27 DETERMINATION OF PREPLACODAL ECTODERM AND SENSORY PLACODES

SALLY A. MOODY

Department of Anatomy and Cell Biology, The George Washington University, Washington, DC

ABSTRACT

During development, the ectoderm adjacent to the neural plate becomes specialized to form numerous peripheral sensory structures. In the vertebrate head, these organs derive from two regions of lateral ectoderm: the neural crest and the cranial sensory placodes. Although the regulation of neural crest development has been studied for decades, only recently have some of the genes involved in placode development been revealed by both work on gene function in model animals and by identifying mutations involved in human craniofacial defects. This chapter reviews recent findings involving the induction and specification of the ectoderm that gives rise to the cranial sensory placodes, it describes the known transcription factors and signaling pathways involved in the regulation of placode fate and initial differentiation, and it identifies some of the human congenital defects that are caused by mutations in these genes.

INTRODUCTION

The vertebrate head contains a number of specialized sensory organs that arise from embryonic ectodermal thickenings called the *cranial sensory placodes* (von Kupffer, 1891; reviewed by Webb and Noden, 1993; Baker and Bonner-Fraser, 2001; Streit, 2004; Brugmann and Moody, 2005; Schlosser, 2005, 2006). During gastrulation, the ectoderm surrounding the anterior neural plate becomes specified to form peripheral sensory structures, a region that is called the *lateral neurogenic zone* (Figure 27.1). The more medial region of this zone, which includes the edge of the neural plate, gives rise to the neural crest, and the more lateral region gives rise to a preplacodal ectoderm (PPE), which later separates into individual cranial sensory placodes (Knouff, 1935; LeDouarin et al., 1986; reviewed by Schlosser and Northcutt, 2000). The cranial sensory placodes are distinct from other ectodermal thickenings (also

Principles of Developemental Genetics

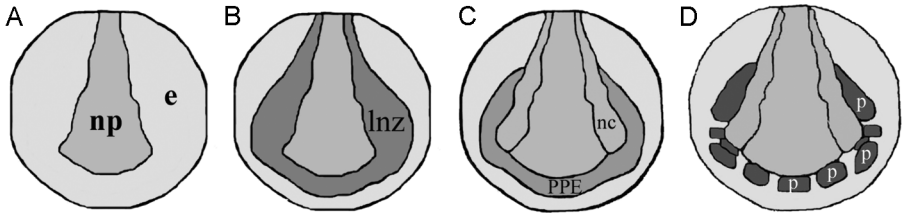

FIGURE 27.1 **The ectodermal domains of the Xenopus embryo at different developmental stages. A,** At gastrulation, the early embryonic ectoderm is divided into the neural plate *(np, blue)* and epidermis *(e, yellow)* domains. **B,** Interactions between these two domains establish a border region *(green)* called *the lateral neurogenic zone (lnz)*, which will give rise to **C,** the medial neural crest *(nc, light green)* and the lateral preplacodal ectoderm *(PPE, darker green)*. **D,** Subsequently, the preplacodal ectoderm breaks up into the individual placodes *(p, dark green)*.

called *placodes*) that form in the nonneural epidermis to give rise to the teeth, hair follicles, and feathers (Pispa and Thesleff, 2003; see also the chapter by Thesleff in this book). During neurulation, signals from underlying tissues Au1
cause the PPE to separate into many discrete placodes, which are histologically recognized as patches of thickened ectoderm and which have distinct developmental fates (Figures 27.1 and 27.2). These placodes will then produce both the structural and neural elements of numerous cranial secretory tissues and sensory organs, including the anterior pituitary gland, the olfactory epithelium, the lens, and the auditory and vestibular organs. In addition, cranial nerve sensory ganglia contain cells derived from both the placodes and the neural crest (see the chapter by Mayor in this book). Thus, the PPE gives rise Au2
to many important structures in the vertebrate head. However, although the

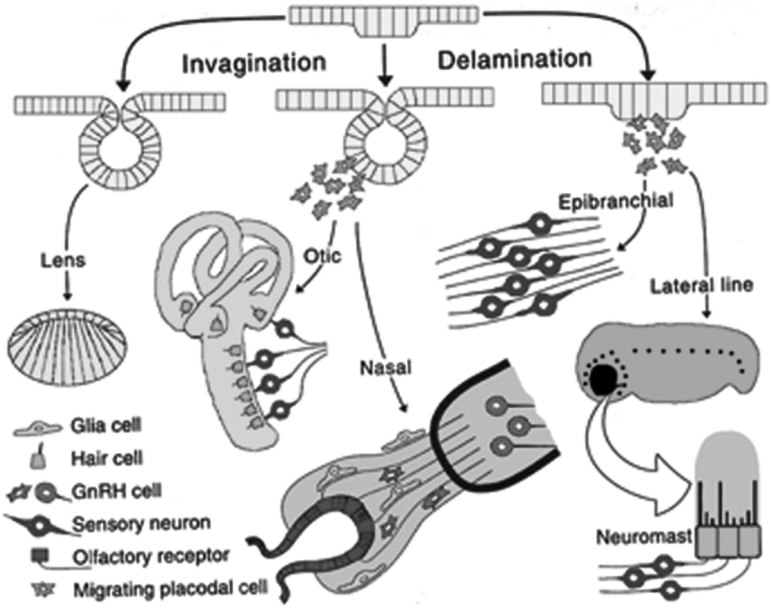

FIGURE 27.2 Placodes give rise to several sensory derivatives. The initial epithelial thickening can invaginate to give rise to a pit (olfactory) or a vesicle (hypophyseal, lens, otic), or cells can simply delaminate and migrate to a secondary position (cranial ganglia, lateral line). Note the numerous cell types that derive from the cranial placodes.Modified from Webb and Noden, 1993 and from Brugmann and Moody, 2005.

cranial placodes have been histologically recognized for more than a century, very little is known about the molecular mechanisms that specify the development of these important sensory precursors. Recent identification of genes that are highly expressed in the PPE and in individual placodes has allowed researchers to begin to reveal the molecular pathways that induce and specify the fate of these important embryonic cells.

I. CRANIAL SENSORY PLACODES GIVE RISE TO DIVERSE STRUCTURES

Structurally, a lot is known about the development of the various cranial sensory placodes. This development is characterized by extensive morphogenetic rearrangements. Some placodes (i.e., hypophyseal, olfactory, lens, otic) invaginate as cup-shaped structures (see Figure 27.2). The olfactory cup, which is also called the *olfactory* or *nasal pit,* further folds to line the nasal cavity as a sensory epithelium. The hypophyseal, lens, and otic cups, however, continue to invaginate, and they eventually pinch off from the surface ectoderm to form epithelial-lined vesicles that then further differentiate into highly specialized structures. In other placodes, the underlying basement membrane of the thickened epithelium becomes fragmented, thereby allowing cells to delaminate from the epithelium. Some migrate within the ectoderm to form patches of sensory organs (e.g., the lateral lines), and some migrate away from the surface ectoderm to coalesce within the head mesenchyme as sensory ganglia.

