

# The preplacodal region: an ectodermal domain with multipotential progenitors that contribute to sense organs and cranial sensory ganglia

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**ABSTRACT** The otic primordium belongs to a group of related structures, the sensory placodes that contribute to the paired sense organs - ear, eye and olfactory epithelium - and to the distal parts of the cranial sensory ganglia. Recent evidence suggests that despite their diversity, all placodes share a common developmental origin and a common molecular mechanism which initiates their formation. At the base of placode induction lies the specification of a unique "placode field", termed the preplacodal region and acquisition of this "preplacodal state" is required for ectodermal cells to undergo otic development. Here I review the molecular mechanisms that sequentially subdivide the ectoderm to give rise to the placode territory.

**KEY WORDS:** *BMP, ear, ectoderm, epibranchial, eye, FGF, olfactory epithelium, sensory placodes, Wnt*

## Introduction

The adult vertebrate inner ear is sophisticated both in structure and function. Responsible for the perception of sound, balance and acceleration it comprises the semicircular canals, cochlea and endolymphatic duct and a large variety of different cell types including hair and supporting cells within the sensory patches. It is therefore remarkable that during development it arises from a simple epithelium, the otic placode, which is first visible around the 10 somite stage next to rhombomeres 5 and 6 of the hindbrain (Bancroft and Bellairs, 1977; Verwoerd, *et al.*, 1981; Haddon and Lewis, 1996; Schlosser and Northcutt, 2000). Subsequently, the placode invaginates and separates from the surface ectoderm to form the otic vesicle, which then undergoes complex morphogenetic events to generate the mature inner ear. However, already long before the otic placode is morphologically distinct, patterning events in the ectoderm are well under way to restrict its formation to the ectoderm next to the future hindbrain and to determine the position of future otic cells in relation to precursors for other sensory placodes (for review: Streit, 2004; Bailey and Streit, 2006; Schlosser, 2006). In particular, classical and recent evidence has highlighted the importance of a unique territory in the head ectoderm that contains precursors for all cranial placodes, including the otic primordium and has therefore been named the preplacodal region (PPR; Jacobson, 1963; for review: Streit, 2004; Bailey and Streit, 2006; Schlosser, 2006). The acquisition of a 'preplacodal state' appears to be a prerequisite for ectoder-

mal cells to become specified as otic precursors (Martin and Groves, 2006).

## The preplacodal region – a common ground state for all sensory placodes

Cranial placodes form an apparently disparate group of structures that contribute to the eye, ear, olfactory epithelium and lateral line (fish, amphibians) and to the distal portions of the cranial sensory ganglia (Fig. 1A). Their derivatives in the adult vary largely in structure, function and in the cell types they produce ranging from simple lens fibre cells to sensory receptor cells like hair cells in the ear or olfactory receptor cells in the nasal epithelium. Their development and derivatives have recently been reviewed extensively elsewhere (Baker and Bronner-Fraser, 2001; Streit, 2004; Schlosser, 2006). Despite their apparent differences they share similarities during early development: all placodes form columnar epithelia next to the neural tube, contain cells that undergo epithelial-mesenchymal transition, contribute to the cranial sensory nervous system and are neurogenic with the exception of the lens. In addition, recent evidence suggests that they initially share a common developmental programme before they diversify and acquire unique identity (see below for discussion; Bailey, *et al.*, 2006) and that cells must go through a

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*Abbreviations used in this paper:* BMP, bone morphogenetic protein; FGF, fibroblast growth factor; PPR, preplacodal region.

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'preplacodal state' before they can respond to placode inducing signals (Martin and Groves, 2006). A continuous placode territory, where all placode precursors reside, can first be identified at neurula stages and is defined by the expression of unique set of molecular markers as well as by common properties of all cells contained in it (Jacobson, 1963; Kozłowski, *et al.*, 1997; Streit, 2002; Bhattacharyya, *et al.*, 2004; Schlosser and Ahrens, 2004; Bailey, *et al.*, 2006; for review: Streit, 2004; Bailey and Streit, 2006; Schlosser, 2006). Here, I review how sequential subdivision of the ectoderm leads to the establishment of the preplacodal region, its functional relevance to placode development and to otic induction in particular and how it becomes subdivided to generate precursors for different placodes.

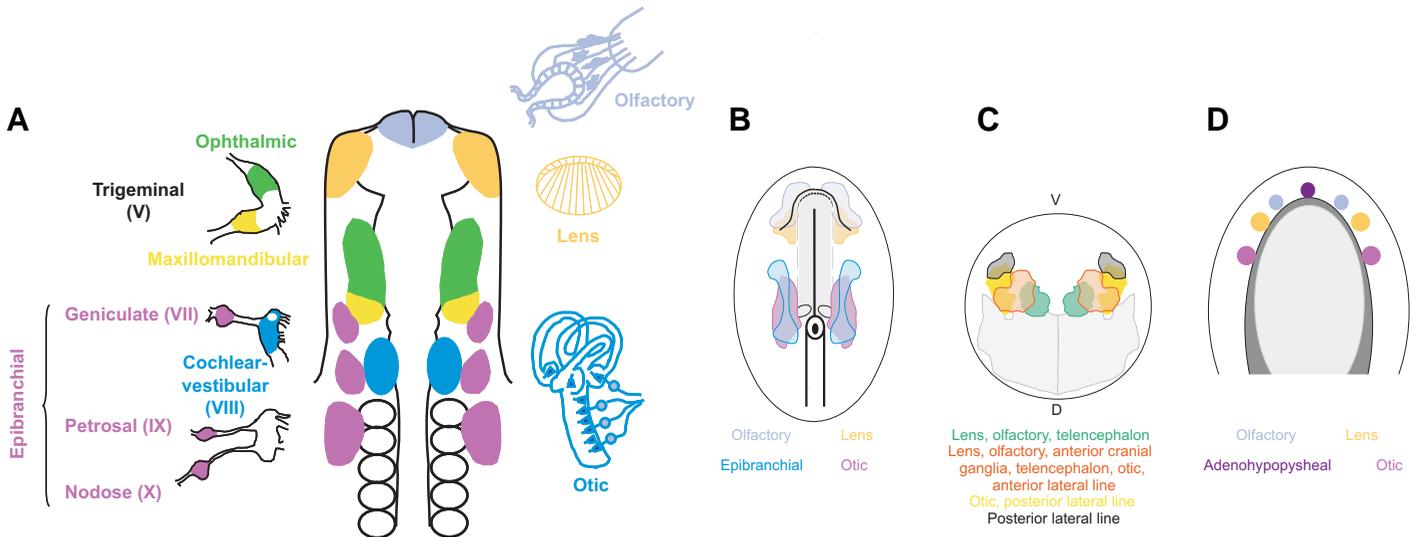
### Subdivision of the ectoderm: changes in gene expression and the segregation of cell fates

