

Review

Induction and specification of cranial placodes

Gerhard Schlosser*

Brain Research Institute, AG Roth, University of Bremen, FB2, PO Box 330440, 28334 Bremen, Germany

Received for publication 6 October 2005; revised 22 December 2005; accepted 23 December 2005

Available online 3 May 2006

Abstract

Cranial placodes are specialized regions of the ectoderm, which give rise to various sensory ganglia and contribute to the pituitary gland and sensory organs of the vertebrate head. They include the adenohypophyseal, olfactory, lens, trigeminal, and profundal placodes, a series of epibranchial placodes, an otic placode, and a series of lateral line placodes. After a long period of neglect, recent years have seen a resurgence of interest in placode induction and specification. There is increasing evidence that all placodes despite their different developmental fates originate from a common panplacodal primordium around the neural plate. This common primordium is defined by the expression of transcription factors of the *Six1/2*, *Six4/5*, and *Eya* families, which later continue to be expressed in all placodes and appear to promote generic placodal properties such as proliferation, the capacity for morphogenetic movements, and neuronal differentiation. A large number of other transcription factors are expressed in subdomains of the panplacodal primordium and appear to contribute to the specification of particular subsets of placodes. This review first provides a brief overview of different cranial placodes and then synthesizes evidence for the common origin of all placodes from a panplacodal primordium. The role of various transcription factors for the development of the different placodes is addressed next, and it is discussed how individual placodes may be specified and compartmentalized within the panplacodal primordium. Finally, tissues and signals involved in placode induction are summarized with a special focus on induction of the panplacodal primordium itself (generic placode induction) and its relation to neural induction and neural crest induction. Integrating current data, new models of generic placode induction and of combinatorial placode specification are presented.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Adenohypophyseal placode; Olfactory placode; Lens placode; Trigeminal placode; Otic placode; Lateral line placodes; Epibranchial placodes; Pituitary; *Xenopus*; Chick; Zebrafish; Mouse; *Six1*; *Six4*; *Eya*; *Dlx3*; *GATA*; *SoxB1*; *Irx*; *ANF*; *Pitx*; *Msx*; *Fox*; *Tbx*; *Pax*; *FGF*; *BMP*; *Wnt*

Introduction

Vertebrates are distinguished from other deuterostomes by their specialized head with an elaborate brain encased in a cartilaginous or bony skull and with complex paired sense organs such as nose, eyes, and ears. Many of these evolutionary innovations of the vertebrate head originate from only two embryonic tissues, the neural crest, and the cranial placodes, which probably evolved in early vertebrates when these ceased to be filter feeders and adopted a new life style as active predators (Northcutt and Gans, 1983; Gans and Northcutt, 1983; see also Northcutt, 1996, 2005; Baker and Bronner-Fraser, 1997a; Holland and Holland, 1999, 2001; Meulemans and Bronner-Fraser, 2004; Schlosser, 2005).

Neural crest and placodes are specialized domains of the embryonic ectoderm which develop similarly in several respects. Both are very versatile embryonic tissues that give rise to multiple non-epidermal cell types including neurons, glia, and secretory cells. Moreover, the development of both tissues involves cell shape changes; these allow placodal and crest cells to migrate and/or to participate in various morphogenetic movements. Finally, both neural crest and placodes develop from populations of cells near the border of the neural plate.

Beyond these similarities, however, cranial placodes develop in a peculiar fashion quite distinct from the neural crest (reviewed for example in Webb and Noden, 1993; Northcutt, 1996; Baker and Bronner-Fraser, 1997a, 2001; Le Douarin and Kalcheim, 1999; Hall, 1999; Kalcheim, 2000; Santagati and Rijli, 2003; Meulemans and Bronner-Fraser, 2004; Huang and Saint-Jeannet, 2004). First, placodes develop exclusively from cranial ectoderm, whereas the neural crest develops in both

* Fax: +49 421 218 4549.

E-mail address: gshloss@uni-bremen.de.

head and trunk. Second, placodes comprise a quite heterogeneous assembly of structures, which form as focal thickenings of the cranial ectoderm at various stages of embryonic development (mostly after neural tube closure) and later invaginate and/or give rise to a subpopulation of migratory cells. The neural crest, in contrast, is an entirely migratory population of cells, which leaves the neural plate border region prior to or during fusion of the neural folds. Third, the developmental potential of placodes is more restricted than of neural crest. While both tissues give rise to secretory cells, neurons, and glia, only neural crest cells can form cartilage and bone, smooth muscle, and pigment cells. And fourth, with few exceptions, neural crest and placodes express different sets of transcription factors indicating that their development is controlled by different gene regulatory networks.

Compared to the neural crest, which has attracted much attention for its versatility and morphogenetic capacity and has been intensely studied ever since its discovery (reviewed in Baker and Bronner-Fraser, 1997b; Le Douarin and Dupin, 2003; Le Douarin and Kalcheim, 1999; Hall, 1999; Mayor and Aybar, 2001; Mayor et al., 1999; Kalcheim, 2000; Aybar and Mayor, 2002; Knecht and Bronner-Fraser, 2002; Santagati and Rijli, 2003; Meulemans and Bronner-Fraser, 2004; Huang and Saint-Jeannet, 2004), placodal development has long been neglected. The last couple of years, however, have seen a resurgence of interest in placode development, spurred by the discovery of many transcription factors with placode-specific expression and by increasing evidence for a common developmental origin of all placodes from a panplacodal primordium (for reviews, see Baker and Bronner-Fraser, 2001; Schlosser, 2002a, 2005; Streit, 2004; Brugmann and Moody, 2005).

The present review focuses on early aspects of placode development addressing in particular the origin of different placodes from such a panplacodal primordium. After providing an overview of different cranial placodes, I review the evidence for their common origin from a panplacodal primordium. Next, I address the role of various transcription factors for placodal development. I then discuss how different placodes may be specified within this primordium and how the latter is finally divided into distinct placodes. Finally, I consider tissues and signals involved in placode induction, concentrating on generic steps of placode induction and their relation to the induction of neural plate and neural crest. Evolutionary implications of our current view of placode development will not be covered but are reviewed elsewhere (Schlosser, 2005).

Development and derivatives of cranial placodes—an overview

Placodes were first discovered as transitory thickenings of cranial ectoderm (van Wijhe, 1883; Froriep, 1885; von Kupffer, 1891, 1895). The cranial placodes, as understood here, include the adeno-hypophyseal, olfactory, lens, trigeminal, and profundal placodes, a series of epibranchial and hypobranchial placodes, an otic placode, and a series of lateral line placodes (Fig. 1) (reviewed in Webb and Noden, 1993; Northcutt, 1996; Baker and Bronner-Fraser, 2001; Schlosser, 2002a, 2005; Streit,

2004). Most of these placodes are present in all vertebrates. However, the neurogenic hypobranchial placodes have only been found in amphibians (Schlosser, 2003; Schlosser and Northcutt, 2000; Schlosser et al., 1999), and the number of epibranchial and lateral line placodes differs for different taxa, with lateral line placodes being completely lost repeatedly, for instance, in amniotes (reviewed in Northcutt, 1992, 1993a,b, 1997; Schlosser, 2002b).

All placodes are specialized areas of the cranial non-neural ectoderm (i.e. ectoderm outside of neural plate and neural crest), where cells undergo pronounced cell shape changes (which may result in thickening, invagination, and/or cell delamination) and which give rise to various non-epidermal cell types. As I have discussed elsewhere (Schlosser, 2002a), placodes are often recognizable as thickenings (regions of columnar epithelium), but this is not always the case. It should be noted that there are some other ectodermal areas—including the amphibian hatching gland and cement gland (Drysdale and Elinson, 1992; Sive and Bradley, 1996) as well as the primordia of teeth, feathers, and hairs (Pispa and Thesleff, 2003)—which also give rise to specialized cell types but are not considered as placodes here because they apparently do not share a common developmental origin or bias (see below) with cranial placodes in the strict sense as enumerated above. Hatching and cement glands, for example, develop from the superficial layer of the bilayered amphibian ectoderm (Drysdale and Elinson, 1992; Sive and Bradley, 1996), while cranial placodes arise from its deep layer (Northcutt and Brändle, 1995; Northcutt et al., 1994; Schlosser and Northcutt, 2000).

There are several generic aspects of placode development shared by different placodes and reflected in the coexpression of many genes in different placodes (see below and McCabe et al., 2004). First, placodes are regions of increased cell proliferation compared to the epidermis (Saka and Smith, 2001; Washausen et al., 2005). Second, the development of placodal derivatives often involves cell shape changes and morphogenetic movements (reviewed in Noden, 1991; Webb and Noden, 1993; Northcutt, 1996; Baker and Bronner-Fraser, 2001), allowing placodes to develop into columnar epithelia, to invaginate, and/or to give rise to various types of migratory cells (neuronal, endocrine, or glial precursor cells or lateral line primordia). And third, all placodes with the exception of adeno-hypophyseal and lens placode are neurogenic (e.g. D'Amico-Martel and Noden, 1983; Ma et al., 1998; Fode et al., 1998; Schlosser and Northcutt, 2000; Andermann et al., 2002; Begbie et al., 2002). The absence of neurogenesis in these two placodes may be due to its active suppression in ectoderm originally biased for a neuronal fate judged by the initial expression and subsequent downregulation of *Xenopus Ngnr-1* in the prospective lens and adeno-hypophyseal ectoderm (Schlosser and Ahrens, 2004).

Aside from these similarities, however, different cranial placodes develop differently and give rise to different sense organs and ganglia, each with a distinct set of derivative cell types. These are briefly summarized in the following paragraphs and in Fig. 1, which also shows the location of cranial placodes in chick and *Xenopus* embryos.

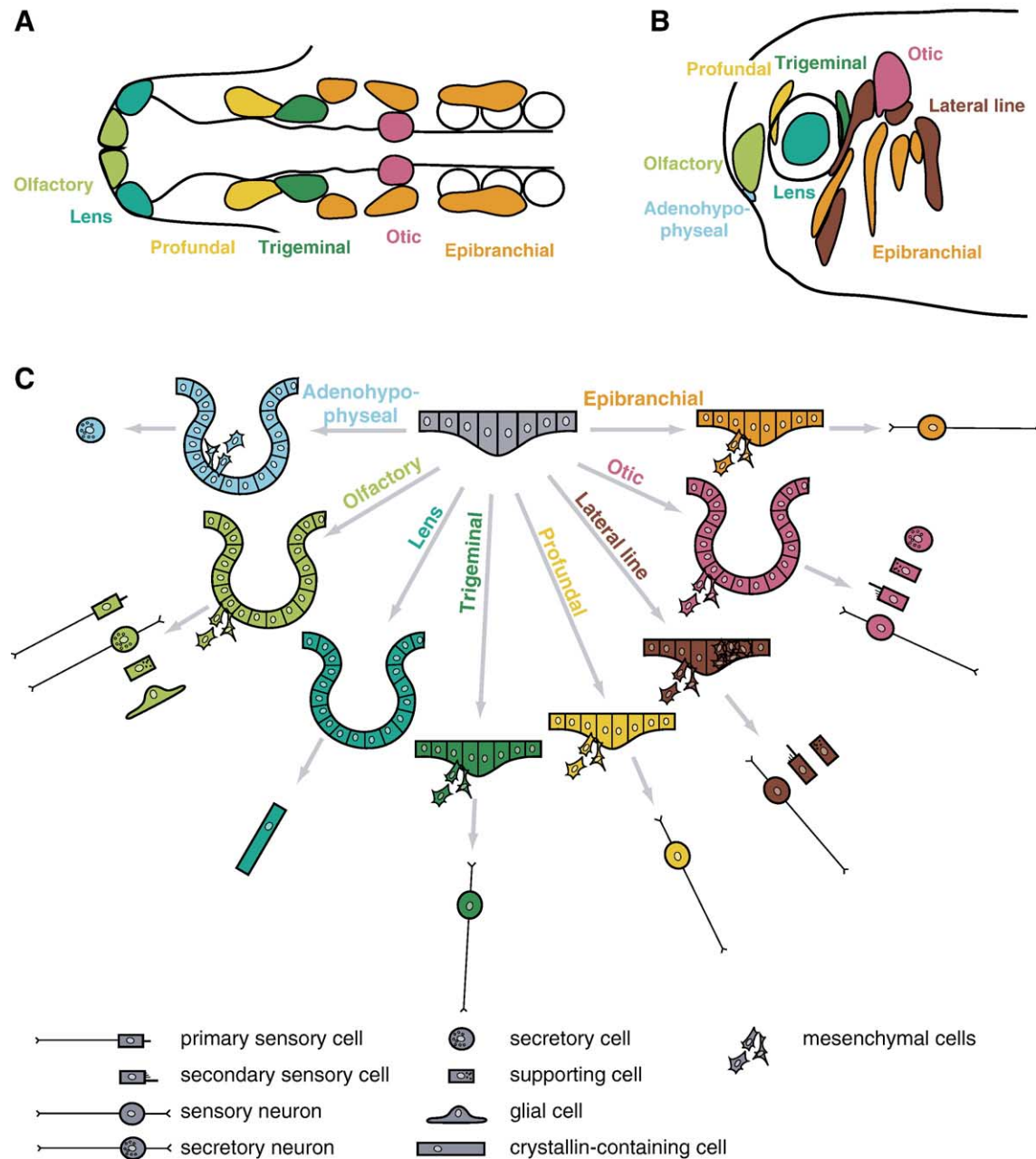


Fig. 1. Cranial placodes in vertebrates. (A) Cranial placodes in a 10- to 13-somite-stage chick embryo (modified from Streit, 2004; based on D'Amico-Martel and Noden, 1983; Bhattacharyya et al., 2004). In amniotes, profundal and trigeminal placode are commonly referred to as ophthalmic and maxillomandibular placode of the trigeminal nerve, respectively. (B) Cranial placodes in a tailbud stage *Xenopus* embryo (modified from Schlosser and Northcutt, 2000). (C) Schematic summary of morphogenesis and cellular derivatives of various cranial placodes. Invagination occurs in adenohypophyseal, olfactory, lens, and otic placodes. Moreover, in all placodes except the lens placode, some cells migrate away from the placodal epithelium as mesenchymal cells to form sensory neurons, secretory cells, or glial cells. In lateral line placodes, another subset of cells migrates along the basement membrane and forms the lateral line primordia (modified from Schlosser, 2005).

Adenohypophyseal placode

The adenohypophyseal placode forms the adenohypophysis, i.e. the anterior lobe of the pituitary gland (Couly and Le Douarin, 1985; Eagleson et al., 1986, 1995; Kawamura and Kikuyama, 1992; El Amraoui and Dubois, 1993a,b; Kouki et al., 2001; Sasaki et al., 2003; Herzog et al., 2003; Uchida et al., 2003; Chapman et al., 2005). The mode of adenohypophysis development differs for different vertebrates, but in most gnathostomes (except teleosts), it involves invagination of the

placode (which thereby forms Rathke's pouch) from the stomodeum. The adenohypophysis is of central importance for the hormonal control of multiple body functions and contains six types of endocrine secretory cells: corticotropes (ACTH), melanotropes (MSH), gonadotropes (LH and FSH), thyrotropes (TSH), lactotropes (prolactin), and somatotropes (somatotropin) (reviewed in Dubois et al., 1997; Kawamura and Kikuyama, 1998; Sheng and Westphal, 1999; Kioussi et al., 1999a; Dasen and Rosenfeld, 2001; Scully and Rosenfeld, 2002; Asa and Ezzat, 2004; Ooi et al., 2004).

Olfactory placode

The olfactory placode is one of the most versatile placodes and is the only one, which retains stem cells capable of forming various differentiated cell types throughout life (reviewed in Crews and Hunter, 1994; Calof et al., 1998; Schwob, 2002; Beites et al., 2005). It invaginates to form the epithelia of the olfactory and vomeronasal organs (e.g. Mendoza et al., 1982; Klein and Graziadei, 1983; Couly and Le Douarin, 1985; Hansen and Zeiske, 1993; Zeiske et al., 2003; reviewed in Brunjes and Frazier, 1986; Farbman, 1994; Reiss and Burd, 1997; Buck, 2004). These epithelia contain secretory cells such as supporting cells and mucus-producing cells as well as primary sensory cells (possessing an axon). The latter are chemoreceptive cells carrying odorant and pheromone receptors and their axons form the olfactory and vomeronasal nerves, respectively. In addition, the olfactory placode is the only placode, which has been demonstrated to give rise to glial cells (Couly and Le Douarin, 1985; Chuah and Au, 1991; Norgren et al., 1992; Ramón-Cueto and Avila, 1998). These include Schwann cells, which ensheath the axons of the olfactory nerve and migrate along this nerve into the olfactory bulb.

Finally, the olfactory placode is believed to give rise to a diverse population of secretory cells releasing neuropeptides such as Neuropeptide Y, FMRFamide, and gonadotropin-releasing hormone (GnRH), which migrate along the olfactory and vomeronasal nerves towards and into the brain (Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989; El Amraoui and Dubois, 1993a,b; Murakami and Arai, 1994; Northcutt and Muske, 1994; Yamamoto et al., 1996; Dellovade et al., 1998; Bless et al., 2005; reviewed in Tarozzo et al., 1995; Daikoku, 1999; Wray, 2002). One subpopulation of the placodally derived GnRH cells forms the terminal nerve (reviewed in Muske and Moore, 1988; Demski, 1993; Von Bartheld, 2004), a distinct ganglionated cranial nerve with neuromodulatory effects on various neurons and neuroendocrine cells including the olfactory receptor cells itself (Eisthen et al., 2000; Abe and Oka, 2000; Park and Eisthen, 2003). Another subpopulation of placodal GnRH cells populates a group of septo-preoptic nuclei and later controls the release of gonadotropins (LH, FSH) from the adenohypophysis (reviewed in Muske, 1993; Parhar, 2002; Somoza et al., 2002; Wray, 2002).

Recent lineage tracing experiments in zebrafish have cast doubt on the olfactory origin of GnRH cells and suggest instead that the septo-preoptic subpopulation originates from the adenohypophyseal placode next to the anteromedial boundary of the olfactory placode, while the terminal nerve subpopulation originates from neural crest cells adjacent to the posterolateral boundary of the olfactory placode (Whitlock et al., 2003; see also Whitlock, 2005). However, due to the close apposition of the olfactory placode to both neural crest and adenohypophyseal placode at the stage when these experiments were performed, inadvertent labeling of olfactory placodal cells cannot be ruled out, and further studies are needed to unequivocally clarify the origin of GnRH cells.

Lens placode

The lens placode is the only placode besides the adenohypophyseal placode that does not form neurons. Instead, it invaginates to form the lens vesicle, which gives rise to the crystallin-accumulating cells of the lens (reviewed in McAvoy, 1980; Piatigorsky, 1981; McAvoy et al., 1999; Cvekl and Piatigorsky, 1996; Ogino and Yasuda, 2000; Chow and Lang, 2001; Lovicu and McAvoy, 2005).

Profundal and trigeminal placodes

The profundal and trigeminal placodes give rise to neuronal precursors, which migrate away from the placode and congregate in the adjacent mesenchyme. Together with neural crest cells, they will form the sensory neurons of the ganglia of the profundal and trigeminal nerves, respectively (Knouff, 1927, 1935; Hamburger, 1961; Ayer-LeLièvre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983; Artinger et al., 1998; Schlosser and Northcutt, 2000). In many gnathostomes, these two nerves are distinct and have separate cranial ganglia (e.g. Allis, 1897; Norris, 1925; Norris and Hughes, 1920; Song and Northcutt, 1991; Northcutt, 1993a; Northcutt and Bemis, 1993; Piotrowski and Northcutt, 1996), but these ganglia fuse during development in amphibians (Northcutt and Brändle, 1995; Schlosser and Roth, 1997). In amniotes, there is only a single ganglion known as the trigeminal or Gasserian ganglion. The latter has, however, an ophthalmic and maxillomandibular subdivision. These subdivisions develop by the coalescence of neural crest cells with an ophthalmic and maxillomandibular placode (Hamburger, 1961; Ayer-LeLièvre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983; Begbie et al., 2002). These placodes are most likely homologues of the profundal and trigeminal placodes of anamniotes, respectively, based on their position and gene expression patterns (for detailed discussion, see Schlosser and Ahrens, 2004; Schlosser and Northcutt, 2000). The sensory neurons derived from the profundal and trigeminal placodes have either free nerve endings or supply receptors of non-placodal origin such as Merkel cells (reviewed in Saxod, 1996) and monitor somatosensory information (touch, temperature, pain) from the oral cavity and the rostralmost face (e.g. Noden, 1980a,b).

Otic placode

The otic placode invaginates to form the otic vesicle, which gives rise to the inner ear and to neuron precursors, which migrate away from the epithelium of the otic vesicle and form the sensory neurons of the vestibulocochlear ganglion nearby (reviewed in Torres and Giráldez, 1998; Fritzsche et al., 1998, 2002; Whitfield et al., 2002; Fekete and Wu, 2002; Noramly and Grainger, 2002; Riley and Phillips, 2003; Barald and Kelley, 2004). The inner ear contains many different specialized epithelial cells including the endolymph-producing secretory cells of the stria vascularis, supporting cells and the mechanosensory hair cells (reviewed in Müller and Littlewood-Evans, 2001; Gao, 2003; Frolenkov et al., 2004; Coffin et al., 2005).

The latter are secondary sensory cells (that is, they do not possess an axon), which are innervated by the sensory neurons of the vestibulocochlear ganglion. Hair cells and supporting cells are concentrated in several distinct sensory areas, which vary in number and position between taxa (see reviews by Fritzsch and Neary, 1998; Bryant et al., 2002; Fritzsch et al., 2002). They are committed to the detection of gravity (utricle and saccular maculae in mammals), angular acceleration (cristae of the semicircular canals), and sound (cochlear organ of Corti in mammals).

Lateral line placodes

The lateral line placodes give rise to all peripheral components of the lateral line system, a sensory system for detection of water movements and electric fields. These components comprise the mechanoreceptive (neuromasts) and electroreceptive receptor organs (ampullary organs or tuberous organs) and the sensory neurons of the lateral line ganglia supplying them (reviewed in Winklbaauer, 1989; Northcutt, 1992, 1997; Smith, 1996; Schlosser, 2002a; Ghysen and Dambly-Chaudiere, 2004; Gibbs, 2004). After leaving the placodal epithelium, the neuron precursors congregate in the nearby mesenchyme to form sensory neurons of the lateral line ganglia, while the remaining placodal cells become lateral line primordia. The latter elongate or migrate between surface ectoderm and basement membrane (all the time being closely tracked by outgrowing neurites from the lateral line ganglia) and finally break up into the primordia of receptor organs. Similar to the sensory areas of the ear, lateral line receptor organs are composed of secondary sensory cells (e.g. hair cells in neuromasts) and secretory supporting cells (reviewed in Flock, 1967; Russell, 1976; Blaxter, 1987; Gibbs, 2004; Coffin et al., 2005). Various numbers of lateral line placodes develop in different vertebrate taxa both rostrally and caudally to the otic placode. Six lateral line placodes were probably primitively present in gnathostomes (Northcutt, 1992, 1993a,b, 1997), but the lateral line system has been reduced to various degrees several times independently in different gnathostome lineages, resulting for example in the loss of subsets of lateral line placodes, loss of receptors of a particular modality (e.g. electroreceptors in frogs), or the complete loss of the entire lateral line system (e.g. in amniotes) (reviewed in Fritzsch, 1989; Northcutt, 1992, 1997; Schlosser, 2002b).

Epibranchial and hypobranchial placodes

The epibranchial placodes derive their name from their position dorsal and slightly caudal to the pharyngeal pouches. They give rise to neuronal precursors, which migrate away from the placodal epithelium to form the sensory neurons of the distal ganglia of the facial (geniculate ganglion), glossopharyngeal (petrosal ganglion), and vagal nerves (nodose ganglia). The neurons in these distal ganglia are viscerosensory and have either free nerve endings or innervate various visceral sensory receptors of non-placodal origin including taste buds (reviewed in Northcutt, 2004). The proximal ganglia of the facial,

glossopharyngeal, and vagal nerves, in contrast, harbor neural crest-derived somatosensory neurons (Yntema, 1937, 1943, 1944; Narayanan and Narayanan, 1980; Ayer-LeLièvre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983; Couly and Le Douarin, 1990; Kious et al., 2002). In amphibians, recently, another type of neurogenic placode was discovered, which develops ventrocaudal to the pharyngeal pouches and has accordingly been termed hypobranchial placodes (Schlosser, 2003; Schlosser and Northcutt, 2000; Schlosser et al., 1999). They give rise to small hypobranchial ganglia of unknown function. However, the development of both epibranchial and hypobranchial placodes from a larger placodal area in the branchial region suggests that hypobranchial placodes may be essentially ventrally displaced epibranchial placodes generating viscerosensory neurons (Schlosser, 2003). Neurogenic hypobranchial placodes or ganglia have not been found in other vertebrates, but a recent study suggests that placodal thickenings ventral to the pharyngeal pouches form also in mammals and are later eliminated by apoptosis (Washhausen et al., 2005).

Origin of all placodes from a panplacodal primordium

The spatiotemporal pattern of cranial placode development during embryogenesis has been described for many taxa in the classical embryological literature (e.g. van Wijhe, 1883; Frieriep, 1885; von Kupffer, 1895, 1900; Landacre, 1910, 1912, 1916, 1926, 1927; Landacre and Conger, 1913; Stone, 1922; Knouff, 1935; Damas, 1944; Romanoff, 1960; Bancroft and Bellairs, 1977; Verwoerd and van Oostrom, 1979; Northcutt and Brändle, 1995; Northcutt et al., 1994; Schlosser and Northcutt, 2000; reviewed in Ariëns-Kappers, 1941; Baker and Bronner-Fraser, 2001). These descriptions continue to be valuable sources of information today. However, the reliance on thickenings as the sole indicators of the presence and extent of placodes necessarily puts some limitations on the usefulness of these accounts for elucidating the earliest stages of placode development. Consequently, it has long been disputed whether all cranial placodes arise by the subdivision of a common primordium (e.g. Platt, 1894; Knouff, 1935; Nieuwkoop, 1963, 1985; Jacobson, 1966; Torres and Giraldez, 1998; Baker and Bronner-Fraser, 2001; Schlosser, 2002a; Streit, 2004) or whether they are essentially unrelated structures which form as individually distinct thickenings at various positions of the head (Stone, 1922; Northcutt and Brändle, 1995; Graham and Begbie, 2000; Begbie and Graham, 2001).

As emphasized before (Schlosser, 2002a, 2005; Schlosser and Ahrens, 2004; Streit, 2004), a panplacodal primordium only exists if two conditions are met. First, all cranial placodes must in fact originate from a contiguous precursor region or pre-placodal region. And second, the pre-placodal region must be biased towards the development of generic placodal properties (shared by the different placodes). Generic placodal bias here must be distinguished from the mere competence to respond to placodal inducers and requires that the pre-placodal region has an autonomous tendency to develop generic placodal properties (without being necessarily already fully specified), for example,

due to the expression of transcription factors promoting proliferation, morphogenesis, or neurogenesis in all placodes.

Fate maps suggest that all placodes originate from a common pre-placodal region

Concerning the first condition, fate maps of early teleost (Kozłowski et al., 1997; Whitlock and Westerfield, 2000; Dutta et al., 2005), amphibian (e.g. Vogt, 1929; Röhlich, 1931; Carpenter, 1937; Fautrez, 1942; Jacobson, 1959; Eagleson and Harris, 1990; Eagleson et al., 1995), and amniote (Couly and Le Douarin, 1985, 1987, 1990; Streit, 2002; Bhattacharyya et al., 2004) embryos indeed suggest that all cranial placodes originate from a horseshoe-shaped pre-placodal region in the outer neural folds and the immediately adjacent ectoderm (Fig. 2), which borders the anterior neural plate rostrally and the cranial neural crest laterally. In teleost and chick embryo, it has been shown that, at late gastrula or early neural plate stages, the areas of origin for particular placodes within this pre-placodal region still show considerable overlap (Figs. 2A, B) and segregate only later (Kozłowski et al., 1997; Whitlock and Westerfield, 2000; Streit, 2002; Bhattacharyya et al., 2004; Dutta et al., 2005). It remains to be seen whether this is also true in *Xenopus*, where, unfortunately, no detailed fate maps of non-neural ectoderm at neural plate stages are currently available.

The pre-placodal region appears to be biased for placode development and, thus, constitutes a panplacodal primordium

Whether the second condition is also met is more controversial. In classical studies, the presence of a common thickening, from which various placodes develop, has often been considered as evidence for a uniform placodal bias of the pre-placodal region. However, this purely morphological criterion has tended to confuse rather than to clarify the question of placodal origins for several reasons. Most importantly, such common thickenings can be identified only

in some vertebrate taxa such as teleosts, anurans, and mammals but not in others such as birds, while the condition in urodeles is debated (discussed in detail in Schlosser, 2002a; Schlosser and Northcutt, 2000). Moreover, several thickenings such as the “primitive placodal thickening” described in anuran embryos by Knouff (1935) correspond to a transitional zone between the highly columnar epithelium of the neural plate/neural crest and the flat, squamosal epithelium of the epidermis. This transitional zone extends throughout head and trunk levels and, thus, cannot be equated with the cranial pre-placodal region (even though it covers parts of it).