In each placode, the cells can adopt a variety of cell fates, including secretory cells, sensory receptor cells, neurons, glia, or supporting cells, depending on their placode of origin (see Figure 27.2). The hypophyseal placode (also called *adenohypophyseal*) first lies on the midline surface of the embryo, and it later occupies the dorsal midline of the oral ectoderm as the stomodeum forms. It then invaginates to form Rathke's pouch, and it pinches off as a vesicle to form the adenohypophysis (anterior pituitary gland), the cells of which secrete a number of peptide hormones. The olfactory placode (also called *nasal*) gives rise to the primary olfactory receptor neurons that detect odorants, glia, mucus-secreting, and structural cells of the olfactory epithelium; in some animals, it also gives rise to the related vomeronasal epithelium, which detects pheromones. In addition, some cells migrate from the olfactory Au3
placode into the hypothalamus to become secretory components of the gonadotropin-releasing hormone system, and some coalesce into a small ganglion that is associated with the terminal nerve. The lens placode gives rise to the lens vesicle in the anterior segment of the eye, which contains the crystalline-secreting cells that focus light on the neural retina. Two placodes contribute neurons to the trigeminal ganglion: the ophthalmic placode (also called *profundal*), which is located dorsal to the eye, and the trigeminal placode (also called *Gasserian* or *maxillomandibular*), which is located just caudal to the eye. These cells are equivalent to dorsal root ganglion cells, and they provide the sensory innervation of the face, the oral cavity, and the scalp. The otic placode gives rise to both the auditory and vestibular parts of the entire inner ear, including the mechanosensory hair cells, the supporting cells, the endolymph secreting cells, the biomineralized otoliths, and the vestibuloacoustic ganglia (see the chapter by Fekete and Sienknecht in this book). A Au4
series of epibranchial placodes forms in the branchial arch ectoderm between adjacent endodermal pharyngeal pouches ventral to the otic placode. Cells

from the epibranchial placodes migrate into the branchial mesenchyme to become neurons in the distal sensory ganglia of three cranial nerves. Those of the facial nerve (called the *geniculate ganglion*) innervate the taste buds; those of the glossopharyngeal nerve (called the *petrosal ganglion*) innervate the taste buds, the heart, and the visceral organs; and those of the vagus nerve (called the *nodose ganglion*) innervate the heart and other visceral organs.

All vertebrates have these placodes in common, but there are numerous species variations (reviewed by Baker and Bronner-Fraser, 2001; Schlosser, 2005, 2006). For example, in some animals, the derivatives of the profundal and trigeminal placodes are maintained as separate sensory ganglia, and, in some, there are up to six separate epibranchial placodes with associated ganglia. In amphibians and fish, there is an additional lateral line sensory system that is specialized for aquatic life. This system consists of islands of sensory organs (receptor and supporting cells as well as the sensory neurons that innervate them) that have striking similarities to inner ear receptor organs and that are distributed across the head and trunk epidermis. Mechanoreceptive neuromast organs detect water turbulence, and electroreceptive ampullary organs detect electrical fields. Lateral line cells derive from a dorsolateral placode located adjacent to the otic placode. In some amphibians, there are also hypobranchial placodes that are located ventral to the second and third pharyngeal pouches that give rise to the hypobranchial ganglia, the function of which is presently unknown (Schlosser, 2003). We do not yet understand the evolutionary mechanisms that have given rise to the diversity of these structures across species (reviewed by Schlosser, 2005; see also the chapter by Swalla in this book), but, as is discussed in the later sections of this chapter, many of the genes involved in placode development are highly conserved from invertebrates to vertebrates.

II. INITIAL FORMATION OF THE PREPLACODAL ECTODERM

The classic descriptions of cranial sensory placode formation proposed that all of these distinct structures derive from a common precursor region called the *PPE,* which forms around the anterior margin of the neural plate (see Figure 27.1). Although there are data that suggest that each placode may be individually induced and specified during development (Graham and Begbie, 2000; Begbie and Graham, 2001), fate mapping and gene expression studies strongly argue that the cranial sensory placodes derive from a common precursor region that is distinct from the neighboring ectodermal fields and that this region is molecularly biased initially toward a general placodal fate (reviewed by Streit, 2004; Ahrens and Schlosser, 2005; Schlosser, 2005, 2006). Because the neural crest and the placodes are both derived from the lateral ectoderm that surrounds the neural plate and because both tissues contribute to the peripheral nervous system, it has been suggested that the placodes might be induced by mechanisms similar to those that induce the neural crest (see also the chapter by Mayor in this book). However, there are several reasons that there also should be distinct differences. The PPE forms lateral to the neural crest and extends around the most rostral tip of the neural plate, whereas neural crest is absent from this region; placodes form only in the head, whereas neural crest cells extend to the caudal regions of the trunk (see Figure 27.1). It has only been in recent years, as a conse-

quence of the cloning and of the characterization of several genes that are highly expressed in the PPE and early placodes, that it has been possible to experimentally examine the mechanisms that induce the PPE and to determine how these compare with those that induce neural crest.

A. The Role of Neural Plate/Nonneural Ectoderm Signaling

Several studies in many animal models demonstrated that the formation of a lateral border zone that gives rise to the neural crest requires an interaction between the neural plate and the nonneural ectoderm as the early neural plate forms (reviewed by Meulemans and Bronner-Fraser, 2004; see also the chapter by Mayor in this book). These interactions appear to initiate the expression of Au8 transcription factors (e.g., *dlx3, msx1 pax3, zic*) that have the following characteristics: (1) they are typical of the lateral border zone; (2) they are necessary for the endogenous expression of "neural crest-specifying" genes; and (3) they cause the ectopic induction of several neural crest markers at the margin of a piece of neural plate grafted into a nonneural ectodermal domain. Evidence of a similar interaction to initiate the development of the PPE is now accumulating. A large body of literature demonstrates that signals from the neural plate are required for the induction of individual placodal structures (reviewed by Baker and Bronner-Fraser, 2001). These studies mostly analyzed late stages using placode-specific markers or morphology to indicate the induction of the tissue of interest (e.g., the otic vesicle, which can be easily identified by histology) and thus do not directly address whether this interaction is necessary for the induction of the panplacodal fate of the PPE. However, recent studies have taken advantage of newly described genes expressed in the early PPE (e.g., *six1, eya1, Xiro1*) to show that neural plate grafts placed into nonneural ectoderm also induce PPE (Woda et al., 2003; Glavic et al., 2004; Ahrens and Schlosser, 2005). Thus, the interaction between the newly formed neural plate and the adjacent nonneural ectoderm specifies a lateral neurogenic zone that commonly gives rise to both the neural crest and the PPE (Figures 27.1 and 27.3). What has not been clear from these studies is whether the interaction specifies two separate domains (neural crest and PPE) or a single, presensory zone that later separates into two fields.

The latter idea is supported by recent studies that indicate that transcription factors expressed in the nonneural ectoderm are required for both neural crest and PPE formation. One factor, *foxi1,* is a member of the Drosophila *forkhead* family. During gastrulation, it is expressed throughout the anterior–ventral embryonic ectoderm, and later it is expressed in a U-shaped domain that surrounds the anterior neural plate (Matsuo-Takasaki et al., 2005). At first, its expression domain extends to the border of the *sox2*-expressing neural plate ectoderm, but later it recedes from the lateral neurogenic zone surrounding the anterior neural plate. In ectodermal (animal cap) explants, *foxi1* expression is induced by bone morphogenetic protein (BMP) and repressed by Chordin, and, in whole embryos, its expression domain is expanded by BMP mRNA injection; this is typical of epidermal genes (see also the chapter by Itoh and Sokol in this book). The knockdown of *foxi1* expres- Au9 sion by the injection of antisense morpholino oligonucleotides (MOs) expands the *sox2*-expressing neural plate domain, but MO also represses the expression of both neural crest *(foxD3)* and placodal *(six1, eya1)* genes, which indicates that its early expression at the neural/nonneural border is required for both