Like neural, neural crest and epidermal precursors, placodal cells are ectodermal derivatives. How and when do cells of different fates segregate? Fate map analysis in zebrafish, *Xenopus*, mouse and chick show that around the time of gastrulation the ectoderm is roughly subdivided into neural and non-neural ectoderm, although a large intermediate region exists in which both fates overlap (Keller, 1975; Keller, 1976; Tam, 1989; Kimmel, *et al.*, 1990; Garcia-Martinez, *et al.*, 1993; Hatada and Stern, 1994; Lawson, 1999; Fernandez-Garre, *et al.*, 2002). This broad separation of cells with different fates is reflected by gene expression (Fig. 2A). Pre-neural markers such as *ERNI* (chick; Streit, *et al.*, 2000), *Sox3* (Penzel, *et al.*, 1997; Rex, *et al.*, 1997; Kudoh, *et al.*, 2004), *Geminin* (*Xenopus*; Kroll, *et al.*, 1998) and *SoxD* (*Xenopus*; Mizuseki, *et al.*, 1998) are concentrated in the future neural domain and gradually decrease towards the non-neural ectoderm, while genes like *Gata2*, *Gata3*, *Dlx-3*, *-5*, *Foxi1* or *Foxi3*, *BMP4* and *Msx1* show the opposite expression pattern (Papalopulu and Kintner, 1993; Akimenko, *et al.*, 1994; Streit, *et*

*al.*, 1998; Pera, *et al.*, 1999; Sheng and Stern, 1999; Streit and Stern, 1999; Luo, *et al.*, 2001; Streit, 2002; Liu, *et al.*, 2003; Solomon, *et al.*, 2003; Ohyama and Groves, 2004; Matsuo-Takasaki, *et al.*, 2005). At this stage, precursors for different placodes, including the otic, are still widely dispersed and intermingled with future neural, epidermal and neural crest cells in the chick (Garcia-Martinez, *et al.*, 1993; Hatada and Stern, 1994; Streit unpublished), although a more restricted distribution has been reported in zebrafish (Kozłowski, *et al.*, 1997).

With the formation of the definitive neural plate (Fig. 2B), neural specific genes like *Sox2* become up-regulated (Rex, *et al.*, 1997; Kishi, *et al.*, 2000), while pre-neural markers either become confined to a broad band of ectoderm surrounding the neural plate (*ERNI*) or remain expressed in both domains (*Sox3*, *Geminin*). Likewise, some non-neural markers become upregulated in (e.g. *Dlx* and *Gata*) or confined to (e.g. *Foxi1*) the ectoderm next to the neural plate. Thus, at early neurula stages a contiguous stripe of ectoderm coexpresses pre-neural and non-neural ectoderm markers and has therefore been termed the 'border' (Streit and Stern, 1999; McLaren, *et al.*, 2003; Woda, *et al.*, 2003; Meulemans and Bronner-Fraser, 2004). Within the border region precursors for neural, neural crest, epidermis and placodes remain interspersed (Kozłowski, *et al.*, 1997; Streit, 2002; Bhattacharyya, *et al.*, 2004).

Shortly thereafter, members of the Six and Eya families of nuclear factors begin to be expressed in a horseshoe-shaped domain surrounding the rostral neural plate from fore- to hindbrain levels (Fig. 2C) (Mishima and Tomarev, 1998; Esteve and Bovolenta, 1999; Sahly, *et al.*, 1999; Kobayashi, *et al.*, 2000; Pandur and Moody, 2000; McLaren, *et al.*, 2003; Bessarab, *et al.*, 2004; Schlosser and Ahrens, 2004; Litsiou, *et al.*, 2005). Simultaneously, precursors for all placodes become concentrated in the *Six/Eya*<sup>+</sup> territory to form a contiguous, unique domain: the preplacodal region (Streit, 2002; Bhattacharyya, *et al.*, 2004) (Fig. 1B). Unlike other factors that have a more widespread expression in the ectoderm (*Dlx3/5* and *Gata3*), *Six1*, *Six4*, *Eya1* and/or *Eya2*



**Fig. 1. Position of sensory placodes at the 10-somite and fate maps at late gastrula and neural plate stages.** (A) Schematic representation of the sensory placodes and their derivatives at the 10 somite stage in the chick embryo (after D'Amico-Martel and Noden, 1983; Bhattacharyya, *et al.*, 2004). (B) Fate map of a 1-somite stage chick embryo (after Streit, 2002; Bhattacharyya, *et al.*, 2004). Grey, neural plate. (C) Fate map of a zebrafish embryo at 50% epiboly (modified from Kozłowski, *et al.*, 1997); grey, neural plate, (Woo and Fraser, 1995). (D) Fate map of salamander embryo (*Ambystoma*) at neural plate stages (modified from Carpenter, 1937). Light grey, neural plate; dark grey, neural folds.

are exclusively expressed in the preplacodal region.

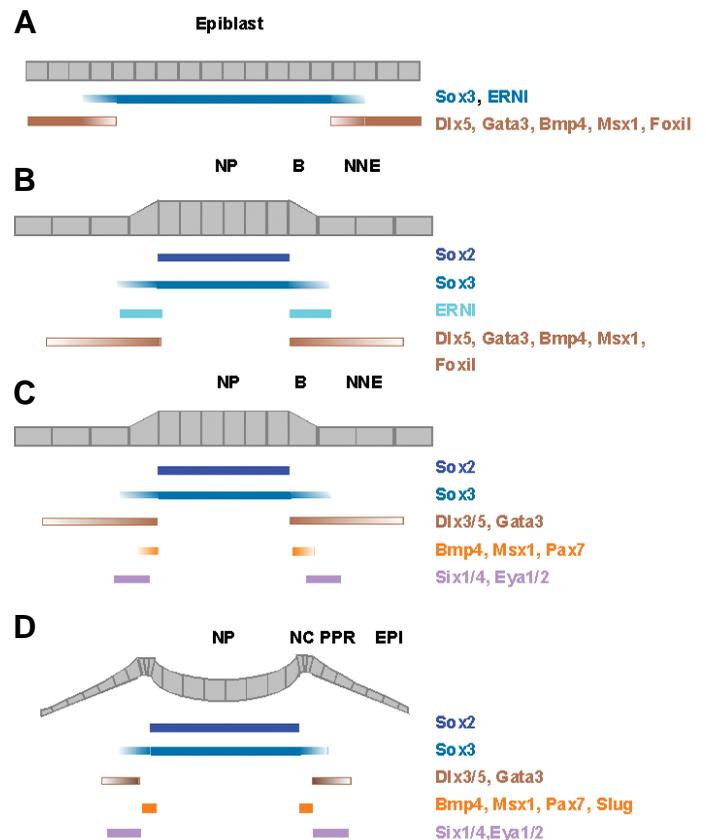
Around the same time, neural crest specific genes such as *Snail2*, *FoxD3* or *Sox10* begin to be expressed in a thin line along most of the neural plate (except its most rostral part, where no neural crest cells are generated; Couly and Le Douarin, 1985; Couly and Le Douarin, 1987). Although some overlap between neural crest and placode precursors is still observed at this stage in chick, both fates are completely segregated by the 4-5 somite stage, when the neural plate has begun to fold and neural folds are morphologically obvious (Fig. 2D) (Streit, 2002; Bhattacharyya, *et al.*, 2004). Thus, by late neurula stages, the preplacodal region is molecularly and cellularly distinct from other ectodermal derivatives.