More convincing evidence for the presence of a generic placodal bias in the pre-placodal region comes from the recent identification of transcription factors, which are initially expressed within this horseshoe-shaped domain, later continue to be expressed in some or all cranial placodes, and have experimentally verified roles for placode development (reviewed in Baker and Bronner-Fraser, 2001; Riley and Phillips, 2003; Streit, 2004; Schlosser, 2005; Schlosser and Ahrens, 2004; Brugmann and Moody, 2005). Many of these transcription factors are either confined to some subregion of the pre-placodal region or extend into ectoderm beyond its limits. However, genes of the *Six1/2* and *Six4/5* subfamilies and of the *Eya* family are expressed in a horseshoe-shaped pattern matching the pre-placodal region at neural plate stages and subsequently continue to be expressed in all cranial placodes (Fig. 3) (*Six1/2* and *Six4/5* genes: Oliver et al., 1995a; Seo et al., 1998a; Ohto et al., 1998; Esteve and Bovolenta, 1999; Kobayashi et al., 2000; Pandur and Moody, 2000; Ghanbari et al., 2001; Ozaki et al., 2001, 2004; Laclef et al., 2003a; Zheng et al., 2003; Li et al., 2003; McLarren et al., 2003; Schlosser and Ahrens, 2004; Bessarab et al., 2004; Zou et al., 2004; *Eya* genes: Abdelhak et al., 1997; Xu et al., 1997a, 1999, 2002a; Kalatzis et al., 1998; Duncan et al., 1997; Mishima and Tomarev, 1998; Sahly et al., 1999; David et al., 2001; McLarren et al., 2003; Schlosser and Ahrens, 2004; Zou et al., 2004). Perturbations of these genes in mutants or by experimental manipulation result in a similar spectrum of

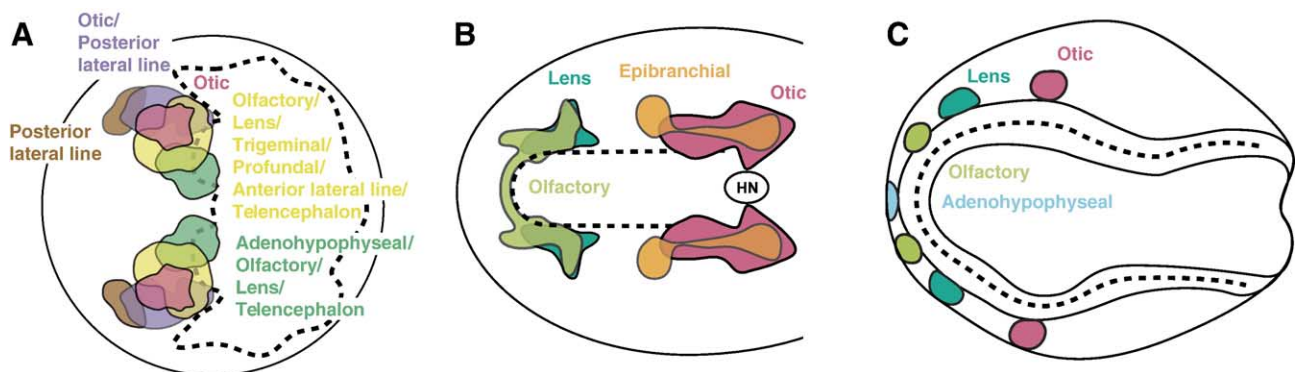


Fig. 2. Fate maps of cranial placodes at late gastrula or neural plate stages. The boundary of the (prospective) neural plate (here defined as the precursor of the entire neural tube, which includes the inner but not the outer neural folds) is indicated by a broken line. All placodes originate from a pre-placodal region in the non-neural ectoderm within and immediately adjacent to the outer neural folds. (A) Fate map of late gastrula zebrafish embryos with substantial overlap between ectodermal regions giving rise to different placodes (modified from Kozłowski et al., 1997). (B) Fate map of neural plate stage chick embryo (0–1 somite stage) with extensive overlap between various prospective placodes (based on data from Streit, 2002; Bhattacharyya et al., 2004). (C) Fate map of neural plate stage salamander (*Ambystoma*) embryo (based on Carpenter, 1937). Recently, otic and lateral line placodes in *Ambystoma* were reported to originate from the outer neural folds themselves and, thus, from slightly more medial positions than depicted here (Northcutt, 1996). HN: Hensen's node.

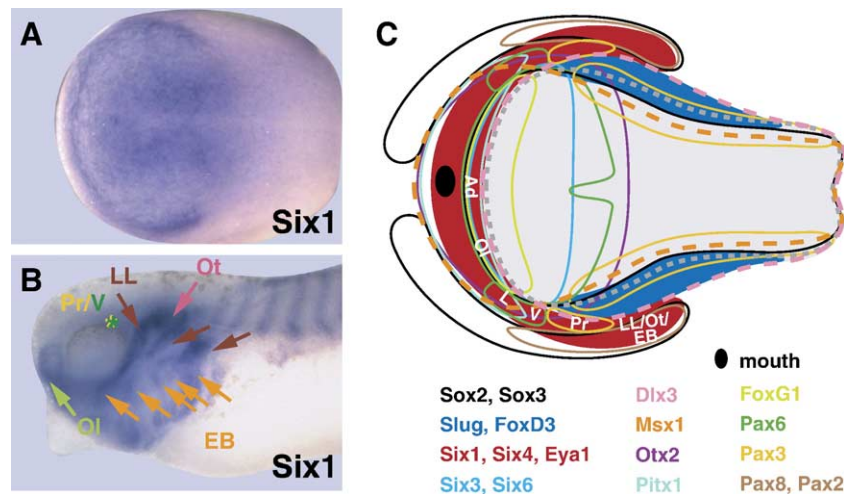


Fig. 3. *Six1*, *Six4*, and *Eya1* define a panplacodal primordium as illustrated for *Xenopus*. (A) *Six1* is first expressed in a horseshoe-shaped domain around the neural plate. (B) At tailbud stages, *Six1* expression continues in all placodes. The position of the fused profundal and trigeminal ganglia is indicated by asterisks (the respective placodes are already disappearing at this stage). (C) Position of the panplacodal primordium (red) relative to the neural plate (gray) and neural crest (blue). The gray broken line indicates the border of the neural plate proper (i.e. the entire ectoderm, which will become incorporated into the neural tube). Solid colored lines enclose areas of expression of various transcription factors, while the broken lines (*Dlx3* and *Msx1*) enclose areas, from which expression is excluded. Note that the region of the outer neural folds immediately peripheral to the hatched gray line is occupied by neural crest cells laterally but by the panplacodal primordium anteriorly. Although no fate map is available, gene expression patterns suggest that placodes arise approximately from the following regions as indicated by white letters: the preoral ectoderm gives rise to the stomodeum with the adenohypophyseal placode and to the olfactory placodes; the lateral domain of *Pax6* expression gives rise to lens and trigeminal placodes; the lateral *Pax3* domain gives rise to the profundal placode; finally, the domain of *Pax8* and *Pax2* expression gives rise to the posterior placodal area, from which otic, lateral line, and epibranchial placodes develop. Expression data are based on the following sources: Bang et al. (1997), Bellefroid et al. (1998), Zygar et al. (1998), Heller and Brändli (1999), Feledy et al. (1999), Ermakova et al. (1999), Hollemann and Pieler (1999), Zuber et al. (1999), Zhou et al. (2000), Sasai et al. (2001), Hartley et al. (2001), Luo et al. (2001a), Schweickert et al. (2001), Schlosser and Ahrens (2004). Abbreviations: Ad: adenohypophyseal placode; EB: epibranchial placodes; L: lens placode; LL: lateral line placodes; Ol: olfactory placode; Ot: otic placode; Pr: profundal placode; V: trigeminal placode.

deficiencies in all placodes, including reductions in cell proliferation and survival and failure of morphogenetic movements and neuronal differentiation as will be discussed in more detail below. *Six1/2*, *Six4/5*, and *Eya* genes are, therefore, currently the most promising candidates for genes biasing the pre-placodal region towards the development of generic placodal properties.

Moreover, many transcription factors involved in regulating some aspect of placode development (including genes of the *Pax*, *Sox*, *Fox*, *Irx*, *Pitx*, and *Tbx* families) are expressed in multiple placodes in a complex pattern of partially overlapping domains. Such a pattern, which will be described in more detail below, is also easier to reconcile with a model according to which different placodes become step by step individualized from a common primordium and may even use some kind of combinatorial code for their specification (Torres and Giráldez, 1998; Schlosser and Ahrens, 2004) than with the alternative model according to which each placode develops completely independently of the others.

To confirm the presence of generic placodal bias in the pre-placodal region experimentally, it has to be shown that development of a normal and positionally appropriate placode from a particular region of the pre-placodal region (e.g. the prospective olfactory region) is more likely when ectoderm in this region is replaced by ectoderm from another part of the pre-placodal region (e.g. the prospective otic region) rather than by ectoderm from outside the pre-placodal region. While this has not yet been explored in a systematic way, experimental evidence in amphibians indeed suggests that, at late neural fold

stages, one part of the pre-placodal ectoderm can substitute to some degree for another, while at least some ectodermal regions outside of the pre-placodal region cannot (for exceptions see, however, Yntema, 1933; Schlosser and Northcutt, 2001). For example, when the prospective olfactory or lens placodes are replaced by prospective otic placodes at these late stages, olfactory and lens placodes form from the replacing ectoderm, while otic placodes form in the reverse experiment (Yntema, 1933; Ikeda, 1937, 1938; Haggis, 1956; Reyer, 1958; Jacobson, 1963a; Henry and Grainger, 1987; Gallagher et al., 1996; Grainger et al., 1997). This suggests that the replacing ectoderm had already acquired some generic bias for placode development in its original position.

Another line of evidence comes from the formation of ectopic placodes after experimental overexpression of transcription factors implicated in development of specific placodes. Such ectopic placodes are often localized exclusively to cranial ectoderm in the vicinity of other placodes, suggesting that proper placode development requires cooperation of these transcription factors with generic placodal factors expressed specifically within the pre-placodal region. For example, overexpression of *Pax6*, *Six3*, or *Sox3* (each expressed in the lens placode in addition to several other placodes) results in the formation of ectopic lenses only in cranial ectoderm (Oliver et al., 1996; Altmann et al., 1997; Loosli et al., 1999; Chow et al., 1999; Köster et al., 2000; Lagutin et al., 2001). On the other hand, however, ectopic otic marker gene expression as well as the formation of ectopic otic vesicles after overexpression of *FoxI1* (Solomon et al., 2003a), *Sox3* (Köster et al., 2000), or

components of various signaling pathways (Gutknecht and Fritschsch, 1990; Lombardo and Slack, 1998; Vendrell et al., 2000; Koebernick et al., 2003) is also found in the trunk and is not cranially restricted, although it is always confined to dorsal ectoderm. The latter findings per se argue neither for nor against a generic placodal bias of the pre-placodal region because it remains currently unclear, whether they are associated with the posterior expansion or ectopic induction of ectoderm with generic placodal bias in these experimental situations or rather reflect the possibility of placode formation independent of generic placodal transcription factors.

***Six1/2*, *Six4/5*, and *Eya*: transcription factors promoting generic aspects of placode development**

The panplacodal distribution of *Six1/2*, *Six4/5*, and *Eya* genes and their known effects on development of multiple placodes make these genes the most promising candidates for regulators of generic placodal properties and suggest that their early expression in the pre-placodal ectoderm provides this ectodermal domain with a generic placodal bias. Here, I briefly review how these genes interact and what evidence supports their role in promoting generic placodal properties.

The Six–Eya–Dach–Pax network

Six genes encode homeodomain transcription factors, which bind directly to DNA (reviewed in Relaix and Buckingham, 1999; Kawakami et al., 2000; Wawersik and Maas, 2000; Hanson, 2001; Epstein and Neel, 2003; Kardon et al., 2004; Brugmann and Moody, 2005). Out of the three metazoan subfamilies of *Six* genes (Kawakami et al., 2000; Bebenek et al., 2004), only *Six1/2* and *Six4/5* but not *Six3/6* subfamily genes exhibit panplacodal expression in vertebrates although details of expression differ between species.

Eya genes encode protein tyrosine phosphatases, which act as transcriptional coactivators of *Six* genes (reviewed in Relaix and Buckingham, 1999; Wawersik and Maas, 2000; Hanson, 2001; Epstein and Neel, 2003; Kardon et al., 2004; Rebay et al., 2005). Due to gene duplication, there are four *Eya* genes in vertebrates, whereas invertebrates have only a single *Eya* gene (Xu et al., 1997a; Zimmerman et al., 1997; Duncan et al., 1997; Borsani et al., 1999; Mazet et al., 2005). All *Eya* genes except *Eya3* are widely expressed in cranial placodes, with each placode expressing at least one *Eya* gene, although the distribution of the different paralogues among placodes differs for different species.

In *Drosophila*, it has first been shown that *sine oculis* and *eyes absent*—homologues of the vertebrate *Six1/2* and *Eya* genes, respectively—form a regulatory network essential for compound eye development together with the nuclear protein *dachshund* and the *Pax6* homologue *eyeless* (reviewed in Gehring and Ikeyo, 1999; Relaix and Buckingham, 1999; Wawersik and Maas, 2000; Kumar and Moses, 2001a; Pappu and Mardon, 2004; Rebay et al., 2005). Mutants in any of these genes disrupt compound eye development (Cheyette et al., 1994; Serikaku and O'Tousa, 1994; Bonini et al., 1993, 1997;

Mardon et al., 1994; Pignoni et al., 1997; Rayapureddi et al., 2003; Tootle et al., 2003). Conversely, these genes synergize in promoting ectopic eye formation after misexpression (Bonini et al., 1997; Pignoni et al., 1997; Chen et al., 1997; Shen and Mardon, 1997; Halder et al., 1998; Niimi et al., 1999). Because the same set of genes is also coexpressed elsewhere in the embryo, a similar regulatory network may operate in other developmental contexts, although this has not been rigorously analyzed yet (Cheyette et al., 1994; Serikaku and O'Tousa, 1994; Bonini et al., 1993, 1998; Pignoni et al., 1997; Suzuki and Saigo, 2000; Kumar and Moses, 2001b; Fabrizio et al., 2003).

In vertebrates, *Six* and *Eya* genes are also coexpressed with *Dachshund* (*Dach*) and *Pax* genes (including *Pax6* but also members of other *Pax* subfamilies such as *Pax2*) in various tissues. These include the kidney, somites, retina, and several placodes (reviewed in Relaix and Buckingham, 1999; Kawakami et al., 2000; Wawersik and Maas, 2000; Hanson, 2001; Baker and Bronner-Fraser, 2001; Kardon et al., 2004; Streit, 2004). Furthermore, some regulatory interactions between these genes as discovered in *Drosophila* appear to be preserved in the vertebrate expression domains, although there are also important differences (reviewed in Relaix and Buckingham, 1999; Kawakami et al., 2000; Wawersik and Maas, 2000; Hanson, 2001; Kardon et al., 2004; Rebay et al., 2005). Most importantly, while in *Drosophila*, the *Pax6* homologue *eyeless* acts as an upstream transcriptional regulator (although subject to feedback regulation) of *eya*, *sine oculis*, and *dach* (Bonini et al., 1997; Halder et al., 1998; Niimi et al., 1999; Bui et al., 2000; Punzo et al., 2002; Pappu et al., 2005), in many tissues of vertebrates including the otic placode *Pax* genes are not upstream of initial expression of these other genes (Xu et al., 1999; Zheng et al., 2003; Ozaki et al., 2004; Purcell et al., 2005; but see Xu et al., 1997a).

At the core of the network are direct physical interactions between *Six* and *Eya* proteins (with conflicting reports for *Six3/6* subfamily members) and between *Eya* and *Dach* (Chen et al., 1997; Pignoni et al., 1997; Heanue et al., 1999; Ohto et al., 1999; Ikeda et al., 2002; Li et al., 2003; Silver et al., 2003; Purcell et al., 2005). The binding of *Eya* to *Six* proteins is required for the translocation of *Eya* into the nucleus where *Eya* then acts as a transcriptional cofactor of *Six* (Ohto et al., 1999). *Dach* acts as another cofactor of *Six*, although it also appears to be able to bind DNA directly (Ikeda et al., 2002; Kim et al., 2002; Li et al., 2003).

Six transcription factors can function either as transcriptional activators or as transcriptional repressors depending on which cofactors they interact with. Upon binding of *Eya* to *Six*, the phosphatase activity of *Eya* is able to turn *Dach* from a corepressor to a coactivator of *Six* (Li et al., 2003), thereby converting *Six* from a transcriptional repressor into an activator (although there are probably additional mechanisms of *Six–Eya* synergy; see reviews of Epstein and Neel, 2003; Rebay et al., 2005). In addition, the transcriptional activity of at least *Six1/2* and *Six3/6* subfamily members can also be modulated by binding to other cofactors including members of the Groucho family of corepressors (some of which are also panplacodally expressed; see, for example, Molenaar et al., 2000) (Kobayashi

et al., 2001; Zhu et al., 2002; López-Rios et al., 2003; Silver et al., 2003; Brugmann et al., 2004). It has therefore been suggested that the mode of action of Six transcription factors is determined by the relative levels of available activating or repressive cofactors (Brugmann et al., 2004; Brugmann and Moody, 2005). The various deficiencies observed in heterozygous human and mouse *Eya* mutants due to haploinsufficiency may be partly attributed to such dependence of normal function on relatively precise protein levels (e.g. Abdelhak et al., 1997; Xu et al., 1999; Zhang et al., 2004).

Promotion of generic placodal properties by *Six1/2*, *Six4/5*, and *Eya* genes

Mutants in mice, humans, and zebrafish suggest an important role of the panplacodally expressed *Six1/2*, *Six4/5*, and *Eya* genes in the development of various placodes. While there is at present little information about *Six2*, *Eya2*, and *Eya3*, mutants of *Six1*, *Six5*, *Eya1*, and *Eya4* have all been associated with defects in placodally derived tissues. As detailed below, *Eya1* and *Six1* represent by far the most thoroughly analyzed cases and have a very similar spectrum of defects in various placodes as well as in other tissues (Table 1). In contrast, *Six4* mouse mutants develop nearly normal and have no obvious placodal

defects (Ozaki et al., 2001). This absence of placodal defects in *Six4* mutants as well as the occurrence of only mild defects in some placodal derivatives of *Six1* and *Eya1* mutants may be due to compensation by other *Six* and *Eya* genes with redundant functions. In favor of this interpretation, recently generated *Six1/Six4* double null mutants show more severe developmental defects in the musculoskeletal system and in several other tissues including placodes than each of the single mutants, although the placodal defects have not yet been characterized in detail (Grifone et al., 2005).

Eya1 and *Six1* were both identified as genes underlying particular forms of inherited deafness in humans known as branchio-otic (BO) or branchio-oto-renal (BOR) syndrome, which are associated with branchial or branchial and renal defects, respectively (Vincent et al., 1997; Abdelhak et al., 1997; Namba et al., 2001; Wayne et al., 2001; Kemperman et al., 2002; Chang et al., 2004; Ruf et al., 2004; Zhang et al., 2004). Mutants in *Eya4* lead to another non-syndromic form of late-onset-inherited deafness (Wayne et al., 2001; Zhang et al., 2004). Studies of mutant mice and zebrafish suggest that these hearing deficits result partly from middle ear defects but also from deficient development of the inner ear from the otic placode. In homozygous mutants of either *Eya1* or *Six1*, only small otic vesicles develop which do not form properly organized semicircular canals or a cochlear duct. The sensory areas of the inner ear are either much smaller or completely absent and do not maintain appropriate levels of expression of neuronal determination and differentiation genes such as *Neurogenin1* and *NeuroD* (Whitfield et al., 1996; Johnson et al., 1999; Xu et al., 1999; Laclef et al., 2003a; Li et al., 2003; Zheng et al., 2003; Ozaki et al., 2004; Zou et al., 2004; Friedman et al., 2005; Kozłowski et al., 2005). While a smaller than usual vestibulocochlear ganglion first forms, it later degenerates by apoptosis in the mouse (Zou et al., 2004; Friedman et al., 2005; Kozłowski et al., 2005).

Interestingly, other placodally derived structures also show extensive developmental deficiencies in *Eya1* and *Six1* mutants. The sensory epithelium derived from the olfactory placode is reduced or missing in mouse *Six1* mutants (Laclef et al., 2003a; Li et al., 2003; Ozaki et al., 2004). Number and size of neuromasts, which develop from lateral line placodes, are reduced in zebrafish *dogear* (*Eya1*) mutants (Whitfield et al., 1996; Kozłowski et al., 2005). However, cranial ganglia including those derived from lateral line and epibranchial placodes appear to be normal in *dogear* zebrafish (Kozłowski et al., 2005). In mouse *Eya1* and *Six1* mutants, the trigeminal and profundal ganglia, which receive contributions from placodes and from neural crest, are reduced in size, whereas the geniculate, petrosal, and nodose ganglion derived from epibranchial placodes are either reduced or completely absent and fail to express the neuronal determination and differentiation genes *Neurogenin2* and *NeuroD* (Xu et al., 1999; Zheng et al., 2003; Zou et al., 2004). Furthermore, reducing *Eya1* protein levels in *Xenopus* by injection of morpholino antisense oligonucleotides leads to reduced expression of neuronal marker genes such as *NeuroD* in all neurogenic placodes (Völker, Stämmler, and Schlosser, unpublished observations). Similarly,

Table 1
Deficits of placodal and pharyngeal derivatives in *Six1* and *Eya1* mutants and morphants

Placodes	<i>Six1</i>	<i>Eya1</i>	<i>Six1/Eya1</i>
Panplacodal markers	+ ^j	+ ^k	
Adenohypophyseal	—	—	+ ^g
Olfactory	+ ^{c,g,h}	+ ^k	
Lens	—	+ ^l	
Trigeminal	+ ⁱ	+ ^{i,k}	
Profundal	+ ⁱ	+ ^{i,k}	
Otic	+ ^{e-i,m}	+ ^{a,b,d,i,k,n-r}	+ ^f
Lateral line	Not determined	+ ^{a,d,k}	
Epibranchial	+ ^{f,i}	+ ^{b,i,k}	
Pharyngeal derivatives (e.g. thymus)	+ ^{e,h,m}	+ ^{c,n,o,q}	

Sources:

- ^a (Whitfield et al., 1996) (zebrafish dogear mutant of *Eya1*).
- ^b (Xu et al., 1999) (mouse *Eya1* mutant).
- ^c (Xu et al., 2002a) (mouse *Eya1* mutant).
- ^d (Kozłowski et al., 2005) (zebrafish dogear mutant of *Eya1*).
- ^e (Laclef et al., 2003a) (mouse *Six1* mutant).
- ^f (Zheng et al., 2003) (mouse *Six1* and *Six1/Eya1* mutants).
- ^g (Li et al., 2003) (mouse *Six1* and *Six1/Eya1* mutants).
- ^h (Ozaki et al., 2004) (mouse *Six1* mutant).
- ⁱ (Zou et al., 2004) (mouse *Six1* and *Eya1* mutants).
- ^j (Brugmann et al., 2004) (*Xenopus Six1* morpholino knockdown).
- ^k Schlosser, unpublished observations (*Xenopus Eya1* morpholino knockdown).
- ^l (Azuma et al., 2000) (human *Eya1* mutants).
- ^m (Ruf et al., 2004) (human *Six1* mutants).
- ⁿ (Namba et al., 2001) (human *Eya1* mutants).
- ^o (Abdelhak et al., 1997) (human *Eya1* mutants).
- ^p (Johnson et al., 1999) (mouse *Eya1* mutants).
- ^q (Chang et al., 2004) (human *Eya1* mutants).
- ^r (Friedman et al., 2005) (mouse *Eya1* mutants).

morpholino-mediated knockdown of *Six1* not only reduces widely expressed placodal genes such as *Eya1* and *Sox11* in *Xenopus*, but also perturbs placodal neurogenesis (Brugmann, 2005; Brugmann et al., 2004). Overexpression of *Eya1* together with *Six1*, in contrast, promotes ectopic neurogenesis (Völker, Stämmler, and Schlosser, unpublished observations).

Finally, structures derived from non-neurogenic placodes are also affected, although more mildly, in *Eya* and *Six* mutants. Lens defects (cataracts) were found in mutants of *Eya1* (human) and *Six5* (mouse) (Azuma et al., 2000; Klesert et al., 2000), whereas the adenohypophysis is almost normal in mouse single mutants of *Eya1* and *Six1* but drastically reduced in double mutants (Zheng et al., 2003).

Underlying this gamut of developmental effects, there appear to be altogether at least three developmental processes affected by *Six* and *Eya* genes, all of which contribute to distinguish the various cranial placodes from the adjacent epidermis (see above). First, placode-derived structures are generally reduced in size or absent in mutants, suggesting that *Six1/2* and *Eya* genes are involved in size regulation. There appear to be two ways in which size is regulated by these genes and which are deficient in mutants: promotion of cell proliferation and inhibition of apoptosis (Xu et al., 1999, 2002a; Zheng et al., 2003; Li et al., 2003; Zou et al., 2004; Kozłowski et al., 2005; Friedman et al., 2005). The pro-proliferative effect of *Six1* and *Eya* has also been implicated in tumorigenesis and may at least partly be attributable to their stimulating effect on known cell cycle control genes such as *CyclinA1* and *cMyc* (Ford et al., 1998; Li et al., 2003; Coletta et al., 2004; Zhang et al., 2005). The anti-apoptotic effect appears to be strongly concentration-dependent since abnormally high levels of *Eya* genes also promote rather than inhibit apoptosis (Clark et al., 2002).

Second, the delamination of sensory ganglion cells from neurogenic placodes and morphogenetic movements (e.g. during formation of the membranous labyrinth) are compromised in mutants. Deficiencies in other tissues in *Six1* and *Eya1* mutant mice including thymus and kidney probably also involve morphogenetic in addition to proliferative defects (Xu et al., 1999, 2002a, 2003; Laclef et al., 2003a; Li et al., 2003; Ozaki et al., 2004). This suggests that *Six1/2* and *Eya* genes are involved in the promotion of cell shape changes, which may also underlie the capacity of *Six1* to enhance the invasiveness of cancer cells, thereby promoting metastasis (Yu et al., 2004). Nothing is presently known about the mechanism by which these genes affect morphogenesis and whether it depends on or is independent of their pro-proliferative effect.

Third, certain pathways of cytodifferentiation are blocked in mutants, for instance, myogenesis in the mesoderm (Heanue et al., 1999; Laclef et al., 2003b) and the formation of neurons and sensory cells in the ectoderm indicating that *Six1/2* and *Eya* genes promote particular pathways of cytodifferentiation. Again, the underlying mechanisms remain to be elucidated, but preliminary data indicate that overexpression of *Six1* or *Eya1* inhibits expression of neuronal differentiation genes in cells with high *Eya1* and *Six1* protein levels, while coinjection of *Six1* and *Eya1* promotes ectopic neurogenesis only in cells of

low *Eya1* and *Six1* protein levels situated immediately adjacent to cells overexpressing high levels of *Eya1* and *Six1* (Brugmann, 2005; Brugmann and Moody, 2005; Völker, Stämmler, and Schlosser, unpublished observations). This suggests that similar to *SoxB1* transcription factors (see below) high levels of *Six* and *Eya* may promote a proliferative neurogenic precursor state but may be incompatible with neuronal differentiation.

Unfortunately, beyond the few genes mentioned, we still know very little about the target genes through which *Six* and *Eya* genes exert their effects. However, the deficiencies observed in mutants and morphants indicate that *Eya* genes and *Six* genes of the *Six1/2* subfamily (and possibly also of the *Six4/5* subfamily although sufficient data are lacking) regulate proliferation, apoptosis, cell shape changes, and neurogenesis in all placodes and, thereby, promote generic placodal properties shared by different placodes. Together with the expression of *Six1/2*, *Six4/5*, and *Eya* genes throughout the pre-placodal region and their subsequent panplacodal expression, this suggests that these genes indeed provide the pre-placodal region with a bias towards generic placodal development thereby defining a panplacodal primordium.

It is not clear whether beyond these roles in promoting expression of a number of generic placodal target genes in all placodes, *Six* and *Eya* genes have additional and more central functions for placodal development. *Six* and *Eya* genes could, for example, also introduce panplacodal bias by defining some kind of generic placodal cell state that predisposes cells to adopt one out of several placodal fates, while preventing adoption of other ectodermal fates. Such a restriction of developmental potential could result, for example, if the positionally appropriate activation of several batteries of genes specific for the different individual placodes would require input of *Six* and *Eya* genes together with multiple other and spatially more restricted transcription factors (as discussed below for transcription factors conferring multiplacodal bias). There is presently little evidence to support such a mode of action for *Six* and *Eya* genes, but this issue requires additional study.

Other transcription factors promoting placode development

Besides the panplacodally expressed *Six1/2*, *Six4/5*, and *Eya* genes, many other transcription factors are now known to affect placode development. However, some of these transcription factors are not confined to the placodal ectoderm, while others are important for only subsets of placodes or particular placodal cell types. In this section, I will briefly review the roles that some of these transcription factors play for placode development. Due to the combination of *cis*-regulatory analyses with functional studies, we are beginning to understand how different transcription factors interact and form networks, but the emerging picture is still not very clear and I will not attempt to summarize it here. Overviews can be found in several recent reviews dealing with specific placodes, viz. the adenohypophyseal (Sheng and Westphal, 1999; Kioussi et al., 1999a; Dasen and Rosenfeld, 2001; Scully and Rosenfeld, 2002; Savage et al.,

2003; Asa and Ezzat, 2004), olfactory (Beites et al., 2005), lens (Ogino and Yasuda, 2000; Chow and Lang, 2001; Bhattacharyya and Bronner-Fraser, 2004), and otic placodes (Noramly and Grainger, 2002; Fekete and Wu, 2002; Riley and Phillips, 2003; Barald and Kelley, 2004). In a separate section below, I will, however, discuss how the spatiotemporal distribution of these transcription factors can provide important clues of how a diverse array of placodes is specified within the panplacodal primordium.

To allow for a structured overview of placodal transcription factors, they will be tentatively sorted into four functional categories here, although there are certainly no sharp dividing lines between them: (1) transcription factors promoting placodal competence; (2) transcription factors promoting placodal neural progenitors; (3) transcription factors acting as positional markers of placode identity; and (4) transcription factors promoting cytodifferentiation of various placodal cell types.

The first group of transcription factors is expressed widely in the pre-placodal region but extends beyond it and covers the entire non-neural ectoderm (here defined restrictively as all ectoderm, which does not become incorporated into either neural tube or neural crest). These genes, which include Distal-less/Dlx homeobox transcription factors (in particular *Dlx3* and *Dlx5*) and some GATA class zinc finger transcription factors (*GATA1*, *GATA2*, *GATA3*), are discussed in more detail below in the section dealing with placode induction. Current evidence suggests that they may play a central role for the development of non-neural ectoderm and may mediate its competence to form various derivatives including epidermis and placodes.

Transcription factors promoting placodal neural progenitors

A second group of transcription factors is approximately expressed in the reverse pattern and besides expression in the pre-placodal ectoderm shows high expression in the neural plate but little expression in the epidermal ectoderm. This group of genes includes *Sox2* and *Sox3*, which together with *Sox1* comprise the *SoxB1* subfamily of HMG box containing transcription factors, as well as homeobox transcription factors *Irxx* to *Irxx6* (also known as *Xiro* or *Ziro* genes in *Xenopus* and zebrafish, respectively) related to *Drosophila Iroquois* (Collignon et al., 1996; Rex et al., 1997; Bosse et al., 1997, 2000; Bellefroid et al., 1998; Gómez-Skarmeta et al., 1998; Wood and Episkopou, 1999; Mizuseki et al., 1998; Kishi et al., 2000; Köster et al., 2000; Cohen et al., 2000; Cheng et al., 2001; Wang et al., 2001; Abu-Elmagd et al., 2001; Ishii et al., 2001; Schlosser and Ahrens, 2004; reviewed in Wegner, 1999; Sasai, 2001; Cavodeassi et al., 2001; Gómez-Skarmeta and Modolell, 2002; St. Amand and Klymkowsky, 2001; Kondoh et al., 2004; Pevny and Placzek, 2005). Although these genes show widespread placodal expression, they do not cover the entire pre-placodal ectoderm. In *Xenopus*, for example, *Irxx1* and *Irxx2* genes are absent from the olfactory placode, while *Sox2* and *Sox3* genes are absent from the profundal and trigeminal placodes.