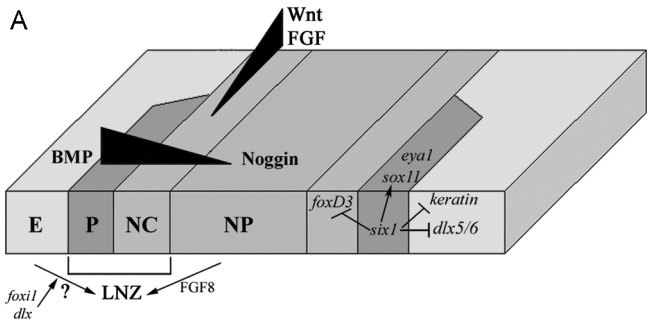

FIGURE 27.3 Several steps are involved in forming the preplacodal ectoderm (PPE). The embryonic ectoderm has been flattened into a sheet and into the four major domains illustrated. *E,* Epidermis; *P,* PPE; *NC,* neural crest; *NP,* neural plate. Inductive interactions are depicted on the left side. First, interactions between the neural plate and the epidermis *(bottom of figure)* cause a lateral neurogenic zone *(LNZ)* to form; this will further divide into neural crest and preplacodal ectoderm. Fibroblast growth factor (FGF)8 is likely one of the responsible signals from the neural plate. The *foxi1* and *dlx* genes are likely to regulate yet-to-be identified signaling factors from the epidermis. In addition, the four ectodermal domains are specified in response to a gradient of bone morphogenetic protein (BMP) signaling, which is antagonized by anti-BMP factors from the midline, such as Noggin. Finally, a gradient of posteriorizing signals (Wnt, FGF) is required for neural crest formation and for the inhibition of PPE formation. On the right side are some of the genes that are expressed in the neural crest, in the PPE, and in the epidermis. Experiments show that *six1* promotes the expression of placode genes *(sox11, eya1)* at the expense of neural crest *(foxD3)* and epidermal *(keratin, dlx)* genes.

derivatives of the lateral neurogenic ectoderm. Similar results have been reported for three *dlx* genes *(dlx3, dlx5, dlx6)*, which are related to Drosophila *distal-less*. They are initially expressed throughout the nonneural ectoderm, and they are induced by BMPs (Luo et al., 2001a, b; Woda et al., 2003). In Xenopus, the initial expression boundaries of *dlx5* and *dlx6* abut the neural plate, whereas the expression boundary of *dlx3* abuts the lateral neurogenic zone. Gain-of-function studies in frog indicate that these genes repress neural plate genes and that they are required for the expression of both neural crest and placode markers during gastrulation stages (Feledy et al., 1999; Beanan and Sargent, 2000; Luo et al. 2001a, Woda et al., 2003). In the chick, *dlx5* expression also represses neural plate markers and promotes neural fold markers (McLarren et al., 2003). These experiments suggest that *dlx* genes promote the formation of the lateral neurogenic border zone. This was confirmed by experiments in which neural plate tissue was grafted into nonneural ectoderm (Woda et al., 2003). Both neural crest and PPE markers were induced when grafts were placed in areas that expressed *dlx* genes, but, when grafts were placed in a region where the activity of all *dlx* genes was downregulated by the expression of a pan-*dlx*-repressor construct, neither marker was induced. The common effects on both neural crest and PPE genes and the timing of the effects reported in these studies indicate that both *foxi1* and *dlx* genes have an early function in subdividing the embryonic ectoderm into neural versus nonneural domains and in establishing a lateral region in the ectoderm surrounding the neural plate that can give rise to both the neural crest and PPE (see Figure 27.3).

The nature of the signal(s) between the neural plate and the nonneural ectoderm that mediate the formation of the lateral neurogenic zone is still uncertain. However, several experiments implicate members of the fibroblast growth factor (FGF) family. Older studies that focused on individual placode

markers or morphology showed a role for FGF3 and FGF8 in a variety of embryos (reviewed by Baker and Bronner-Fraser, 2001). More recent studies in Xenopus and chick using PPE molecular markers showed that FGF8, which is expressed in the anterior neural plate, is involved (Ahrens and Schlosser, 2005; Litsiou et al., 2005). Experimental manipulations that increased FGF8 levels in nonneural ectoderm induced PPE markers, and a reduction of FGF signaling either with a general FGF receptor inhibitor or with FGF8-specific MOs repressed PPE markers. However, studies in zebrafish show that, although FGF8 and FGF3 are required for otic placode induction, they are not required for the expression of some PPE marker genes (Phillips et al., 2001; Maroon et al., 2002; Leger and Brand, 2002, Liu et al., 2003; Solomon et al., 2004). Therefore, it remains to be determined whether all vertebrates share a common PPE induction by a neural plate source of FGF8. Au23

Interestingly, the studies mentioned previously showed that FGF8 alone can not induce PPE marker genes. The effectiveness of FGF8 for inducing PPE genes depended on the concomitant reduction of the level of BMP signaling in the nonneural ectoderm (Ahrens and Schlosser, 2005). For example, although beads coated with FGF8 implanted in the nonneural ectoderm could induce low levels of PPE gene expression, combining FGF8 with Noggin caused a dramatic induction. The same results were found after grafting animal cap ectoderm that expressed either FGF8 alone or in combination with Noggin. Thus, in addition to FGF signaling, PPE induction also appears to require reduced BMP signaling.

B. The Role of Bone Morphogenetic Protein Signaling

The vertebrate central nervous system forms in the embryonic ectoderm largely as a consequence of the dorsal expression of several molecules that antagonize the signaling of BMPs, which are highly expressed in ventral ectoderm (see also the chapter by Itoh and Sokol in this book). Several studies indicate Au10
that, although high concentrations of BMP antagonists such as Noggin and Chordin induce neural plate formation, intermediate concentrations induce neural crest formation (see also the chapter by Mayor in this book). These Au11
results led to the idea that a concentration gradient of BMP patterns the embryonic ectoderm into several subdomains, with epidermis forming at the high end of the gradient, neural plate forming at the low end of the gradient, and neural crest forming at intermediate levels (see Figure 27.3). This gradient may be established by the expression of BMP antagonists in the dorsal midline mesoderm that diffuse laterally through both the mesoderm and the adjacent ectoderm or the local expression of antagonists in the underlying tissues.

Because the PPE develops between the neural crest and the epidermis, it was proposed that it is also likely to form at an intermediate—and perhaps even lower—level of BMP signaling (Baker and Bronner-Fraser, 2001). It became possible to experimentally test this hypothesis when molecular markers of the PPE *(six, eya)* became available (Esteve and Bovolenta, 1999; Kobayashi et al., 2000; Pandur and Moody, 2000; David et al., 2001; Ghanbari et al., 2001; Bessarab et al., 2004). First, the injection of mRNAs encoding BMP antagonists Noggin and Cerberus induced *six1* expression in Xenopus animal cap explants (Brugmann et al., 2004), which demonstrated that BMP signaling needs to be reduced in the embryonic ectoderm for PPE gene expression. When explants were cultured in different concentrations of Noggin

protein, *six1* and *eya1* were highly expressed at very low concentrations (1 to 5 ng/mL), and their expression was dramatically reduced as Noggin concentration increased to intermediate levels that induced a neural crest gene *(foxD3)* or to high levels that induced a neural plate gene *(sox2)*. These studies indicate that genes that are characteristic of the three early neurogenic fields (i.e., neural plate, neural crest, and PPE) are most strongly induced in ectodermal explants at different concentrations of BMP antagonist. However, two observations are not concordant with a gradient model: (1) ventral nonneural ectoderm transplanted into the neural plate, which is a locale of presumably high BMP antagonist expression, strongly expresses *six1* (Ahrens and Schlosser, 2005); and (2) BMP4 mRNA levels are relatively high along the neural plate border (Hemmati-Brivanlou and Thomsen, 1995; Streit and Stern, 1999). In none of these studies has the level of BMP protein in the areas of PPE gene expression been measured, so the issue requires further testing. Nonetheless, experiments in whole embryos confirm that PPE gene expression requires a reduction in the level of BMP signaling. The endogenous expression domains of PPE marker genes are reduced when BMP4 is expressed in the lateral neurogenic zone, and they are expanded when BMP signaling is reduced in that zone by the expression of either a dominant-negative BMP receptor or BMP antagonists (Brugmann et al., 2004; Glavic et al., 2004; Ahrens and Schlosser, 2005).