### Transcription factors that position the neural plate border

As outlined above, a number of transcription factors are co-expressed at the border of neural and non-neural ectoderm before the onset of definitive neural crest and placode markers and are therefore likely to function upstream of preplacodal genes. Indeed, some of these have been implicated in controlling the position of the border and appear to be required for the specification of border derivatives. *Msx1* is a direct mediator of BMP signalling and as such functions in promoting epidermal and repressing neural character during early stages of *Xenopus* development, thereby positioning the border between both tissues (Suzuki, *et al.*, 1997; Feledy, *et al.*, 1999; Phillips, *et al.*, 2006). However, at late gastrula/early neurula stages *Msx1* is more specifically involved in neural crest cell formation; it is both required for and sufficient to induce neural crest cells at the border of the neural plate (Tribulo, *et al.*, 2003; Monsoro-Burq, *et al.*, 2005; Khadka, *et al.*, 2006; Phillips, *et al.*, 2006). Like *Msx1*, members of the *Dlx* family also counteract neural plate formation, but in addition both seem to play antagonistic roles during the specification of neural crest and placode precursors. Misexpression of *Dlx5*, *Dlx3* or constitutively active *Dlx3* represses neural and neural crest cells, while promoting the expression of preplacodal *Six1* and *-4* (Luo, *et al.*, 2001; McLaren, *et al.*, 2003; Woda, *et al.*, 2003). In contrast, overexpression of dominant negative *Dlx3* shifts the neural plate border laterally or results in a complete loss of preplacodal gene expression (Woda, *et al.*, 2003), while in zebrafish, knockdown or deletion of *dlx3b* and *-4b* (b380 mutants) leads to a severe reduction of olfactory, trigeminal and otic placodes (Solomon and Fritz, 2002; Kaji and Artinger, 2004). However, when *MsxB*, *C* and *E* function is blocked in b380 mutants, placode development is restored indicating that in the absence of *Dlx* function *Msx* proteins repress placode formation (Phillips, *et al.*, 2006). Thus, *Dlx* protein function is required for normal placode formation, but its activity in the border region opposes *Msx1* function. Since their expression partially overlaps fine tuning of their function maybe achieved through direct protein-protein interaction: *Dlx* and *Msx* proteins can form heterodimers that block their function as transcriptional activator or repressor (Zhang, *et al.*, 1997). Thus, on cellular level relative expression levels of *Dlx* and *Msx* proteins may determine cell fate choices.

The winged helix transcription factor *Foxil* has mainly been studied in relation to its function in early otic development. However, recent evidence indicates that it may have an even

earlier function in positioning the border between neural and non-neural ectoderm. In *Xenopus* and fish, *Foxil* is initially expressed widely, but then becomes rapidly confined to a stripe of ectoderm surrounding the neural plate (Solomon, *et al.*, 2003; Matsuo-Takasaki, *et al.*, 2005; Hans, *et al.*, 2007). A similar expression is observed in mouse for *Foxi3* (Ohyama and Groves, 2004). In fish, its requirement for otic and epibranchial placode specification has been demonstrated in *Foxil* mutants (*foo/foo*, *hearsay*, and *no soul*; Lee, *et al.*, 2003; Nissen, *et al.*, 2003; Solomon, *et al.*, 2003), while other sensory placodes have not been investigated in detail. Two recent studies suggest that both *Foxil* and *Dlx3b* are required in ectodermal cells to respond to the otic inducing factor FGF8 (Hans, *et al.*, 2004; Hans, *et al.*, 2007): misexpression of FGF8



**Fig. 2. Changes in gene expression from gastrula to early somite stages.** Diagrams show a cross section through chick embryos at gastrula (A), head process (B), 1-2-somite (C) and 4-5-somite (D) stages. (A) At gastrula stages, the epiblast is roughly subdivided into neural and non-neural territories. (B) The neural plate can be identified morphologically expressing definitive neural markers (*Sox2*), while preneural markers (*ERNI*) become confined to the border, where non-neural markers become upregulated (*Dlx5*, *Gata3*, *BMP4*, *Msx1*, *Foxil*). (C) Preplacodal markers begin to be expressed (*Six1*, *Six4*, *Eya2*); there is some overlap between *BMP4*, *Msx1* and *Pax7*, which are later confined to the neural folds where neural crest cells arise. (D) Neural folds are morphologically distinct and express neural crest cells markers (*Slug*, *Pax7*). There is no overlap between neural crest and preplacodal genes. NP, neural plate; B, border; NNE, non-neural ectoderm; NC, neural crest; PPR, preplacodal region; EPI, future epidermis.

leads to an enlarged otic vesicle only within the normal ear field, where both genes are co-expressed. In contrast, loss of both genes completely abolishes the formation of otic structures (Solomon, *et al.*, 2004; Hans, *et al.*, 2007). Experiments in *Xenopus* demonstrate an even earlier role for Foxil; loss of *Foxila* function leads to an expansion of the neural plate at gastrula stages, while its overexpression suppresses neural development while simultaneously promoting epidermal character (Matsuo-Takasaki, *et al.*, 2005). It should be noted that so far an early role for Foxi class genes has not been demonstrated in mouse; this may be due the difference in Foxi gene expression in different species. Together, these findings identify Foxil as one of the early players in ectodermal patterning involved in setting the border between neural and non-neural ectoderm and as a prerequisite for otic and epibranchial development. Future experiments will need to address its potential role in the formation of other placodes and its epistatic relation to genes specific for the placode territory.

Thus, members of the Dlx, Msx and Foxi family are expressed early and in a broad domain and regulate neural crest and placode specific genes. They are therefore at the top of a hierarchy controlling the specification of cells that arise from the border region and are likely to be intracellular mediators of the signalling pathways that pattern the ectoderm and position the placode territory next to the cranial neural plate.

### The Six/Eya/Dach network in placode development

Among the many transcription factors expressed in the preplacodal region, only members of the Six and Eya families match precisely the location of all placode precursors and are subsequently maintained in all placodes, but lost from non-placodal ectoderm. They have been implicated in multiple processes during placode formation and are therefore likely candidates to be involved in defining the placode territory at early developmental stages.

#### Six and Eya genes in sensory organ formation

Their importance in sensory organ development was initially reported in *Drosophila*, where their homologues *sine oculis* (so) and *eyes absent* (eya) form a non-linear, regulatory network that together with *dachshund* (dac) control eye development and photoreceptor cell specification (for review: Kumar and Moses, 2001; Donner and Maas, 2004; Pappu and Mardon, 2004; Rebay, *et al.*, 2005). Loss of any of these genes results in defects or absence of the eye (Bonini, *et al.*, 1993; Cheyette, *et al.*, 1994; Mardon, *et al.*, 1994; Quiring, *et al.*, 1994; Serikaku and O'Tousa, 1994), while their overexpression leads to ectopic eye formation in restricted positions of other imaginal discs (Halder, *et al.*, 1995; Bonini, *et al.*, 1997; Chen, *et al.*, 1997; Pignoni, *et al.*, 1997; Shen and Mardon, 1997; Weasner, *et al.*, 2007). So, *eya* and *dac* regulate each others' expression and function downstream of the Pax6 homologue *eyeless* (ey): their expression and eye-inducing ability depends on the presence of functional Ey (Halder, *et al.*, 1998; Niimi, *et al.*, 1999; Bui, *et al.*, 2000; Punzo, *et al.*, 2002; Pappu, *et al.*, 2005; for review: Kumar and Moses, 2001; Pappu and Mardon, 2004).