In the neural plate, *SoxB1* and *Irxx* genes appear to have similar functions in maintaining cells in a neural progenitor state, while at the same time preventing terminal neuronal

differentiation (Bellefroid et al., 1998; Mizuseki et al., 1998; Pevny et al., 1998; Gómez-Skarmeta et al., 1998; Kishi et al., 2000; de la Calle-Mustienes et al., 2002; Itoh et al., 2002; Bylund et al., 2003; Graham et al., 2003; Lecaudey et al., 2004; Ferri et al., 2004; Zhao et al., 2004). However, *Sox1* has also been reported to directly promote neuronal differentiation (Pevny et al., 1998; Kan et al., 2004).

The roles of these genes for placode development are less well understood but may be quite similar as concerns placodal neurogenesis. Zebrafish *Irxx* genes are required for neurogenesis in the profundal/trigeminal placode (Itoh et al., 2002). Preliminary data also indicate that *Sox3* may be similarly required for neurogenesis in at least the epibranchial placodes, while overexpression of *Sox3* appears to prevent migration and differentiation of neurons from different neurogenic placodes (Abu-Elmagd et al., 2001; Scotting, personal communication; Schlosser, unpublished observations). Moreover, *SoxB1* genes and probably *Irxx* genes as well (although this requires more thorough analysis) are strongly expressed in the placodal ectoderm but downregulated as soon as placodally derived neurons migrate away and express neuronal determination and differentiation genes (Abu-Elmagd et al., 2001; Schlosser and Ahrens, 2004). Taken together, this suggests that *SoxB1* and *Irxx* genes are likely to be important for regulating placodal neurogenesis probably by promoting a neural progenitor state similar to their function in the neural plate. However, neither *Irxx* nor *SoxB1* genes are expressed in all neurogenic placodes, suggesting that neuronal progenitor states may be regulated differently in different placodes.

In addition, *Irxx* and *SoxB1* genes are likely to play other and possibly more fundamental roles in placodal development, which require further investigation. As discussed in more detail below, the exclusion of some *Irxx* genes from the rostralmost neural plate and placodes makes them attractive candidates for defining a posterior equivalence group of placodes. *Irxx1* was indeed shown to be required for maintaining expression of various placodal transcription factors including the panplacodal factor *Six1* and transcription factors with more restricted expression (*Sox2*, *Pax2*), while overexpression of *Irxx1* leads to an expansion of the placodal expression domains of these genes (Glavic et al., 2004). Overexpression of *Sox2* even results in ectopic expression of *Eya1* and *Pax6* and in ectopic formation of lens and otic placodes (Köster et al., 2000). In the lens placode, *SoxB1* genes have been shown to cooperate with *Pax6* and *L-Maf* in the regulation of lens-specific cytodifferentiation including direct regulation of crystallin expression (Kamachi et al., 1998, 2001; Muta et al., 2002; Shimada et al., 2003; reviewed in Bhattacharyya and Bronner-Fraser, 2004; Cvekl et al., 2004; Kondoh et al., 2004), while in the otic placode *Sox2* is required for sensory organ formation (Kiernan et al., 2005).

Transcription factors acting as positional markers of placode identity

A third, large and heterogeneous group of placodal transcription factors comprise genes which are confined to

relatively large but anteroposteriorly (and sometimes dorso-ventrally) restricted subregions of the panplacodal primordium (plus possibly other parts of the ectoderm). These genes often continue to be expressed in subsets of placodes at later stages, and they are required for development of the placodes, in which they are expressed. Thus, they are candidates for positional markers involved in defining placode identity. Often, these transcription factors have a relatively broad spectrum of effects on placodal development and regulate proliferation of progenitor cells, differentiation, and morphogenesis in a position-specific manner. Transcription factors of this category comprise various homeobox (viz. *Otx*, *Emx*, *Six3/6*, *ANF*, *Pitx*, and *Pax* genes), winged helix (*Fox*), and T-box (*Tbx*) transcription factors. As a caveat, it must, however, be pointed out that many of these genes (and *Otx* genes in particular) are expressed in large domains extending beyond the panplacodal primordium and often are essential for development of a number of additional tissues. Therefore, some of their documented effects on placode development may be indirect, and additional studies are needed to clearly distinguish between direct and indirect modes of action.

Otx genes

Otx homeobox genes show anteriorly restricted expression in all germ layers from gastrulation onwards. Their ectodermal expression includes the prospective fore- and midbrain and the prospective adenohipophyseal, olfactory, and lens placodes; at later stages, they are also expressed in the otic placode (Simeone et al., 1993; Pannese et al., 1995; Kablar et al., 1996). *Otx* genes control a multitude of unrelated target genes (including, for example, pituitary hormones; Acampora et al., 1998) and are thought to play a central role in head specification (reviewed in Acampora et al., 2000; Boyl et al., 2001; Boncinelli and Morgan, 2001; Simeone and Acampora, 2001; Simeone et al., 2002). Blocking *Otx2* function in early embryogenesis results in lack of many head structures including the fore- and midbrain and the adenohipophyseal, olfactory, and lens placodes (Acampora et al., 1995; Matsuo et al., 1995; Gammill and Sive, 2001) and in deficient development of the otic vesicle (Acampora et al., 1996; Fritzsche et al., 2001).

Emx genes

Emx homeobox genes show a more restricted anterior expression than *Otx* genes in the prospective forebrain as well as in the olfactory and otic placodes (Simeone et al., 1992; Morita et al., 1995; Kablar et al., 1996; Kawahara and Dawid, 2002). They have multiple roles in regulating proliferation, differentiation, migration, and regional subdivision of the forebrain (reviewed in Cecchi and Boncinelli, 2000; Cecchi, 2002). Placodal defects of *Emx2* mutants (Pellegriani et al., 1996; Yoshida et al., 1997) have not been well characterized, but the failure of the olfactory nerve to connect to the olfactory bulbs in such mutants is possibly due to deficient axon guidance of placode-derived olfactory receptor neurons since *Emx* proteins are known to be localized to these axons, where they regulate protein translation (Briata et al., 1996; Nedelec et al., 2004).

Six3/6 genes

In contrast to the *Six1/2* and *Six4/5* genes already discussed above, expression of *Six3/6* is restricted to the anterior neural plate (prospective forebrain) and the immediately adjacent non-neural ectoderm including the prospective adenohipophyseal, olfactory, and lens placodes (Oliver et al., 1995b; Loosli et al., 1998; Kobayashi et al., 1998; Zuber et al., 1999; Jean et al., 1999; López-Ríos et al., 1999; Zhou et al., 2000; Bernier et al., 2000). *Six3/6* genes promote the development of forebrain, retina, and the rostral placodes in multiple ways: they inhibit Wnt and BMP signaling (Lagutin et al., 2003; Gestri et al., 2005); promote cell proliferation by various mechanisms including sequestering of the cell cycle inhibitor Geminin and transcriptional repression of the cell cycle inhibitor *p27Kip1* (Kobayashi et al., 1998; Zuber et al., 1999; Li et al., 2002; Del Bene et al., 2004; Gestri et al., 2005); delay neuronal differentiation (Gestri et al., 2005); and bias cell fate choices (Oliver et al., 1996; Loosli et al., 1999; Bernier et al., 2000; Lagutin et al., 2001). Loss of function of either *Six3* or *Six6* results in loss or size reduction of forebrain, retina, and the adenohipophyseal, olfactory, and lens placodes (Zuber et al., 1999; Li et al., 2002; Carl et al., 2002; Lagutin et al., 2003).

ANF genes

The *ANF* homeobox genes (also known as *HesX1* or *Rpx* in mammals) also show anteriorly restricted expression at neural plate stages in the prospective forebrain and in adjacent ectoderm of the anterior neural folds including the prospective adenohipophyseal, olfactory, and possibly lens placodes, but their placodal expression soon becomes confined to the adenohipophyseal placode (Zaraisky et al., 1992, 1995; Mathers et al., 1995; Kazanskaya et al., 1997; Ermakova et al., 1999; Hermesz et al., 1996; Thomas and Beddington, 1996). *ANF* genes have been implicated in both patterning and size regulation of forebrain and adenohipophysis and need to be downregulated in order to permit cytodifferentiation of neurons or of several of the adenohipophyseal neuroendocrine cell types (Ermakova et al., 1999; Dasen et al., 2001). *ANF* (*HesX1*) mutants accordingly show strong reduction or aberrant development of the forebrain, adenohipophyseal, olfactory, and lens placodes (Dattani et al., 1998).

Pitx genes

Pituitary homeobox (*Pitx*) genes are expressed (with some rather subtle differences between the different paralogues) in the ectoderm immediately rostral to the neural plate including the prospective adenohipophyseal, olfactory, and lens placodes (Semina et al., 1996; Gage and Camper, 1997; Lanctot et al., 1997; Hollemann and Pieler, 1999; Essner et al., 2000; Chang et al., 2001; Schweickert et al., 2001; Pommereit et al., 2001; Boorman and Shimeld, 2002; Uchida et al., 2003; Zilinski et al., 2005; Dutta et al., 2005). Besides their function in regulating left–right asymmetries, limb development, and craniofacial development, vertebrate *Pitx* genes play important roles for adenohipophyseal and lens development (Lamonerie et al., 1996; Szeto et al., 1996, 1999; Lin et al., 1999; Gage et al., 1999; Suh et al., 2002; Kiousi et al., 2002; Briata et al., 2003;

Shi et al., 2005; Dutta et al., 2005). They promote proliferation of different adenohypophyseal cell populations, as well as the expression of various adenohypophysis-specific transcription factors and hormones, and loss of *Pitx* genes results in reduced expression of many pituitary transcription factors, reduced numbers of adenohypophyseal cells, and in compromised lens development.

Msx genes

In contrast to the genes discussed so far, *Msx* homeobox genes show relatively widespread expression in the lateral neural plate, neural crest, and non-neural ectoderm including many prospective placodes with some interspecific differences (for example, *Msx1* is excluded from adenohypophyseal and olfactory placodes in *Xenopus* but not in mice) (Hill et al., 1989; Robert et al., 1989; Mackenzie et al., 1991; Ekker et al., 1992; Maeda et al., 1997; Suzuki et al., 1997; Streit and Stern, 1999a; Feledy et al., 1999; Gong and Kiba, 1999; Onitsuka et al., 2000). As direct BMP target genes with antineural effects, *Msx* genes have been implicated in early ectodermal patterning (Suzuki et al., 1997; Feledy et al., 1999). In *Xenopus*, where *Msx* genes are excluded from the anterior ectoderm, they inhibit anterior development and promote epidermal development in non-neuralized ectoderm but neural crest development in neuralized ectoderm (Suzuki et al., 1997; Gong and Kiba, 1999; Feledy et al., 1999; Yamamoto et al., 2001; Tribulo et al., 2003; Monsoro-Burq et al., 2005). They are thought to exert their effects by multiple mechanisms such as preventing exit from the cell cycle and promoting apoptosis (Marazzi et al., 1997; Hu et al., 2001; Liu et al., 2004; Tribulo et al., 2004). Loss of *Msx* function results in complex craniofacial defects, but despite strong *Msx* expression in several placodes (e.g. otic placode), no severe placodal defects have been described in mutants (Satokata and Maas, 1994; Satokata et al., 2000; Houzelstein et al., 1997; reviewed in Bendall and Abate-Shen, 2000; Alappat et al., 2003) so the role of *Msx* for placodal development is currently unclear.

Pax genes

There are four different subfamilies of *Pax* genes, transcription factors with a paired-type DNA-binding domain (Dahl et al., 1997; Miller et al., 2000; Chi and Epstein, 2002). Members of the *Pax2/5/8*, *Pax3/7*, and *Pax6* subfamilies, but not of the *Pax1/9* subfamily, play important roles for placode development. *Pax* genes affect multiple developmental processes including proliferation, cytodifferentiation, cell adhesion, and signaling, and while they generally tend to maintain an undifferentiated state, they are also known to promote the formation of specific subpopulations of differentiated cells (reviewed in Mansouri et al., 1996; Dahl et al., 1997; Dressler and Woolf, 1999; Mansouri, 1998; Gehring and Ikeo, 1999; Underhill, 2000; van Heyningen and Williamson, 2002; Simpson and Price, 2002; Epstein, 2000; Chi and Epstein, 2002). Because *Pax* genes interact in a regulatory network with the panplacodally expressed *Eya*, *Six*, and *Dach* genes but are themselves expressed in a more regionally restricted manner, they were proposed to have a special role in conferring placode

identity (Baker and Bronner-Fraser, 2000, 2001; Streit, 2002, 2004). However, since each *Pax* gene is still expressed in multiple placodes (see Schlosser and Ahrens, 2004), *Pax* genes alone cannot suffice to specify placode identity but must cooperate with other transcription factors (see below).

Pax6 is widely expressed in the neural plate including the prospective forebrain as well as in the adjacent rostral non-neural ectoderm that will give rise to the adenohypophyseal, olfactory, lens, and trigeminal placodes (Püschel et al., 1992a; Li et al., 1994; Grindley et al., 1995; Hirsch and Harris, 1997a; Murakami et al., 2001; Schlosser and Ahrens, 2004). *Pax6* promotes the formation of rostral ectodermal structures such as forebrain, eyes, and rostral placodes (reviewed in Gehring and Ikeo, 1999; van Heyningen and Williamson, 2002; Simpson and Price, 2002; Kondoh et al., 2004; Bhattacharyya and Bronner-Fraser, 2004). For example, *Pax6* directly binds to the enhancer of various lens crystallin genes and in combination with other transcription factors regulates their expression (review in Piatigorsky, 1998; Cvekl et al., 2004; Kondoh et al., 2004). While *Pax6* overexpression leads to ectopic formation of lens placodes (Altmann et al., 1997; Chow et al., 1999), *Pax6* mutants have reduced or missing lens and olfactory placodes due to deficient proliferation and differentiation and altered adhesive properties, which lead to the exclusion of *Pax6*^{−/−} cells from placodes (Hogan et al., 1986; Hill et al., 1991; Grindley et al., 1995; Ashery-Padan et al., 2000; van Raamsdonk and Tilghman, 2000; Dimanlig et al., 2001; Collinson et al., 2001, 2003). In addition, the adenohypophyseal placode develops abnormally in *Pax6* mutants due to patterning defects (ventralization resulting in decrease of lactotropes and somatotropes) (Bentley et al., 1999; Kiousi et al., 1999b).

Pax3 and *Pax7* genes are expressed in the lateral neural plate (subsequently in dorsal neural tube) and neural crest as well as in the profundal placode (Goulding et al., 1991; Bang et al., 1997, 1999; Stark et al., 1997; Seo et al., 1998b; Baker et al., 1999; McCauley and Bronner-Fraser, 2002; Schlosser and Ahrens, 2004). Besides playing crucial roles in myogenesis, dorsal neural tube patterning, and at various stages of neural crest development (reviewed in Mansouri, 1998; Epstein, 2000; Chi and Epstein, 2002), *Pax3* may be important for establishing neuron identity in the profundal placode (Baker and Bronner-Fraser, 2000; Baker et al., 2002). In *Pax3* mutants, various cranial ganglia are strongly hypoplastic including the profundal ganglion (i.e. the ophthalmic division of the trigeminal ganglionic complex), but to what extent this is due to defects of neural crest or profundal placode development has not been determined (Epstein et al., 1991; Tremblay et al., 1995).

The three vertebrate genes of the *Pax2/5/8* subfamily (*Pax2*, *Pax5*, *Pax8*) show partly overlapping and partly distinct expression at multiple sites and are involved in the development of many different structures including the retina, kidney, thyroid, thymus, midbrain–hindbrain boundary, and spinal cord (reviewed in Dahl et al., 1997; Dressler and Woolf, 1999; Chi and Epstein, 2002). In addition, *Pax2* and *Pax8* are expressed in the posterior placodal area at neural plate stages, with expression continuing into the otic and epibranchial placodes (Püschel et al., 1992b; Rinkwitz-Brandt et al., 1996;

Heller and Brändli, 1997, 1999; Herbrand et al., 1998; Pfeffer et al., 1998; Groves and Bronner-Fraser, 2000; Streit, 2002; Lawoko-Kerali et al., 2002; Li et al., 2004a; Schlosser and Ahrens, 2004). *Pax2* has been suggested to control epibranchial neuron identity (Baker and Bronner-Fraser, 2000), and *Pax2* and *Pax8* have crucial and partially redundant functions in otic placode development (reviewed in Whitfield et al., 2002; Noramly and Grainger, 2002; Riley and Phillips, 2003). No otic defects were reported for *Pax8* mutants (Mansouri et al., 1998), but *Pax2* mutants in the mouse exhibit compromised morphogenesis, sensory organ formation, and neurogenesis from the medial otic placode (Favor et al., 1996; Torres et al., 1996; Burton et al., 2004), while *Pax2* mutants in the zebrafish are merely deficient in lateral inhibition during hair cell development (Riley et al., 1999). In contrast, the otic placode fails to form in zebrafish if both *Pax8* and *Pax2* are compromised (Hans et al., 2004; Mackereth et al., 2005).

Fox genes

Several members of the large *Fox* family of winged helix transcription factors are placodally expressed and play important roles for various aspects of placode development (review in Carlsson and Mahlapuu, 2002; Lehmann et al., 2003; Pohl and Knöchel, 2005). The *FoxE* subfamily members *FoxE3* and *FoxE4* (also known as *Lens1*) are expressed in the lens placode where they promote proliferation and prevent differentiation, which occurs prematurely in mutants (Kenyon et al., 1999; Blixt et al., 2000; Brownell et al., 2000; Zilinski et al., 2004).

The *FoxG* subfamily member *FoxG1* (previously known as *BF-1*) is expressed in the anteriormost neural plate, giving rise to the telencephalon and in all placodes and was shown to be required to maintain proliferative neural progenitors in the ventral telencephalon and olfactory placode and to affect neuronal cell fate choices (Tao and Lai, 1992; Xuan et al., 1995; Toresson et al., 1998; Bourguignon et al., 1998; Dou et al., 1999; Hatini et al., 1999; Hardcastle and Papalopulu, 2000; Ohyama and Groves, 2004; Hanashima et al., 2004; Muzio and Mallamaci, 2005; Martynoga et al., 2005).

FoxI subfamily members are expressed in various subsets of posterior placodes (with some interspecific differences) including otic, lateral line, and epibranchial placodes (Hulander et al., 1998; Pohl et al., 2002, 2005; Nissen et al., 2003; Solomon et al., 2003a,b; Lee et al., 2003; Schlosser and Ahrens, 2004). In zebrafish, overexpression of *FoxI1* induces ectopic expression of otic and epibranchial placodal markers (Lee et al., 2003; Solomon et al., 2003a), while *FoxI1* mutants are deficient in the formation of viscerosensory neurons from epibranchial placodes (Lee et al., 2003) and have severely reduced otic placodes probably partly due to the failure of otic *Pax8* induction (Nissen et al., 2003; Solomon et al., 2003a, 2004; Hans et al., 2004; Mackereth et al., 2005). However, mouse mutants of *FoxI1* have much milder defects of otic placode development (Hulander et al., 1998, 2003).

T-box genes

Finally, several *T-box* genes (reviewed in Papaioannou, 2001; Wilson and Conlon, 2002; Showell et al., 2004; Naiche et

al., 2005) are involved in placode development. *Tbx1* is expressed in the pharyngeal pouches, pharyngeal arches, and in the otic placode, and its disruption causes complex craniofacial defects (known as DiGeorge syndrome in humans) which include hypoplasia of the inner ear and reduction of its sensory areas (Bollag et al., 1994; Chapman et al., 1996; Jerome and Papaioannou, 2001; Sauka-Spengler et al., 2002; Vitelli et al., 2003; Piotrowski et al., 2003; Raft et al., 2004; Moraes et al., 2005). In the otic placode, *Tbx1* has been suggested to favor sensory organ differentiation at the expense of neuronal differentiation (Vitelli et al., 2003; Raft et al., 2004). Little is known about the role of other placodally expressed *T-box* genes for placode development, such as *Tbx2*, *Tbx3*, and *Eomesodermin* (Chapman et al., 1996; Gibson-Brown et al., 1998; Ryan et al., 1998; Dheen et al., 1999; Ruvinsky et al., 2000; Takabatake et al., 2000, 2002; Schlosser and Ahrens, 2004).

In summary, the “positional” transcription factors discussed in this section tend to be expressed early and often in relatively large regions. They usually have multiple effects on placodal development, affecting proliferation, differentiation, and morphogenesis. Many of these transcription factors (e.g. *Six3/6*, many *Pax* and *Fox* genes) keep cells in an undifferentiated state, although they may nevertheless bias cell fate decisions. This happens usually in cooperation with other transcription factors with different spatiotemporal expression patterns allowing the spatially restricted activation of transcription factors, which are specifically dedicated to the control of particular pathways of cytodifferentiation. This latter category of transcription factors will be discussed next.

Transcription factors promoting cytodifferentiation of placodal cell types

The fourth and final group of transcription factors comprise proteins, which regulate particular pathways of cytodifferentiation. They are encoded by a large number of basic helix–loop–helix (bHLH)-related genes, LIM-, POU-, and Paired like-class homeobox genes, as well as basic leucine zipper (bZIP) genes and will be summarized here, taking the different placodal cell types in turn.

Cytodifferentiation in the lens placode

Determination and differentiation of lens fibers require many different transcription factors. However, of all transcription factors, which are essential for lens fiber differentiation, only L-Maf and several other bZIP proteins have a lens-specific expression and, thus, are thought to play a central role for lens fiber development (reviewed in Ogino and Yasuda, 2000; Chow and Lang, 2001; Reza and Yasuda, 2004; Cvekl et al., 2004; Bhattacharyya and Bronner-Fraser, 2004). In particular, these genes are known to cooperate with broadly expressed transcription factors like *Pax6* and *SoxB1* in the activation of various crystallins.

Cytodifferentiation in the adenohypophyseal placode

Cytodifferentiation of the various neuroendocrine cells of the adenohypophysis is likewise governed by combinations of

several transcription factors (reviewed in Watkins-Chow and Camper, 1998; Sheng and Westphal, 1999; Kioussi et al., 1999a; Dasen and Rosenfeld, 2001; Scully and Rosenfeld, 2002; Savage et al., 2003; Zhu and Rosenfeld, 2004; Asa and Ezzat, 2004). Some of these transcription factors such as Six3, ANF (HesX1/Rpx), Pitx, Pax6, and the LIM homeodomain proteins Lhx3, Lhx4, and Islet1 have general roles in early development of the adenohypophysis but later show spatially restricted expression and may participate in cell type specification. Other transcription factors are more specifically required for particular subpopulations of adenohypophyseal cells. For example, the POU class homeobox transcription factor Pit1 is required for the differentiation of somatotropes, lactotropes, and thyrotropes; the zinc finger transcription factor GATA2 is required for the differentiation of gonadotropes and thyrotropes; the T-box transcription factor Tbx19 (T-pit) and the bHLH transcription factor NeuroD are required for the differentiation of melanotropes and corticotropes; and the steroid receptor FTZ-F1 (SF-1) is required for gonadotropes.

Cytodifferentiation in the neurogenic placodes

Cytodifferentiation of placodal neurons and sensory cells is now known to be under the control of transcription factors of the bHLH superfamily related to the *Drosophila atonal* and *achaete-scute* genes as is the case in the neural plate and neural crest. Some of these genes operate as neuronal determination (or proneural) genes, which are expressed in proliferative cells and initiate a cascade of transcription factors leading to neuronal specification.

Many proneural genes play a dual role in determining a generic neuronal fate and participating in the assignment of neuronal or sensory subtype identity (reviewed in Guillemot, 1999; Brunet and Ghysen, 1999; Hassan and Bellen, 2000; Bertrand et al., 2002). Consequently, specification of different placodally derived neuronal and sensory cell types depends on different proneural genes. *Neurogenin1* (*Ngn1*) or *Neurogenin2* (*Ngn2*), for example, are expressed in different subsets of neurogenic placodes and are essential for the determination of placodal neurons, although details differ between species (Fode et al., 1998; Ma et al., 1998, 2000; Schlosser and Northcutt, 2000; Cau et al., 2002; Begbie et al., 2002; Andermann et al., 2002; reviewed in Korzh and Strähle, 2002; Bertrand et al., 2002). In mammals, *Ngn1* is involved in neuronal determination in the profundal/trigeminal and otic placodes, while *Ngn2* is required for determination of epibranchial-placode-derived neurons (Ma et al., 1998, 2000; Fode et al., 1998). However, other neuronal determination genes are involved in determination of olfactory receptor neurons and otic hair cells, viz. *Ash1* (e.g. *Mash1* in the mouse) and *Ath1* (e.g. *Math1* in the mouse), respectively (Cau et al., 1997; Bermingham et al., 1999; Chen et al., 2002; Murray et al., 2003; Woods et al., 2004; reviewed in Bertrand et al., 2002; Kelley, 2002; Bryant et al., 2002; Gao, 2003; Beites et al., 2005).

On the one hand, neuronal determination genes activate bHLH genes that control generic aspects of neuronal differentiation, such as *NeuroD* (reviewed in Lee, 1997; Cho

and Tsai, 2004), which is expressed not only in various parts of the neural tube but also in all neurogenic placodes (Schlosser and Northcutt, 2000; Andermann et al., 2002). Other types of helix–loop–helix (HLH) genes, viz. the *HES* (hairy/enhancer of split related), *Id*, and *COE* (Collier/Olf1/EBF) genes, also have expression domains in neural tube, neural crest, and placodes (e.g. Wang et al., 1997; Garel et al., 1997; Jen et al., 1997; Dubois et al., 1998; Bally-Cuif et al., 1998; Vasiliauskas and Stern, 2000; Koyano-Nakagawa et al., 2000; Cau et al., 2000; Zheng et al., 2000; Zine et al., 2001; Pozzoli et al., 2001; Kee and Bronner-Fraser, 2001a,b, 2005; Burns and Vetter, 2002; Liu and Harland, 2003; Meulemans et al., 2003; Light et al., 2005). These genes antagonize (e.g. *Hes1*, *Id* genes; reviewed in Yokota, 2001; Bertrand et al., 2002; Tzeng, 2003; Ruzinova and Benezra, 2003) or synergize (e.g. *Hes6*, *COE* genes; reviewed in Dubois and Vincent, 2001; Bertrand et al., 2002; Liberg et al., 2002) with neuronal determination and differentiation genes.

On the other hand, neuronal determination genes activate further transcription factors that control neuronal or sensory subtype specification, but this requires cooperation with other placodally expressed transcription factors in particular LIM-class (e.g. *Islet1*; Korzh et al., 1993; Li et al., 2004b; Radde-Gallwitz et al., 2004) and Paired like-class (e.g. *Phox2a*, *Phox2b*; Tiveron et al., 1996; Pattyn et al., 1997; Guo et al., 1999; Begbie et al., 2002; Talikka et al., 2004) homeodomain transcription factors (reviewed in Bertrand et al., 2002; Shirasaki and Pfaff, 2002; Brunet and Pattyn, 2002; Allan and Thor, 2003). Among the latter are, for example, POU class IV homeodomain transcription factors such as *Brn3a* and *Brn3c*, which promote maturation and survival of neurons in the trigeminal and vestibulocochlear ganglia and hair cell differentiation, respectively (Erkman et al., 1996; McEvilly et al., 1996; Xiang et al., 1997, 1998; Huang et al., 1999, 2001; Eng et al., 2004; reviewed in Latchman, 1999; Bryant et al., 2002; Gao, 2003).

Specification of different placodes in the panplacodal primordium—insights from *Xenopus*

Recently, increasing availability of data on gene expression during early placode development revealed a surprising molecular complexity in the early panplacodal primordium indicating that, despite its apparent morphological uniformity and its uniform expression of transcription factors promoting generic placodal development, it is already divided into various molecularly non-equivalent subdomains. In particular, many transcription factors that are restricted to subsets of placodes at subsequent stages already show properly regionalized expression at neural plate stages (for example, the various *Pax* genes discussed above). This suggests that the panplacodal primordium is already subdivided at neural plate or early neural fold stages into subregions differentially biased for several different types of placodes.

To elucidate the potential role of various transcription factors in placode specification, it is useful to consider their spatiotemporal expression pattern in relation to the developing

placodes in some detail. I will do this here focusing on *Xenopus* for which both a detailed description of placode development and extensive information on gene expression are available (see Figs. 3–6 and Schlosser and Ahrens, 2004; Schlosser and Northcutt, 2000). Most of the transcription factors described have also been cloned from chick, mouse, and zebrafish and, generally, have similar distributions there (see above). It must be emphasized that we still know relatively little about the precise role of many of these transcription factors during placode development and how they interact with each other. As summarized above, most of them have been shown experimentally to be required for some aspects of placode development, but, in many cases, it still needs to be clarified, which of these effects are directly due to their activity within pre-placodal and placodal cells rather than in adjacent tissues. At present, therefore, most of the transcription factors discussed should merely be considered promising candidates for factors, which probably participate at some level in the regulatory networks underlying placode specification.