C. The Role of Anterior–Posterior Axis Signaling

Although *six1* and *eya1* can be induced in animal cap explants simply in response to the appropriate concentration of BMP antagonist, this does not occur in the intact embryo. The ectopic expression of Noggin or Chordin in nonneural ectoderm in either chick or frog does not induce the ectopic expression of placode markers (Brugmann et al., 2004; Glavic et al., 2004; Ahrens and Schlosser, 2005; Litsiou et al., 2005). However, there is one exception: if a secondary axis with an endogenous anterior–posterior (AP) axis was induced by ectopically expressed Noggin or Chordin or by grafting the Organizer, *six1* was expressed only at the anterior pole of the ectopic AP axis Au12 (Brugmann et al., 2004; Ahrens and Schlosser, 2005). These results indicate that PPE induction is linked to the formation of the AP axis.

It had previously been demonstrated that induction of the neural crest, which extends from midbrain levels to the caudal end of the spinal cord, requires signaling pathways that establish the posterior axis of the neural plate (FGF, Wnt, and retinoic acid; see also the chapter by Mayor this book). Therefore, perhaps the PPE and the placodes, which are confined to the head, are negatively regulated by these posterior signaling molecules. In both animal cap explants and whole embryos, it was demonstrated that the repression of either Wnt or FGF signaling expanded the *six1* expression domain, whereas the activation of Wnt or FGF pathways repressed it (Brugmann et al., 2004). Similarly, the late expression of *foxi1* in the U-shaped domain surrounding the neural plate was also expanded by Wnt antagonists (Matsuo-Takasaki et al., 2005). Experiments in the chick confirmed that combined signaling is required for PPE induction; the expression of *six4* and *eya2* require the reduction of both BMP and Wnt signaling (Litsiou et al., 2005). Finally, it is interesting to note the following: (1) the dorsal endomesoderm that is required for PPE induction in Xenopus is a source of Cerberus (Ahrens and Schlosser, 2005), a secreted protein that inhibits BMP, Wnt, and Nodal signaling and

Au13

that is necessary for the formation of the head (Piccolo et al., 1999); and (2) the anterior neural plate is also a source of Wnt inhibitors (Bradley et al., 2000; Pera and De Robertis, 2000). Together, these studies indicate that, although neural crest induction requires posteriorizing signals, the PPE only develops in the absence of these signals. In fact, the differential response of neural crest and PPE to posteriorizing factors provides a simple explanation of why neural crest does not form in the most anterior tip of the head and of why sensory placodes do not form in the trunk (see Figure 27.3).

Taken together, these studies suggest that interactions between the neural plate and nonneural ectoderm define a new neurogenic region called the *lateral neurogenic zone;* this term is used to distinguish it from the *border zone,* a term that is sometimes used to refer to the lateral border of the neural plate, which gives rise to the neural crest. It is not clear whether this lateral neurogenic zone is initially competent to give rise to both neural crest and placodal derivatives and then becomes divided into two separately specified domains or whether the two tissues are distinct from the onset. In support of the first idea are the following observations: (1) the earliest known genes expressed in the lateral neurogenic ectoderm (*foxi1* and *dlx* genes) affect both neural crest and PPE markers in a similar manner; (2) although *six1* and *eya1* are highly expressed at Noggin concentrations lower than those required for a neural crest gene, there is significant overlap in the dose–response curves for the two sets of marker genes (Brugmann et al., 2004); (3) single-cell–mapping studies demonstrated that cells fated to give rise to otic placode are intermingled with rather than separate from future neural crest precursors (Streit, 2002); and (4) the expression domains of several neural crest and placodal marker genes partially overlap (McLarren et al., 2003; Glavic et al., 2004; Schlosser and Ahrens, 2004). In support of the second idea are the following observations: (1) in explant studies, the specification and loss of ectodermal competence for placode markers occur much later than they do for neural crest markers; and (2) in neural plate grafting experiments, placode markers are induced in the surrounding nonneural ectoderm of the host, whereas neural crest markers are induced primarily in the lateral edge of the graft (Ahrens and Schlosser, 2005). Further study of the molecular genetic mechanisms that dictate neural crest versus PPE/placodal fate will be necessary to resolve this issue.

III. GENES THAT SPECIFY PREPLACODAL ECTODERM FATE

Work performed during the past decade has described the expression of a large number of transcription factors in the various placodes of many different animals (reviewed by Baker and Bronner-Fraser, 2001; Schlosser and Ahrens, 2004; Schlosser, 2005, 2006). However, only a few of these genes are expressed throughout the entire PPE from the outset of its formation. In particular, members of two gene families *(six* and *eya)* are candidates for specifying the early preplacodal state, because they are expressed in the characteristic horseshoe-shaped domain that surrounds the anterior neural plate, which corresponds with the morphologic description of the PPE in the early embryo (see Figure 27.1; Esteve and Bovolenta, 1999; Kobayashi et al., 2000; Pandur and Moody, 2000; David et al., 2001; Ghanbari et al., 2001; reviewed by Schlosser and Ahrens, 2004). Recent functional studies indicate that these two families are necessary for the specification of the PPE and its derivatives.

A. *six* Genes

Vertebrate *six* genes are highly related to Drosophila *sine oculis* (*so*). Although *so* is essential for fly visual system formation (Cheyette et al., 1994; Serikaku and O'Tousa, 1994), vertebrate *six* genes play major roles in eye, muscle, kidney and craniofacial development (Kawakami et al., 1996; Brodbeck and Englert, 2004). All *six/so* proteins contain a highly conserved *six*-type homeodomain, which binds DNA, and an adjacent *six* domain (SD), which appears to increase DNA binding specificity by interacting with cofactors (Pignoni et al., 1997; Kawakami et al., 2000; Kobayashi et al., 2001). Vertebrate *six* genes have been grouped into three subfamilies *(six1/six2; six3/six4; six5/six6)* on the basis of sequence variations in both the homeodomain and the SD regions (Kawakami et al., 2000). *six1* and *six2* are most closely related to the fly *so,* but neither is known to play a major role in eye development; rather, *six3* and *six6* are critical for vertebrate eye development (see also the chapter by Vetter in this book). Au15

Three *six* genes *(six1, six2, six4)* are expressed in vertebrate PPE, placodes, and/or placode derivatives. However, the expression patterns across vertebrates are not identical (reviewed by Brugmann and Moody, 2005). It is not clear whether the differences are the result of true species variation or whether incomplete descriptions from diverse experimental techniques and different developmental stages make the patterns appear disparate. In general, *six1* and *six2* are expressed in the PPE, the placodes (except lens, which expresses *six3*), the lateral line organs, the muscle precursors, the kidneys, the genitalia, and the limb buds. *six4* is typically expressed in the PPE, the placodes, the muscle precursors, the kidneys, the brain, and the eye. It will be very important to fully describe the developmental expression patterns of these *six* genes across all of the animal models and humans to fully understand their roles in placode development and congenital syndromes.

Several experiments indicate that *six1* has a central role in PPE/placode development. First, several loss-of-function studies indicate that *six1* is a required gene. In humans, *six1* mutations lead to some cases of branchio-otic (BO) and branchio-oto-renal (BOR) syndromes, which are autosomal-dominant developmental disorders that are characterized by craniofacial defects and hearing loss (BO, BOR) and by additional malformations of the kidney and the urinary tract (BOR; Ruf et al., 2004). Likewise, *six1*-null mutant mice exhibit severe defects in the development of the nose, the thymus, the skeletal muscles, and the kidneys; in addition, all components of the inner ear fail to form as a result of increased cell death and reduced proliferation in the otic epithelium (Oliver et al., 1995; Laclef et al., 2003; Zheng et al., 2003; Ozaki et al., 2004). Consistent with these mammalian mutations is that the knockdown of *six1* via MO injection in Xenopus embryos results in the loss of early PPE marker gene expression and the expansion of adjacent epidermal *(keratin)* and neural crest *(foxD3)* markers (Brugmann et al., 2004). Second, the increased expression of wild-type *six1* by mRNA injection into the precursors of the lateral neurogenic zone in Xenopus embryos expands the expression domains of other early PPE genes *(sox11* and *eya1)*, and it represses the adjacent epidermal and neural crest domains. These results demonstrate that elevated *six1* expression in the lateral neurogenic zone promotes PPE gene expression at the expense of epidermal and neural crest genes (Figures 27.3 and 27.4).

