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Lens Specification Is the Ground State of All Sensory Placodes, from which FGF Promotes Olfactory Identity

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Summary

The sense organs of the vertebrate head comprise structures as varied as the eye, inner ear, and olfactory epithelium. In the early embryo, these assorted structures share a common developmental origin within the preplacodal region and acquire specific characteristics only later. Here we demonstrate a fundamental similarity in placodal precursors: in the chick all are specified as lens prior to acquiring features of specific sensory or neurogenic placodes. Lens specification becomes progressively restricted in the head ectoderm, initially by FGF and subsequently by signals derived from migrating neural crest cells. We show that FGF8 from the anterior neural ridge is both necessary and sufficient to promote olfactory fate in adjacent ectoderm. Our results reveal that placode precursors share a common ground state as lens and progressive restriction allows the full range of placodal derivatives to form.

Introduction

Since Spemann's original experiments (Spemann, 1901), lens formation has remained an important model for studying inductive interactions during embryogenesis. He suggested that the optic vesicle, the future retina, is the source of lens-inducing signals sufficient to generate lenses in competent ectoderm. This idea has since been revised to propose a multistep model for lens induction. In amphibians, key events in lens formation occur around late gastrula/early neurula stages, before the optic vesicle contacts the presumptive lens ectoderm. Molecular interactions at these preplacode stages are ill defined, whereas the molecular control of lens placode formation (which occurs after optic vesicle contact) is relatively well understood (Grainger, 1996; Ogino and Yasuda, 2000; Lang, 2004). Ultimately, formation of a functional eye depends on the mutual interaction between the differentiating lens vesicle and the optic cup (Coulombre and Coulombre, 1963; Yamamoto and Jeffery, 2000; Lang, 2004).

One of the key factors in early lens formation is the transcription factor Pax6, whose ability to induce ectopic eyes is conserved throughout the animal kingdom (Halder et al., 1995; Chow et al., 1999; Gehring and Ikeo, 1999; Donner and Maas, 2004). *Pax6* is expressed prior to lens placode formation in the presumptive lens ectoderm (PLE) and is required cell autonomously in the PLE for its transition to a morphological placode (Walther and Gruss, 1991; Grindley et al., 1995; Ashery-Padan et al., 2000). Downstream of Pax6, different transcription factors of the Sox, Maf, Fox, and Six families cooperate with Pax6 and with each other to activate the lens program, initially manifested by the expression of lens-specific crystallins (Kamachi et al., 1995, 1998, 2001; Ogino and Yasuda, 1998; Muta et al., 2002; Zhu et al., 2002; Shimada et al., 2003). BMP signaling in the optic vesicle and in the PLE is necessary for gene expression specific for the lens placode (Furuta and Hogan, 1998; Wawersik et al., 1999; Faber et al., 2002), while FGF signaling is required in the placode (Faber et al., 2001). Thus, our current molecular understanding of lens induction is most complete for the transition from preplacode to placode stages, while the events that initiate this process (upstream of preplacodal Pax6) are unknown.

At early neurula stages, cells fated to become lens are part of a unique domain in the cranial ectoderm which contains the precursors for all sensory placodes and thus has been termed the "preplacodal region" (Kozlowski et al., 1997; Streit, 2002, 2004; Bhattacharyya et al., 2004; Bailey and Streit, 2006; Schlosser, 2006). This region expresses a unique set of genes belonging to the Six, Eya, and Dach families (Bailey and Streit, 2006; Schlosser, 2006), which have been implicated in its own formation and in the control of various aspects of sensory organ development (Xu et al., 1999; Li et al., 2003; Zheng et al., 2003; Kawakami et al., 2000; Hanson, 2001). Within the preplacodal region, precursors for different placodes are initially interspersed, but then separate to form individual placodes at discrete positions along the neural tube (Kozlowski et al., 1997; Streit, 2002; Bhattacharyya et al., 2004). In the adult, placode derivatives contribute to the cranial sensory nervous system giving rise to structures as diverse as the lens, the inner ear, the olfactory epithelium, the adenohypophysis, and the sensory ganglia, and generate a large variety of different cell types (Bailey and Streit, 2006; Schlosser, 2006). Among these, the adenohypophysis and the lens are the only nonneurogenic placodes, the latter being the simplest placode, which produces an anterior epithelial sheet and a posterior mass of lens fibers.

Despite their eventual cellular and functional diversity, it has been proposed that all placodes initially share a common developmental program (Jacobson, 1966; Torres and Giraldez, 1998; Streit, 2004; Bailey and Streit, 2006; Schlosser, 2006). In this model, an early step in placode specification entails the induction of the preplacodal region, which would represent a unique state through which cells have to pass before becoming mature placodes. So far, the strongest support for this model comes from a recent study of otic induction