Overview of placode development in *Xenopus*

Fig. 4 summarizes placodal development in *Xenopus* (Schlosser and Ahrens, 2004; Schlosser and Northcutt, 2000) and relates some of the molecular subdivisions of the panplacodal primordium at neural plate and fold stages (Figs. 3C, 4A, B) to the morphologically recognizable units, which become apparent after neurulation. Immediately after neural tube closure, all prospective placodes with exception of the lens are thickened. However, at early tailbud stages, the various placodal thickenings are still very closely apposed to each other (Figs. 4C, D). The olfactory placode and the stomodeal thickening, which includes the prospective adenohypophyseal placode, together form a contiguous anterior placodal area. Profundal and trigeminal placodes are fused but can be distinguished by their distinct gene expression patterns. Finally, a posterior placodal area can be recognized probably containing the prospective otic, lateral line, epi- and hypobranchial placodes. The posterior placodal area initially forms two ventral

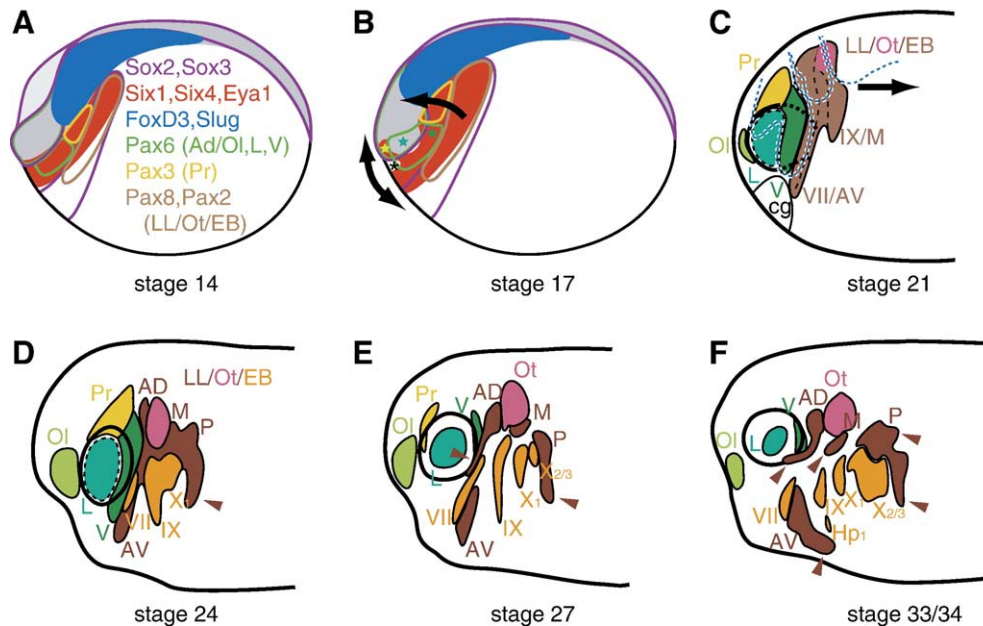


Fig. 4. Summary of placodal development in *Xenopus laevis* in lateral views (modified from Schlosser and Ahrens, 2004; stages after Nieuwkoop and Faber, 1967). (A, B) Gene expression domains (colored outlines) during neural plate (A) and neural fold stages (B). The panplacodal primordium (red) is apposed to the neural plate (gray) anteriorly and to neural crest (blue) laterally (see also Fig. 3C). Arrows in panel B indicate shifts of placodal expression domains due to neural fold elevation (dorsal shift) and wedge-like expansion of anterior ectoderm accompanying the formation of the optic vesicles. Green stars identify three areas of *Pax6* expression that will contribute (from anterior to posterior) to adenohypophyseal and olfactory placode (light green), lens placode (blue green), and trigeminal placode (dark green). *Six1* and *Eya1* expressions are downregulated at neural fold stages in the regions of prospective lens placode and cement gland (black asterisk). (C–F) Development of placodes after neural tube closure until late tailbud stages. Drawings are based on reconstructions of ectodermal thickenings from serial sections (after Schlosser and Ahrens, 2004; Schlosser and Northcutt, 2000). The adenohypophyseal placode, which is located medial to the ventral part of the olfactory placode, is not shown in these lateral views. Various green colors identify placodes or prospective placodes expressing *Pax6*, yellow identifies the profundal placode expressing *Pax3*, and brown, pink, and orange jointly identify the posterior placodal area expressing *Pax2* and *Pax8*, with pink being reserved for the subregion forming the otic placode/vesicle and orange for the subregions forming the epibranchial placodes. At stage 21, the posterior placodal area is divided into an anterior and a posterior subregion of thickened ectoderm, separated ventrally by an indentation and dorsally by a region of thinner ectoderm (between broken black lines), while the prospective otic placode (pink) is identifiable as a particularly prominent thickening. The broken blue lines in panel C indicate neural crest streams as reconstructed from serial sections (Schlosser and Northcutt, 2000). The arrow in panel C indicates that the posterior placodal area expands posteriorly at early tailbud stages. Brown arrowheads in panels D–F indicate developing lateral line primordia. Abbreviations: Ad/Ol: anterior placodal area, from which adenohypophyseal (Ad) and olfactory placodes (Ol) develop; AV: anteroventral lateral line placode; cg: cement gland; Hp₁: first hypobranchial placode; L: prospective lens placode (hatched outline), lens placode or lens (invagination of placode between stage 27 and 33/34); LL/Ot/EB: posterior placodal area, from which lateral line (LL), otic (Ot), and epibranchial (EB) placodes develop; M: middle lateral line placode; Ol: olfactory placode; Ot: otic placode or vesicle (invagination of placode between stage 24 and 33/34); P: posterior lateral line placode; Pr: profundal placode; V: trigeminal placode; VII: facial epibranchial placode; IX: glossopharyngeal epibranchial placode; X₁: first vagal epibranchial placode; X_{2/3}: second and third vagal epibranchial placodes (fused).

	st 13-16	st 24-26	References		st 13-16	st 24-26	References
Six1			Pandur and Moody (2000), Ghanbari et al. (2001), Schlosser and Ahrens (2004)	Ir1			Gómez-Skarmeta et al. (1998); Xiro1, Glavic et al. (2004); Xiro1, Schlosser and Ahrens (2004); Xiro1
Six2			Ghanbari et al. (2001)	Ir2			Gómez-Skarmeta et al. (1998); Xiro2
Six4			Ghanbari et al. (2001), Schlosser and Ahrens (2004)	Ir3			Bellefroid et al. (1998); Xiro3
Six3			Zhou et al. (2000), Ghanbari et al. (2001)	Gbx2a, 2b			von Bubnoff et al. (1996), Tour et al. (2001, 2002)
Six6			Zuber et al. (1999), Ghanbari et al. (2001)	Otx2			Pannese et al. (1995), Kablar et al. (1996), Zygar et al. (1998)
Eya1			David et al. (2001), Schlosser and Ahrens (2004)	Otx1, Otx4			Kablar et al. (1996)
Grg4, Grg5			Molenaar et al. (2000)	Otx5			Vignall et al. (2000)
Dlx3			Dirksen et al. (1994); Xdl2, Feledy et al. (1999), Luo et al. (2001a,b), Schlosser and Ahrens (2004)	Emx2			Pannese et al. (1998)
Dlx5			Papalopulu and Kintner (1993); Xdl3, Luo et al. (2001a,b)	Nkx5.1			Bayramov et al. (2004)
Dlx6			Luo et al. (2001a)	ANF1, ANF2			Zarasky et al. (1992, 1995), Ermakova et al. (1999), Mathers et al. (1995)
GATA1, 2, 3			Kelley et al. (1994), Walmsley et al. (1994), Read et al. (1998)	Pitx1			Hollemann and Pieler (1999), Chang et al. (2001), Schweickert et al. (2001)
NZFB			Kim et al. (2003)	Pitx2b, 2c			Schweickert et al. (2001)
Dmrt4			Huang et al. (2005)	Pitx3			Pommereit et al. (2001)
Sox2, Sox3			Mizuseki et al. (1998), Penzel et al. (1997), Zygar et al. (1998), Schlosser and Ahrens (2004)	Msx1			Maeda et al. (1997), Suzuki et al. (1997), Feledy et al. (1999), Schlosser and Ahrens (2004)
Sox11			Brugmann et al. (2004)	Msx2, 2B			Gong and Kiba (1999), Onitsuka et al. (2000)
Sox9			Spokony et al. (2002), Saint-Germain et al. (2004)	Pax6			Hirsch and Harris (1997a), Zygar et al. (1998), Schlosser and Ahrens (2004)
Sox10			Honoré et al. (2003), Aoki et al. (2003)	Pax3			Bang et al. (1997, 1999), Schlosser and Ahrens (2004)

Fig. 5.

	st 13-16	st 24-26	References		st 13-16	st 24-26	References
Pax2			Heller and Brändli (1997), Schlosser and Ahrens (2004)	Ngnr1			Ma et al. (1996), Schlosser and Northcutt (2000) Schlosser and Ahrens (2004)
Pax5			Heller and Brändli (1999)	NeuroD			Lee et al. (1995), Schlosser and Northcutt (2000)
Pax8			Heller and Brändli (1999), Schlosser and Ahrens (2004)	ATH1			Kim et al. (1997): their "trigeminal" expression is most likely anterior lateral line
FoxK1			Pohl and Knöchel (2004)	ATH3			Takebayashi et al. (1997), Burns and Vetter (2002)
FoxG1			Dirksen and Jamrich (1995): XFKH4, Bourguignon et al. (1998): BF-1, Eagleson and Dempewolf (2002): BF-1	ATH5			Kanekar et al. (1997), Burns and Vetter (2002)
Foxl1a, b, c			Lef et al. (1994): XFD-2, XFD-2', Pohl et al., (2002), Schlosser and Ahrens (2004)	EBF2			Dubois et al. (1998): COE2, Burns and Vetter (2002)
Foxl2			Pohl et al. (2005)	EBF3			Pozzoli et al. (2001), Burns and Vetter (2002)
FoxE4			Kenyon et al. (1999): Xlen1	Id2			Zhang et al., (1995): Id1la, Liu and Harland (2003)
Tbx1			Ataliotis et al. (2005)	Id3			Wilson and Mohun (1995): Idx, Zhang et al., (1995): Id1a, Liu and Harland (2003)
Tbx2			Hayata et al. (1999), Takabatake et al. (2000, 2002), Schlosser and Ahrens (2004)	Id4			Liu and Harland (2003)
Tbx3			Takabatake et al. (2000, 2002), Schlosser and Ahrens (2004)	HES6			Koyano-Nakagawa et al. (2000)
Eomesodermin			Ryan et al. (1998)	ESR6e			Deblandre et al. (1999)
Lhx3			Taira et al. (1993)	Hairy2a, 2b			Turner and Weintraub (1994), Davis et al. (2001), Tsuiji et al. (2003)
Lmx1B			Haldin et al. (2003)	HRT-1			Pichon et al. (2002)
MafB			Ishibashi and Yasuda (2001)	MyT1			Bellefroid et al. (1996), Schlosser and Northcutt (2000)
L-Maf			Ishibashi and Yasuda (2001)	Brn3d			Hutcheson and Vetter (2001)
Phox2a, 2b			Talikka et al. (2004)	Brn3a			Hirsch and Harris (1997b)

extensions, which most likely give rise to the facial and glossopharyngeal epibranchial placode, the first hypobranchial placode, and an anterior subset of lateral line placodes (Fig. 4C). In contrast, the vagal epibranchial placodes, the second hypobranchial placode, and a posterior subset of lateral line placodes probably arise from a secondary posterior expansion of the posterior placodal area, which forms only at mid-tailbud stages (Fig. 4D). Separate placodes—recognizable either as distinct thickenings or as areas of disrupted basement membranes—develop from these placodal thickenings at later tailbud stages (Figs. 4E, F) and subsequently give rise to the various sensory organs and ganglia as described above.

*Transcription factor expression during early *Xenopus* placode development*

The expression pattern of a large number of transcription factors in various subregions of the panplacodal primordium at neural plate/fold stages and their subsequent expression in the different placodes at tailbud stages are summarized in Fig. 5 (which also lists the relevant references). Fig. 6 gives a schematic overview over how their expression domains are positioned relative to each other at neural plate/fold stages, whereas Fig. 3C shows this more realistically for a few important transcription factors. Although a detailed fate map of the ectoderm in neural plate stage *Xenopus* embryos is not yet available and only the approximate position of prospective placodes relative to these expression domains is known, some general patterns are evident.

Most transcription factors are expressed in multiple placodes

First, with few exceptions (e.g. *Pax3* expression in the prospective profundal placode), placodally expressed transcription factors are not restricted to a single prospective placode at neural plate stages but rather extend through larger areas, from which multiple placodes originate (Figs. 5, 6). Whereas many transcription factors are expressed in a contiguous domain covering several adjacent placodes, transcription factors specifically involved in governing neuronal differentiation (e.g. *NeuroD*, *ATH3*, *EBF2*, *EBF3*, *MyT1*, *Brn3d*) are often absent from the regions of the prospective non-neurogenic adeno-hypophyseal and lens placodes. Unfortunately, important details of these expression patterns, which are crucial for further

elucidating mechanisms of placode specification, are still unknown. For instance, it is largely unclear to what extent transcription factors with overlapping expression domains are in fact coexpressed at equal levels in individual cells or whether there is instead a mosaic of cells, which preferentially or exclusively express one or the other transcription factor (as has been suggested to be the case for *Dlx5* and *Pax6* expression in the rostral part of the pre-placodal region in chick embryos; Bhattacharyya et al., 2004).

Transcription factor expression domains are centered around a rostral and a caudal focus

Second, the majority of those transcription factors, which are not panplacodally expressed, have expression domains centered around either the anterior placodal area (with prospective adeno-hypophyseal and olfactory placodes) or the posterior placodal area (with prospective lateral line, otic and epibranchial placodes) (Fig. 6), each of which is also apparent as a morphological unit (Schlosser and Northcutt, 2000). Only at later stages are these two areas further subdivided (see below). Beyond its implications for models of placode specification (see below), this pattern is suggestive of a particular trajectory of placode evolution, which can only be sketched here for space constraints. Briefly, it is most compatible with an evolutionary scenario, in which all placodes evolved from only two placodes, one rostrally and one caudally, in the ancestor of extant vertebrates (these in turn may have evolved from a single rostral protoplacode as discussed in Schlosser, 2005). These two placodes subsequently split into the adeno-hypophyseal, olfactory, and lens placodes and the otic, lateral line, and epi- and hypobranchial placodes, respectively, while the trigeminal and profundal placodes budded off at some time from either the rostral or the caudal group of placodes.

Nested and partly overlapping expression of transcription factors suggests a combinatorial mode of placode specification

Third, while most expression domains include either the anterior or the posterior placodal area or both, their spatial extension differs (Fig. 6). The resulting nested domains of transcription factors subdivide the panplacodal primordium into different areas characterized by the expression of different combinations of transcription factors. The prospective lens placode, for example, shares expression of many transcription

Fig. 5. Expression of transcription factors and cofactors in the placodal ectoderm of neural plate (stage 13–16) and tailbud (stage 24–26) stage *Xenopus* embryos (stages after Nieuwkoop and Faber, 1967). Approximate borders of prospective placodal areas at neural plate stages are indicated by gray lines, whereas borders of definite placodes at tailbud stages are indicated by black lines. Faint colors indicate weak expression. In some cases, spatial restriction of gene expression to parts of a placode is indicated by corresponding partial coloring. N, C, and E indicate that a gene is also expressed in neural plate or tube (N), neural crest (C), or epidermis (E). Question marks indicate that gene expression in a particular placode at a particular stage has either been not described or cannot be determined unambiguously from published information. Hypobranchial placodes are not listed separately but tend to share gene expression patterns with epibranchial placodes. Transcription factors that are very widely or universally expressed throughout the embryo or that are associated with particular signaling cascades (e.g. Smads or Lef/Tcf) are not included even if they have placodal expression domains. Abbreviations: Ad: adeno-hypophyseal placode; EB: epibranchial placodes; L: lens placode; LL: lateral line placodes; LL/Ot/EB: posterior placodal area, from which lateral line, otic, and epibranchial placodes develop; Ol: olfactory placode; Ot: otic placode; Pr: profundal placode; V: trigeminal placode. Expression domains are based on the references listed in the right column (in addition to references cited elsewhere in the text, this include Aoki et al., 2003; Bayramov et al., 2004; Bellefroid et al., 1996; Davis et al., 2001; Deblandre et al., 1999; Dirksen and Jamrich, 1995; Eagleson and Dempewolf, 2002; Haldin et al., 2003; Hayata et al., 1999; Hirsch and Harris, 1997b; Honoré et al., 2003; Hutcheson and Vetter, 2001; Kanekar et al., 1997; Kim et al., 1997, 2003; Lef et al., 1994; Pannese et al., 1998; Papalopulu and Kintner, 1993; Penzel et al., 1997; Pichon et al., 2002; Pohl and Knöchel, 2004; Saint-Germain et al., 2004; Spokony et al., 2002; Taira et al., 1993; Takebayashi et al., 1997; Tour et al., 2001; Tsuji et al., 2003; Turner and Weintraub, 1994; Vignali et al., 2000; von Bubnoff et al., 1996; Wilson and Mohun, 1995; Zhang et al., 1995).

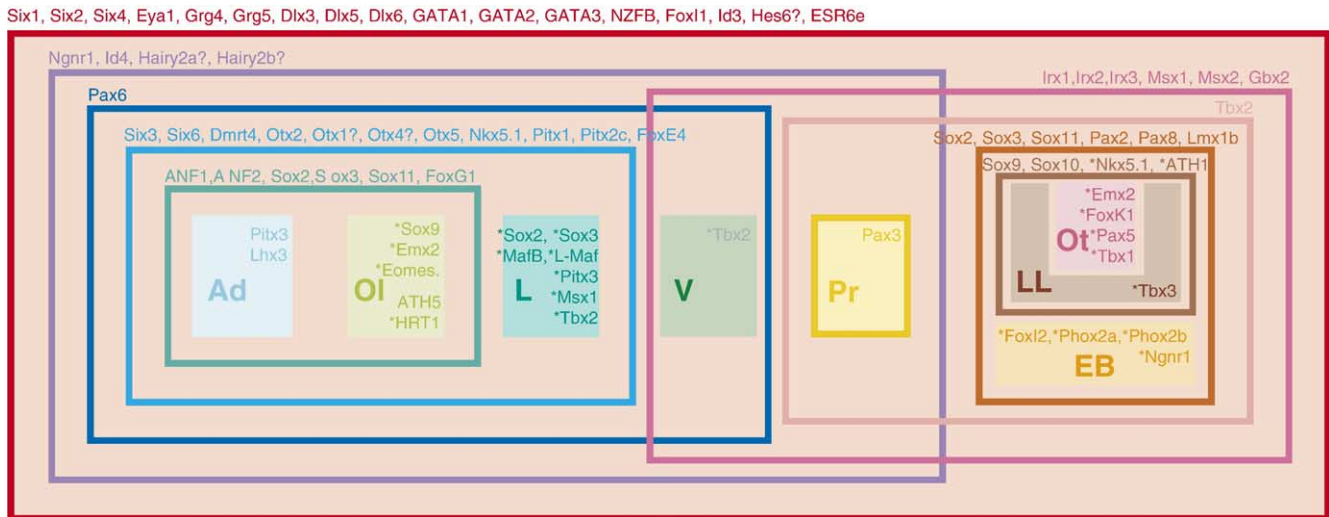


Fig. 6. Schematic summary of transcription factor expression domains in the placodal ectoderm of neural plate stage *Xenopus* embryos. Transcription factors that control neuronal determination and differentiation and are expressed in all neurogenic placodes but excluded from adenohypophyseal and lens placodes are not included. The position of various prospective placodes within the panplacodal primordium (faint red) is indicated by colored rectangles. Colored lines enclose expression domains of the transcription factors listed at neural plate stages. Listing of transcription factors without asterisk refers to their expression domains established at neural plate stages, while listing of transcription factors preceded by asterisks refers to expression domains established at later stages. Question marks indicate tentative assignments because precise domain boundaries cannot be determined unambiguously from published information. For simplicity, some details of expression are ignored (for example, *Msx1* and *Msx2* are strongly expressed only in the dorsal part of the indicated region). For details and references, see Fig. 5. Note that there are two foci of transcription factor expression, one centered on an anterior placodal area (with prospective adenohypophyseal and olfactory placodes), the other centered on a posterior placodal area (with prospective lateral line, otic, and epibranchial placodes). However, the spatial extension of various expression domains differs resulting in two nested hierarchies, which are overlapping in the regions of profundal and trigeminal placodes. For detailed explanation, see text. Abbreviations: Ad: adenohypophyseal placode; EB: epibranchial placodes; L: lens placode; LL: lateral line placodes; Ol: olfactory placode; Ot: otic placode; Pr: profundal placode; V: trigeminal placode.

factors including *Six3*, *Six6*, *Pitx1*, *FoxE4*, and *Nkx5.1* with the anterior placodal area but is distinguished from the latter by its lack of expression of other genes such as *ANF1*, *Sox2*, *Sox3*, and *FoxG1*. Moreover, some anteriorly centered transcription factors overlap with some of the posteriorly centered transcription factors in the regions, where the profundal and trigeminal placodes form. This results in unique combinations of transcription factors in each of these domains: the trigeminal placode forms, where the anterior *Pax6* domain overlaps with the posteriorly centered domains of, for instance, *Irx1*, *Msx1*, and *Gbx2*, whereas the profundal placode develops posterior to the *Pax6* domain in a region expressing *Tbx2* in addition to *Irx1*, *Msx1*, and *Gbx2*.

Even though the precise role of many of these transcription factors for placode specification remains to be elucidated, the partly nested and partly overlapping expression in the preplacodal ectoderm of such a large number of transcription factors likely to be involved in placode specification supports combinatorial models of placode specification (Torres and Giráldez, 1998). More specifically, it suggests that the expression domains of various transcription factors covering multiple prospective placodes may define nested hierarchies of multiplacodal areas—ectodermal regions biased for the development of particular set of placodes. For example, within the region expressing *Six1*, *Six4*, and *Eya1* biased for generic placodal development, the ectodermal area expressing *ANF* and *FoxG1* genes may be biased to form either adenohypophyseal and olfactory placodes, the more comprehensive ectodermal region expressing *Six3*, *Six6*, *Pitx1*, *FoxE4*, and *Nkx5.1* may be

biased to form adenohypophyseal, olfactory, or lens placodes, whereas the region expressing *Pax2* and *Pax8* may be biased to form lateral line, otic, or epibranchial placodes.

How may a transcription factor covering multiple prospective placodes promote multiplacodal bias? One possibility is that it contributes to the activation of target genes specifically required in all of the placodes derived from a particular multiplacodal area but not in others (akin to the role of *Six* and *Eya* genes in the promotion of generic placodal properties proposed above). However, there is at present little evidence for the existence of many effector (e.g. cytodifferentiation) genes shared, for example, among lens, olfactory, and adenohypophyseal placodes to the exclusion of other placodes. An alternative and more plausible scenario is illustrated schematically in Fig. 7. A transcription factor may also introduce a multiplacodal bias in binding to *cis*-regulatory regions of target genes, each of which promotes development of a different individual placode but will only be fully activated after binding of additional transcription factors with spatially more restricted distribution. An increasing number of studies provide evidence for this type of combinatorial action of transcription factors at *cis*-regulatory regions of placodal target genes, among them the well-studied regulation of lens crystallins by *Pax6*, *Sox2*, and *L-Maf* already mentioned (reviewed in Piatigorsky, 1998; Bhattacharyya and Bronner-Fraser, 2004; Cvekl et al., 2004; Kondoh et al., 2004).

Some empirical support for the existence of multiplacodally biased regions comes from recent studies in the zebrafish, where *pitx3* initially defines a broad anterior placodal region (similar to *Xenopus Pitx1*, *Pitx2b*, and *Pitx2c*; see Fig. 3C), giving rise to

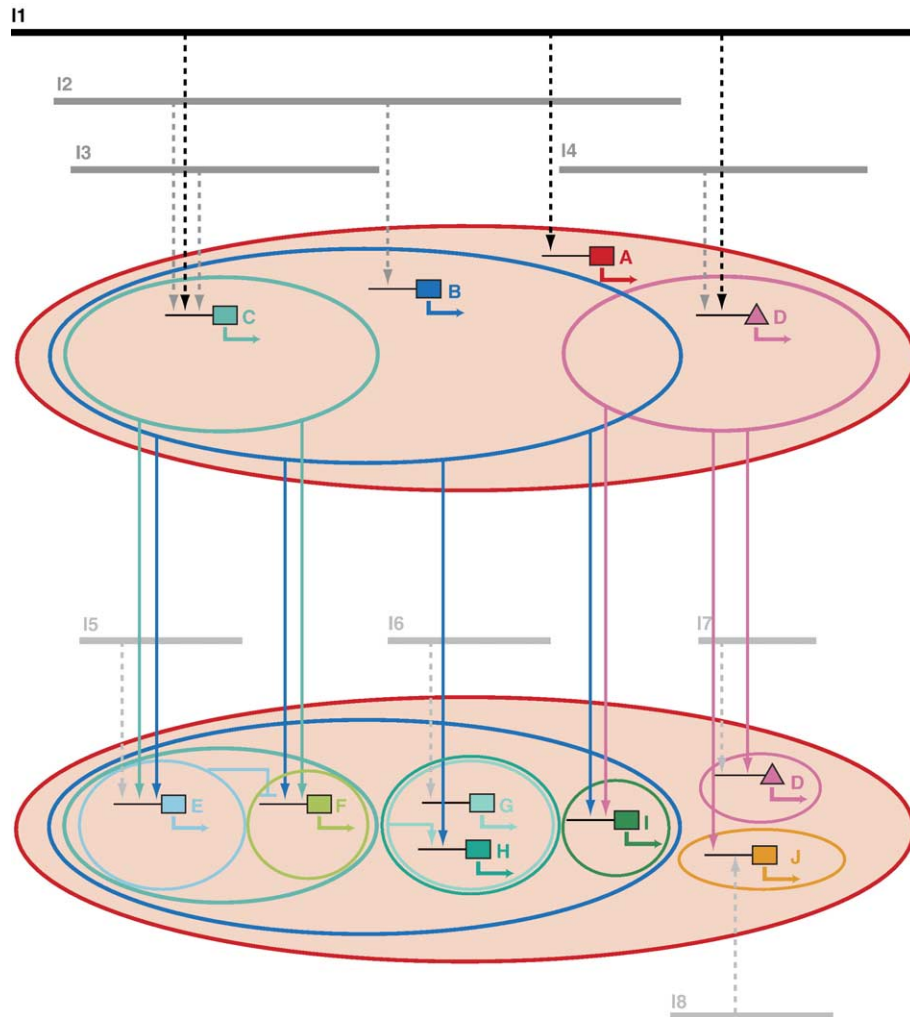


Fig. 7. Combinatorial multistep model for placode induction and specification. Expression of various transcription factor genes involved in placode specification (colored rectangles and triangles, A–J) may be regulated by particular combinations of upstream factors including inducing signaling molecules (I1–I8) as well as other transcription factors, which bind to their *cis*-regulatory region. Upstream factors may be activating (arrows; broken arrows are used for inducing signals because these act indirectly via transcription factors downstream of signaling cascades) or repressing (bars). In the hypothetical case illustrated, upstream factors are assumed to be singly necessary but only jointly sufficient for activation of the downstream gene. The expression domain of a transcription factor (enclosed by colored lines; the red area represents the panplacodal primordium) is determined by the spatial extent of the expression domains of inducers and transcription factors acting upstream (indicated by black and gray lines for inducers and colored ellipses for transcription factors). The expression of transcription factors specific for individual placodes (lower panel) depends on panplacodal (black), multiplacodal (dark gray), and placode-specific (light gray) inducers, which may act either directly or indirectly (i.e. mediated by other transcription factors with a panplacodal, multiplacodal, or placode-specific distribution). Some transcription factors (as illustrated for D), which show placode-specific expression at later stages, may initially be more broadly expressed but then become restricted because they require input from more localized inducers for sustained activation. This model implies that different placodes share some but not all of the inducers and transcription factors involved in their specification. Moreover, it proposes that at early stages of placode specification (upper panel) there are nested and partially overlapping regions differentially biased for development of different sets of placodes. Multiplacodal bias is introduced by transcription factors (such as B–D) which cover multiple prospective placodes and activate various placode-specific transcription factors, without being by themselves sufficient for their activation. Panplacodally expressed transcription factors such as A may, in addition, activate genes involved in the regulation of generic placodal processes such as proliferation, cell shape changes, and neurogenesis (not shown).

adenohypophyseal, olfactory, lens, and trigeminal placodes (Dutta et al., 2005; Zilinski et al., 2005). Moreover, *pitx3* is required both for the expression of several pituitary-specific genes and for lens fiber cell specification (Dutta et al., 2005). Whether and where lens or adenohypophyseal placodes form within the *pitx3*-positive region appears to depend mainly on the level of hedgehog signaling with high levels of hedgehog emanating from the axial midline determining a medial position of the adenohypophyseal placode during normal development (Karlstrom et al., 1999; Kondoh et al., 2000; Varga et al., 2001; Herzog et al., 2003; Sbrogna et al., 2003). When hedgehog is

overexpressed in the prospective lens ectoderm, the latter also adopts an adenohypophyseal phenotype (Dutta et al., 2005). Blocking hedgehog signaling in the region of the prospective adenohypophyseal placode, conversely, leads to the rostromedial expansion of the lens placode at the expense of the adenohypophyseal placode (Karlstrom et al., 1999; Kondoh et al., 2000; Varga et al., 2001; Dutta et al., 2005; Zilinski et al., 2005). Taken together, this suggests that the *pitx3* expressing ectodermal domain constitutes an “equivalence domain” (Dutta et al., 2005) or a multiplacodal area, biased for the differentiation of adenohypophyseal and lens placodes (and possibly

olfactory and trigeminal placodes as well, although this has not been analyzed). However, whether there is in fact a nested hierarchy of such multiplacodal areas, as the model predicts, and whether otic, lateral line, and epibranchial placodes comprise a similar multiplacodal area in the posterior still remain to be confirmed experimentally.

Dynamic changes of expression patterns result in placode-specific combinations of transcription factors

Fourth, the further differentiation of prospective placodes and the subdivision of initially molecularly equivalent areas (such as the anterior or posterior placodal area) involves various dynamic changes of gene expression after neural plate stages (Fig. 5). On the one hand, new transcription factors and other genes, which may be involved in the regulation of placode-specific processes of cytodifferentiation and morphogenesis, are upregulated in a placode-specific manner (for example, *Lhx3* in the adenohypophyseal placode, *Ath5* in olfactory placodes, *MafB* in the lens placode, *FoxK1* in the otic placode, *Tbx3* in the lateral line placodes, *Phox2* genes in the epibranchial placodes; for other genes, see, for example, Baker et al., 1995; Shi et al., 1998; Vokes and Krieg, 2000; Golub et al., 2000; Novoselov et al., 2003). On the other hand, existing transcription factor domains may expand (for example, the upregulation of *Sox2* and *Sox3* in the lens placode or of *FoxG1* in the otic, lateral line, and epibranchial placodes) or retract (for example, the exclusion of *Pitx2* genes from the olfactory and lens placodes, of *FoxE4* genes from the adenohypophyseal and olfactory placodes, and of *Dlx3*, *Six1*, *Six4*, and *Eya1* from the lens placode). The latter may result either from differential local downregulation or from cell sorting. This will be discussed in more detail in the section below, dealing with individualization of placodes.

The appearance of placode-specific combinations of transcription factors is the first indication for the separation of the various multiplacodal areas into individual placodes. However, it does not imply that these molecularly distinct regions are already fully specified or even committed for a particular placodal fate because they may still require localized signals from adjacent tissues for consolidation of present or induction of additional transcription factors.