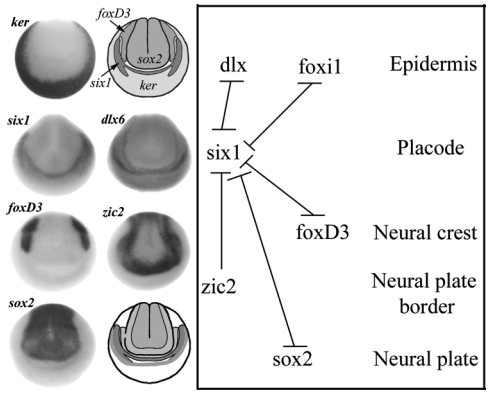

FIGURE 27.4 Boundaries between the four ectodermal domains may be formed by mutually repressive interactions. The left column shows Xenopus embryos that are stained for endogenous mRNA domains at neural plate stages to demonstrate the four ectodermal domains. *ker,* Epidermis; *six1*, preplacodal ectoderm (PPE); *foxD3*, neural crest; *sox2*, neural plate. A summary diagram of these domains is shown on top of the next column of embryos (color coding is the same as it was for Figures 27.1 and 27.3). In addition, the expression domains of two border genes *(zic2, dlx6)* are shown, and, on the bottom of that column, there is a summary of the relative expression domains for neural plate genes *(blue)*, neural plate border genes *(aqua)*, PPE genes *(green)*, and epidermis border genes *(orange)*. Experiments, which are summarized in the box to the right, demonstrate that the PPE gene, *six1,* may maintain preplacode fate in part through the mutual repression of genes expressed in the other domains and in the border zones of other domains.Data from Brugmann et al., 2004, and from Brugmann and Moody, unpublished observations. Au20 Au21

The functional roles of *six2* and *six4,* which are also expressed in the PPE, have yet to be described in any detail, although they are frequently used as placode marker genes in a number of animal models. To our knowledge, no human syndromes have been assigned to mutations in *six2* or *six4,* and phenotypes of *six2* null mice have not yet been reported. *six4* null mice do not have obvious craniofacial defects or hearing loss (Ozaki et al., 2001), but this lack of phenotype may be the result of redundant functions between the family members. For example, a recent report indicates that double *six1/six4* null mutant mice have more severe defects than either of the single mutants (Grifone et al., 2005), although the placode defects were not characterized. However, because *six1, six2,* and *six4* have distinct roles in myogenic differentiation and in kidney development (Ohto et al., 1998; Spitz et al., 1998; Fougerousse et al., 2002; Xu et al., 2003; Brodbeck and Englert, 2004; Himeda et al., 2004; Takasota et al., 2004), it is predicted that they will have distinct functions in PPE/placode development as well. Therefore, it will be important to perform both gain- and loss-of-function studies with *six2* and *six4,* both alone and in combination with *six1,* to better understand their functions in PPE and placode development.

B. *eya* Genes

There are four vertebrate *eya* genes that are homologues of Drosophila *eyes absent* (*eya*); the latter plays an essential role in fly eye development as a cofactor for *so* (Bonini et al., 1993). Vertebrate *eya* genes are expressed in multiple embryonic tissues, including the eyes, the somites, the kidneys, and the hypaxial muscle precursors; *eya1, eya2,* and *eya4* are also expressed in the PPE and the placodes (Abdelhak et al., 1997; Xu et al., 1997; Sahly

et al., 1999; David et al., 2001). In Xenopus and zebrafish, *eya1* expression is remarkably similar to that of *six1* (Sahly et al., 1999; David et al., 2001), which suggests that it has an important role in PPE/placode development. The *eya* proteins do not bind to DNA, but they are characterized by a highly conserved protein/protein-binding domain called the *eya* domain (ED), which is located at the C-terminus of the protein. In Drosophila, the ED participates in protein/protein binding with the SD of the *so* protein (Pignoni et al., 1997), and, in vertebrates, the interaction between the *six1* SD and the *eya1* ED domains is essential for *eya1* nuclear translocation and for exerting the transcriptional function of the complex (Ohto et al., 1999; Ikeda et al., 2002). However, *eya1* can bind to several proteins in addition to *six1*. It can act as a cofactor for other *six* proteins (*six2, six4,* and *six5;*Heanue et al., 1999; Ohto et al., 1999), and recent protein interaction studies in Drosophila identified several other potential *eya* binding partners (Giot et al., 2003; Database of Interacting Proteins Web site). Recent work has shown that *eya* functions as a phosphatase; this activity is necessary for Drosophila eye development (Rayapureddi et al., 2003; Tootle et al., 2003), and it is thought to regulate whether the *six1-Dach* complex (described later) acts as a transcriptional repressor or activator (Li et al., 2003). There is also evidence that *eya1* is a substrate for mitogen-activated protein kinase in the receptor tyrosine kinase signaling pathway (Hsiao et al., 2001). These potential multiple cellular functions will need to be kept in mind when evaluating the consequences of *eya* mutations in animal models and human congenital syndromes.

Experimental data for the role of *eya* proteins in PPE/placode development are most abundant for *eya1*. Several human cases have been identified that harbor *eya1* mutations, and these mutations cause some cases of BO and BOR syndromes (Abdelhak et al., 1997; Kumar et al., 1997; Rodriguez-Soriano, 2003; Spruijt et al., 2006), some cases of oto-facio-cervical syndrome (Rickard, 2001; Estefania et al., 2006), and isolated defects in the anterior segment of the eye (Azuma et al., 2000). Often the defects lie in the ED, where they act to inhibit the interaction between *eya1* and *six* proteins (Buller et al., 2001; Ozaki et al., 2002). The *eya1* mutant mice show defects in the inner ear, some of the cranial ganglia, the thymus, the thyroid, the parathyroid, the kidney, and the skeletal muscles (Abdelhak et al., 1997; Johnson et al., 1999; Xu et al., 1997, 1999, 2002). The *dogeared (dog)* mutation in zebrafish (which is caused by a point mutation in the ED) and *eya1* knockdown by MO result in defects of the inner ear and of the lateral line, but the defects appear to affect the cell survival of the sensory cell precursors rather than to establish the PPE (Kozlowski et al., 2005). The analysis of the effects of two zebrafish *eya1* mutants *(aal* and *dog)* on the anterior pituitary, which is derived from the hypophyseal placode, showed that three of the four cell lineages are dependent on *eya1* expression but not *six1* expression (Nica et al., 2006). To date, mutations in *eya2* have not been reported in humans, and mutations in *eya4* are involved in nonsyndromic deafness, which is suggestive of developmental defects in the otic placode (Wayne et al., 2001; Zhang et al., 2004). The potential roles of these genes in PPE/placode development is ripe for further experimentation.

C. Are there Other *six/eya* Interacting Proteins Involved in Preplacodal Ectoderm Fate Specification?

It is well documented that *six* proteins bind, via their SDs, to several proteins that lack the ability to bind to DNA, and there is evidence that some of these

proteins modulate *six* function as either coactivators or corepressors (Zhu et al., 2002; Tessmar et al., 2002; Giot et al., 2003). In fact, a gene network that includes *pax, six, eya,* and *fox* genes has been described to be essential in eye, lens, muscle, and kidney development (reviewed by Bhattacharyya and Bronner-Fraser, 2004; Brodbeck and Englert, 2004). In Drosophila, yeast two-hybrid experiments have identified 24 proteins in addition to *eya* that are likely to specifically bind to *so* (Giot et al., 2003; Kenyon et al., 2005). Because *so* belongs to the same *six* gene subfamily as vertebrate *six1/six2,* several of these proteins may have important roles in PPE fate specification. Recent work indicates that *six1/six2* can act as both transcriptional activators and repressors, depending on the presence of either *eya* or *groucho* cofactors (Silver et al., 2003), and that these two proteins in combination with *six1* differentially influence PPE development (Brugmann et al., 2004).