In vertebrates, six Six genes have been identified (Six1-6), while there are only four Eya genes (Eya1-4) (for review: Kawakami,

*et al.*, 2000; Wawersik and Maas, 2000; Hanson, 2001; Rebay, *et al.*, 2005). Of those Six1, Six4, Eya1 and Eya2 are found in the pre-placodal region, while combinations of different family members are coexpressed in mature placodes (Mishima and Tomarev, 1998; Esteve and Bovolenta, 1999; Sahly, *et al.*, 1999; Kobayashi, *et al.*, 2000; Pandur and Moody, 2000; McLarren, *et al.*, 2003; Bessarab, *et al.*, 2004; Schlosser and Ahrens, 2004; Litsiou, *et al.*, 2005). Of the Eya gene family, only Eya3 is never found in any placode. As in the fly, they are often colocalised with members of the Pax gene family (e.g. Pax6: lens, olfactory; Pax2: otic and epibranchial; Pax3: trigeminal) although their regulatory relationship appears to be more complicated. For example, in the mouse olfactory ectoderm initial expression of Six3, Eya1 and Dach1 proteins is Pax6 independent, while their maintenance in the placode requires Pax6 (Purcell, *et al.*, 2005). Likewise, in the presumptive lens ectoderm, *Pax6* is controlled by Six3 but once the placode is formed *Six3* expression depends on Pax6 activity (Purcell, *et al.*, 2005; Liu, *et al.*, 2006). In the ear *Pax2*, *Eya1* and *Six1* are expressed in partially overlapping domains; *Eya1* and *Six1* expression is independent of Pax2, while *Six1* depends on Eya1 function (Zheng, *et al.*, 2003; Burton, *et al.*, 2004). In the preplacodal region *Six* and *Eya* transcripts are present prior to the onset of *Pax* gene and are therefore likely to act independently.

The importance of Six and Eya genes for normal placode development has been demonstrated through loss-of-function in mouse, zebrafish and humans. Eya1 and Six1 have been studied extensively and play a role in the formation of most placode derivatives (see below), reflecting their widespread expression in the preplacodal region. Likewise, mutations in Eya4 and Six5 are associated with defects in placode derivatives (Klesert, *et al.*, 2000; Wayne, *et al.*, 2001; Zhang, *et al.*, 2004), while information about Six2 and Eya2 is very sparse. Mice lacking Eya2 function have been generated, however, their placodal phenotype has not been described in detail (Grifone, *et al.*, 2007). So far, no placode phenotype has been described for Six4 mutant mice (Ozaki, *et al.*, 2001; Grifone, *et al.*, 2005); one possible explanation may be functional redundancy between genes of the same family that are normally co-expressed. In support of this, Six1 and -4 double knock-out mice show a more severe muscle, kidney and trigeminal ganglion phenotype than Six1 mutants alone (Grifone, *et al.*, 2005; Konishi, *et al.*, 2006; Kobayashi, *et al.*, 2007). However, it is not known whether this is also the case for other placodes.

Mice heterozygous for Eya1 display a phenotype very similar to an inherited form of deafness in humans, the Branchio-Oto-Renal (BOR) syndrome, a form of conductive hearing loss due to defects in middle ear development (Abdelhak, *et al.*, 1997; Xu, *et al.*, 1999). Mice completely lacking Eya1 function have severe inner ear defects (Johnson, *et al.*, 1999; Xu, *et al.*, 1999; Li, *et al.*, 2003; Zou, *et al.*, 2004; Friedman, *et al.*, 2005; Zou, *et al.*, 2006): otic development arrests at vesicle stages, sensory patches remain small and while cochlear-vestibular neurons initially form, they later undergo apoptosis. In addition, the trigeminal ganglion is reduced in size, epibranchial placode derived petrosal, geniculate and nodose ganglia are missing or greatly reduced and fail to express neuronal determination genes. Zebrafish dogeared mutants (Eya1) also show ear defects and the development of the lateral line placodes is impaired, however cranial ganglia are generally unaffected (Kozlowski, *et al.*, 2005; Whitfield, 2005). Eya1 mutations in humans are also associated with congenital

eye defects (Azuma, *et al.*, 2000), although these have not been described in mice. Finally, mutations in the *eya*-homologous region of *Eya4* lead to late-onset deafness in humans (Wayne, *et al.*, 2001; Zhang, *et al.*, 2004; Schonberger, *et al.*, 2005).

Like *Eya1*, *Six1* has been implicated in normal development of the inner ear and mutations in human *Six1* cause BOR syndrome like *Eya1* mutations (Ruf, *et al.*, 2004). Mice lacking *Six1* function display very similar phenotypes to *Eya1* mutant mice: otic vesicles are small, lack the cochlea and semicircular canals and do not form a cochlear-vestibular ganglion (Laclef, *et al.*, 2003; Li, *et al.*, 2003; Zheng, *et al.*, 2003; Ozaki, *et al.*, 2004). In addition, trigeminal and epibranchial placode derived neurons are reduced or absent and development of the olfactory epithelium is impaired. In zebrafish, *Six1* promotes the formation of hair cells by increasing their proliferation, while inhibiting neurogenesis by inducing apoptosis (Bricaud and Collazo, 2006). Finally, *Six5* mutations lead to cataract formation in the lens (Klesert, *et al.*, 2000; Sarkar, *et al.*, 2000; Bateman, *et al.*, 2006) and are associated with BOR syndrome in humans (Hoskins, *et al.*, 2007).

The widespread defects in almost all placode derivatives in *Six1* and/or *Eya1* mutants argues for a conserved function of this network during sensory placode formation or for an involvement at very early stages development, maybe in the preplacodal region. Unfortunately, none of the above studies has addressed this issue. In *Xenopus*, *Six1* function has been assessed at preplacodal stages (Brugmann, *et al.*, 2004), where it promotes the expression of other preplacodal genes like *Eya1*, while repressing neural, neural crest and epidermal fates. These findings point to a potentially early role of *Six* and *Eya* proteins in ectodermal patterning by establishing the preplacodal region and conferring common preplacodal properties (see below). However, further studies are required to determine their precise role at these early stages.

#### **Molecular function and targets of the Six/Eya/Dach network**

*Six*, *Eya* and *Dach* proteins are thought to interact physically and to act as a transcription factor complex to activate downstream target genes (for review: Relaix and Buckingham, 1999; Kawakami, *et al.*, 2000; Wawersik and Maas, 2000; Hanson, 2001; Silver and Rebay, 2005). *Six* genes encode homeodomain DNA binding proteins (Seo, *et al.*, 1999; Kawakami, *et al.*, 2000) that can act either as transcriptional activators or repressors depending on the recruitment of appropriate cofactors. One group of such cofactors are the *Dach* proteins (Mardon, *et al.*, 1994; Hammond, *et al.*, 1998; Davis, *et al.*, 1999), nuclear factors which together with other repressors inhibit target gene transcription. In addition, *Dach* proteins themselves seem to bind DNA (Ikeda, *et al.*, 2002) and modulate BMP signalling by interacting with *Smad4* (Wu, *et al.*, 2003; Kida, *et al.*, 2004). *Eya* proteins represent transcriptional coactivators that are recruited to DNA via their interaction with *Six* proteins (Ohto, *et al.*, 1999; Silver, *et al.*, 2003). Recently, *Eya* proteins have been shown to have catalytic activity as protein phosphatases and this activity appears to be required for their function as activators (Li, *et al.*, 2003; Rayapureddi, *et al.*, 2003; Tootle, *et al.*, 2003). Direct binding has indeed been shown for *Eya* and *Dach* and *Six* and *Eya* proteins (Chen, *et al.*, 1997; Pignoni, *et al.*, 1997; Ohto, *et al.*, 1999; Ikeda, *et al.*, 2002; Li, *et al.*, 2003; Silver, *et al.*, 2003) and nuclear translocation of *Eya* protein is dependent on its interaction with

members of the *Six* family (Ohto, *et al.*, 1999). Furthermore, *Groucho* repressors have been shown to bind *Six* proteins, in particular *Six3* directly and thus modulate its activity (Kobayashi, *et al.*, 2001; Zhu, *et al.*, 2002).