In *Xenopus*, molecularly distinct individual placodes appear at the following schedule. Profundal and trigeminal placodes are already molecularly distinct at neural plate stages, immediately followed by their terminal neuronal differentiation (Chitnis et al., 1995; Lee et al., 1995; Ma et al., 1996; Schlosser and Ahrens, 2004; Schlosser and Northcutt, 2000). Adenohypophyseal, olfactory, and lens placodes acquire separate identities at approximately neural fold stages (e.g. Zarásky et al., 1995; Kenyon et al., 1999; Ishibashi and Yasuda, 2001; Burns and Vetter, 2002; Pommereit et al., 2001) but continue to require localized inductive signals for proper patterning and differentiation (reviewed in Grainger, 1996; Calof et al., 1996; Plendl et al., 1999; Kioussi et al., 1999a; Ogino and Yasuda, 2000; Chow and Lang, 2001; Dasen and Rosenfeld, 2001; Baker and Bronner-Fraser, 2001; Scully and Rosenfeld, 2002; Fisher and Grainger, 2004; Lang, 2004; Beites et al., 2005). Finally, the posterior placodal area while being already capable of forming

ectopic otic-like vesicles at neural plate stages (Gallagher et al., 1996) only subdivides into molecularly distinct otic, lateral line, and epibranchial placodes much later at mid-tailbud stages (e.g. Baker et al., 1995; Vokes and Krieg, 2000; Shi et al., 1998; Golub et al., 2000; Novoselov et al., 2003; Talikka et al., 2004; Ataliotis et al., 2005; Schlosser and Ahrens, 2004). The requirement for localized inductive signals for proper patterning of the otic vesicle into different sensory areas even persists into later tailbud stages (Kil and Collazo, 2001; see also Yntema, 1933, 1939; Harrison, 1945; Ginsburg, 1946, 1995; Detwiler and van Dyke, 1951; reviewed in Baker and Bronner-Fraser, 2001; Whitfield et al., 2002; Noramly and Grainger, 2002; Riley and Phillips, 2003; Barald and Kelley, 2004). Thus, somewhat surprisingly, placodes with very similar function such as the profundal and trigeminal placodes appear to diverge very early regarding their dependence on specific transcriptional regulators, whereas the functionally heterogeneous rostral and caudal groups of placodes appear to rely for much longer on common regulatory factors.

Individualization of placodes requires formation of stable boundaries and physical separation

The individualization of different placodes from larger fields involves the subdivision of these fields by the differential suppression of initially overlapping transcription factors in different subdomains and/or by the local upregulation of new transcription factors (e.g. in response to locally confined inducers). Consequently, mutually exclusive expression domains of certain transcription factors are established (Fig. 8A), which then control the development of placode-specific pathways of cytodifferentiation or morphogenesis. For example, the subdivision of the anterior placodal region, which initially coexpresses *Pitx2c*, *FoxE4*, and several other genes and which gives rise to adenohypophyseal, olfactory, and lens placodes, involves the exclusion of *Pitx2c* from the olfactory and lens and of *FoxE4* from the adenohypophyseal and olfactory territory together with the upregulation of *Lhx3* in the adenohypophyseal placode, of *ATH5* in the olfactory placode, and of *MafB* in the lens placode. Such subdivisions of a larger field of cells require mechanisms to establish stable boundaries and to keep cells with distinct identities separate from each other. The formation of stable boundaries between gene expression domains must then be accompanied or followed by processes leading to the physical separation of the individual placodes.

Formation of boundaries by switch-like cell fate decisions and compartmentalization

Two different types of mechanisms probably play a role for boundary formation: switch-like cell fate decisions and compartmentalization. Switch-like cell fate decisions abolish or prevent coexpression of transcription factors in individual cells. Sharpening of boundaries results from the differential upregulation of one but downregulation of the other transcription factor in cells located in different regions, for example due to (direct or indirect) mutual transcriptional repression (Fig. 8B). Compartmentalization

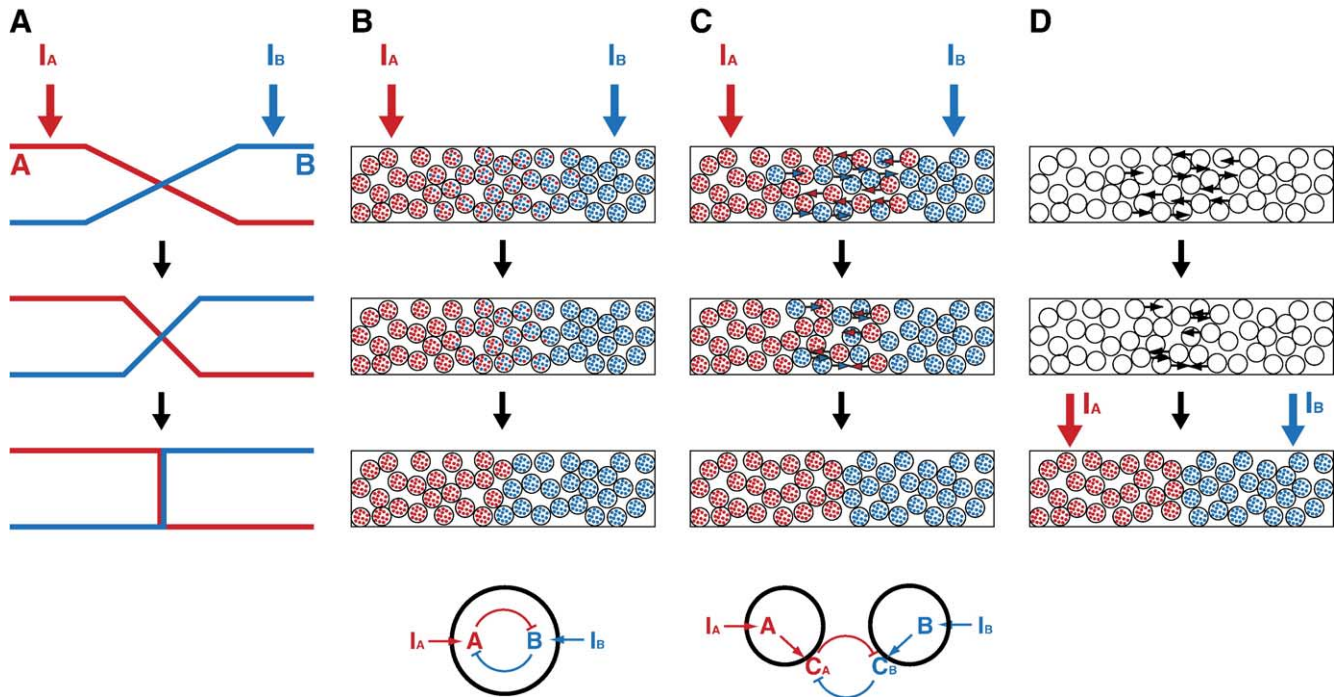


Fig. 8. Establishment of sharp boundaries between differentially specified regions by switch-like cell fate decisions or compartmentalization. (A) Transcription factors A and B, which are involved in specification of two different domains (e.g. neural crest and placodes or two different individual placodes), may be induced by two different inducers (I_A and I_B , respectively) and have initially overlapping expression domains followed by sharpening of the boundary. (B) Overlapping expression domains as depicted in panel A may be due to coexpression of A and B proteins (red and blue dots) in individual cells (circles). Sharpening of the boundary in this case involves the differential upregulation of one but downregulation of the other transcription factor in individual cells (switch-like cell fate decisions), for example, due to their direct or indirect mutual transcriptional repression (lower panel). (C) Alternatively or additionally, overlapping expression domains as depicted in panel A may be due to mixing of cells expressing different transcription factors. Sharpening of the boundary may then involve the sorting of cells by non-random cell movements (arrows) (compartmentalization), for example mediated by mutually repelling cell adhesion molecules (C_A , C_B), activated by the different transcription factors (lower panel). (D) However, occurrence of cell movements per se does not necessarily imply cell sorting as depicted in panel C since random cell movements (arrows) may also occur before ectodermal areas are differentially specified to express different transcription factors.

on the other hand abolishes or prevents intermingling of cells expressing different transcription factors. Sharpening of boundaries results from the sorting of different cells by cell movements possibly mediated by cell adhesion molecules, which are mutually repellent and activated by the different transcription factors (Fig. 8C). This may result in the formation of separate compartments, i.e. adjacent domains of cells, which are lineage-restricted and do not mix. Due to the occurrence of random cell movements in epithelia, the maintenance of stable boundaries between different adjacent epithelial domains probably always requires some sort of compartmentalization. Of course, switch-like cell fate decisions and compartmentalization are not mutually exclusive mechanisms, and they may cooperate in the establishment of stable boundaries.

Both of these mechanisms are involved in boundary formation during development of the central nervous system (CNS) and in other developmental contexts (reviewed in Dahmann and Basler, 1999; Simeone, 2000; Irvine and Rauskolb, 2001; Wurst and Bally-Cuif, 2001; Redies and Puelles, 2001; Kiecker and Lumsden, 2005), but, unfortunately, we still know very little about their role and relative importance during the formation of individual placodes from the panplacodal primordium.

At present, there is no direct evidence for the involvement of switch-like cell fate decisions in the subdivision of the panplacodal primordium. However, several transcription factors

expressed in adjacent domains in the pre-placodal ectoderm have been shown to be involved in boundary formation in central nervous system development due to mutually antagonistic effects. These include *Otx2* and *Gbx2* (Millet et al., 1999; Broccoli et al., 1999; Katahira et al., 2000; Martinez-Barbera et al., 2002; Tour et al., 2002), *Six3* and *Irx3* (Kobayashi et al., 2002), as well as *Pax6* and *Pax2* (Schwarz et al., 1999, 2000; Matsunaga et al., 2000). Moreover, in the case of *Pax6* and *Pax2*, there is evidence for direct mutual repression of these transcription factors (Schwarz et al., 2000). It is tempting to speculate that these transcription factors play a similar role during the subdivision of the panplacodal primordium. Although placodal *Pax2* and *Pax6* expression domains are partly separated by a *Pax3* domain, *Pax2* expression extends laterally around the *Pax3* domain and forms a common border with *Pax6* rostrally (see Fig. 3C). *Otx2* and *Gbx2* as well as *Six3* and *Irx* genes, in turn, are attractive candidates for defining the boundary between an anterior multiplacodal area including adeno-hypophyseal, olfactory, and lens placodes and a posterior multiplacodal area including the remaining (i.e. profundal, trigeminal, otic, lateral line, and epibranchial) placodes. Experimental perturbations that lead to rostral shifts of the expression of some *Irx* genes in the CNS of zebrafish have indeed been shown to similarly affect their placodal expression (Itoh et al., 2002), suggesting that CNS and placodal expression domains are regulated in a similar

fashion. However, additional experiments are needed to clarify the role of these genes for placode formation.

Presently, there is also no conclusive evidence for a role of compartmentalization in the subdivision of the panplacodal primordium. However, several recent studies in chick and zebrafish embryos, which have followed the fate of individually labeled cells or of small groups of cells in various subregions of the pre-placodal ectoderm, have suggested that such mechanisms may be of greater importance than previously thought (Whitlock and Westerfield, 2000; Streit, 2002; Bhattacharyya et al., 2004; Dutta et al., 2005). These studies revealed a surprising degree of mixing of cells, which later contribute to different placodes at neural plate and neural fold stages. Prospective otic placode cells are interspersed with prospective epibranchial placode cells posteriorly (Streit, 2002), whereas prospective olfactory placode cells are intermingled with prospective lens placode cells and at least in the zebrafish also with adenohypophyseal placode cells anteriorly (Bhattacharyya et al., 2004; Dutta et al., 2005). Mixing of cells destined for different parts of the inner ear has also been found within the otic placode/vesicle of *Xenopus* after neural tube closure, but whether precursors of different placodes are intermingled at earlier stages has not been investigated (Kil and Collazo, 2001). Extensive cell movements then lead to the segregation of precursors for different placodes at late neural fold and early neural tube stages in the chick (Streit, 2002; Bhattacharyya et al., 2004).

These fate mapping studies could, however, not establish to what degree this segregation of cells fated for different placodes involves the directed sorting of cells already specified for a specific placodal fate (Fig. 8C) or rather is due to random movement of cells, which are only specified for a particular placodal fate later (Fig. 8D). The timing of specification of various placodes in the chick was analyzed in other experimental studies involving explantation and ectopic grafts (reviewed in Baker and Bronner-Fraser, 2001). Such studies failed to provide a clear picture regarding the specification of lens and adenohypophyseal placodes, but they demonstrated that specification of the olfactory and otic placodes takes place at stages at which little or no intermingling of precursors for different placodes are seen anymore in fate mapping studies (Street, 1937; Herbrand et al., 1998; Groves and Bronner-Fraser, 2000). This does not rule out that cell sorting occurs (for instance of cells, which are already biased but not specified for a particular placodal fate), but it indicates that cell sorting may not be required to account for the segregation of cells fated for different placodes observed in recent fate mapping studies, which may instead be due predominantly to random cell movements of unspecified cells.

Additional studies are, thus, needed to firmly establish to what extent cell sorting contributes to the sharpening of boundaries between different placodes and whether individual placodes subsequently form lineage-restricted compartments. That cell sorting may at least play some role is suggested by some further recent observations. First, the overwhelming majority of cells individually labeled in the rostral placodal domain of zebrafish bud stage embryos later contributed only to a single placode despite strong mixing of adenohypophyseal,

olfactory, and lens placode precursor cells in some regions (Dutta et al., 2005). Second, there is some evidence that initially overlapping but later mutually exclusive transcription factors such as *Pax6* and *Dlx5* in the chick are initially expressed in a mosaic-, salt-, and pepper-like pattern (although it remains to be shown that their expression is complementary), suggesting that there is in fact intermingling of cells biased or specified in a non-equivalent fashion (Bhattacharyya et al., 2004; see also Streit, 2002). Third, lens precursor cells, which ectopically overexpress *Dlx5* or which lack *Pax6* expression, are excluded from the lens (Collinson et al., 2000; Bhattacharyya et al., 2004), suggesting that these transcription factors mediate cell sorting, possibly via the documented ability of *Pax6* and *Dlx* genes to promote expression of cell adhesion molecules (Chalepakakis et al., 1994; Holst et al., 1997; Stoykova et al., 1997; Meech et al., 1999; Davis et al., 2003; Tyas et al., 2003; Rouzankina et al., 2004; McKeown et al., 2005).

Unfortunately, while placodes are known to be distinguished from the adjacent epidermis by the expression of several cell adhesion molecules (Thiery et al., 1982; Levi et al., 1987; Richardson et al., 1987; Miragall et al., 1989; Simonneau et al., 1992; Prouty and Levitt, 1993; Brown et al., 1998; David and Wedlich, 2000; Xu et al., 2002b; Novince et al., 2003), virtually nothing is known about differential expression of cell adhesion molecules in the pre-placodal ectoderm, which could mediate its compartmentalization into different placodes.

Finally, while the studies reviewed here have documented extensive cell movements in the posterior as well as in the anterior part of the pre-placodal ectoderm, they leave open how far anteriorly and posteriorly these movements extend. It will be a challenging task for future studies to relate cell movements in the pre-placodal ectoderm to patterns of transcription factor expression in order to establish whether any of the larger multiplacodal areas defined by molecular subdivisions of the pre-placodal ectoderm at neural plate and fold stages (for example the anterior or posterior placodal area) already forms a lineage-restricted compartment before individual placodes segregate.

Physical separation of individual placodes

Because different placodes form distinct entities at tailbud stages, which occupy widely separate positions in the head ectoderm, the initial regionalization of the panplacodal primordium by the local up- and downregulation of transcription factors and possibly by the restriction of cell movements can only be a first step in the subdivision of the panplacodal primordium into distinct placodes. This must be followed by additional steps leading to the physical separation of placodes. How this is achieved is little understood, but several different processes are likely to be involved.

Downregulation of placodal and upregulation of epidermal transcription factors in parts of the pre-placodal ectoderm accompanied by cell sorting is likely to play at least some role because the pre-placodal ectoderm is known to give rise not only to placodes but also to epidermal regions in the vicinity of placodes, including for example the epidermis of the pharyngeal grooves and the stomodeum (Ishii et al., 2001; Xu et al., 2002a;

Streit, 2002; Laclef et al., 2003a; Schlosser, 2003; Bhattacharyya et al., 2004; Dutta et al., 2005).

In addition, morphogenetic movements may be important. The separation of the adenohypophyseal and olfactory placodes from the remaining placodes, for example, has been suggested to be mediated by the elevation of the neural folds during neurulation (Schlosser and Ahrens, 2004). Neural fold elevation may dorsally displace the rostralmost placodes relative to the others because the rostralmost part of the pre-placodal ectoderm extends until the tip of the outer neural folds, while more posterior parts of the pre-placodal ectoderm are positioned further laterally (due to the occupation of the outer neural folds by neural crest cells; see Fig. 3C). Expansion of the anterior non-neural ectoderm (see, for example, Carpenter, 1937), associated with the bulging out of the retina, may additionally contribute to this separation. Finally, apoptosis has also been suggested to contribute to the physical separation of placodes, while increased proliferation within the placodes makes them morphologically more apparent (Washausen et al., 2005).

Induction of placodes

As detailed above, generic placodal genes such as *Six1*, *Six4*, and *Eya1* begin to be panplacodally expressed at neural plate stages. In addition, many other transcription factors, which contribute to the development of various placodes, are expressed in partly nested and partly overlapping patterns often from neural plate stages on before placode-specific transcription factors appear later in development. Since specification of each placode appears to depend on multiple of these transcription factors, each of which is expressed in a different spatiotemporal pattern, placode induction itself is likely to be a complex multistep process, which relies on different inducers for the induction of panplacodally expressed transcription factors (generic placode induction) and for the induction of transcription factors with a more restricted distribution. Some of these restricted inductive processes are probably shared between adjacent placodes that depend on the same transcription factor, but others will be placode-specific. Moreover, the early molecular subdivision of the panplacodal primordium, as discussed here for neural plate stage *Xenopus* embryos, together with experimental evidence for the early specification of some placodes already during neural plate stages in frogs (Zwilling, 1940, 1941; Bando, 1930; Choi, 1931; Ginsburg, 1995; Gallagher et al., 1996), indicates that some of these more restricted inductive processes occur early and in parallel with generic placode induction.

Induction of individual placodes—a brief overview

Placode induction has been most thoroughly investigated for the adenohypophyseal, lens, and otic placode. Each of these placodes and the olfactory and lateral line placodes require early (gastrula–neural plate stage) signals from the endomesoderm, while signals from the anterior neural plate or tube are necessary for at least some and often multiple subsequent steps of placode development (e.g. Zwilling, 1940; Kohan, 1944; Raven and

Kloos, 1945; Yntema, 1950; Jacobson, 1963a,b,c; Orts-Llorca and Jimenez-Collado, 1971; Henry and Grainger, 1990; Giraldez, 1998; Mendonsa and Riley, 1999; Gleiberman et al., 1999; Ladher et al., 2000a, 2005; reviewed in Jacobson, 1966; Grainger, 1996; Reiss and Burd, 1997; Kioussi et al., 1999a; Ogino and Yasuda, 2000; Baker and Bronner-Fraser, 2001; Chow and Lang, 2001; Dasen and Rosenfeld, 2001; Schlosser, 2002a, 2005; Noramly and Grainger, 2002; Scully and Rosenfeld, 2002; Riley and Phillips, 2003; Fisher and Grainger, 2004; Barald and Kelley, 2004; Streit, 2004; Lang, 2004; Bhattacharyya and Bronner-Fraser, 2004; Rizzoti and Lovell-Badge, 2005). In contrast, there is at present only evidence for a neural-plate-derived signal required for induction of the profundal placode (Stark et al., 1997) and for pharyngeal-pouch-derived signals required for the induction of epibranchial placodes (Begbie et al., 1999; Holzschuh et al., 2005; Nechiporuk et al., 2005), although signals from additional tissues are probably involved at least in case of the latter (Nechiporuk et al., 2005; Scotting, personal communication).

Multiple signaling pathways are involved in the induction of the different placodes. Induction of the adenohypophyseal placode initially requires hedgehog signaling from axial midline tissues and subsequently depends on Wnt, BMP4, and FGF signals from the prospective diencephalon and on sonic hedgehog from the oral ectoderm before intrinsic signaling gradients of BMP2 and Wnt4 are established, which pattern the adenohypophyseal primordium (Treier et al., 1998; Ericson et al., 1998; Takuma et al., 1998; Sbrogna et al., 2003; Herzog et al., 2003, 2004; reviewed in Sheng and Westphal, 1999; Kioussi et al., 1999a; Baker and Bronner-Fraser, 2001; Dasen and Rosenfeld, 2001; Scully and Rosenfeld, 2002; Rizzoti and Lovell-Badge, 2005).

Induction of olfactory and lens placodes, in contrast, is blocked by hedgehog signaling (Cornesse et al., 2005; Dutta et al., 2005). However, it is still unknown which endomesodermal- and/or neural-plate-derived signals are positively involved in the early induction of olfactory and lens placodes. Retinoic acid, FGFs, and BMPs from the adjacent frontonasal mesenchyme (largely neural-crest-derived) and the olfactory ectoderm itself all play important roles for subsequent development of the olfactory epithelium (e.g. La Mantia et al., 2000; Kawauchi et al., 2004; reviewed in Calof et al., 1996; Plendl et al., 1999; Beites et al., 2005). BMPs (BMP4 and possibly BMP7) and FGFs from the optic cup also have been implicated in lens induction, while BMP expression in the lens placode itself may be required for subsequent stages of lens development and FGFs and IGFs appear to be essential for promoting lens fiber differentiation (Furuta and Hogan, 1998; Wawersik et al., 1999; Faber et al., 2001; reviewed in Chow and Lang, 2001; Lang, 2004; Fisher and Grainger, 2004; Lovicu and McAvoy, 2005).

Induction of the otic placode depends on an early FGF signal from the endomesoderm followed by additional FGF signals emanating from the hindbrain (which may synergize with a Wnt signal in amniotes), although the FGFs involved in these various steps differ between different vertebrates (Represa et al., 1991; Lombardo and Slack, 1998; Ladher et al., 2000a, 2005; Vendrell et al., 2000; Phillips et al., 2001, 2004; Maroon et al., 2002;

Léger and Brand, 2002; Liu et al., 2003; Alvarez et al., 2003; Wright and Mansour, 2003; Wright et al., 2004; Solomon et al., 2004; reviewed in Whitfield et al., 2002; Noramly and Grainger, 2002; Riley and Phillips, 2003; Barald and Kelley, 2004). Further patterning of the otic vesicle requires additional signals including Wnt and sonic hedgehog from the adjacent notochord and neural tube (Liu et al., 2002; Riccomagno et al., 2002, 2005).

Induction of neurogenesis in the epibranchial placodes depends on both BMP and FGF signals from the adjacent pharyngeal pouch endoderm (Begbie et al., 1999; Holzschuh et al., 2005; Nechiporuk et al., 2005), while additional factors, which are most likely not endodermally derived, are required for the induction of earlier markers of the epibranchial placodes such as *FoxI1* and *Sox3* (Nechiporuk et al., 2005; Scotting, personal communication).

Because most studies of placode induction so far have focused on a particular placode, we unfortunately still know very little about inductive processes shared between different placodes or about generic placode induction, i.e. the induction of the panplacodal primordium itself. However, many of the transcription factors used as markers in studies of induction of a particular placode are also expressed in adjacent placodes, suggesting that at least some of the inductive events identified for a particular placode are in fact shared among several placodes. For example, *Pax8*, *Pax2*, and *FoxI1*, which have been regarded as otic placode markers, are expressed more broadly at neural plate stages in the posterior placodal area, which gives rise to otic, epibranchial, and lateral line placodes (*Pax8*, *Pax2*) or even in the entire panplacodal primordium (*FoxI1*) (Schlosser and Ahrens, 2004). Therefore, some of the signals implicated in the early induction of the otic placode may in fact be more general inducers of the posterior placodal area, which may only later be regionalized by more localized inductive signals from the pharyngeal pouches and hindbrain, which induce transcription factors specific for epibranchial and otic placodes, respectively.

Models for generic placode induction

Concerning generic placode induction, the orderly and stereotypical positioning of the neural crest, panplacodal primordium, and epidermis around the anterior neural plate in a quasi-concentric fashion (except for the rostral absence of neural crest cells) suggests that the processes underlying induction of these four ectodermal tissues are in some way linked (Fig. 9A). Our understanding of neural and neural crest induction has made great advances in the last decade, and several excellent reviews synthesize these issues (Chang and Hemmati-Brivanlou, 1998a; Weinstein and Hemmati-Brivanlou, 1999; Streit and Stern, 1999b; Mayor et al., 1999; Harland, 2000; Wilson and Edlund, 2001; Mayor and Aybar, 2001; Aybar and Mayor, 2002; Knecht and Bronner-Fraser, 2002; Gammill and Bronner-Fraser, 2003; Meulemans and Bronner-Fraser, 2004; Wu et al., 2003; Yanfeng et al., 2003; Huang and Saint-Jeannet, 2004; Niehrs, 2004; De Robertis and Kuroda, 2004; Stern, 2005). Neural induction, essentially, occurs during gastrulation by the interplay of various signaling molecules

from the organizer including FGFs, Wnt antagonists, and BMP antagonists. While the precise mode of action of these signals is still debated and may differ somewhat for different vertebrates, in each case, a sink of BMP signaling is finally created in the dorsal midline and stable induction of the neural plate appears to require BMP signaling to be below a certain low threshold level. However, how the induction of other ectodermal cell fates is tied to neural induction is more controversial and several models have been proposed (Fig. 9A).

The first model, which I will refer to as the “Delay model”, suggests that a neurally inducing signal travels slowly from the dorsal midline (e.g. via homoiogenetic induction in the ectoderm involving a relay mechanism), while the ectodermal competence to respond to it changes in a cell-autonomous fashion, resulting in the induction of an orderly progression of different ectodermal fates (Nieuwkoop et al., 1985; Albers, 1987).

The second model, here labeled the “Gradient model”, proposes that a gradient of a morphogen such as BMP, which is established in the ectoderm during gastrulation, induces different ectodermal fates at different threshold concentrations (Sasai and De Robertis, 1997; Neave et al., 1997; Nguyen et al., 1998; Marchant et al., 1998; Mayor and Aybar, 2001; Mayor et al., 1999; Aybar and Mayor, 2002; Tribulo et al., 2004; Glavic et al., 2004; Brugmann et al., 2004).

The third model (which is compatible with the Gradient model, but does not require a gradient), termed the “Neural plate border state model”, proposes that first a neural plate border state is induced between neural plate and epidermis. Additional signals from the underlying mesoderm as well as from neural plate and epidermis then induce neural crest medially and placodal fates laterally within this border region (Streit and Stern, 1999a; Baker and Bronner-Fraser, 2001; McLaren et al., 2003; Woda et al., 2003; Meulemans and Bronner-Fraser, 2004; Glavic et al., 2004; Brugmann et al., 2004; Litsiou et al., 2005). Different versions of this model differ regarding whether the border region is induced by a morphogen gradient (Glavic et al., 2004; Brugmann et al., 2004), by epidermal–neural interactions (Streit and Stern, 1999a; McLaren et al., 2003; Woda et al., 2003; Glavic et al., 2004), or by initial induction of a border fate throughout the dorsal ectoderm followed by induction of a proper neural plate in its central part (Morgan and Sargent, 1997; Streit and Stern, 1999a; Litsiou et al., 2005).

Finally, the fourth model, here referred to as “Binary competence model”, suggests that signals from the organizer-derived axial mesoderm induce a dorsal ectodermal region (the border of which may be set at a particular threshold of a morphogen gradient), which maintains competence to form neural plate and neural crest (with a neural default fate), while further ventrally ectoderm loses neural/neural crest competence with a cell-autonomous time course but retains competence to adopt epidermal or placodal fates (with an epidermal default fate). Subsequently, signals from the epidermis and paraxial or intermediary mesoderm induce neural crest at the border of the neural/neural crest competence region, whereas signals from the neural plate and dorsolateral mesoderm induce the panplacodal primordium at the border of the epidermal/placodal competence region (Ahrens and Schlosser, 2005).

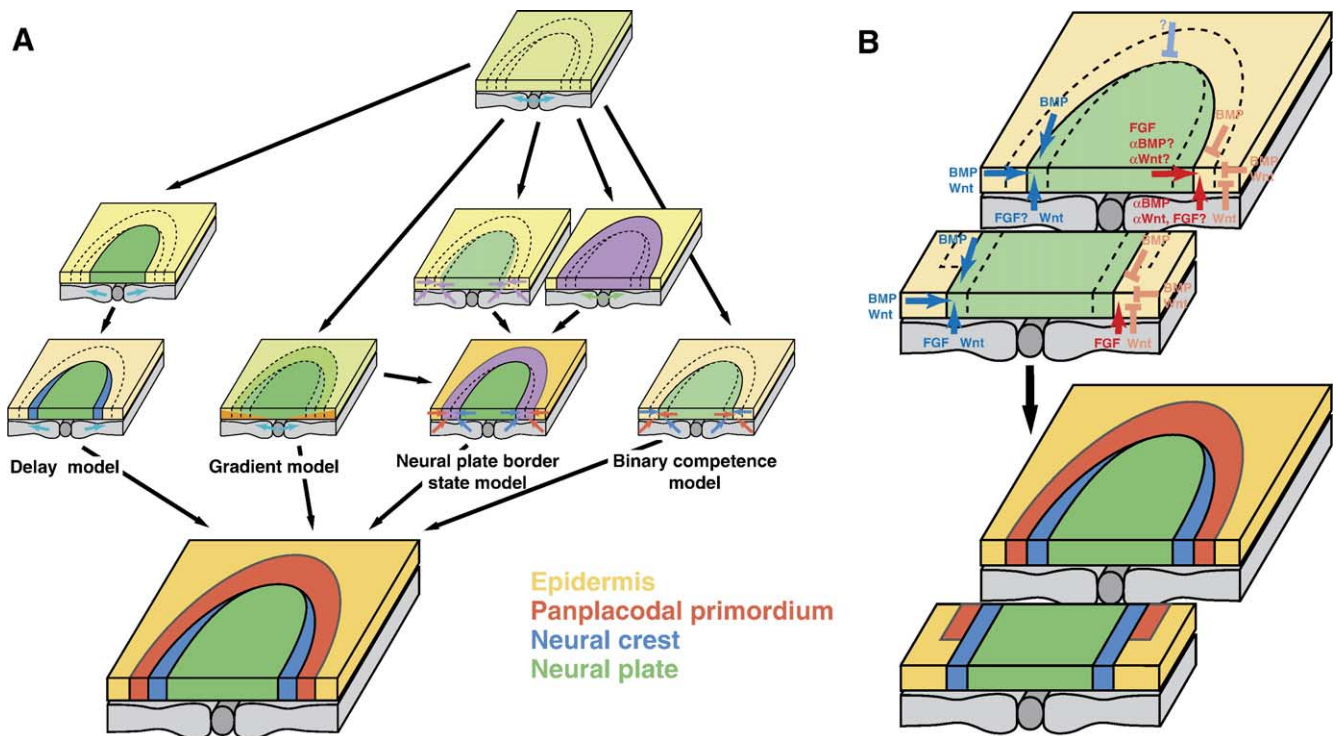


Fig. 9. Models for generic placode induction. (A) Four models have been proposed to explain the positioning of the neural crest, panplacodal primordium, and epidermis around the anterior neural plate. All models share the assumption that in a first step neural induction occurs by a signal (turquoise arrows) from the axial mesoderm (dark gray circle). The Delay model suggests that the slowly spreading neural inducer then reaches more lateral ectoderm after a time delay, during which ectodermal competence has changed in an autonomous fashion (more yellowish color). The Gradient model assumes that neural induction establishes a gradient of a morphogen (e.g., BMP; orange) followed by the specification of different ectodermal fates at different threshold concentrations. The Neural plate border state model proposes that a special neural plate border region (pink) is induced first followed by the induction of neural crest and panplacodal primordium by additional signals from adjacent ectoderm and mesoderm (blue and red arrows). Different versions of this model suggest (from left to right) that the neural plate border is established (1) by a morphogen gradient (orange), (2) by epidermal–neural interactions (pink arrows), or (3) by initial induction of a border state throughout the dorsal ectoderm followed by induction of a proper neural plate in the center (green arrows). The Binary competence model suggests that neural induction establishes a dorsal ectodermal competence region (possibly at a particular threshold of a BMP gradient), which maintains competence (light green) to form neural plate and neural crest and has a neural default fate, while ventral ectoderm cell-autonomously loses neural/neural crest competence but retains competence (light yellow) to adopt epidermal or placodal fates and has an epidermal default fate. Subsequently, additional signals from adjacent ectoderm and mesoderm induce neural crest and panplacodal primordium (left side; blue arrows and bars) and panplacodal primordium (right side; red arrows and bars) from the perspective of the Binary competence model. In addition to the cranial neural plate, its continuation into upper trunk levels is depicted. Whereas signals from prospective epidermis, neural plate border region, and paraxial mesoderm including Wnts, FGFs, and BMPs induce neural crest at the border of the neural/neural crest competence region, FGFs together with BMP and Wnt antagonists from the anterior neural plate and the cranial dorsolateral endomesoderm induce the panplacodal primordium at the border of the epidermal/placodal competence region. Because neural crest is specified earlier than the panplacodal primordium, it may escape the action of BMP and Wnt antagonists from the neural plate. Note that in this model differential ectodermal competence of the dorsal and ventral ectodermal territories defines the ventral limit of neural crest induction and the dorsal limit of generic placode induction. The ventral and posterior limits of generic placode induction are probably imposed by the extent of the signaling center producing BMP and Wnt antagonists in the cranial dorsolateral endomesoderm, which protects the overlying ectoderm from BMP and Wnt signals only in the dorsolateral head region. In contrast, the dorsal limits of neural crest induction may be set by thresholds of availability of the signals (Wnts, BMP, FGF) required for neural crest induction, while the anterior limits of crest induction are probably imposed by an unknown repressing signal that is rostrally confined.