As described above, *six1* expression in the Xenopus lateral neurogenic zone upregulates PPE marker genes and reduces the expression domains of genes that mark the adjacent epidermis and neural crest. To determine whether these effects of *six1* are executed via transcriptional activation or repression, activating *(six1VP16)* and repressing *(six1EnR) six1* constructs were expressed in the lateral neurogenic zone (Brugmann et al., 2004). These experiments demonstrated the following: (1) that *keratin* expression in the epidermis is repressed by *six1* both directly and indirectly, because wild-type *six1* and both activating and repressing constructs reduced its domain; (2) that PPE genes are transcriptionally activated by *six1,* because the effect of *six1VP16* mimicked wild-type *six1,* and the effect of *six1EnR* was the reverse; and (3) that *foxD3* expression in the neural crest is transcriptionally repressed by *six1,* because the effect of *six1EnR* mimicked wild-type *six1,* and the effect of *six1VP16* was the reverse. These interpretations are supported by experiments in which wild-type *six1* was coexpressed with either a known coactivator *(eya1)* or a known corepressor *(groucho)*. The coexpression of wild-type *six1* with *eya1* gave identical results to those obtained with the *six1VP16* construct for every marker gene examined, and the coexpression of wild-type *six1* with *groucho* mimicked the results obtained with the *six1EnR* construct. Because both *eya1* and *groucho* are endogenously expressed in the lateral neurogenic zone, these data indicate that *six1* functions in PPE development as both a transcriptional activator and a repressor, depending on the cofactor with which it interacts.

Because the Drosophila interactome data indicate that there are several other potential *six1* cofactors (Giot et al., 2003; Kenyon et al., 2005), there may be multiple modifiers of *six* transcriptional activity that are developmentally relevant to PPE/placode development. In addition, there are likely to be protein regulators that modify *six1* function by binding to or modifying the activity of *eya*. For example, *dac* has an important role in Drosophila eye development in cooperation with *eya* and *so* (Chen et al., 1997). *dac* can bind to both *eya* and DNA, but it does not have a direct interaction with *so*. Vertebrate Dach is expressed widely in embryonic tissues, including placodes (reviewed in Schlosser, 2006), and it can regulate the transcriptional effectiveness of *six/eya* complexes (Heanue et al., 1999; Ikeda et al., 2002; Li et al., 2003). However, a specific role for Dach or for other potential *six/eya* cofactors in PPE/placode development remains to be discovered. It is anticipated that the genome, proteome, and interactome databases of lower organisms (in particular *Caenorhabditis elegans* and Drosophila) will provide important

clues regarding which additional factors are important in PPE/placode development. These types of investigations will allow for functional studies in vertebrate animal models, and they are likely to identify new causal genes in human congenital syndromes that affect the cranial sensory organs.

IV. MAINTAINING THE BOUNDARIES OF THE PREPLACODAL ECTODERM AND OTHER ECTODERMAL DOMAINS

As the neural plate is induced and the lateral neurogenic zone is established, several transcription factors become expressed along the neural plate border. It has been proposed that some of these, which are called *neural plate border-specifying genes (dlx, msx, pax, zic)*, interpret the neural inductive and anterior–posterior signals in the locale of the lateral neurogenic zone and that they in turn activate neural crest fate-specifying genes (Meulemans and Bronner-Fraser, 2004). For example, *zic* genes, which are the vertebrate homologues of Drosophila *odd-paired,* become restricted to the lateral edges of the neural plate, and they are required for cranial neural crest formation (Nakata et al., 1997, 1998; Brewster et al., 1998; Kuo et al., 1998; Mizuseki et al., 1998). However, it is possible that these genes additionally function to maintain the boundaries between the various ectodermal domains by interacting with genes expressed in the adjacent domains. In chick, for example, *msx1* and *pax7,* which are required for neural crest formation, are first expressed in a broad stripe that becomes restricted to the neural folds as PPE genes are expressed in the lateral part of that stripe. This observation suggests that interactions between *msx1*/*pax7* and PPE genes lead to the segregation of fate domains (reviewed by Streit, 2004). Refinements in the domains of the genes that are first expressed in overlapping zones and then expressed in discrete stripes around the neural plate also have been described in Xenopus (reviewed by Schlosser and Ahrens, 2004; Schlosser, 2006). This pattern of broad, overlapping zones resolving to discrete domains via mutually repressive interactions is reminiscent of the establishment of segmental boundaries in Drosophila (see also chapter by Kenyon in this book). Au16

To date, only a few of the neural plate border-specifying genes have been investigated for their potential roles in affecting PPE formation. As discussed previously, *dlx3, dlx5,* and *dlx6* appear to be necessary for the initial formation of the lateral neurogenic zone and for the expression of both neural crest and PPE markers. The domains of these genes later resolve into stripes that border the PPE by late neural plate stages. The increased expression of either all three genes (Woda et al., 2003) or of *dlx5* or *dlx6* singly (Brugmann et al., 2004) in the lateral neurogenic zone of intact Xenopus embryos reduced the size of the PPE. Increased *zic2* expression also repressed the PPE while expanding the neural crest domains, and, interestingly, *six1* in turn repressed the expression domains of *dlx5, dlx6,* and *zic2* (Brugmann et al., 2004). These results suggest that at least some neural plate border-specifying genes *(dlx, zic)* and at least one placode fate-specifying gene *(six1)* mutually interact to maintain separate ectodermal domains. In support of this idea is that fact that other genes expressed in the various domains (e.g., *foxi1* in epidermis, *sox2* in neural plate, *foxD3* in neural crest) also have mutually repressive interactions with *six1* (Brugmann et al., 2004; Matsuo-Takasaki et al., 2005). Thus, after the

fates of the four major ectodermal domains are specified by the expression of region-specific fate-specifying transcription factors, these factors may continue as maintenance factors to preserve the boundaries between these domains (see Figure 27.4).

However, it should be kept in mind that the types of experiments that have been performed to date do not sufficiently control the timing or spatial localization of overexpression and loss of function. Many of these genes likely have changing roles as the embryonic ectoderm becomes specified to different regional fates. For example, *foxi1* is initially expressed throughout the nonneural ectoderm, and it is required for the expression of later PPE marker genes (Matsuo-Takasaki et al., 2005). However, during neural plate stages when the PPE is fully established, the *foxi1* expression domain is mostly lateral to those of *six* and *eya,* and overexpression at this time, which is controlled by a hormone-inducible construct, represses them. Thus, early *foxi1* expression may be required to specify the lateral neurogenic zone, and later expression may maintain the border between the PPE and the epidermis. Further manipulations involving the use of constructs that can be temporally and spatially controlled need to be performed to fully understand the molecular interactions that both establish and maintain the boundaries between the different ectodermal zones.

V. PLACODE IDENTITY AND ONSET OF DIFFERENTIATION

After the PPE is established as a separate domain in the embryonic ectoderm with distinct boundaries from the other ectodermal domains, the tissue undergoes several steps of differentiation. First, under the inductive influences of underlying tissues, the PPE subdivides into individual placodes with different fates (see Figures 27.1 and 27.2). Concomitantly, the placodes express different sets of transcription factors that likely reflect their acquisition of identity. The placodes then undergo the morphogenetic movements that will produce their wide range of sensory organ structures and cellular phenotypes. What is known about how these steps are accomplished is reviewed in detail elsewhere (Baker and Bronner-Fraser, 2001; Schlosser and Ahrens, 2004; Schlosser, 2005, 2006). Here, we focus instead on four sets of genes that are expressed in nearly every placode during the initial steps of differentiation in the following temporal order: (1) *six* and *eya*; (2) *sox*; (3) *pax*; and (4) proneural determination and differentiation (*basic-Helix-Loop-Helix [bHLH]*) genes (Figure 27.5). Do these genes constitute a regulatory network that controls the onset of placode differentiation?