So far only a few direct target genes have been identified, among them *cMyc* and *CyclinA1* and *-D1*, all involved in cell cycle control (Coletta, *et al.*, 2004; Yu, *et al.*, 2006). Indeed, both *Six1* and *Eya2* appear to promote tumorigenesis by enhancing proliferation (Coletta, *et al.*, 2004; Zhang, *et al.*, 2005; Yu, *et al.*, 2006), while high levels of *Eya2* seem to trigger apoptosis (Clark, *et al.*, 2002). In the otic vesicle, loss of *Eya1* and *Six1* leads to reduced proliferation, while in *Drosophila* loss of either *so*, *dac* or *eya* initially results in overgrowth followed by cell death (Bonini, *et al.*, 1993; Pignoni, *et al.*, 1997; Xu, *et al.*, 1999; Li, *et al.*, 2003; Ozaki, *et al.*, 2004; Kozlowski, *et al.*, 2005; Zou, *et al.*, 2006). In zebrafish, *Six1* plays opposite roles in hair cells and otic neurons that arise from common sensory patches. *Six1* induces apoptosis in neuronal precursors, but promotes proliferation in sensory hair cells (Bricaud and Collazo, 2006) thereby regulating the balance between both cell types. Thus, the *Six/Eya/Dach* network may control the number of placode precursors during early stages of development, differential proliferation and apoptosis during morphogenesis and the number of precursors for different cell types within placodes.

Although data in *Drosophila* show that the *Six/Eya/Dach* cassette can induce cell fate changes by making non-eye cells adopt an eye fate, the exact molecular mechanisms of how they operate during this process are still unknown (Bonini, *et al.*, 1997; Pignoni, *et al.*, 1997; Shen and Mardon, 1997; Weasner, *et al.*, 2007). *Eyeless* is directly regulated by *sine oculis*, however further targets have not been identified. In vertebrates, functional *Six* and *Eya* are required for myogenesis (Heanue, *et al.*, 1999) for review Relaix and Buckingham, 1999), but the evidence that they control cell specification without affecting proliferation during placode development is very poor. As mentioned above, misexpression of *Six1* promotes preplacodal gene expression (Brugmann, *et al.*, 2004), but by itself or in combination with *Eya* is insufficient to generate mature placodes or to activate *Pax* genes (Christophorou and Streit, unpublished). Since only cells within the preplacodal region are competent to respond to placode inducing signals, one potential role of *Six* and *Eya* genes may be to impart competence to such inducing factors.

In summary, there is considerable evidence for a crucial role of the *Six/Eya/Dach* network in various aspects of placode development, however in many cases the precise molecular mechanisms remain to be identified. Characterisation of direct targets in different cellular contexts will be an important step to understand their function.

#### **Signalling pathways inducing the neural plate border and the preplacodal region**

Formation of the preplacodal region is initiated through a series of events that first define the border of the neural plate and subsequently subdivide the border into placode and neural crest precursors. This is achieved through interactions with surrounding tissues – neural plate, future epidermis and the underlying head mesoderm – which secrete factors that promote or attenuate placode formation. Thus, different signalling pathways converge

to position the placode territory in the head ectoderm next to the neural plate.

### FGF pathway

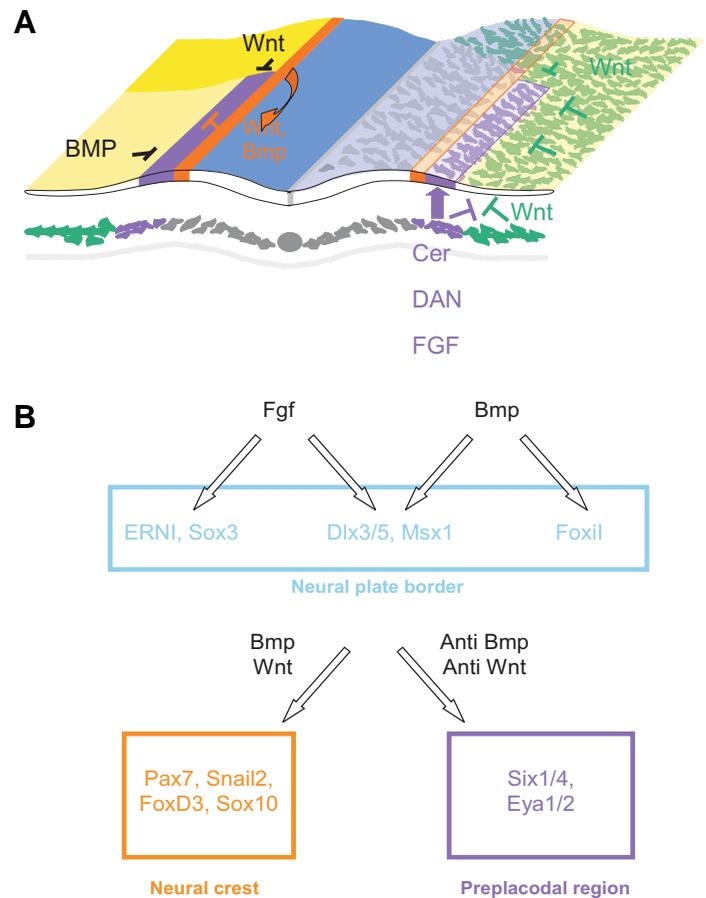
Several observations implicate FGFs as one of the factors that initiate the formation of the border region. In the chick, misexpression of FGF8 rapidly induces ectopic expression of a set of genes normally coexpressed in the border: *ERNI*, *Sox3*, *Dlx5* and *Msx1* (Streit and Stern, 1999; Streit, *et al.*, 2000; Litsiou, *et al.*, 2005). However, FGF alone is not sufficient to generate any of the cell types that arise from the border: neural crest and placodes (Mayor, *et al.*, 1997; LaBonne and Bronner-Fraser, 1998; Monsoro-Burq, *et al.*, 2003; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). In contrast, FGF inhibition using the antagonist SU5402 or dominant negative receptors shows that active signalling through the FGF pathway is required for at least some of the border genes (*Sox3*, *ERNI*; Streit, *et al.*, 2000) and for the generation of border derivatives (Mayor, *et al.*, 1997; LaBonne and Bronner-Fraser, 1998; Monsoro-Burq, *et al.*, 2003; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). Together, these findings argue for a role of FGFs in promoting border character in ectodermal cells as a prerequisite to generate neural crest and placode cells. Accordingly, FGFs are expressed in the head mesoderm and trunk paraxial mesoderm that comes to underlie the border region and in *Xenopus* at the edge of the neural plate (Niswander and Martin, 1992; Shamim and Mason, 1999; Streit and Stern, 1999; Ahrens and Schlosser, 2005).

