a rich history of over a century of experimental embryological experiments (reviewed in [5,6]). These experiments utilised chick, amphibian and fish model systems as their paradigm and suggested that signals act from the tissues adjacent or subjacent the posterior portion of the PPR, namely the cranial paraxial mesoderm (CPM) and the neural ectoderm/hindbrain, were responsible for the induction of the inner ear. The current model for otic induction is summarised in Fig. 1.

In the molecular age, genetic markers have allowed a greater resolution of the signalling events that induce the inner ear. One marker, and the earliest marker for the inner ear, is *Pax2* or its close family member *Pax8* [34,35]. In almost all vertebrates, *Pax2/8* expression can be first seen in the non-neural ectoderm abutting the neural folds, just rostral to the first somite, as the neural folds begin to elevate [36–45]. As the neural plate closes, *Pax2* expression expands laterally. This initial domain of expression encompasses a larger patch of non-neural ectoderm then one would expect if *Pax2* were purely an otic marker. Indeed, elegant lineage labelling experiments performed in the chick suggested that the initial *Pax2* expression domain gave rise to both epibranchial and otic placodal progenitors [18]. Subsequent genetic labelling experiments, performed in mouse and zebrafish, supported this data further adding to the idea that the domain marked by *Pax2* expression represented a pool of progenitors for the otic and epibranchial placodes and, in the case of fish, anterior lateral line [46–48]. This domain has been termed the otic-epibranchial progenitor region (OEPD – a term that is used through the rest of this review), the pre-otic region or the posterior placodal area (PPA) [2,7–9]. Consideration of the OEPD has provided a way to understand the hierarchical organisation of signalling during the induction of the inner ear.

The gradual restriction of the otic placode from the OEPD is apparent from a number of experiments. One of the clearest is the ability of explanted ectoderm to express various inner ear markers [9,49]. In these sets of experiments, the presumptive inner ear ectoderm is removed from the embryo, freed of underlying tissues and then placed in culture. The idea is that isolation of the ectoderm assesses the extent of otic placode specification. Chick inner ear ectoderm explanted between 4 and 6 somites stage can only express *Pax2*, an OEPD marker. Importantly, ectoderm taken at this stage cannot express a marker for the otic placode proper, known as

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