Several recent studies have begun to shed light on the timing of generic placode induction, on the tissues and signals involved, and on the role of ectodermal competence (McLarren et al., 2003; Woda et al., 2003; Brugmann et al., 2004; Glavic et al., 2004; Ahrens and Schlosser, 2005; Litsiou et al., 2005). In combination with studies on neural and neural crest induction, these new data allow a tentative evaluation of the models just described even though much remains to be clarified.

Time window of generic placode induction

The time window of generic placode induction was determined by grafting and extirpation experiments in *Xenopus*.

These demonstrated that expression of *Six1* in the panplacodal primordium is specified at late neural plate stages and is committed at neural fold stages, while non-neural (belly) ectoderm remains competent to express *Six1* until tailbud stages (Ahrens and Schlosser, 2005). In contrast, neural plate and neural crest are specified at the end of gastrulation in *Xenopus* accompanied by a decline in competence for neural and neural crest induction in non-neural ectoderm (Kintner and Dodd, 1991; Servetnick and Grainger, 1991; Mayor et al., 1995; Mancilla and Mayor, 1996). This delay of generic placodal induction relative to neural and neural crest induction is compatible with most of the proposed models but is difficult to reconcile with a Gradient model.

Tissues involved in generic placode induction

Several tissues have been identified as signaling sources for generic placode induction. Both the anterior neural plate and the cranial dorsolateral mesoderm, which includes mesodermal precursors for the heart and pharyngeal arches (Rosenquist, 1970; Keller, 1976; Redkar et al., 2001), are required for generic placode induction in *Xenopus* and chick embryos, while axial mesoderm was shown to be dispensable at neural plate stages in *Xenopus* (Woda et al., 2003; Glavic et al., 2004; Ahrens and Schlosser, 2005; Litsiou et al., 2005). Dorsolateral endomesoderm is also able to induce several panplacodal markers (*Six1*, *Six4*, *Eya2*, *Dach1*) ectopically in the extraembryonic ectoderm of chick embryos but cannot induce *Six1* in *Xenopus* belly ectoderm (Ahrens and Schlosser, 2005; Litsiou et al., 2005). In contrast, neural plate induces several panplacodal markers in both species (*Eya1*, *Six1* in *Xenopus* belly ectoderm; *Dach1*, *Six1* in chick extraembryonic ectoderm) but cannot induce other panplacodal genes (*Eya2*, *Six4*) in the chick (Woda et al., 2003; Glavic et al., 2004; Ahrens and Schlosser, 2005; Litsiou et al., 2005). The somewhat divergent results in frog and chick may be due to the fact that the competence of the responding ectoderm in both cases is non-equivalent, with neural competence still present in chick extraembryonic ectoderm but not in *Xenopus* belly ectoderm (Kintner and Dodd, 1991; Servetnick and Grainger, 1991; Litsiou et al., 2005; Ahrens and Schlosser, 2005). Alternatively, there may be interspecific differences in the inductive capacities of endomesoderm and neural plate. Additional experiments are needed to decide between these possibilities. The finding that generic placode induction depends on neural plate and dorsolateral endomesoderm but is independent of axial mesoderm strongly argues against the Delay model and is also difficult to reconcile with a simple Gradient model because it suggests that generic placode induction requires other signaling sources than the organizer-derived axial mesoderm.

Signals involved in generic placode induction

Several signals from the underlying endomesoderm and the neural plate contribute to generic placode induction (Fig. 9B). First, high BMP levels inhibit induction of panplacodal genes such as *Six1*, while inhibition of BMP signaling adjacent to their normal expression domain promotes their expansion without inducing them ectopically (Brugmann et al., 2004; Glavic et al., 2004; Ahrens and Schlosser, 2005; Litsiou et al., 2005). In addition, *Six1* is induced in animal cap ectoderm of *Xenopus* in response to intermediate levels of BMP4 (Brugmann et al., 2004). However, the latter experiments do not necessarily support the Gradient model postulating a requirement of intermediate BMP levels for generic placode induction. Although no strong induction of the neural plate marker *Sox2* was reported at intermediate BMP4 levels, it cannot be ruled out that partial neuralization of animal caps (with weak *Sox2* expression in restricted domains) occurs only at intermediate BMP4 levels, which may be a prerequisite for

permitting the epidermal–neural interactions required for *Six1* induction.

Several observations in fact argue against a requirement of intermediate BMP levels for placode induction. Manipulations of BMP levels either by BMP4 injections or by mutation of BMP pathway components, which presumably lead to shallower than normal BMP gradients with broader domains of intermediary BMP concentrations, only result in expansion of neural crest domains, whereas placodal marker genes are merely displaced but not expanded contrary to the predictions of the Gradient model (Neave et al., 1997; Nguyen et al., 1998). Furthermore, when neurally competent ectoderm (e.g. animal caps) is transplanted to the pre-placodal region of neural plate stage amphibian embryos, the graft is completely neuralized and does not develop into placodal tissue (Holtfreter, 1933; Albers, 1987; Bastidas et al., 2004; Ahrens and Schlosser, 2005). This indicates that effective BMP levels in the pre-placodal region are low enough to permit neural induction in competent ectoderm. Conversely, *Xenopus* belly ectoderm, which has lost neural competence, is induced to express *Six1*, when grafted into the neural plate (Ahrens and Schlosser, 2005), where it is exposed to strong BMP inhibition from the axial mesoderm (Smith and Harland, 1992; Lamb et al., 1993; Sasai et al., 1995).

Taken together, current evidence indicates that high BMP levels are incompatible with generic placode induction without supporting a role for intermediate levels of BMP. Because BMP is strongly expressed in the pre-placodal region at neural plate stages (Fainsod et al., 1994; Hemmati-Brivanlou and Thomsen, 1995; Schmidt et al., 1995; Streit et al., 1998; Streit and Stern, 1999a; Faure et al., 2002), diffusible BMP inhibitors from the neural plate (e.g. *noggin*; Knecht and Harland, 1997) and/or the dorsolateral endomesoderm (e.g. the multifunctional BMP/Wnt/nodal antagonist *Cerberus*; Bouwmeester et al., 1996; Chapman et al., 2002) are probably required for generic placode induction. In contrast, induction of the neural crest requires at least some BMP signaling (Liem et al., 1995; Morgan and Sargent, 1997; Nguyen et al., 1998, 2000; Marchant et al., 1998; Selleck et al., 1998; Tribulo et al., 2003).

In addition to BMP antagonists, FGF signals were shown to be required for generic placode induction. In *Xenopus*, FGF8—most likely from the anterior neural plate—is required for *Six1* induction throughout the pre-placodal ectoderm and in combination with BMP inhibitors is sufficient to induce *Six1* ectopically in rostroventral ectoderm (Ahrens and Schlosser, 2005). In chick embryos, FGFs can induce some panplacodal markers (*Eya2*) directly but promote the induction of others (*Six4*) only when followed by activity of BMP and Wnt antagonists (Litsiou et al., 2005). One interpretation of these findings is that early FGF signals from organizer-derived mesoderm promote the formation of a neural plate border identity, while later FGF signals from the prospective heart mesoderm initiate expression of some panplacodal markers (Streit and Stern, 1999a; Litsiou et al., 2005). However, it is equally possible that FGF signals from the neural plate and/or the dorsolateral mesoderm (e.g. Christen and Slack, 1997; Shamim and Mason, 1999; Ahrens and Schlosser, 2005) are specifically required for the induction of panplacodal markers,

some of which need BMP and Wnt antagonists for their subsequent maintenance.

In contrast to *Xenopus*, in amniotes, *FGF8* is expressed in a restricted domain of cranial endoderm but absent from the early neural plate (Crossley and Martin, 1995; Adamska et al., 2002; Ladher et al., 2005), while in zebrafish, *FGF8* is required for otic placode induction but dispensable for the induction of panplacodal genes elsewhere (Shanmugalingam et al., 2000; Léger and Brand, 2002; Solomon et al., 2004). Therefore, other FGF family members are probably involved in generic placode induction in amniotes and teleosts. Such phylogenetically flexible roles of FGFs during placode evolution were also demonstrated for FGFs involved in otic placode induction (see above). While FGFs have also been implicated in neural crest induction, different tissues (in particular paraxial mesoderm) probably serve as main sources of the FGF signal involved in neural crest induction (Mayor et al., 1997; La Bonne and Bronner-Fraser, 1998; Villanueva et al., 2002; Monsoro-Burq et al., 2003, 2005).

Finally, generic placode induction is inhibited by canonical Wnt signaling in both *Xenopus* and chick embryos, whereas ectopic attenuation of Wnt signaling in the trunk region in combination with BMP inhibition results in extension of panplacodal gene expression into the trunk (Brugmann et al., 2004; Litsiou et al., 2005; Huang et al., 2005). This suggests that suppression of canonical Wnt signaling in the head by Wnt antagonists secreted by the cranial endomesoderm and possibly the anterior neural plate (Bouwmeester et al., 1996; Leyns et al., 1997; Glinka et al., 1998; Piccolo et al., 1999; Duprez et al., 1999; Ladher et al., 2000b; Pera and De Robertis, 2000; Bradley et al., 2000; Chapman et al., 2002) is also required for generic placode induction. Conversely, Wnt signaling from the trunk epidermis and mesoderm (e.g. Christian and Moon, 1993; McGrew et al., 1997; Chang and Hemmati-Brivanlou, 1998b; Bang et al., 1999; Garcia-Castro et al., 2002; Schubert et al., 2002; Litsiou et al., 2005) prevents generic placode induction in the trunk. In contrast, neural crest induction requires canonical Wnt signaling, for example, from the adjacent epidermis or underlying mesoderm (Mayor et al., 1997; Chang and Hemmati-Brivanlou, 1998b; La Bonne and Bronner-Fraser, 1998; Bang et al., 1999; Garcia-Castro et al., 2002; Villanueva et al., 2002; Lewis et al., 2004; Bastidas et al., 2004; Wu et al., 2005; Sato et al., 2005; Monsoro-Burq et al., 2005). Wnt overexpression even promotes neural crest formation at the expense of the pre-placodal region (Litsiou et al., 2005).

In conclusion, it is now clear that neither generic placode induction nor neural crest induction occurs simply as a corollary of dorsal midline signaling during neural induction contrary to the predictions of the Delay and Gradient models (Fig. 9A). Induction of both the panplacodal primordium and the neural crest depends instead on multiple signals from the underlying mesoderm as well as from the adjacent ectoderm, which is compatible with the Neural plate border state and Binary competence models. In order to compare these latter two models, the role of ectodermal competence for neural crest and generic placode induction needs to be considered in more detail.

Distribution of competence for generic placode induction

Importantly, when neural plate is juxtaposed with non-neural (prospective epidermal) belly ectoderm in *Xenopus*, *Six1* is only induced in the non-neural ectoderm but not in the neural plate, indicating that competence to express panplacodal genes is restricted to non-neural ectoderm but is absent from the neural plate at neural plate stages (Glavic et al., 2004; Ahrens and Schlosser, 2005). In contrast, in avian embryos, *Six1* induction was observed on the neural as well as on the non-neural side of the border of anterior neural plate grafts (Litsiou et al., 2005). However, this does not necessarily imply that neural plate in avian embryos is competent to express panplacodal genes. An alternative possibility is, due to the more gradual progression of neural specification along the anteroposterior axis in avian embryos, that the posterior side of the neural plate grafts (i.e. the side oriented towards Hensen's node) may not have been fully neurally specified at the time of transplantation (stages 5–7).

Juxtaposition of neural plate and non-neural ectoderm is also known to induce neural crest cells (Rollhäuser-ter-Horst, 1979; Moury and Jacobson, 1989, 1990; Selleck and Bronner-Fraser, 1995; Liem et al., 1995; Dickinson et al., 1995; Mancilla and Mayor, 1996; Basch et al., 2000; Litsiou et al., 2005). Whereas competence for the induction of panplacodal genes appears to be restricted to the non-neural ectoderm, neural crest induction was reported to occur on both sides of the boundary between neural plate and non-neural ectoderm (Moury and Jacobson, 1990; Selleck and Bronner-Fraser, 1995; Mancilla and Mayor, 1996). However, while evidence for neural crest induction on the neural side is strong, data presented to support neural crest induction on the non-neural side are inconclusive.

In the study by Moury and Jacobson (1990), cells derived from pigmented non-neural ectoderm were reported in cranial and spinal ganglia (but never as melanocytes), and these were regarded as neural-crest-derived cells. However, it is unclear whether any of these cells were indeed neural-crest-derived since cranial ganglia also receive a placodal contribution (as the authors acknowledge), and their Fig. 7 indicates that they erroneously interpreted posterior cranial ganglia as “spinal ganglia”. In the study by Selleck and Bronner-Fraser (1995), neural plates were grafted into a region of non-neural ectoderm at the border of area pellucida and opaca that is neurally competent and can be neuralized by neural plate grafts (Pera et al., 1999). Thus, the neural crest cells induced in the host ectoderm next to the graft may have formed in a neuralized portion of the host ectoderm rather than from non-neural ectoderm (as explicitly acknowledged by the authors). In the study by Mancilla and Mayor (1996), the neural plate graft was probably incompletely labeled and bigger than indicated by the authors (their Figs. 3c, d). Estimating the size of their graft by the distribution of scattered cells staining for the graft-specific fluorescent lineage tracer, which are detectable peripheral to the line supposed to demarcate the graft boundary in their published figure, the graft presumably contained all induced neural crest cells. This interpretation is in accordance with their observation that competence for neural crest formation is completely lost in non-neural ectoderm at neural plate stages (Mancilla and

Mayor, 1996). Recent experiments from my own laboratory (Ahrens and Schlosser, unpublished observations) also reveal induction of neural crest markers (*Slug*, *Foxd3*) only on the neural but not on the non-neural side after grafting anterior neural plate into belly ectoderm in early neural plate stage *Xenopus* embryos. Moreover, several studies have only reported induction of neural crest markers on the neural side of the boundary (Liem et al., 1995; Dickinson et al., 1995; Basch et al., 2000). Finally, single cell lineage studies in neural fold stage chick embryos found cells that can contribute to neural tube, neural crest, and epidermis or to neural tube and neural crest only, while cells that contribute to neural crest and epidermis only were never found, again suggesting that neural crest cells can only be induced from neural ectoderm (Selleck and Bronner-Fraser, 1995).

Taken together, current evidence suggests that as a result of neural induction during gastrulation the ectoderm is initially divided into two territories, which differ both in default fate and competence. The dorsomedial ectoderm has a neural default fate and is competent to form neural crest cells in response to proper signals from the adjacent epidermis and underlying mesoderm, while the ventrolateral ectoderm (also referred to as non-neural ectoderm here) has an epidermal default fate and is competent to form the panplacodal primordium in response to signals from the adjacent neural plate and underlying mesoderm. This scenario supports the Binary competence model outlined above but is in conflict with models proposing that neural crest and placodes are induced from a common neural plate border state.

On the other hand, precursors for neural crest and placodal cells are still extensively intermingled at neural fold stages in chick embryos (Selleck and Bronner-Fraser, 1995; Streit, 2002), and this seems to fit better with predictions of Neural plate border state models. However, cell mixing does not necessarily imply the existence of a common neural plate border state. An alternative interpretation, which is fully compatible with the Binary competence model, is that expression boundaries between transcription factors, which promote neural/neural crest competence and those which promote epidermal/placodal competence, may be initially unsharp and overlapping due to coexpression in single cells and/or intermingling of cells before they become refined by mutual transcriptional repression and/or cell sorting (see Fig. 8). Mutual transcriptional repression has indeed been observed between placodal *Six1* and the neural-crest-specific transcription factor *FoxD3* (Brugmann et al., 2004), although it is still unclear whether this is due to direct or indirect interactions. Nevertheless, more detailed analyses of how the developmental potential of single cells at the neural plate border is sequentially restricted will be necessary to conclusively decide between the Binary competence and Neural plate border state models.

Molecular basis for placodal competence in the ectoderm

The molecular mechanisms underlying differences in ectodermal competence are still poorly understood. However, some members of the *Dlx* and *GATA* families appear to be

central for the development of non-neural ectoderm and may be involved in defining its state of competence. At the end of gastrulation, *Dlx3* and/or *Dlx5* as well as *GATA1*, *GATA2*, and *GATA3* are widely expressed in the non-neural ectoderm but are excluded from the developing neural plate (Akimenko et al., 1994; Dirksen et al., 1994; Yang et al., 1998; Depew et al., 1999; Acampora et al., 1999; Feledy et al., 1999; Pera et al., 1999; Quint et al., 2000; Luo et al., 2001a,b; McLaren et al., 2003; Schlosser and Ahrens, 2004; Walmsley et al., 1994; Kelley et al., 1994; Neave et al., 1995; Read et al., 1998; Sheng and Stern, 1999). Which *Dlx* gene corresponds most closely to the border of the non-neural ectoderm in the restrictive sense used here (i.e. ectoderm, which does not become incorporated into either neural tube or neural crest) differs between vertebrates. In zebrafish and *Xenopus*, it would be *Dlx3* but in amniotes *Dlx5*. The pre-placodal region expresses particularly high levels of *Dlx3* and *Dlx5* at neural plate stages, and subsequently high expression of these genes persists in olfactory and otic placodes. Although *GATA2* and *GATA3* genes were reported to be excluded from parts of the pre-placodal ectoderm at neural plate stages (Read et al., 1998), they are later expressed at least in adenohypophyseal, olfactory, and otic placodes (George et al., 1994; Read et al., 1998; Dasen et al., 1999; Sheng and Stern, 1999; Karis et al., 2001; Lillevali et al., 2004; Lawoko-Kerali et al., 2002).

Several lines of evidence suggest that—besides their multiple developmental functions at later stages (for a review, see Bendall and Abate-Shen, 2000; Merlo et al., 2000; Beanan and Sargent, 2000; Panganiban and Rubenstein, 2002; Patient and McGhee, 2002; Hecklen-Klein et al., 2005)—genes from both families may be critical in determining the border between a non-neural (epidermal–placodal) and neural (neural plate–neural crest) competence region in the ectoderm. Canonical Wnt signaling is initially required for the exclusion of *Dlx3* from the dorsal ectoderm during early gastrulation (Beanan et al., 2000). Both *GATA* and *Dlx* genes also respond to BMP signaling, although only *GATA* but not *Dlx* genes are immediate BMP response genes (Friedle and Knöchel, 2002; Feledy et al., 1999). Consequently, during subsequent gastrulation, both *Dlx* and *GATA* genes are stably activated in a ventrally confined pattern due to dorsal inhibition of BMP signaling by the organizer (Maeno et al., 1996; Zhang and Evans, 1996; Feledy et al., 1999; Pera et al., 1999; Luo et al., 2001a,b; McLaren et al., 2003; Woda et al., 2003).

Both *Dlx* and *GATA* genes repress neural differentiation in early embryos (Xu et al., 1997b; Shibata et al., 1998; Pera et al., 1999; Feledy et al., 1999; McLaren et al., 2003; Woda et al., 2003). Conversely, these genes are required for normal development of many derivatives of the non-neural ectoderm. *GATA* factors are important for proper epidermal (Kaufman et al., 2003) and placodal development: whereas *GATA2* is required for the differentiation of gonadotropes and thyrotropes from the adenohypophyseal placode (Steger et al., 1994; Gordon et al., 1997; Dasen et al., 1999), *GATA3* is important for inner ear morphogenesis and proper differentiation of auditory neuroblasts (Karis et al., 2001; Lawoko-Kerali et al., 2004). Similarly, *Dlx3* and *Dlx5* are required for proper

development of the epidermis (Morasso et al., 1996) and of the olfactory, profundal/trigeminal, and otic placodes (Acampora et al., 1999; Depew et al., 1999; Merlo et al., 2002; Solomon and Fritz, 2002; Woda et al., 2003; Long et al., 2003; Levi et al., 2003; Liu et al., 2003; McLarren et al., 2003; Solomon et al., 2004; Kaji and Artinger, 2004; Givens et al., 2005). Most importantly, the ability of belly ectoderm to ectopically express the panplacodal gene *Six1* in response to neural plate grafts is abolished if *Dlx3* activity is blocked (Woda et al., 2003). This suggests that *Dlx3* is required for making non-neural ectoderm responsive to generic placode inducers.

Gain of function studies show, however, that *Dlx3* and *Dlx5* are unable to activate epidermal or many placode-specific genes by themselves (less is known about *GATA* factors) (Feledy et al., 1999; McLarren et al., 2003; Woda et al., 2003). Taken together with the requirement of *Dlx3* for responding to placode inducers, this suggests that these *Dlx* genes may function mainly as competence factors, which help to create a permissive environment for non-neural fates and to confer responsiveness to inducers of specialized non-neural fates such as placodes. However, after overexpression of *Dlx3*, *Dlx5*, or *Dlx6*, expression of the panplacodal gene *Six1* is inhibited (Woda et al., 2003; Bruggmann et al., 2004). This could be due to the fact that after *Dlx* overexpression the neural plate is juxtaposed with ectoderm, in which ectopic *Dlx* has suppressed neural fate but which otherwise is non-equivalent to normal non-neural ectoderm and does not have placodal competence. This would suggest that competence itself depends on the cooperation of several components, for example, synergy of *Dlx* with *GATA* genes, although this speculation remains to be tested.

Interestingly, juxtaposition of *Dlx*-expressing ectoderm with neural plate is also necessary for the induction of neural crest and for lateral neural plate derivatives (e.g. Rohon Beard cells), although *Dlx* expression alone is not sufficient but requires cooperation with other epidermally derived factors (Woda et al., 2003; McLarren et al., 2003; Kaji and Artinger, 2004). However, in contrast to its cell-autonomous requirement for placode induction, *Dlx* promotes neural crest and lateral neural plate fates in a non-cell autonomous fashion (McLarren et al., 2003; Kaji and Artinger, 2004), suggesting that *Dlx3* is not involved in promoting competence for neural crest induction but rather is required in the non-neural ectoderm together with other factors for the production of neural-crest-inducing signals.

In summary, current knowledge of the tissues, signals, and distribution of competence involved in generic placode induction most strongly supports the Binary competence model outlined above. It is summarized and compared with neural crest induction in Fig. 9B. During gastrulation, various signaling molecules including FGFs, Wnt antagonists, and BMP antagonists emanating from the organizer (prospective axial mesoderm) establish a dorsal ectodermal domain (possibly defined by a certain BMP threshold) competent for adopting neural or neural crest fates (neural being the default fate), whereas the remaining ventral ectodermal domain is competent to adopt epidermal or placodal fates (with epidermis being the default fate). Signals from the prospective epidermis, neural plate border region, and the paraxial mesoderm including Wnts,

FGFs, and BMPs then induce neural crest at the border of the neural/neural crest competence region at the end of gastrulation, while FGFs together with BMP and Wnt antagonists from the anterior neural plate and the cranial dorsolateral endomesoderm induce generic placodal markers such as *Six1* and *Eya1* at the border of the epidermal/placodal competence region in the head at neural plate and neural fold stages.

Conclusions

Our understanding of placode development has made significant progress in the last years. Evidence is accumulating that all cranial placodes originate from a common panplacodal primordium. We also have made great strides in elucidating the role of tissues and signaling molecules involved in the multistep induction of different placodes from this primordium as well as the role of many transcription factors involved in the specification and differentiation of placodes. However, these advances must not obscure the fact that we are only beginning to understand the intricacies of placode development.

In conclusion of this review, I want to briefly outline a few of the many important questions that remain to be tackled. First, even for the most well-studied model organisms, we still lack complete and detailed fate and specification maps of the prospective placodal ectoderm at gastrula and neurula stages. However, this information is urgently needed to understand when and how different placodes are specified. Second, we still know very little about how the different transcription factors involved in placode specification and differentiation interact and which target genes they affect. Unraveling the regulatory networks controlling placode development will require increasing integration of experimental gain and loss of function studies with *cis*-regulatory analyses and microarray techniques. Third, the mechanisms underlying the subdivision of the panplacodal primordium into different placodes are largely obscure. For example, what role do mutual transcriptional repression or cell sorting by differential adhesion play for the sharpening of boundaries between different placodes? And to what extent are morphogenetic movements and apoptosis involved in the subdivision of the panplacodal primordium? Fourth and finally, it is presently unclear which inductive events and subsequent pathways of specification and differentiations are unique to an individual placode and which are shared between different placodes or even between placodes and other ectodermal tissues. Addressing this important and complex question is still hindered by the narrow focus of most studies on a single placode. It is likely to be resolved only with an increasing awareness that the early developmental history of different placodes is intricately intertwined.

Acknowledgments

I thank Katja Ahrens for useful comments on the manuscript and Dr. Paul J. Scotting for sharing data prior to publication. This work was supported by grants SCHL 450/5-1, 5-3, and 5-4 of the German Science Foundation.