During the initiation of placode separation, *six, six2, and six4* expression is maintained in all of the individual placodes except the lens (Pandur and Moody, 2000; Ghanbari et al., 2001; Schlosser and Ahrens, 2004); lens expresses *six3* instead (reviewed by Bhattacharyya and Bronner-Fraser, 2004). The *eya* genes are also expressed in all placodes, with some variation, depending on the animal and the placode (reviewed by Schlosser, 2006). Because *six* and *eya* genes continue to be expressed in all placodes, they are unlikely to be involved in the acquisition of the identity of the individual placodes. Instead, there is evidence that they are involved in the regulation of the initiation of differentiation. Expression patterns suggest that *six1* might maintain undifferentiated placodal cells in a "stem/progenitor" state (Brugmann

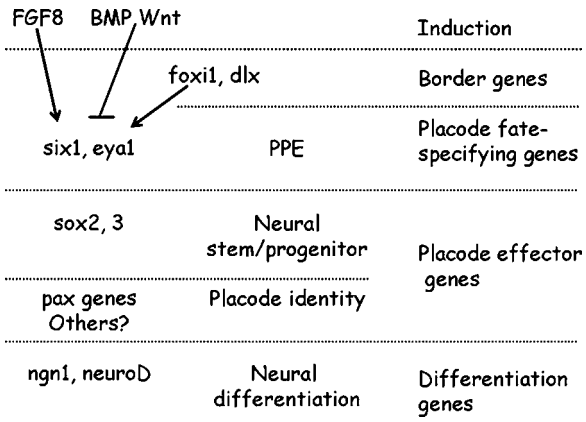

FIGURE 27.5 A model of the gene regulatory cascade that may regulate preplacodal ectoderm (PPE) formation and initial placode differentiation. First, a combination of signaling factors from the neural plate (fibroblast growth factor 8), the ventral epidermis (bone morphogenetic protein), the posterior end of the embryo (Wnt), and the epidermis border (regulated by *foxi1* and *dlx* genes) cause the formation of the PPE and the expression of the *six* and *eya* genes. Individual placodes form as *sox* genes, which may regulate the production of neural stem and progenitor cells in the neurogenic placodes, and *pax* genes, which may regulate placode identity, are expressed. As differentiation is initiated, neural progenitors express *neurogenin*-type genes; the subsequent expression of *neuroD*-type genes defines the precursors of different neural cell types.

and Moody, 2005). First, *six1* is downregulated as placodal cells show morphologic signs of differentiation, such as delaminating from the epithelium and coalescing into ganglia (Pandur and Moody, 2000). Second, *six1* expression is complementary to that of *bHLH* differentiation markers (*ngn1* and *neuroD;*Schlosser and Northcutt, 2000), which suggests that *six1* may need to be downregulated for placodal cells to differentiate. By contrast, *six2*, *six4*, and *eya1* continue to be expressed in differentiating placode-derived structures, including the cranial ganglia, which suggests that they may have later roles in placode differentiation, as has been suggested for mouse *six4* in other tissues (Niiya et al., 1998; Ohto et al., 1998).

Because *six1* is expressed earlier than *sox* and *pax* genes in the PPE and the placodes and because it is expressed earlier than and in a complementary pattern to *bHLH* genes during placode differentiation, its function after establishing the PPE fate may be to maintain subsets of placode cells in an undifferentiated state by repressing these other genes. If this is true, then *six1* gain of function should repress genes that are involved in initiating differentiation (see Figure 27.5), and it should promote continued cell proliferation. Preliminary data from our laboratory indicates that the overexpression of *six1* after the establishment of the PPE (using a hormone-inducible construct) reduces the expression of several of these later-expressed placode genes (Brugmann and Moody, unpublished observations). Studies in other systems Au17
indicate that *six* genes keep progenitor cells in a proliferative state before cell type specification. The loss of *six1* in mice appears to decrease proliferation, which results in apoptosis (Li et al., 2003; Ozaki et al., 2004). In humans, *six1* overexpression occurs in hyperproliferating cell populations (e.g., primary breast cancers and metastatic lesions; Ford et al., 1998). These authors showed that human *six1* overexpression allows DNA damage to go unchecked by causing an attenuation of the DNA damage-induced G_2

checkpoint. In a more recent study, the overexpression of *six1* was shown to influence cellular proliferation by directly activating the transcription of cyclin A1, a tissue-restricted cyclin that is expressed in the embryonic mammary gland but not in the differentiated adult mammary gland (Coletta et al., 2004). These studies suggest that *six1* may maintain cells in an immature state by influencing cell cycle regulation.

sox genes have been generally described as playing a role in the initial specification of the neural plate and neural stem cells (reviewed by Wegner, 1999; Moody and Je, 2002). Several experiments in several vertebrates indicate that *sox2* functions to maintain the neural stem cell state, and its premature inhibition causes neural cells to prematurely delaminate from the proliferative zone, exit the cell cycle, and terminally differentiate (Mizuseki et al., 1998; Kishi et al., 2000; Graham et al., 2003). Although *sox2* and *sox3* are well-known markers of the vertebrate neural plate, they are additionally expressed in subdomains of the PPE after the onset of *six1* and *eya1* expression before the morphologic segregation of the individual placodes (Schlosser and Ahrens, 2004). It is possible that the *sox* genes are only expressed in the neural stem/progenitor cells of the neurogenic placodes and that they are thus responsible for the initiation of a neural differentiation pathway. However, *sox2* and *sox3* also play important roles in lens placode development, which has no neural derivatives (Kamachi et al., 1998), and they are not expressed in the profundal or trigeminal placodes, which give rise to sensory neurons (Schlosser and Ahrens, 2004). Therefore, they obviously have other functions in nonneural cells, and there must be other genes that initiate neurogenesis, at least in some placodes. One potential candidate is *sox11,* which is expressed in both the neural plate and PPE and which is upregulated by *six1* (Brugmann et al., 2004).

Members of the *pax* gene family have multiple roles in early developmental processes and organogenesis (Mansouri et al., 1999). In neural crest development, *pax3* and *pax7* have a role in specifying the neural plate border and, subsequently, the neural crest fate (Meulemans and Bronner-Fraser, 2004); whether they have a role in PPE specification has not yet been tested. However, after the PPE forms, several *pax* genes are expressed in restricted regions just before the placodes begin to separate (Baker and Bronner-Fraser, 2001; Schlosser and Ahrens, 2004). Individual placodes express different combinations of *pax* genes: *pax2, pax5,* and *pax8* are expressed in the otocyst; *pax6* is expressed in the olfactory, lens, and trigeminal placodes; and *pax3* is expressed in the ophthalmic/profundal placode. Transplantation experiments in the chick indicate that the onset of *pax* expression correlates with the acquisition of placode identity (Baker et al., 1999; Baker and Bronner-Fraser, 2000), thereby leading these authors to propose that the combination of *pax* genes expressed by an individual placode (the "*pax* code") may provide identity to that placode. However, many other transcription factors are also differentially expressed during the period when placodes separate, which suggests that they may also influence placode identity (Baker and Bronner-Fraser, 2001; Schlosser and Ahrens, 2004; Schlosser, 2006). For example, some early genes are expressed rather ubiquitously through the PPE zone (e.g., *six1, six4, eya1, dlx3*) but become repressed in the lens placode; this loss of expression may contribute to the lens fate. Other early genes (e.g., *Xiro1, foxi1, tbx2*) become restricted to only the most posterior placodes during placode separation, and thus they may influence posterior placode identities. There are many examples from loss-of-function studies that identify the genes that are required for the

formation of individual placodes (Baker and Bronner-Fraser, 2001; Ahrens and Schlosser, 2004; Schlosser, 2006). However, because many of these genes are Au24 expressed both broadly during early stages and in specific placodes during later stages, it is difficult to determine whether the genes are involved in initial placode identity or in later differentiation processes. This will be an important issue to address using temporally and spatially controlled constructs.