In addition, FGFs seem to play a role in preplacodal induction at slightly later stages. FGF signalling from surrounding tissues (head mesoderm in chick, neural plate in *Xenopus*) is required for the induction of preplacodal markers, while ectopic expression of FGF8 promotes expression of *Eya2*, but not of any other placode specific gene (Brugmann, *et al.*, 2004; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). Thus, FGFs play a dual role in the supporting placode formation: initially they promote the expression of border genes and later initiate expression of a subset of preplacodal markers.

### BMP pathway

Modulation of BMP signalling has been widely implicated in early ectodermal patterning (Wilson, *et al.*, 1997; Marchant, *et al.*, 1998; Barth, *et al.*, 1999; Tribulo, *et al.*, 2003; for review Sasai and De Robertis, 1997; Aybar and Mayor, 2002; Stern, 2005). Indeed, *Foxil* is dependent on BMP signalling in fish and frogs (Matsuo-Takasaki, *et al.*, 2005; Phillips, *et al.*, 2006). In zebrafish, *Foxil* expression is reduced or lost in BMP7 and BMP2a mutants, while it is downregulated in *Xenopus* in the presence of the BMP antagonist Chordin. In contrast, overexpression of BMP4 causes an expansion of *Foxil* at the expense of neural tissue. Likewise, BMP signalling is required for *Dlx* gene expression in chick, frog and fish (Nguyen, *et al.*, 1998; Feledy, *et al.*, 1999; Pera, *et al.*, 1999; Luo, *et al.*, 2001), while *Msx1* is a direct target of BMP signalling and mediates its ability to promote epidermis (Suzuki, *et al.*, 1997). These findings implicate BMP activity, like FGF signalling, in the regulation of border specific genes.

One model mainly based on experiments in *Xenopus* suggests that a gradient of BMP activity within the ectoderm acts to



**Fig. 3. Model for induction of the preplacodal region. (A)** The diagram shows a cross section through a chick embryo at the 2-4 somite stage viewed from anterior towards posterior (modified from Litsiou, *et al.*, 2005). Ectodermal signals that influence the position of the preplacodal region are schematised on the left, whereas mesoderm derived signals are shown on the right. The preplacodal region (purple) is surrounded by inhibitory signals from the lateral (light yellow; BMP) and posterior (yellow; Wnt) ectoderm, from the neural folds (orange; Wnt, BMP) and from the lateral and posterior mesoderm (green; Wnt). FGF, Wnt antagonists and BMP antagonists (purple) from the mesoderm underlying the preplacodal region protect the overlying ectoderm from these inhibitory signals and allow the formation of placode precursors. **(B)** Signals and transcription factors in the border and preplacodal region. FGFs and BMPs act upstream of border specific transcription factors. Once the border is established, levels of BMP and Wnt signalling determine whether border cells generate neural crest or placode cells.

allocate different cell fates for review (Sasai and De Robertis, 1997; Aybar and Mayor, 2002; Vonica and Brivanlou, 2006). In support of this idea, *Xenopus* animal caps treated with different concentrations of BMP antagonists form epidermis in the presence of high levels of BMP activity, while neural crest and preplacodal cells are generated at intermediate and neural plate at low levels (Wilson, *et al.*, 1997; Tribulo, *et al.*, 2003; Brugmann, *et al.*, 2004; Glavic, *et al.*, 2004). Likewise, zebrafish mutants with reduced BMP activity (and thus a shallower gradient) show a relatively larger expansion of the neural crest territory than of the neural plate (Nguyen, *et al.*, 1998; Barth, *et al.*, 1999). However, the placode territory is merely displaced,

but not expanded and different placodes are affected differentially arguing against a simple gradient model (Neave, *et al.*, 1997; Nguyen, *et al.*, 1998).

In chick, the main region sensitive to modulation of BMP signalling is the neural plate border itself (Streit and Stern, 1999). Here, misexpression of BMP antagonists leads to a shift of the border towards the non-neural ectoderm, while misexpression of BMP4 narrows the neural plate and shifts the border medially. In contrast, modulation of the BMP pathway away from the border does not have any effect. Likewise, local reduction of BMP signalling close to the preplacodal region expands this territory in chick and *Xenopus*, but is not sufficient to induce it ectopically in future epidermis (Glavic, *et al.*, 2004; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). One possible explanation to reconcile these differences is that *Xenopus* animal caps may contain border territory and are therefore particularly sensitive to changes in BMP activity.

Thus, modulation of BMP activity and loss- or gain-of-function experiments for border specific transcription factors show the same effect: they shift the border of the neural plate. It is therefore likely that BMP signalling acts via mediators such as Foxil, Dlx and Msx to alter preplacodal gene expression indirectly.

#### Wnt pathway

As discussed above, both FGF and BMP pathways modulate the expression of preplacodal genes: FGF8 activates *Eya2*, while inhibition of BMP signalling expands preplacodal markers (Brugmann, *et al.*, 2004; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). However, even the combination of FGF and BMP antagonists is not sufficient to induce preplacodal character in ectoderm away from the endogenous placode territory or in the future trunk ectoderm (Brugmann, *et al.*, 2004; Ahrens and Schlosser, 2005; Litsiou, *et al.*, 2005). Like inhibition of BMP, misexpression of Wnt antagonists leads to an expansion of preplacodal gene expression at the expense of future epidermis (Brugmann, *et al.*, 2004; Litsiou, *et al.*, 2005). Interestingly, *Six1*, *-4* and *Eya2* also extend into the trunk ectoderm, where placode formation is not normally observed. In contrast, activation of canonical Wnt signalling represses preplacodal gene expression suggesting that Wnt attenuation allows the specification of placode precursors (Litsiou, *et al.*, 2005). Furthermore, a combination of FGF with Wnt and BMP antagonists induces an ectopic preplacodal region in naïve ectoderm in the absence of neural and mesodermal tissue indicating that these factors promote placode character directly (Litsiou, *et al.*, 2005). Thus, temporal and spatial integration of all three signals is important to generate placode precursors.

Unlike placode precursors, neural crest cells require canonical Wnt signalling (for review: Aybar and Mayor, 2002; Knecht and Bronner-Fraser, 2002). These findings suggest that at the border of the neural plate exposure to different levels of Wnt activity determines whether cells adopt placode or neural crest cell fates. Indeed, activation of Wnt signalling expands neural crest markers into the placode territory, while its inhibition has the opposite effect (Litsiou, *et al.*, 2005). In this context it is interesting that at slightly later stages when the otic placode begins to form, Wnt signalling promotes placode formation: Pax2<sup>+</sup> cells that activate the pathway become otic, while those

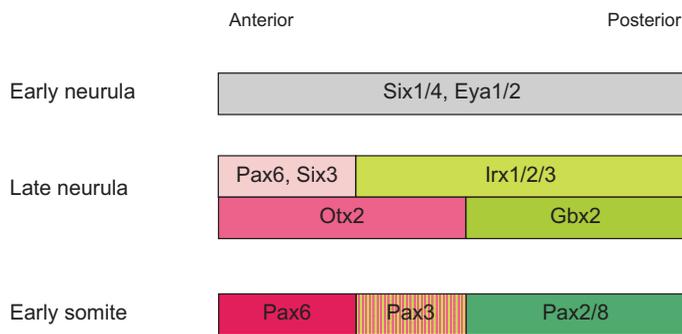
that do not, develop into epidermis (Ohyama, *et al.*, 2006). These findings highlight that interpretation of the same signalling pathway is highly context dependent and is determined by the developmental history of individual cells.