References

- Abdelhak, S., Kalatzis, V., Heilig, R., Compain, S., Samson, D., Vincent, C., Weil, D., Cruaud, C., Sahly, I., Leibovici, M., Bitner-Grindzicz, M., Francis, M., Lacombe, D., Vigneron, J., Charachon, R., Boven, K., Bedbeter, P., Van Regemorter, N., Weissenbach, J., Petit, C., 1997. A human homologue of the *Drosophila* eyes absent gene underlies Branchio-Oto-Renal (BOR) syndrome and identifies a novel gene family. *Nat. Genet.* 15, 157–164.
- Abe, H., Oka, Y., 2000. Modulation of pacemaker activity by salmon gonadotropin-releasing hormone (sGnRH) in terminal nerve (TN)-GnRH neurons. *J. Neurophysiol.* 83, 3196–3200.
- Abu-Elmagd, M., Ishii, Y., Cheung, M., Rex, M., Le Rouedec, D., Scotting, P.J., 2001. cSox3 expression and neurogenesis in the epibranchial placodes. *Dev. Biol.* 237, 258–269.
- Acampora, D., Mazan, S., Lallemand, Y., Avantaggiato, V., Maury, M., Simeone, A., Brulet, P., 1995. Forebrain and midbrain regions are deleted in *Otx2*^{−/−} mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* 121, 3279–3290.
- Acampora, D., Mazan, S., Avantaggiato, V., Barone, P., Tuorto, F., Lallemand, Y., Brulet, P., Simeone, A., 1996. Epilepsy and brain abnormalities in mice lacking the *Otx1* gene. *Nat. Genet.* 14, 218–222.
- Acampora, D., Mazan, S., Tuorto, F., Avantaggiato, V., Tremblay, J.J., Lazzaro, D., di Carlo, A., Mariano, A., Macchia, P.E., Corte, G., Macchia, V., Drouin, J., Brulet, P., Simeone, A., 1998. Transient dwarfism and hypogonadism in mice lacking *Otx1* reveal prepubescent stage-specific control of pituitary levels of GH, FSH and LH. *Development* 125, 1229–1239.
- Acampora, D., Merlo, G.R., Paleari, L., Zerega, B., Postiglione, M.P., Mantero, S., Bober, E., Barbieri, O., Simeone, A., Levi, G., 1999. Craniofacial, vestibular and bone defects in mice lacking the *Distal-less*-related gene *Dlx5*. *Development* 126, 3795–3809.
- Acampora, D., Gulisano, M., Simeone, A., 2000. Genetic and molecular roles of *Otx* homeodomain proteins in head development. *Gene* 246, 23–35.
- Adamska, M., Herbrand, H., Adamski, M., Kruger, M., Braun, T., Bober, E., 2002. FGFs control the patterning of the inner ear but are not able to induce the full ear program. *Mech. Dev.* 109, 303–313.
- Ahrens, K., Schlosser, G., 2005. Tissues and signals involved in the induction of placodal *Six1* expression in *Xenopus laevis*. *Dev. Biol.* 288, 40–59.
- Akimenko, M.A., Ekker, M., Wegner, J., Lin, W., Westerfield, M., 1994. Combinatorial expression of three zebrafish genes related to *distal-less*: part of a homeobox gene code for the head. *J. Neurosci.* 14, 3475–3486.
- Alappat, S., Zhang, Z.Y., Chen, Y.P., 2003. *Msx* homeobox gene family and craniofacial development. *Cell Res.* 13, 429–442.
- Albers, B., 1987. Competence as the main factor determining the size of the neural plate. *Dev. Growth Differ.* 29, 535–545.
- Allan, D.W., Thor, S., 2003. Together at last: bHLH and LIM-HD regulators cooperate to specify motor neurons. *Neuron* 38, 675–677.
- Allis, E.P., 1897. The cranial muscles and cranial and first spinal nerves in *Amia calva*. *J. Morphol.* 12, 487–769.
- Altman, C.R., Chow, R.L., Lang, R.A., Hemmati-Brivanlou, A., 1997. Lens induction by Pax-6 in *Xenopus laevis*. *Dev. Biol.* 185, 119–123.
- Alvarez, Y., Alonso, M.T., Vendrell, V., Zelarayan, L.C., Chamero, P., Theil, T., Bosl, M.R., Kato, S., Maconochie, M., Riethmacher, D., Schimmang, T., 2003. Requirements for FGF3 and FGF10 during inner ear formation. *Development* 130, 6329–6338.
- Andermann, P., Ungos, J., Raible, D.W., 2002. Neurogenin1 defines zebrafish cranial sensory ganglia precursors. *Dev. Biol.* 251, 45–58.
- Aoki, Y., Saint-Germain, N., Gyda, M., Magner-Fink, E., Lee, Y.H., Credidio, C., Saint-Jeannet, J.P., 2003. *Sox10* regulates the development of neural crest-derived melanocytes in *Xenopus*. *Dev. Biol.* 259, 19–33.
- Ariëns-Kappers, J., 1941. Kopflaploden bei Wirbeltieren. *Ergeb. Anat. Entwickl. Gesch.* 33, 370–412.
- Artinger, K.B., Fedtsova, N., Rhee, J.M., Bronner-Fraser, M., Turner, E., 1998. Placodal origin of *Brn-3*-expressing cranial sensory neurons. *J. Neurobiol.* 36, 572–585.
- Asa, S.L., Ezzat, S., 2004. Molecular basis of pituitary development and cytogenesis. *Front. Horm. Res.* 32, 1–19.
- Ashery-Padan, R., Marquardt, T., Zhou, X., Gruss, P., 2000. Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. *Genes Dev.* 14, 2701–2711.
- Ataliotis, P., Ivins, S., Mohun, T.J., Scambler, P.J., 2005. *XTbx1* is a transcriptional activator involved in head and pharyngeal arch development in *Xenopus laevis*. *Dev. Dyn.* 232, 979–991.
- Aybar, M.J., Mayor, R., 2002. Early induction of neural crest cells: lessons learned from frog, fish and chick. *Curr. Opin. Genet. Dev.* 12, 452–458.
- Ayer-LeLièvre, C.S., Le Douarin, N.M., 1982. The early development of cranial sensory ganglia and the potentialities of their component cells studied in quail–chick chimeras. *Dev. Biol.* 94, 291–310.
- Azuma, N., Hirakiyama, A., Inoue, T., Asaka, A., Yamada, M., 2000. Mutations of a human homologue of the *Drosophila* eyes absent gene (*EYA1*) detected in patients with congenital cataracts and ocular anterior segment anomalies. *Hum. Mol. Genet.* 9, 363–366.
- Baker, C.V.H., Bronner-Fraser, M., 1997a. The origins of the neural crest. Part II: an evolutionary perspective. *Mech. Dev.* 69, 13–29.
- Baker, C.V.H., Bronner-Fraser, M., 1997b. The origins of the neural crest. Part I: embryonic induction. *Mech. Dev.* 69, 3–11.
- Baker, C.V.H., Bronner-Fraser, M., 2000. Establishing neuronal identity in vertebrate neurogenic placodes. *Development* 127, 3045–3056.
- Baker, C.V.H., Bronner-Fraser, M., 2001. Vertebrate cranial placodes: I. Embryonic induction. *Dev. Biol.* 232, 1–61.
- Baker, C.V.H., Sharpe, C.R., Torpey, N.P., Heasman, J., Wylie, C.C., 1995. A *Xenopus* c-kit related receptor tyrosine kinase expressed in migrating stem cells of the lateral line system. *Mech. Dev.* 50, 217–228.
- Baker, C.V., Stark, M.R., Marcelle, C., Bronner-Fraser, M., 1999. Competence, specification and induction of Pax-3 in the trigeminal placode. *Development* 126, 147–156.
- Baker, C.V., Stark, M.R., Bronner-Fraser, M., 2002. Pax3-expressing trigeminal placode cells can localize to trunk neural crest sites but are committed to a cutaneous sensory neuron fate. *Dev. Biol.* 249, 219–236.
- Bally-Cuif, L., Dubois, L., Vincent, A., 1998. Molecular cloning of *Zco2*, the zebrafish homolog of *Xenopus* *Xco2* and mouse *EBF-2*, and its expression during primary neurogenesis. *Mech. Dev.* 77, 85–90.
- Bancroft, M., Bellairs, R., 1977. Placodes of the chick embryo studied by SEM. *Anat. Embryol. (Berl.)* 151, 97–108.
- Bando, K., 1930. Untersuchungen über die Entstehung des Hörbläschens aus einem in die Haut gesteckten Ektodermstück bei der Amphibienlarve. *Jpn. J. Med. Sci., Pt. 1. Anat.* 22, 45–63.
- Bang, A.G., Papalopulu, N., Kintner, C., Goulding, M.D., 1997. Expression of Pax-3 is initiated in the early neural plate by posteriorizing signals produced by the organizer and by posterior non-axial mesoderm. *Development* 124, 2075–2085.
- Bang, A.G., Papalopulu, N., Goulding, M.D., Kintner, C., 1999. Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from posterior nonaxial mesoderm. *Dev. Biol.* 212, 366–380.
- Barald, K.F., Kelley, M.W., 2004. From placode to polarization: new tunes in inner ear development. *Development* 131, 4119–4130.
- Basch, M.L., Selleck, M.A.J., Bronner-Fraser, M., 2000. Timing and competence of neural crest formation. *Dev. Neurosci.* 22, 217–227.
- Bastidas, F., De Calisto, J., Mayor, R., 2004. Identification of neural crest competence territory: role of Wnt signaling. *Dev. Dyn.* 229, 109–117.
- Bayramov, A.V., Martynova, N.Y., Eroshkin, F.M., Ermakova, G.V., Zarskiy, A.G., 2004. The homeodomain-containing transcription factor *X-nkx-5.1* inhibits expression of the homeobox gene *Xanf-1* during the *Xenopus laevis* forebrain development. *Mech. Dev.* 121, 1425–1441.
- Beanan, M.J., Sargent, T.D., 2000. Regulation and function of *Dlx3* in vertebrate development. *Dev. Dyn.* 218, 545–553.
- Beanan, M.J., Feledy, J.A., Sargent, T.D., 2000. Regulation of early expression of *Dlx3*, a *Xenopus* anti-neural factor, by beta-catenin signaling. *Mech. Dev.* 91, 227–235.
- Bebenek, I.G., Gates, R.D., Morris, J., Hartenstein, V., Jacobs, D.K., 2004. Sine oculis in basal Metazoa. *Dev. Genes Evol.* 214, 342–351.
- Begbie, J., Graham, A., 2001. The ectodermal placodes: a dysfunctional family. *Philos. Trans. R. Soc. London, Ser. B Biol. Sci.* 356, 1655–1660.
- Begbie, J., Brunet, J.-F., Rubenstein, J.L.R., Graham, A., 1999. Induction of the epibranchial placodes. *Development* 126, 895–902.

- Begbie, J., Ballivet, M., Graham, A., 2002. Early steps in the production of sensory neurons by the neurogenic placodes. *Mol. Cell. Neurosci.* 21, 502–511.
- Beites, C.L., Kawauchi, S., Crocker, C.E., Calof, A.L., 2005. Identification and molecular regulation of neural stem cells in the olfactory epithelium. *Exp. Cell Res.* 306, 309–316.
- Bellefroid, E.J., Bourguignon, C., Hollemann, T., Ma, Q., Anderson, D.J., Kintner, C., Pieler, T., 1996. X-Myt1, a *Xenopus* C2HC-type zinc finger protein with a regulatory function in neuronal differentiation. *Cell* 87, 1191–1202.
- Bellefroid, E.J., Kobbe, A., Gruss, P., Pieler, T., Gurdon, J.B., Papalopulu, N., 1998. Xiro3 encodes a *Xenopus* homolog of the *Drosophila* Iroquois genes and functions in neural specification. *EMBO J.* 17, 191–203.
- Bendall, A.J., Abate-Shen, C., 2000. Roles for Msx and Dlx homeoproteins in vertebrate development. *Gene* 247, 17–31.
- Bentley, C.A., Zidehsarai, M.P., Grindley, J.C., Parlow, A.F., Barth-Hall, S., Roberts, V.J., 1999. Pax6 is implicated in murine pituitary endocrine function. *Endocrine* 10, 171–177.
- Bermingham, N.A., Hassan, B.A., Price, S.D., Vollrath, M.A., Ben Arie, N., Eatock, R.A., Bellen, H.J., Lysakowski, A., Zoghbi, H.Y., 1999. Math1: an essential gene for the generation of inner ear hair cells. *Science* 284, 1837–1841.
- Bernier, G., Panitz, F., Zhou, X.L., Hollemann, T., Gruss, P., Pieler, T., 2000. Expanded retina territory by midbrain transformation upon overexpression of Six6 (Optx2) in *Xenopus* embryos. *Mech. Dev.* 93, 59–69.
- Bertrand, N., Castro, D.S., Guillemot, F., 2002. Proneural genes and the specification of neural cell types. *Nat. Rev., Neurosci.* 3, 517–530.
- Bessarab, D.A., Chong, S.W., Korzh, V., 2004. Expression of zebrafish six1 during sensory organ development and myogenesis. *Dev. Dyn.* 230, 781–786.
- Bhattacharyya, S., Bronner-Fraser, M., 2004. Hierarchy of regulatory events in sensory placode development. *Curr. Opin. Genet. Dev.* 14, 520–526.
- Bhattacharyya, S., Bailey, A.P., Bronner-Fraser, M., Streit, A., 2004. Segregation of lens and olfactory precursors from a common territory: cell sorting and reciprocity of Dlx5 and Pax6 expression. *Dev. Biol.* 271, 403–414.
- Blaxter, J.H.S., 1987. Structure and development of the lateral line. *Biol. Rev.* 62, 471–514.
- Bless, E.P., Walker, H.J., Yu, K.W., Knoll, J.G., Moenter, S.M., Schwarting, G.A., Tobet, S.A., 2005. Live view of gonadotropin-releasing hormone containing neuron migration. *Endocrinology* 146, 463–468.
- Blixt, A., Mahlapuu, M., Aitola, M., Peltö-Huikko, M., Enerback, S., Carlsson, P., 2000. A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. *Genes Dev.* 14, 245–254.
- Bollag, R.J., Siegfried, Z., Cebra-Thomas, J.A., Garvey, N., Davison, E.M., Silver, L.M., 1994. An ancient family of embryonically expressed mouse genes sharing a conserved protein motif with the T locus. *Nat. Genet.* 7, 383–389.
- Boncinelli, E., Morgan, R., 2001. Downstream of Otx2, or how to get a head. *Trends Genet.* 17, 633–636.
- Bonini, N.M., Leiserson, W.M., Benzer, S., 1993. The eyes absent gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. *Cell* 72, 379–395.
- Bonini, N.M., Bui, Q.T., Gray-Board, G.L., Warrick, J.M., 1997. The *Drosophila* eyes absent gene directs ectopic eye formation in a pathway conserved between flies and vertebrates. *Development* 124, 4819–4826.
- Bonini, N.M., Leiserson, W.M., Benzer, S., 1998. Multiple roles of the eyes absent gene in *Drosophila*. *Dev. Biol.* 196, 42–57.
- Boorman, C.J., Shimeld, S.M., 2002. Cloning and expression of a Pitx homeobox gene from the lamprey, a jawless vertebrate. *Dev. Genes Evol.* 212, 349–353.
- Borsani, G., DeGrandi, A., Ballabio, A., Bulfone, A., Bernard, L., Banfi, S., Gattuso, C., Mariani, M., Dixon, M., Donnai, D., Metcalfe, K., Winter, R., Robertson, M., Axton, R., Brown, A., van Heyningen, V., Hanson, I., 1999. EYA4, a novel vertebrate gene related to *Drosophila* eyes absent. *Hum. Mol. Genet.* 8, 11–23.
- Bosse, A., Zulch, A., Becker, M.B., Torres, M., Gomez-Skarmeta, J.L., Modolell, J., Gruss, P., 1997. Identification of the vertebrate Iroquois homeobox gene family with overlapping expression during early development of the nervous system. *Mech. Dev.* 69, 169–181.
- Bosse, A., Stoykova, A., Nieselt-Struwe, K., Chowdhury, K., Copeland, N.G., Jenkins, N.A., Gruss, P., 2000. Identification of a novel mouse Iroquois homeobox gene, Irx5, and chromosomal localisation of all members of the mouse Iroquois gene family. *Dev. Dyn.* 218, 160–174.
- Bourguignon, C., Li, J., Papalopulu, N., 1998. XBF-1, a winged helix transcription factor with dual activity, has a role in positioning neurogenesis in *Xenopus* competent ectoderm. *Development* 125, 4889–4900.
- Bouwmeester, T., Kim, S.-H., Sasai, Y., Lu, B., Derobertis, E.M., 1996. Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* 382, 595–601.
- Boyl, P.P., Signore, M., Annino, A., Barbera, J.P.M., Acampora, D., Simeone, A., 2001. Otx genes in the development and evolution of the vertebrate brain. *Int. J. Dev. Neurosci.* 19, 353–363.
- Bradley, L., Sun, B., Collins-Racie, L., LaVallie, E., McCoy, J., Sive, H., 2000. Different activities of the frizzled-related proteins frzb2 and sizzled2 during *Xenopus* anteroposterior patterning. *Dev. Biol.* 227, 118–132.
- Briata, P., Di Blas, E., Gulisano, M., Mallamaci, A., Iannone, R., Boncinelli, E., Corte, G., 1996. EMX1 homeoprotein is expressed in cell nuclei of the developing cerebral cortex and in the axons of the olfactory sensory neurons. *Mech. Dev.* 57, 169–180.
- Briata, P., Ilengo, C., Corte, G., Moroni, C., Rosenfeld, M.G., Chen, C.Y., Gherzi, R., 2003. The Wnt/beta-catenin → Pitx2 pathway controls the turnover of Pitx2 and other unstable mRNAs. *Mol. Cell* 12, 1201–1211.
- Broccoli, V., Boncinelli, E., Wurst, W., 1999. The caudal limit of Otx2 expression positions the isthmus organizer. *Nature* 401, 164–168.
- Brown, J.W., Beck-Jefferson, E., Hilfer, S.R., 1998. A role for neural cell adhesion molecule in the formation of the avian inner ear. *Dev. Dyn.* 213, 359–369.
- Brownell, I., Dirksen, M., Jamrich, M., 2000. Forkhead Foxe3 maps to the dysgenetic lens locus and is critical in lens development and differentiation. *Genesis* 27, 81–93.
- Brugmann, S.A., 2005. The induction and function of *Xenopus* Six1 in cranial placode development. PhD thesis. The George Washington University Columbian College of Arts and Sciences.
- Brugmann, S.A., Moody, S.A., 2005. Induction and specification of the vertebrate ectodermal placodes: precursors of the cranial sensory organs. *Biol. Cell* 97, 303–319.
- Brugmann, S.A., Pandur, P.D., Kenyon, K.L., Pignoni, F., Moody, S.A., 2004. Six1 promotes a placodal fate within the lateral neurogenic ectoderm by functioning as both a transcriptional activator and repressor. *Development* 131, 5871–5881.
- Brunet, J.F., Ghysen, A., 1999. Deconstructing cell determination: proneural genes and neuronal identity. *Bioessays* 21, 313–318.
- Brunet, J.F., Pattyn, A., 2002. Phox2 genes—From patterning to connectivity. *Curr. Opin. Genet. Dev.* 12, 435–440.
- Brunjes, P.C., Frazier, L.L., 1986. Maturation and plasticity in the olfactory system of vertebrates. *Brain Res.* 396, 1–45.
- Bryant, J., Goodyear, R.J., Richardson, G.P., 2002. Sensory organ development in the inner ear: molecular and cellular mechanisms. *Br. Med. Bull.* 63, 39–57.
- Buck, L.B., 2004. Olfactory receptors and odor coding in mammals. *Nutr. Rev.* 62, S184–S188.
- Bui, Q.T., Zimmerman, J.E., Liu, H., Gray-Board, G.L., Bonini, N.M., 2000. Functional analysis of an eye enhancer of the *Drosophila* eyes absent gene: differential regulation by eye specification genes. *Dev. Biol.* 221, 355–364.
- Burns, C.J., Vetter, M.L., 2002. Xath5 regulates neurogenesis in the *Xenopus* olfactory placode. *Dev. Dyn.* 225, 536–543.
- Burton, Q., Cole, L.K., Mulheisen, M., Chang, W., Wu, D.K., 2004. The role of Pax2 in mouse inner ear development. *Dev. Biol.* 272, 161–175.
- Bylund, M., Andersson, E., Novitsch, B.G., Muhr, J., 2003. Vertebrate neurogenesis is counteracted by Sox1–3 activity. *Nat. Neurosci.* 6, 1162–1168.
- Calof, A.L., Hagiwara, N., Holcomb, J.D., Mumm, J.S., Shou, J., 1996. Neurogenesis and cell death in olfactory epithelium. *J. Neurobiol.* 30, 67–81.

- Calof, A.L., Mumm, J.S., Rim, P.C., Shou, J., 1998. The neuronal stem cell of the olfactory epithelium. *J. Neurobiol.* 36, 190–205.
- Carl, M., Loosli, F., Wittbrodt, J., 2002. Six3 inactivation reveals its essential role for the formation and patterning of the vertebrate eye. *Development* 129, 4057–4063.
- Carlsson, P., Mahlapuu, M., 2002. Forkhead transcription factors: key players in development and metabolism. *Dev. Biol.* 250, 1–23.
- Carpenter, E., 1937. The head pattern in *Amblystoma* studied by vital staining and transplantation methods. *J. Exp. Zool.* 75, 103–129.
- Cau, E., Gradwohl, G., Fode, C., Guillemot, F., 1997. Mash1 activates a cascade of bhlh regulators in olfactory neuron progenitors. *Development* 124, 1611–1621.
- Cau, E., Gradwohl, G., Casarosa, S., Kageyama, R., Guillemot, F., 2000. Hes genes regulate sequential stages of neurogenesis in the olfactory epithelium. *Development* 127, 2323–2332.
- Cau, E., Casarosa, S., Guillemot, F., 2002. Mash1 and Ngn1 control distinct steps of determination and differentiation in the olfactory sensory neuron lineage. *Development* 129, 1871–1880.
- Cavodeassi, F., Modolell, J., Gomez-Skarmeta, J.L., 2001. The Iroquois family of genes: from body building to neural patterning. *Development* 128, 2847–2855.
- Cecchi, C., 2002. Emx2: a gene responsible for cortical development, regionalization and area specification. *Gene* 291, 1–9.
- Cecchi, C., Boncinelli, E., 2000. Emx homeogenes and mouse brain development. *Trends Neurosci.* 23, 347–352.
- Chalepakakis, G., Wijnholds, J., Giese, P., Schachner, M., Gruss, P., 1994. Characterization of Pax-6 and Hoxa-1 binding to the promoter region of the neural cell adhesion molecule L1. *DNA Cell Biol.* 13, 891–900.
- Chang, C.B., Hemmati-Brivanlou, A., 1998a. Cell fate determination in embryonic ectoderm. *J. Neurobiol.* 36, 128–151.
- Chang, C., Hemmati-Brivanlou, A., 1998b. Neural crest induction by Xwnt7B in *Xenopus*. *Dev. Biol.* 194, 129–134.
- Chang, W.Y., Khosrow-Shahian, F., Chang, R., Crawford, M.J., 2001. xPitx1 plays a role in specifying cement gland and head during early *Xenopus* development. *Genesis* 29, 78–90.
- Chang, E.H., Menezes, M., Meyer, N.C., Cucci, R.A., Vervoort, V.S., Schwartz, C.E., Smith, R.J., 2004. Branchio-oto-renal syndrome: the mutation spectrum in EYA1 and its phenotypic consequences. *Hum. Mutat.* 23, 582–589.
- Chapman, D.L., Garvey, N., Hancock, S., Alexiou, M., Agulnik, S.I., Gibson-Brown, J.J., Cebra-Thomas, J., Bollag, R.J., Silver, L.M., Papaioannou, V.E., 1996. Expression of the T-box family genes, Tbx1–Tbx5, during early mouse development. *Dev. Dyn.* 206, 379–390.
- Chapman, S.C., Schubert, F.R., Schoenwolf, G.C., Lumsden, A., 2002. Analysis of spatial and temporal gene expression patterns in blastula and gastrula stage chick embryos. *Dev. Biol.* 245, 187–199.
- Chapman, S.C., Sawitzke, A.L., Campbell, D.S., Schoenwolf, G.C., 2005. A three-dimensional atlas of pituitary gland development in the zebrafish. *J. Comp. Neurol.* 487, 428–440.
- Chen, R., Amoui, M., Zhang, Z., Mardon, G., 1997. Dachshund and eyes absent proteins form a complex and function synergistically to induce ectopic eye development in *Drosophila*. *Cell* 91, 893–903.
- Chen, P., Johnson, J.E., Zoghbi, H.Y., Segil, N., 2002. The role of Math1 in inner ear development: uncoupling the establishment of the sensory primordium from hair cell fate determination. *Development* 129, 2495–2505.
- Cheng, C.W., Hui, C., Strahle, U., Cheng, S.H., 2001. Identification and expression of zebrafish Iroquois homeobox gene irx1. *Dev. Genes Evol.* 211, 442–444.
- Cheyette, B.N., Green, P.J., Martin, K., Garren, H., Hartenstein, V., Zipursky, S.L., 1994. The *Drosophila* sine oculis locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* 12, 977–996.
- Chi, N., Epstein, J.A., 2002. Getting your Pax straight: Pax proteins in development and disease. *Trends Genet.* 18, 41–47.
- Chitnis, A., Henrique, D., Lewis, J., Ish-Horowicz, D., Kintner, C., 1995. Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene Delta. *Nature* 375, 761–766.
- Cho, J.H., Tsai, M.J., 2004. The role of BETA2/NeuroD1 in the development of the nervous system. *Mol. Neurobiol.* 30, 35–47.
- Choi, M.H., 1931. Determination of the ear and side-specificity of the ear region in amphibian embryos. *Folia Anat. Jpn.* 9, 315–332.
- Chow, R.L., Lang, R.A., 2001. Early eye development in vertebrates. *Annu. Rev. Cell Dev. Biol.* 17, 255–296.
- Chow, R.L., Altmann, C.R., Lang, R.A., Hemmati-Brivanlou, A., 1999. Pax6 induces ectopic eyes in a vertebrate. *Development* 126, 4213–4222.
- Christen, B., Slack, J.M.W., 1997. Fgf-8 is associated with anteroposterior patterning and limb regeneration in *Xenopus*. *Dev. Biol.* 192, 455–466.
- Christian, J.L., Moon, R.T., 1993. Interactions between Xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Gene Dev.* 7, 13–28.
- Chuah, M.I., Au, C., 1991. Olfactory Schwann cells are derived from precursor cells in the olfactory epithelium. *J. Neurosci. Res.* 29, 172–180.
- Clark, S.W., Fee, B.E., Cleveland, J.L., 2002. Misexpression of the eyes absent family triggers the apoptotic program. *J. Biol. Chem.* 277, 3560–3567.
- Coffin, A., Kelley, M., Manley, G.A., Popper, A.N., 2005. Evolution of sensory hair cells. In: Manley, G.A., Popper, A.N., Fay, R.R. (Eds.), *Evolution of the Vertebrate Auditory System*. Springer, New York.
- Cohen, D.R., Cheng, C.W., Cheng, S.H., Hui, C.C., 2000. Expression of two novel mouse Iroquois homeobox genes during neurogenesis. *Mech. Dev.* 91, 317–321.
- Coletta, R.D., Christensen, K., Reichenberger, K.J., Lamb, J., Micomnaco, D., Huang, L., Wolf, D.M., Muller-Tidow, C., Golub, T.R., Kawakami, K., Ford, H.L., 2004. The Six1 homeoprotein stimulates tumorigenesis by reactivation of cyclin A1. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6478–6483.
- Collignon, J., Sockanathan, S., Harker, A., Cohen-Tannoudji, M., Norris, D., Rastan, S., Stevanovic, M., Goodfellow, P.N., Lovell-Badge, R., 1996. A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. *Development* 122, 509–520.
- Collinson, J.M., Hill, R.E., West, J.D., 2000. Different roles for Pax6 in the optic vesicle and facial epithelium mediate early morphogenesis of the murine eye. *Development* 127, 945–956.
- Collinson, J.M., Quinn, J.C., Buchanan, M.A., Kaufman, M.H., Wedden, S.E., West, J.D., Hill, R.E., 2001. Primary defects in the lens underlie complex anterior segment abnormalities of the Pax6 heterozygous eye. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9688–9693.
- Collinson, J.M., Quinn, J.C., Hill, R.E., West, J.D., 2003. The roles of Pax6 in the cornea, retina, and olfactory epithelium of the developing mouse embryo. *Dev. Biol.* 255, 303–312.
- Cornesse, Y., Pieler, T., Hollemann, T., 2005. Olfactory and lens placode formation is controlled by the hedgehog-interacting protein (Xhip) in *Xenopus*. *Dev. Biol.* 277, 296–315.
- Couly, G.F., Le Douarin, N.M., 1985. Mapping the early neural primordium in quail–chick chimeras: I. Developmental relationships between placodes, facial ectoderm, and prosencephalon. *Dev. Biol.* 110, 422–439.
- Couly, G.F., Le Douarin, N.M., 1987. Mapping of the early neural primordium in quail–chick chimeras: II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. *Dev. Biol.* 120, 198–214.
- Couly, G., Le Douarin, N.M., 1990. Head morphogenesis in embryonic avian chimeras: evidence for a segmental pattern in the ectoderm corresponding to the neuromeres. *Development* 108, 543–558.
- Crews, L., Hunter, D., 1994. Neurogenesis in the olfactory epithelium. *Perspect. Dev. Neurobiol.* 2, 151–161.
- Crossley, P.H., Martin, G.R., 1995. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 121, 439–451.
- Cvekl, A., Piatigorsky, J., 1996. Lens development and crystallin gene expression: many roles for Pax-6. *Bioessays* 18, 621–630.
- Cvekl, A., Yang, Y., Chauhan, B.K., Cveklava, K., 2004. Regulation of gene expression by Pax6 in ocular cells: a case of tissue-preferred expression of crystallins in lens. *Int. J. Dev. Biol.* 48, 829–844.
- Dahl, E., Koseki, H., Balling, R., 1997. Pax genes and organogenesis. *Bioessays* 19, 755–765.
- Dahmann, C., Basler, K., 1999. Compartment boundaries: at the edge of development. *Trends Genet.* 15, 320–326.