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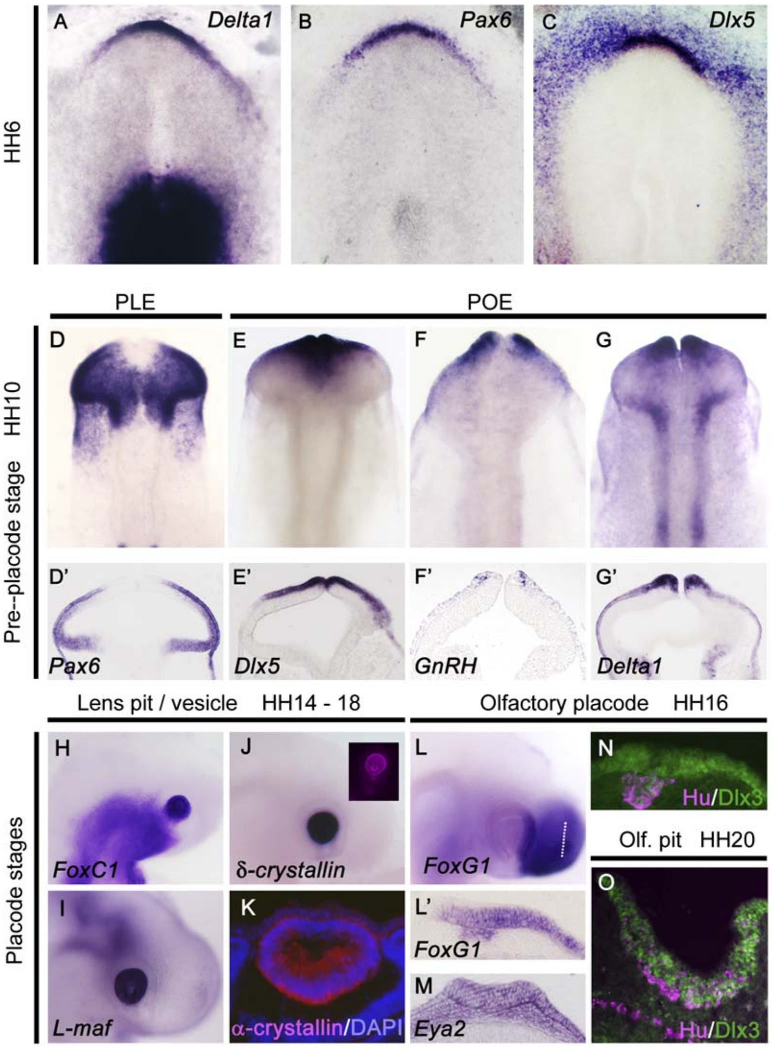

Figure 1. Molecular Markers of Preplacode and Placode Stages

(A–C) At HH6, *Delta1* (A) and *Pax6* (B) are expressed outside the anterior neural plate, while *Dlx5* (C) surrounds the entire neural plate.

(D–G) At HH10, the presumptive lens ectoderm (PLE) occupies the region overlying the optic vesicles, while the presumptive olfactory ectoderm (POE) surrounds the anterior neuropore. The PLE is marked by *Pax6* (D and D′), while the POE is labeled by *Dlx5* (E and E′), *GnRH* (F and F′), and *Delta1* (G and G′).

(D′ and E′) Transverse sections through embryos shown in (D)–(G).

(H–K) The lens placode (H) and vesicle (I–K) express *FoxC1* (H), *L-maf* (I), *δ-crystallin* transcript (J) and protein ([J], inset), and α-crystallin (K).

(L–O) At HH16, the olfactory placode expresses *FoxG1* (L and L′), *Eya2* (M), and Dlx3 protein (N); a subset of migrating cells is Hu^{+} (N). The line in (L) indicates the plane of section in (L′) and (M)–(O).

(O) At HH20, the olfactory pit expresses Dlx3, and a subset of cells also expresses Hu.

showing that the acquisition of preplacodal-like character is required for cells to respond to an otic-inducing signal (FGF; Martin and Groves, 2006). Although the terms “placode bias” or “generic placode character” have been used to describe the preplacodal state, the exact properties of cells in the placode territory remain ill defined.

Surprisingly, we find that the entire preplacodal region is initially specified as lens tissue. This implies that “lens” is a default state of the preplacodal territory, which must therefore be repressed in the nonlens domains. FGF from the anterior neural ridge initiates lens suppression and simultaneously imparts olfactory fates to neighboring cells. However, FGF alone is not sufficient to restrict lens formation to its correct position in the embryo; we show that neural crest cells inhibit lens specification in vitro, while their ablation in vivo results in ectopic lens formation.

Results

All Sensory Placodes Are Initially Specified as Lens

To characterize the regional identity and developmental state of different parts of the preplacodal region over time, we first analyzed the expression of molecular markers specific for individual presumptive and mature placodes. At head fold stages (HH6), the chick preplacodal region expresses *Six1*, *Six4*, and *Eya2* (Streit, 2004; Schlosser, 2006). Within this domain, *Pax6*, *Delta1*, and *Dlx5* overlap in the anterior placode territory (Figures 1A–1C; Bhattacharyya et al., 2004); however, by the ten-somite stage, *Pax6* (Figures 1D and 1D′) is concentrated in the presumptive lens ectoderm, while *Dlx5* and *Delta1* (Figures 1E, 1E′, 1G, and 1G′) are strongly expressed in the presumptive olfactory region (POE), which also expresses *gonadotropin releasing hormone* (*GnRH*) transiently (Figures 1F and F′). The otic region is *Pax2*$^{+}$ and *Sox10*$^{+}$ (Cheng et al., 2000; Groves and Bronner-Fraser, 2000), while the trigeminal territory expresses Pax3 (see Figure S1 in the Supplemental Data available with this article online). At stage HH16, all placodes have acquired their typical morphology; the olfactory placode expresses *FoxG1* (Figures 1L and 1L′), *Eya2* (Figure 1M), and *FGF8* (Karabagli et al., 2002b), is strongly Dlx3^{+}, and has begun to generate Hu^{+} neurons, a subpopulation of which is Dlx3^{+} (Figure 1N; stage HH20: Figure 1O). The lens expresses high levels of *L-maf* (Figure 1I), *FoxC1* (Figure 1H), *δ-crystallin*