#### A model for induction of the preplacodal region

The data summarised above highlight that induction of the preplacodal region is a multi-step process, which requires the integration of different signals produced by different tissues. The following model tries to integrate tissue interactions and signalling pathways (Fig. 3). As a first step, a border territory is set up in the early neurula ectoderm between future neural and epidermal cells. Genes specific for this region are under the control of FGF and/or BMP signalling. *BMP4* and *-7* are expressed in the non-neural ectoderm and transcript levels and phospho-smad activity are highest at the edge of the neural plate (Fainsod, *et al.*, 1994; Streit, *et al.*, 1998; Streit and Stern, 1999; Faure, *et al.*, 2002), which in turn may lead to the upregulation of some border genes (*Dlx*, *Msx*, *Foxil*). FGFs emanating from the organiser and the mesoderm underlying the border maintain the expression of *Sox3* and ERNI and cooperate with BMPs to promote *Dlx* and *Msx* gene expression. Once established the border gives rise to two different cell types: neural crest and placodes. *BMP4* and *-7* transcripts concentrate in the forming neural folds (Fainsod, *et al.*, 1994; Liem, *et al.*, 1995), where Wnts begin to be expressed; together they promote formation of neural crest cells. The future heart mesoderm expands anteriorly and comes to underlie the placode territory (Kimmel and Warga, 1988; Keller and Tibbetts, 1989; Tam, *et al.*, 1997; Redkar, *et al.*, 2001; Hochgreb, *et al.*, 2003). This tissue expresses FGF4, the BMP antagonist DAN and the Wnt inhibitor Cerberus (Ogita, *et al.*, 2001), while more lateral and posterior mesoderm contains high levels of *Wnt8c* (Hume and Dodd, 1993; Litsiou, *et al.*, 2005). *Wnt6* is found in the trunk, but not the head ectoderm (Garcia-Castro, *et al.*, 2002; Schubert, *et al.*, 2002). Thus, the preplacodal region is surrounded by inhibitory factors at its medial, lateral and posterior edges. Signals from the heart mesoderm protect the overlying ectoderm from these inhibitory influences and allow it to adopt placode fate.

#### Anterior-posterior patterning of the preplacodal region

Within the preplacodal region precursors for different placodes are intermingled, although some separation of individual populations along the anterior posterior axis is already apparent. Precursors for anterior placodes (adenohypophysis, olfactory, lens) are located in the rostral preplacodal region, while precursors for posterior placodes (trigeminal, epibranchial, otic, lateral line) are restricted more caudally (D'Amico-Martel and Noden, 1983; Couly and Le Douarin, 1985; Couly and Le Douarin, 1988; Kozłowski, *et al.*, 1997; Streit, 2002; Bhattacharyya, *et al.*, 2004; Litsiou, *et al.*, 2005). This approximate subdivision is reflected by the onset of regionally restricted expression of transcription factors (and few other genes), shortly after the induction of the placode territory. As development proceeds, the preplacodal region becomes molecularly divided in successively smaller sub-domains such that by the time placodes can be identified morphologically each appears to have a unique transcription factor code (Torres and Giraldez, 1998; Bailey and Streit, 2006; Schlosser, 2006). These changes



**Fig. 4. Anterior-posterior patterning of the preplacodal region.** At early neurula stages preplacodal markers are expressed along the entire length of the preplacodal region (anterior to the left, posterior to the right). Soon thereafter, regionalised gene expression is apparent: *Pax6*, *Six3* and *Otx2* are concentrated anteriorly, while *Ir1-3* and *Gbx2* are restricted posteriorly. At early somite stages *Pax3* is upregulated in the ophthalmic part of the trigeminal region and *Pax2* and *-8* in the otic/epibranchial territory. Note: the relative boundaries of gene expression are inferred from data in the literature that show in situ hybridisation with a single gene (references see text). Double in situ hybridisation is required to confirm this model.

in gene expression have recently been reviewed in detail in *Xenopus* (Schlosser, 2006) and therefore only some of the early aspects of patterning will be highlighted below.

#### Transcription factors in anterior-posterior patterning

At neurula stages, the rostral preplacodal region begins to express *Otx2*, *Six3*, *Pitx3*, *Dmbx1* and *Pax6*, while its caudal part is characterised by *Ir1*, *-2* and *-3* and *Gbx2* (Li, *et al.*, 1994; Bally-Cuif, *et al.*, 1995; Oliver, *et al.*, 1995; Pannese, *et al.*, 1995; Hirsch and Harris, 1997; Bellefroid, *et al.*, 1998; Gomez-Skarmeta, *et al.*, 1998; Shamim and Mason, 1998; Goriely, *et al.*, 1999; Zhou, *et al.*, 2000; Glavic, *et al.*, 2002; Gogoi, *et al.*, 2002; Matsumoto, *et al.*, 2004; Dutta, *et al.*, 2005; Zilinski, *et al.*, 2005; Liu, *et al.*, 2006; for review: Schlosser, 2006). Although these patterns appear to be roughly complementary, closer inspection reveals that different transcripts do not share the same rostro-caudal boundary (Fig. 4). Rather pairs of transcription factors have boundaries at different levels: *Six3* expression abuts *Ir1*, while *Otx2* and *Gbx2* abut at slightly more posterior levels. These patterns are very reminiscent of their expression in the neural plate, where the same pairs of genes control the subdivision of the brain into different compartments (for review: Martinez, 2001; Nakamura, 2001; Hidalgo-Sanchez, *et al.*, 2005). *Gbx2* and *Otx2* are involved in positioning the midbrain-hindbrain boundary (Broccoli, *et al.*, 1999; Millet, *et al.*, 1999; Katahira, *et al.*, 2000; Acampora, *et al.*, 2001; Li and Joyner, 2001), while *Six3* and *Ir3* define a boundary in the forebrain that later corresponds to the zona limitans intrathalamica (Kobayashi, *et al.*, 2002).

At early somite stages, members of the Pax gene family become expressed in more restricted domains within the preplacodal region (Bang, *et al.*, 1997; Hirsch and Harris, 1997; Li, 1994 #195; Stark, *et al.*, 1997; Heller and Brandli, 1999; Groves and Bronner-Fraser, 2000). *Pax3* is detected in the ophthalmic part of the trigeminal, *Pax8* in the otic and *Pax2* epibranchial, otic and lateral line territory. Together Pax genes cover the entire placode region in non-overlapping patterns except *Pax2* and *-8*

which are co-expressed in the future otic placode. Interestingly, in the neural plate *Pax2* and *-6* represent another pair of transcription factors that position a boundary, in this case between future diencephalon and mesencephalon (Okafuji, *et al.*, 1999; Schwarz, *et al.*, 1999; Matsunaga, *et al.*, 2000; Schwarz, *et al.*, 2000). As it is the case for *Six3/Ir3* and *Otx2/Gbx2*, in the brain *Pax2* and *-6* negatively cross regulate each other, leading to sharpening of the molecular boundary and separation of different cell fates. It is tempting to speculate that the same molecular mechanisms that pattern the brain also operate to impart regional identity to the placodes.