Finally, *bHLH* transcription factors, which were first identified in Drosophila for playing essential roles in neurogenesis (reviewed in Jan and Jan, 1993; Guillemot, 1999), promote the generation of neural progenitors, cause neural progenitors to exit the cell cycle, and promote neuronal differentiation (Lee et al., 1995; Bertrand et al., 2002; Ohnuma and Harris, 2003). They can be grouped into two classes: those that are expressed early in the neural fate cascade (the determination factors such as *neurogenin* [*ngn*]) and those that are expressed later in the cascade (the differentiation factors such as *neuroD*). The expression of *ngn1* and *neuroD* in placodes and placodal derivatives has been extensively studied in Xenopus (Schlosser and Northcutt, 2000; Schlosser and Ahrens, 2004). In several placodes, *ngn1* expression is detected first in the inner ectodermal layer as soon as the individual placodes form and later in the prospective ganglion cells that are delaminating and migrating away from the placode; *ngn1* expression is lost as the coalescing ganglion cells differentiate into neurons and glia. The expression of *neuroD* occurs later than that of *ngn1*; it is first seen in scattered cells within the inner ectodermal layer, and it remains expressed in most or all of the placode-derived ganglion cells. This sequence supports the general notion that *ngn1* acts early in the differentiation pathway and that it is followed by *neuroD*. Several studies suggest that neurogenic *bHLH* factors play important roles as regulators of neural differentiation in placodes after the acquisition of placode identity (see Figure 27.5; reviewed in Schlosser, 2006). The identity of similar factors for the nonneural derivatives of the placodes has not yet been studied in detail.

This simplistic scheme of gene expression sketches out a potential gene regulatory network for the initial steps of PPE/placode development (see Figure 27.5), but many details are still missing. By contrast, a gene network for neural crest specification has been proposed (Meulemans and Bronner-Fraser, 2004). First, BMP, Wnt, and FGF signals are necessary for the initial induction of neural crest fate. Next, these growth factor signals activate neural plate border-specifying genes, which in turn activate neural crest fate-specifying genes. These then regulate a large number of neural crest effector genes, which regulate differentiation pathways. Is there a similar gene network that regulates placode development and the onset of differentiation? First, PPE induction involves many of the same signaling molecules, but, whereas neural crest requires posteriorizing signals, these repress PPE. Second, there is evidence that some of the neural plate border-specifying genes that promote neural crest negatively regulate the PPE (see Figure 27.4). Third, the one PPE fate-specifying gene that has been studied to date *(six1)* seems to regulate at least some placode identity genes (*sox, pax;* perhaps these can be considered to be "placode effector" genes) and "neural differentiation" genes (*ngn1, neuroD;* see Figure 27.5; Brugmann and Moody, unpublished observations). Clearly, however, a great deal of work remains to be done to identify Au18 the full list of genes that are involved at each step of this putative network and to determine precisely how they interact to regulate the various aspects of PPE and placode development.

VI. FUTURE DIRECTIONS

Determining the molecular mechanisms of placode gene function is important for both understanding normal development and interpreting human congenital syndromes. First, the differential roles of all of the *six* proteins in PPE/placode development need to be determined. All three *(six1, six2, six4)* are expressed during placode development, and one affected locus (BOS3) in BO and BOR patients contains *six1, six4,* and *six6* (Ruf et al., 2004). Second, a comprehensive understanding of which genes are able to interact with *six* genes as cofactors is needed. The penetrance of BO and BOR syndromes is variable, and studies in *six1* heterozygous mice suggest that there are additional modifier genes that influence *six1* activity or function, thereby modulating the mutant phenotype (Xu et al., 2003; Ruf et al., 2004). The recent interactome data from *C. elegans* and *Drosophila* indicate that there are several proteins yet to be experimentally tested that could potentially influence *six* and *eya* functions. Third, identifying and understanding the function of all of the genes involved in PPE specification and placode differentiation pathways will have a major impact on craniofacial tissue repair efforts. Elucidating the basic molecular mechanisms by which PPE cells are induced and transformed from the embryonic ectoderm into numerous differentiated cell types and how the process differs from that described for the closely related neural crest will be critical for designing techniques for sensory organ replacement from various stem and progenitor cell sources. Although we are at the very beginning of identifying the mechanisms that regulate PPE specification and initial placode differentiation, future work to elucidate the gene regulatory pathways reviewed herein may make it possible to repair craniofacial defects that result from birth defects, trauma, and disease.

SUMMARY

- The cranial sensory placodes arise from a PPE that is lateral to the anterior neural plate, and they give rise to a large number of specialized sensory organs.
- Three steps are necessary to induce and appropriately position the PPE: (1) interactions between the neural plate and the epidermis, perhaps involving FGF8; (2) the appropriate level of neural inductive (anti-BMP) signaling; and (3) the repression of posteriorizing signals (Wnt, FGF).
- The *six* and *eya* genes play important roles in specifying the PPE fate. In particular, *six1* positively regulates the expression of other PPE markers and negatively regulates the adjacent neural crest and epidermis. Importantly, *six1* does so both as a transcriptional activator and a transcriptional repressor, depending on the available cofactors, including *eya1* and *groucho*.
- Interactions between neural plate and epidermis border-specifying genes (e.g., *zic, dlx, foxi1*) and PPE genes refine the borders of the various ectodermal domains (i.e., epidermis, PPE, neural crest, neural plate).
- The *six* and *eya* genes may function upstream of putative neural stem genes *(sox)*, placode identity genes *(pax)*, and neural differentiation genes *(bHLH)* to regulate the onset of placode identity and differentiation.

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GLOSSARY

Adenohypophysis
The anterior pituitary gland, derived from the hypophyseal placode, that has cells that secrete a number of peptide hormones.

Branchio-otic syndrome
An autosomal-dominant syndrome in humans that presents with branchial cleft fistulas and hearing loss. Some cases are caused by mutations in the *eya1* gene, and some are caused by mutations in the *six1* gene.

Branchio-otic-renal syndrome
An autosomal-dominant syndrome in humans that presents with craniofacial defects, hearing loss, and renal or urinary tract defects. Some cases are caused by mutations in the *eya1* gene, and some are caused by mutations in the *six1* gene.

Lateral neurogenic zone
A region of the embryonic ectoderm that surrounds the anterior neural plate and that gives rise to the neural crest and to the preplacodal ectoderm.

Neurulation
The process by which the flat, disc-shaped neural plate ectoderm folds into an elongated tube, thereby becoming the precursor tissue of the central nervous system.

Organizer
The region of the vertebrate embryo that become the source of signaling molecules that dorsalize both the mesoderm and the ectoderm.

Oto-facio-cervical syndrome
Patients present with hearing loss; a long, narrow face; and various facial and cervical structural anomalies. Some cases are caused by mutations in the *eya1* gene.

Preplacodal ectoderm
The region of the embryonic ectoderm that surrounds the anterior neural plate and that is characterized by the expression of the *six* and *eya* genes, which will give rise to all of the cranial sensory placodes.

Stomodeum
An anterior region of the embryonic ectoderm that invaginates to contact the anteriormost end of the endoderm (i.e., the pharynx). This contact will eventually perforate and form the mouth.

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RECOMMENDED RESOURCES

Database of Interacting Proteins (DIP): http: dip.doe-mbi.ucla.edu/dip/Main.cgi.
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