Loss of *Otx2* function results in severe defects in the head including the brain, olfactory and lens placode as well as patterning of the otic vesicle (Acampora, *et al.*, 1995; for review: Acampora, *et al.*, 2001). However, because of the severe fore- and midbrain defects, it has been difficult to assess its direct function in placode development without the availability of tissue specific knock outs. Mice deficient in *Gbx2* (Lin, *et al.*, 2005) and *Pax2* function show patterning defects in the otic vesicle (Torres, *et al.*, 1996; Burton, *et al.*, 2004), while loss of *Six3* or *Pax6* affects lens and olfactory development (Hogan, *et al.*, 1988; Quinn, *et al.*, 1996; Grindley, *et al.*, 1997; Lagutin, *et al.*, 2003; Liu, *et al.*, 2006). In *Xenopus*, *Ir1* is required for the expression of the early preplacodal marker *Six1* and later placode specific genes like *Sox2* and *Pax2* (Glavic, *et al.*, 2004). Thus, mutation in or loss of any of these genes leads to defects in placode development, although their role in early patterning of the preplacodal region remains elusive probably due to functional redundancy with other members of the same family that are expressed in similar patterns (Schlosser, 2006).

#### Signalling pathways in anterior-posterior patterning

In the neural plate, regional identity is initially set up through the graded activity of Wnts, FGF and retinoic acid, all of which possess posteriorising activity and control some of the transcription factors described above (for review: Yamaguchi, 2001; Wilson and Houart, 2004; Kiecker and Lumsden, 2005; Rhinn, *et al.*, 2006). Do the same signalling pathways control anterior posterior patterning in the preplacodal region? Experiments in *Xenopus* revealed that the formation of neural crest cells indeed requires Wnt and retinoic acid activity and that anterior neural folds, which normally do not generate neural crest cells, do so in the presence of these factors (Villanueva, *et al.*, 2002). In chick and *Xenopus*, the expression of preplacodal markers can be expanded into the trunk ectoderm in the presence of Wnt antagonists (Brugmann, *et al.*, 2004; Litsiou, *et al.*, 2005). In contrast, the zebrafish mutants masterblind and headless, in which Wnt signalling is overactivated, show a loss of anterior placodes (lens, olfactory), but an expansion of trigeminal neurons around the anterior neural plate (Kim, *et al.*, 2000; Heisenberg, *et al.*, 2001). Thus, differential activation of the Wnt pathway along the rostro-caudal axis influences patterning of the preplacodal region suggesting that the mechanisms that allocate regional identity in the neural plate may act more globally to pattern the entire ectoderm.

#### Cells in the preplacodal region share a common developmental programme

As outlined at the beginning of this review, placodes form diverse structures with different functional properties and a vari-

ety of different cell types. Yet, they have variously been grouped together as a family and considered as related structures. Does this grouping reflect a meaningful developmental concept and does the preplacodal region represent a 'basic placode state'? At least two conditions need to be fulfilled. First, at some point along their developmental history placode cells should acquire a unique state that distinguishes them from other ectodermal derivatives. This is clearly the case (see above): placode precursors occupy a unique region in the embryonic ectoderm surrounding the cranial neural plate and they express a unique set of molecular markers. Furthermore, the preplacodal region is induced by a specific combination of tissues and signals that is different from the signals that induce neural, neural crest and epidermal precursors. Second and more importantly, the 'preplacode state' should be a unique property of placode precursors and be a prerequisite for cells to become mature placodes.

Recent experiments on otic induction in the chick embryo provide strong evidence that indeed ectodermal cells have to acquire preplacode character before they are responsive to the otic inducer FGF (Martin and Groves, 2006). Only cells within the preplacodal territory are responsive to FGF, while future epidermal cells are not. Anterior epiblast from chick gastrula stage embryos normally never gives rise to the otic placode, but is competent to do so when transplanted into the ectoderm next to the hindbrain, where the otic placode normally forms. Likewise, explants of anterior epiblast do not express otic markers when cultured in isolation or when treated with FGF. However, when the same tissue is first transplanted into the preplacodal region for a brief period (ca. 8 hrs), it initiates gene expression characteristic for this territory (*Eya2*, *Dlx*) and when explanted can now respond to FGF by expressing otic markers. These experiments support the idea that the preplacodal region has unique properties and suggest that the acquisition of preplacodal character is an essential step in otic induction and a prerequisite for cells to form mature placodes.

But do all placode precursors share a common developmental history? Recent evidence indicates that at the very least they initially share common characteristics: unlike any other part of the ectoderm, the entire preplacodal region is specified as lens (Bailey, *et al.*, 2006). When the preplacodal region is subdivided into four portions along the anterior posterior axis and cultured in neutral environment, no markers specific for olfactory, trigeminal or otic placodes are expressed – the tissue is not specified. However, in the same experiment, explants from all parts of the preplacodal region, even cells that normally never contribute to the lens, but to the otic placode, form lens-like structures. These explants follow the same sequence and timing of gene expression as observed during normal lens development: they initially express *Pax6*, followed by *L-maf* and *Foxc1* and finally,  $\delta$ - and  $\alpha$ -crystallin. Thus, all placode cells regardless of their ultimate fate initially possess lens character suggesting that in normal development placode induction is intimately linked with lens suppression. The lens probably represents the simplest placode derivative: it only generates two cell types, lens fibre and lens epithelial cells. The only other non-neurogenic placode, the anterior pituitary, which develops in the anterior midline, is easily transformed into lens in the absence of sonic hedgehog from underlying axial structures (Sbrogna, *et al.*, 2003; Dutta, *et al.*, 2005; Zilinski, *et al.*, 2005). In contrast to the lens and anterior pituitary, all other

placodes give rise to sensory neurons and/or sensory cells. Thus, lens suppression must be accompanied by the acquisition of neurogenic properties in non-lens placodes. Together, these results provide good support for the concept of a common ground state for all sensory placodes and for the importance of the preplacodal region in the developmental history of placode cells.

What are the lens repressing signals? FGFs appear to be the main players to initiate lens repression: activation of the FGF pathway inhibits the expression of the presumptive lens marker *Pax6* (Bailey, *et al.*, 2006). FGFs (FGF3, FGF10, FGF19 depending on species) also play an important role in otic induction (Ladher, *et al.*, 2000; Vendrell, *et al.*, 2000; Leger and Brand, 2002; Maroon, *et al.*, 2002; Wright and Mansour, 2003) and lens specification is abolished in preplacodal explants in the presence of FGF2, while the otic marker *Pax2* is induced (Bailey and Streit, unpublished). In contrast, exposure to FGF8 is sufficient to induce olfactory character from lens specified ectoderm (Bailey, *et al.*, 2006). Finally, FGF signalling (FGF3, -8) has been implicated in epibranchial placode specification and in the generation of the adenohypophysis, a placode forming in the anterior midline (Herzog, *et al.*, 2004; Nechiporuk, *et al.*, 2007; Nikaido, *et al.*, 2007; Sun, *et al.*, 2007). Thus, FGFs play a key role in restricting lens fate and in simultaneously inducing other placodes. The next important question to address is how the activation of the same signalling pathway in the preplacodal region elicits different responses leading to the formation of placodes with different identities.

## Future perspectives

In summary, prior to the appearance of morphological placodes induction of the preplacodal region is an essential process, which imparts unique identity to all placode precursors. The signalling pathways involved are similar to those implicated in neural and neural crest induction, however, timing and levels differ. Recent evidence argues that all placodes initially share a common ground state as lens and thus possess a common developmental history, before they diversify later. Surprisingly, FGF signalling appears to play a role during the induction of different placodes from this ground state. The future challenge is to unravel how different pathways cooperate with FGF signalling to impart placode identity and to establish networks of transcription factors that control this process.

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