- Daikoku, S., 1999. The olfactory origin of luteinizing hormone-releasing hormone (LHRH) neurons. A new era in reproduction physiology. *Arch. Histol. Cytol.* 62, 107–117.
- Damas, H., 1944. Recherches sur le développement de *Lampetra fluviatilis* L. Contribution à l'étude de la céphalogenèse des vertébrés. *Arch. Biol.* 55, 1–284.
- D'Amico-Martel, A., Noden, D.M., 1983. Contributions of placodal and neural crest cells to avian cranial peripheral ganglia. *Am. J. Anat.* 166, 445–468.
- Dasen, J.S., Rosenfeld, M.G., 2001. Signaling and transcriptional mechanisms in pituitary development. *Annu. Rev. Neurosci.* 24, 327–355.
- Dasen, J.S., O'Connell, S.M., Flynn, S.E., Treier, M., Gleiberman, A.S., Szeto, D.P., Hooshmand, F., Aggarwal, A.K., Rosenfeld, M.G., 1999. Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. *Cell* 97, 587–598.
- Dasen, J.S., Barbera, J.P., Herman, T.S., Connell, S.O., Olson, L., Ju, B., Tollkuhn, J., Baek, S.H., Rose, D.W., Rosenfeld, M.G., 2001. Temporal regulation of a paired-like homeodomain repressor/TLE corepressor complex and a related activator is required for pituitary organogenesis. *Genes Dev.* 15, 3193–3207.
- Dattani, M.T., Martinez-Barbera, J.P., Thomas, P.Q., Brickman, J.M., Gupta, R., Martensson, I.L., Toresson, H., Fox, M., Wales, J.K., Hindmarsh, P.C., Krauss, S., Beddington, R.S., Robinson, I.C., 1998. Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat. Genet.* 19, 125–133.
- David, R., Wedlich, D., 2000. *Xenopus* cadherin-6 is expressed in the central and peripheral nervous system and in neurogenic placodes. *Mech. Dev.* 97, 187–190.
- David, R., Ahrens, K., Wedlich, D., Schlosser, G., 2001. *Xenopus Eya1* demarcates all neurogenic placodes as well as migrating hypaxial muscle precursors. *Mech. Dev.* 103, 189–192.
- Davis, R.L., Turner, D.L., Evans, L.M., Kirschner, M.W., 2001. Molecular targets of vertebrate segmentation: two mechanisms control segmental expression of *Xenopus hairy2* during somite formation. *Dev. Cell* 1, 553–565.
- Davis, J., Duncan, M.K., Robison Jr., W.G., Piatigorsky, J., 2003. Requirement for Pax6 in corneal morphogenesis: a role in adhesion. *J. Cell Sci.* 116, 2157–2167.
- de la Calle-Mustienes, E., Glavic, A., Modolell, J., Gomez-Skarmeta, J.L., 2002. Xiro homeoproteins coordinate cell cycle exit and primary neuron formation by upregulating neuronal-fate repressors and downregulating the cell-cycle inhibitor XGadd45-gamma. *Mech. Dev.* 119, 69.
- De Robertis, E.M., Kuroda, H., 2004. Dorsal–ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell Dev. Biol.* 20, 285–308.
- Deblandre, G.A., Wettstein, D.A., KoyanoNakagawa, N., Kintner, C., 1999. A two-step mechanism generates the spacing pattern of the ciliated cells in the skin of *Xenopus* embryos. *Development* 126, 4715–4728.
- Del Bene, F., Tessmar-Raible, K., Wittbrodt, J., 2004. Direct interaction of *geminin* and *Six3* in eye development. *Nature* 427, 745–749.
- Dellovade, T.L., Pfaff, D.W., Schwanzel-Fukuda, M., 1998. The gonadotropin-releasing hormone system does not develop in Small-Eye (Sey) mouse phenotype. *Brain Res. Dev. Brain Res.* 107, 233–240.
- Demski, L.S., 1993. Terminal nerve complex. *Acta Anat. (Basel)* 148, 81–95.
- Depew, M.J., Liu, J.K., Long, J.E., Presley, R., Meneses, J.J., Pedersen, R.A., Rubenstein, J.L., 1999. *Dlx5* regulates regional development of the branchial arches and sensory capsules. *Development* 126, 3831–3846.
- Detwiler, S.R., van Dyke, R.H., 1951. Recent experiments on the differentiation of the labyrinth in *Amblystoma*. *J. Exp. Zool.* 118, 389–405.
- Dheen, T., Sleptsova-Friedrich, I., Xu, Y., Clark, M., Lehrach, H., Gong, Z., Korzh, V., 1999. Zebrafish *tbx-c* functions during formation of midline structures. *Development* 126, 2703–2713.
- Dickinson, M.E., Selleck, M.A.J., McMahon, A.P., Bronner-Fraser, M., 1995. Dorsalization of the neural tube by the non-neural ectoderm. *Development* 121, 2099–2106.
- Dimanlig, P.V., Faber, S.C., Auerbach, W., Makarenkova, H.P., Lang, R.A., 2001. The upstream ectoderm enhancer in Pax6 has an important role in lens induction. *Development* 128, 4415–4424.
- Dirksen, M.L., Jamrich, M., 1995. Differential expression of fork head genes during early *Xenopus* and zebrafish development. *Dev. Genet.* 17, 107–116.
- Dirksen, M.L., Morasso, M.I., Sargent, T.D., Jamrich, M., 1994. Differential expression of a Distal-less homeobox gene *Xdll-2* in ectodermal cell lineages. *Mech. Dev.* 46, 63–70.
- Dou, C.L., Li, S., Lai, E., 1999. Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cereb. Cortex* 9, 543–550.
- Dressler, G.R., Woolf, A.S., 1999. Pax2 in development and renal disease. *Int. J. Dev. Biol.* 43, 463–468.
- Drysdale, T.A., Elinson, R.P., 1992. Cell migration and induction in the development of the surface ectodermal pattern of the *Xenopus laevis* tadpole. *Dev. Growth Differ.* 34, 51–59.
- Dubois, L., Vincent, A., 2001. The COE–Collier/Olf1/EBF–transcription factors: structural conservation and diversity of developmental functions. *Mech. Dev.* 108, 3–12.
- Dubois, P.M., el Amraoui, A., Heritier, A.G., 1997. Development and differentiation of pituitary cells. *Microsc. Res. Tech.* 39, 98–113.
- Dubois, L., Bally-Cuif, L., Crozatier, M., Moreau, J., Paquereau, L., Vincent, A., 1998. *Xcoe2*, a transcription factor of the col/olf-1/ebf family involved in the specification of primary neurons in *Xenopus*. *Curr. Biol.* 8, 199–209.
- Duncan, M.K., Kos, L., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., Tomarev, S.I., 1997. Eyes absent: a gene family found in several metazoan phyla. *Mamm. Genome* 8, 479–485.
- Duprez, D., Leyns, L., Bonnin, M.A., Lapointe, F., Etchevers, H., De Robertis, E.M., Le Douarin, N., 1999. Expression of *Frzb-1* during chick development. *Mech. Dev.* 89, 179–183.
- Dutta, S., Dietrich, J.E., Aspöck, G., Burdine, R.D., Schier, A., Westerfield, M., Varga, Z.M., 2005. *pitx3* defines an equivalence domain for lens and anterior pituitary placode. *Development* 132, 1579–1590.
- Eagleson, G.W., Harris, W.A., 1990. Mapping of the presumptive brain regions in the neural plate of *Xenopus laevis*. *J. Neurobiol.* 21, 427–440.
- Eagleson, G.W., Dempewolf, R.D., 2002. The role of the anterior neural ridge and *Fgf-8* in early forebrain patterning and regionalization in *Xenopus laevis*. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* 132, 179–189.
- Eagleson, G.W., Jenks, B.G., Van Overbeeke, A.P., 1986. The pituitary adrenocorticotropes originate from neural ridge tissue in *Xenopus laevis*. *J. Embryol. Exp. Morphol.* 95, 1–14.
- Eagleson, G., Ferreira, B., Harris, W.A., 1995. Fate of the anterior neural ridge and the morphogenesis of the *Xenopus* forebrain. *J. Neurobiol.* 28, 146–158.
- Eisthen, H.L., Delay, R.J., Wirsig-Wiechmann, C.R., Dionne, V.E., 2000. Neuromodulatory effects of gonadotropin releasing hormone on olfactory receptor neurons. *J. Neurosci.* 20, 3947–3955.
- Ekker, M., Akimenko, M.A., Bremiller, R., Westerfield, M., 1992. Regional expression of 3 homeobox transcripts in the inner ear of zebrafish embryos. *Neuron* 9, 27–35.
- El Amraoui, A., Dubois, P.M., 1993a. Experimental evidence for an early commitment of gonadotropin-releasing hormone neurons, with special regard to their origin from the ectoderm of nasal cavity presumptive territory. *Neuroendocrinology* 57, 991–1002.
- El Amraoui, A., Dubois, P.M., 1993b. Experimental evidence for the early commitment of the presumptive adenohypophysis. *Neuroendocrinology* 58, 609–615.
- Eng, S.R., Lanier, J., Fedtsova, N., Turner, E.E., 2004. Coordinated regulation of gene expression by *Brn3a* in developing sensory ganglia. *Development* 131, 3859–3870.
- Epstein, J.A., 2000. Pax3 and vertebrate development. *Methods Mol. Biol.* 137, 459–470.
- Epstein, J.A., Neel, B.G., 2003. Signal transduction: an eye on organ development. *Nature* 426, 238–239.
- Epstein, D.J., Vekemans, M., Gros, P., 1991. *Splotch* (Sp2H), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell* 67, 767–774.
- Ericson, J., Norlin, S., Jessell, T.M., Edlund, T., 1998. Integrated FGF and BMP signaling controls the progression of progenitor cell differentiation and the emergence of pattern in the embryonic anterior pituitary. *Development* 125, 1005–1015.
- Erkman, L., McEvilly, R.J., Luo, L., Ryan, A.K., Hooshmand, F., O'Connell, S.M., Keithley, E.M., Rapaport, D.H., Ryan, A.F., Rosenfeld, M.G., 1996.

- Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. *Nature* 381, 603–606.
- Ermakova, G.V., Alexandrova, E.M., Kazanskaya, O.V., Vasiliev, O.L., Smith, M.W., Zaraisky, A.G., 1999. The homeobox gene, *Xanf-1*, can control both neural differentiation and patterning in the presumptive anterior neuroectoderm of the *Xenopus laevis* embryo. *Development* 126, 4513–4523.
- Essner, J.J., Branford, W.W., Zhang, J., Yost, H.J., 2000. Mesendoderm and left–right brain, heart and gut development are differentially regulated by *pitx2* isoforms. *Development* 127, 1081–1093.
- Esteve, P., Bovolenta, P., 1999. *cSix4*, a member of the six gene family of transcription factors, is expressed during placode and somite development. *Mech. Dev.* 85, 161–165.
- Faber, S.C., Dimanlig, P., Makarenkova, H.P., Shirke, S., Ko, K., Lang, R.A., 2001. FGF receptor signaling plays a role in lens induction. *Development* 128, 4425–4438.
- Fabrizio, J.J., Boyle, M., Dinardo, S., 2003. A somatic role for eyes absent (*eya*) and sine oculis (*so*) in *Drosophila* spermatocyte development. *Dev. Biol.* 258, 117–128.
- Fainsod, A., Steinbeisser, H., De Robertis, E.M., 1994. On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. *EMBO J.* 13, 5015–5025.
- Farbman, A.I., 1994. Developmental biology of olfactory sensory neurons. *Semin. Cell Biol.* 5, 3–10.
- Faure, S., de Santa, B.P., Roberts, D.J., Whitman, M., 2002. Endogenous patterns of BMP signaling during early chick development. *Dev. Biol.* 244, 44–65.
- Fautrez, J., 1942. La signification de la partie céphalique du bourrelet de la plaque médullaire chez les urodèles. Localisation des ébauches présomptives des microplacodes des nerfs crâniens et de la tête au stade neurale. *Bull. Cl. Sci., Acad. R. Belg.* 28, 391–403.
- Favor, J., Sandulache, R., Neuhauser-Klaus, A., Pretsch, W., Chatterjee, B., Senft, E., Wurst, W., Blanquet, V., Grimes, P., Spörle, R., Schughart, K., 1996. The mouse *Pax2*^{Neu} mutation is identical to a human *Pax2* mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye and kidney. *Proc. Natl. Acad. Sci. U. S. A.* 93, 13870–13875.
- Fekete, D.M., Wu, D.K., 2002. Revisiting cell fate specification in the inner ear. *Curr. Opin. Neurobiol.* 12, 35–42.
- Feledy, J.A., Beanan, M.J., Sandoval, J.J., Goodrich, J.S., Lim, J.H., Matsuo-Takasaki, M., Sato, S.M., Sargent, T.D., 1999. Inhibitory patterning of the anterior neural plate in *Xenopus* by homeodomain factors *Dlx3* and *Msx1*. *Dev. Biol.* 212, 455–464.
- Ferri, A.L., Cavallaro, M., Braida, D., Di Cristofano, A., Canta, A., Vezzani, A., Ottolenghi, S., Pandolfi, P.P., Sala, M., DeBiasi, S., Nicolis, S.K., 2004. *Sox2* deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development* 131, 3805–3819.
- Fisher, M., Grainger, R.M., 2004. Lens induction and determination. In: Lovicu, F.J., Robinson, M.L. (Eds.), *Development of the Ocular Lens*. Cambridge Univ. Press, New York.
- Flock, A., 1967. Ultrastructure and function in the lateral line organs. In: Cahn, P. (Ed.), *Lateral Line Detectors*. Indiana Univ. Press, Bloomington, pp. 163–197.
- Fode, C., Gradwohl, G., Morin, X., Dierich, A., Lemeur, M., Goridis, C., Guillemot, F., 1998. The bHLH protein neurogenin 2 is a determination factor for epibranchial placode-derived sensory neurons. *Neuron* 20, 483–494.
- Ford, H.L., Kabingu, E.N., Bump, E.A., Mutter, G.L., Pardee, A.B., 1998. Abrogation of the G2 cell cycle checkpoint associated with overexpression of *HSIX1*: a possible mechanism of breast carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 95, 12608–12613.
- Friedle, H., Knöchel, W., 2002. Cooperative interaction of *Xvent-2* and *GATA-2* in the activation of the ventral homeobox gene *Xvent-1B*. *J. Biol.* 8.
- Friedman, R.A., Makmura, L., Biesiada, E., Wang, X., Keithley, E.M., 2005. *Eya1* acts upstream of *Tbx1*, *Neurogenin 1*, *NeuroD* and the neurotrophins *BDNF* and *NT-3* during inner ear development. *Mech. Dev.* 122, 625–634.
- Fritzsche, B., 1989. Diversity and regression in the amphibian lateral line and electrosensory system. In: Coombs, S., Görner, P., Münz, H. (Eds.), *The Mechanosensory Lateral Line*. Springer, New York, pp. 99–114.
- Fritzsche, B., Neary, T., 1998. The octavolateralis system of mechanosensory and electrosensory organs. In: Heatwole, H., Dawley, E.M. (Eds.), *Amphibian Biology*, vol. 3. Surrey Beatty and Sons, Australia, pp. 878–922.
- Fritzsche, B., Barald, K.F., Lomax, M.I., 1998. Early embryology of the vertebrate ear. In: Rubel, E.W., Popper, A.N., Fay, R.R. (Eds.), *Development of the Auditory System*. Springer, New York, pp. 80–145.
- Fritzsche, B., Signore, M., Simeone, A., 2001. *Otx1* null mutant mice show partial segregation of sensory epithelia comparable to lamprey ears. *Dev. Genes Evol.* 211, 388–396.
- Fritzsche, B., Beisel, K.W., Jones, K., Farinas, I., Maklad, A., Lee, J., Reichardt, L.F., 2002. Development and evolution of inner ear sensory epithelia and their innervation. *J. Neurobiol.* 53, 143–156.
- Frolenkov, G.I., Belyantseva, I.A., Friedman, T.B., Griffith, A.J., 2004. Genetic insights into the morphogenesis of inner ear hair cells. *Nat. Rev., Genet.* 5, 489–498.
- Froriep, A., 1885. Über Anlagen von Sinnesorganen am Facialis, Glossopharyngeus und Vagus, über die genetische Stellung des Vagus zum Hypoglossus, und über die Herkunft der Zungenmuskulatur. *Arch. Anat. Physiol.* 1885, 1–55.
- Furuta, Y., Hogan, B.L., 1998. BMP4 is essential for lens induction in the mouse embryo. *Genes Dev.* 12, 3764–3775.
- Gage, P.J., Camper, S.A., 1997. Pituitary homeobox 2, a novel member of the bicoid-related family of homeobox genes, is a potential regulator of anterior structure formation. *Hum. Mol. Genet.* 6, 457–464.
- Gage, P.J., Suh, H., Camper, S.A., 1999. Dosage requirement of *Pitx2* for development of multiple organs. *Development* 126, 4643–4651.
- Gallagher, B.C., Henry, J.J., Grainger, R.M., 1996. Inductive processes leading to inner ear formation during *Xenopus* development. *Dev. Biol.* 175, 95–107.
- Gammill, L.S., Bronner-Fraser, M., 2003. Neural crest specification: migrating into genomics. *Nat. Rev., Neurosci.* 4, 795–805.
- Gammill, L.S., Sive, H., 2001. *otx2* expression in the ectoderm activates anterior neural determination and is required for *Xenopus* cement gland formation. *Dev. Biol.* 240, 223–236.
- Gans, C., Northcutt, R.G., 1983. Neural crest and the origin of vertebrates: a new head. *Science* 220, 268–274.
- Gao, W.Q., 2003. Hair cell development in higher vertebrates. *Curr. Top. Dev. Biol.* 57, 293–319.
- Garcia-Castro, M.I., Marcelle, C., Bronner-Fraser, M., 2002. Ectodermal Wnt function as a neural crest inducer. *Science* 297, 848–851.
- Garel, S., Marin, F., Mattei, M.G., Vesque, C., Vincent, A., Charnay, P., 1997. Family of *Ebf/Olf-1*-related genes potentially involved in neuronal differentiation and regional specification in the central nervous system. *Dev. Dyn.* 210, 191–205.
- Gehring, W.J., Ikeo, K., 1999. *Pax 6*-mastering eye morphogenesis and eye evolution. *Trends Genet.* 15, 371–377.
- George, K.M., Leonard, M.W., Roth, M.E., Lieu, K.H., Kioussis, D., Grosveld, F., Engel, J.D., 1994. Embryonic expression and cloning of the murine *GATA-3* gene. *Development* 120, 2673–2686.
- Gestri, G., Carl, M., Appolloni, I., Wilson, S.W., Barsacchi, G., Andreazzoli, M., 2005. *Six3* functions in anterior neural plate specification by promoting cell proliferation and inhibiting *Bmp4* expression. *Development* 132, 2401–2413.
- Ghanbari, H., Seo, H.C., Fjose, A., Brändli, A.W., 2001. Molecular cloning and embryonic expression of *Xenopus Six* homeobox genes. *Mech. Dev.* 101, 271–277.
- Ghysen, A., Dambly-Chaudière, C., 2004. Development of the zebrafish lateral line. *Curr. Opin. Neurobiol.* 14, 67–73.
- Gibbs, M.A., 2004. Lateral line receptors: where do they come from developmentally and where is our research going? *Brain Behav. Evol.* 64, 163–181.
- Gibson-Brown, J.J., Agulnik, I., Silver, L.M., Papaioannou, V.E., 1998. Expression of T-box genes *Tbx2–Tbx5* during chick organogenesis. *Mech. Dev.* 74, 165–169.
- Ginsburg, A.S., 1946. Specific differences in the determination of the internal ear and other ectodermal organs in certain urodela. *C. R. Acad. Sci. URSS* 54, 557–560.
- Ginsburg, A.S., 1995. Determination of the labyrinth in different amphibian species and its correlation with determination of the other ectoderm derivatives. *Roux's Arch. Dev. Biol.* 204, 351–358.

- Giraldez, F., 1998. Regionalized organizing activity of the neural tube revealed by the regulation of *lhx1* in the otic vesicle. *Dev. Biol.* 203, 189–200.
- Givens, M.L., Rave-Harel, N., Goonewardena, V.D., Kurotani, R., Berdy, S.E., Swan, C.H., Rubenstein, J.L., Robert, B., Mellon, P.L., 2005. Developmental regulation of gonadotropin-releasing hormone gene expression by the *MSX* and *DLX* homeodomain protein families. *J. Biol. Chem.* 280, 19156–19165.
- Glavic, A., Maris, H.S., Gloria, F.C., Bastidas, F., Allende, M.L., Mayor, R., 2004. Role of BMP signaling and the homeoprotein *Iroquois* in the specification of the cranial placodal field. *Dev. Biol.* 272, 89–103.
- Gleiberman, A.S., Fedtsova, N.G., Rosenfeld, M.G., 1999. Tissue interactions in the induction of anterior pituitary: role of the ventral diencephalon, mesenchyme, and notochord. *Dev. Biol.* 213, 340–353.
- Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., Niehrs, C., 1998. *Dickkopf-1* is a member of a new family of secreted proteins and functions in head induction. *Nature* 391, 357–362.
- Golub, R., Adelman, Z., Clementi, J., Weiss, R., Bonasera, J., Servetnick, M., 2000. Evolutionarily conserved and divergent expression of members of the FGF receptor family among vertebrate embryos, as revealed by FGFR expression patterns in *Xenopus*. *Dev. Genes Evol.* 210, 345–357.
- Gómez-Skarmeta, J.L., Modolell, J., 2002. *Iroquois* genes: genomic organization and function in vertebrate neural development. *Curr. Opin. Genet. Dev.* 12, 403–408.
- Gómez-Skarmeta, J.L., Glavic, A., de la Calle-Mustienes, E., Modolell, J., Mayor, R., 1998. *Xiro*, a *Xenopus* homolog of the *Drosophila iroquois* complex genes, controls development at the neural plate. *EMBO J.* 17, 181–190.
- Gong, S.G., Kiba, A., 1999. The role of *Xmxs-2* in the anterior–posterior patterning of the mesoderm in *Xenopus laevis*. *Differentiation* 65, 131–140.
- Gordon, D.F., Lewis, S.R., Haugen, B.R., James, R.A., McDermott, M.T., Wood, W.M., Ridgway, E.C., 1997. *Pit-1* and *GATA-2* interact and functionally cooperate to activate the thyrotropin beta-subunit promoter. *J. Biol. Chem.* 272, 24339–24347.
- Goulding, M.D., Chalepakis, G., Deutsch, U., Erselius, J.R., Gruss, P., 1991. *Pax-3*, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* 10, 1135–1147.
- Graham, A., Begbie, J., 2000. Neurogenic placodes: a common front. *Trends Neurosci.* 23, 313–316.
- Graham, V., Khudyakov, J., Ellis, P., Pevny, L., 2003. *SOX2* functions to maintain neural progenitor identity. *Neuron* 39, 749–765.
- Grainger, R.M., 1996. New perspectives on embryonic lens induction. *Semin. Cell Dev. Biol.* 7, 149–155.
- Grainger, R.M., Mannion, J.E., Cook Jr., T.L., Zygar, C.A., 1997. Defining intermediate stages in cell determination: acquisition of a lens-forming bias in head ectoderm during lens determination. *Dev. Genet.* 20, 246–257.
- Grifone, R., Demignon, J., Houbon, C., Souil, E., Niro, C., Seller, M.J., Hamard, G., Maire, P., 2005. *Six1* and *Six4* homeoproteins are required for *Pax3* and *Mrf* expression during myogenesis in the mouse embryo. *Development* 132, 2235–2249.
- Grindley, J.C., Davidson, D.R., Hill, R.E., 1995. The role of *Pax-6* in eye and nasal development. *Development* 121, 1433–1442.
- Groves, A.K., Bronner-Fraser, M., 2000. Competence, specification and commitment in otic placode induction. *Development* 127, 3489–3499.
- Guillemot, F., 1999. Vertebrate *bHLH* genes and the determination of neuronal fates. *Exp. Cell Res.* 253, 357–364.
- Guo, S., Brush, J., Teraoka, H., Goddard, A., Wilson, S.W., Mullins, M.C., Rosenthal, A., 1999. Development of noradrenergic neurons in the zebrafish hindbrain requires BMP, FGF8, and the homeodomain protein *soulless/Phox2a*. *Neuron* 24, 555–566.
- Gutknecht, D., Fritsch, B., 1990. Lithium can transform ear placodes of *Xenopus* into multiple otic vesicles connected by tubes. *Naturwissenschaften* 77, 235–237.
- Haggis, A.J., 1956. Analysis of the determination of the olfactory placode in *Amblystoma punctatum*. *J. Embryol. Exp. Morphol.* 4, 120–138.
- Halder, G., Callaerts, P., Flister, S., Walldorf, U., Kloter, U., Gehring, W.J., 1998. *Eyeless* initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. *Development* 125, 2181–2191.
- Haldin, C.E., Nijjar, S., Masse, K., Barnett, M.W., Jones, E.A., 2003. Isolation and growth factor inducibility of the *Xenopus laevis* *Lmx1b* gene. *Int. J. Dev. Biol.* 47, 253–262.
- Hall, B.K., 1999. *The Neural Crest in Development and Evolution*. Springer, New York.
- Hamburger, V., 1961. Experimental analysis of the dual origin of the trigeminal ganglion in the chick embryo. *J. Exp. Zool.* 148, 91–123.
- Hanashima, C., Li, S.C., Shen, L., Lai, E., Fishell, G., 2004. *Foxg1* suppresses early cortical cell fate. *Science* 303, 56–59.
- Hans, S., Liu, D., Westerfield, M., 2004. *Pax8* and *Pax2a* function synergistically in otic specification, downstream of the *Foxl1* and *Dlx3b* transcription factors. *Development* 131, 5091–5102.
- Hansen, A., Zeiske, E., 1993. Development of the olfactory organ in the zebrafish, *Brachydanio rerio*. *J. Comp. Neurol.* 333, 289–300.
- Hanson, I.M., 2001. Mammalian homologues of the *Drosophila* eye specification genes. *Semin. Cell Dev. Biol.* 12, 475–484.
- Hardcastle, Z., Papalopulu, N., 2000. Distinct effects of *XBF-1* in regulating the cell cycle inhibitor *p27^{XIC1}* and imparting a neural fate. *Development* 127, 1303–1314.
- Harland, R., 2000. Neural induction. *Curr. Opin. Genet. Dev.* 10, 357–362.
- Harrison, R.G., 1945. Relations of symmetry in the developing embryo. *Trans. Conn. Acad. Arts Sci.* 36, 277–330.
- Hartley, K.O., Hardcastle, Z., Friday, R.V., Amaya, E., Papalopulu, N., 2001. Transgenic *Xenopus* embryos reveal that anterior neural development requires continued suppression of BMP signaling after gastrulation. *Dev. Biol.* 238, 168–184.
- Hassan, B.A., Bellen, H.J., 2000. Doing the MATH: is the mouse a good model for fly development? *Genes Dev.* 14, 1852–1865.
- Hatini, V., Ye, X., Balas, G., Lai, E., 1999. Dynamics of placodal lineage development revealed by targeted transgene expression. *Dev. Dyn.* 215, 332–343.
- Hayata, T., Kuroda, H., Eisaki, A., Asashima, M., 1999. Expression of *Xenopus* T-box transcription factor, *Tbx2* in *Xenopus* embryo. *Dev. Genes Evol.* 209, 625–628.
- Heanue, T.A., Reshef, R., Davis, R.J., Mardon, G., Oliver, G., Tomarev, S., Lassar, A.B., Tabin, C.J., 1999. Synergistic regulation of vertebrate muscle development by *Dach2*, *Eya2*, and *Six1*, homologs of genes required for *Drosophila* eye formation. *Gene Dev.* 13, 3231–3243.
- Heicklen-Klein, A., McReynolds, L.J., Evans, T., 2005. Using the zebrafish model to study GATA transcription factors. *Semin. Cell Dev. Biol.* 16, 95–106.
- Heller, N., Brändli, A., 1997. *Xenopus Pax-2* displays multiple splice forms during embryogenesis and pronephric kidney development. *Mech. Dev.* 69, 83–104.
- Heller, N., Brändli, A.W., 1999. *Xenopus Pax-2/5/8* orthologues: novel insights into *Pax* gene evolution and identification of *Pax-8* as the earliest marker for otic and pronephric cell lineages. *Dev. Genet.* 24, 208–219.
- Hemmati-Brivanlou, A., Thomsen, G.H., 1995. Ventral mesodermal patterning in *Xenopus* embryos: expression patterns and activities of *BMP-2* and *BMP-4*. *Dev. Genet.* 17, 78–89.
- Henry, J.J., Grainger, R.M., 1987. Inductive interactions in the spatial and temporal restriction of lens-forming potential in embryonic ectoderm of *Xenopus laevis*. *Dev. Biol.* 124, 200–214.
- Henry, J.J., Grainger, R.M., 1990. Early tissue interactions leading to embryonic lens formation in *Xenopus laevis*. *Dev. Biol.* 141, 149–163.
- Herbrand, H., Guthrie, S., Hadrys, T., Hoffmann, S., Arnold, H.H., Rinkwitz-Brandt, S., Bober, E., 1998. Two regulatory genes, *cnkx5-1* and *cpax2*, show different responses to local signals during otic placode and vesicle formation in the chick embryo. *Development* 125, 645–654.
- Hermesz, E., Mackem, S., Mahon, K.A., 1996. *Rpx*: a novel anterior-restricted homeobox gene progressively activated in the prechordal plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development* 122, 41–52.
- Herzog, W., Zeng, X., Lele, Z., Sonntag, C., Ting, J.W., Chang, C.Y., Hammerschmidt, M., 2003. Adenohypophysis formation in the zebrafish and its dependence on sonic hedgehog. *Dev. Biol.* 254, 36–49.
- Herzog, W., Sonntag, C., von der, H.S., Roehl, H.H., Varga, Z.M., Hammerschmidt, M., 2004. *Fgf3* signaling from the ventral diencephalon

- is required for early specification and subsequent survival of the zebrafish adenohypophysis. *Development* 131, 3681–3692.
- Hill, R.E., Jones, P.F., Rees, A.R., Sime, C.M., Justice, M.J., Copeland, N.G., Jenkins, N.A., Graham, E., Davidson, D.R., 1989. A new family of mouse homeobox-containing genes: molecular structure, chromosomal location, and developmental expression of Hox-7.1. *Genes Dev.* 3, 26–37.
- Hill, R.E., Favor, J., Hogan, B.L., Ton, C.C., Saunders, G.F., Hanson, I.M., Prosser, J., Jordan, T., Hastie, N.D., van, H.V., 1991. Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* 354, 522–525.
- Hirsch, N., Harris, W.A., 1997a. *Xenopus* Pax-6 and retinal development. *J. Neurobiol.* 32, 45–61.
- Hirsch, N., Harris, W.A., 1997b. *Xenopus* brn-3.0, a pou-domain gene expressed in the developing retina and tectum-not regulated by innervation. *Invest. Ophthalmol. Visual Sci.* 38, 960–969.
- Hogan, B.L., Horsburgh, G., Cohen, J., Hetherington, C.M., Fisher, G., Lyon, M.F., 1986. Small eyes (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse. *J. Embryol. Exp. Morphol.* 97, 95–110.
- Holland, L.Z., Holland, N.D., 1999. Chordate origins of the vertebrate central nervous system. *Curr. Opin. Neurobiol.* 9, 596–602.
- Holland, L.Z., Holland, N.D., 2001. Evolution of neural crest and placodes: amphioxus as a model for the ancestral vertebrate? *J. Anat.* 199, 85–98.
- Hollemann, T., Pieler, T., 1999. Xpitx-1: a homeobox gene expressed during pituitary and cement gland formation of *Xenopus* embryos. *Mech. Dev.* 88, 249–252.
- Holst, B.D., Wang, Y., Jones, F.S., Edelman, G.M., 1997. A binding site for Pax proteins regulates expression of the gene for the neural cell adhesion molecule in the embryonic spinal cord. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1465–1470.
- Holtfrete, J., 1933. Der Einfluss von Wirtsalter und verschiedenen Organbezirken auf die Differenzierung von angelagertem Gastrulaektoderm. *Roux's Arch. Entwicklungsmech. Org.* 127, 619–775.
- Holzschuh, J., Wada, N., Wada, C., Schaffer, A., Javidan, Y., Tallafuss, A., Bally-Cuif, L., Schilling, T.F., 2005. Requirements for endoderm and BMP signaling in sensory neurogenesis in zebrafish. *Development* 132, 3731–3742.
- Honoré, S.M., Aybar, M.J., Mayor, R., 2003. Sox10 is required for the early development of the prospective neural crest in *Xenopus* embryos. *Dev. Biol.* 260, 79–96.
- Houzelstein, D., Cohen, A., Buckingham, M.E., Robert, B., 1997. Insertional mutation of the mouse Msx1 homeobox gene by an nlacZ reporter gene. *Mech. Dev.* 65, 123–133.
- Hu, G., Lee, H., Price, S.M., Shen, M.M., Abate-Shen, C., 2001. Msx homeobox genes inhibit differentiation through upregulation of cyclin D1. *Development* 128, 2373–2384.
- Huang, X., Saint-Jeannet, J.P., 2004. Induction of the neural crest and the opportunities of life on the edge. *Dev. Biol.* 275, 1–11.
- Huang, E.J., Zang, K., Schmidt, A., Saulys, A., Xiang, M., Reichardt, L.F., 1999. POU domain factor Brn-3a controls the differentiation and survival of trigeminal neurons by regulating Trk receptor expression. *Development* 126, 2869–2882.
- Huang, E.J., Liu, W., Fritsch, B., Bianchi, L.M., Reichardt, L.F., Xiang, M.Q., 2001. Brn3a is a transcriptional regulator of soma size, target field innervation and axon pathfinding of inner ear sensory neurons. *Development* 128, 2421–2432.
- Huang, X., Hong, C.S., O'Donnell, M., Saint-Jeannet, J.P., 2005. The doublesex-related gene, XDmrt4, is required for neurogenesis in the olfactory system. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11349–11354.
- Hulander, M., Wurst, W., Carlsson, P., Enerback, S., 1998. The winged helix transcription factor Fkh10 is required for normal development of the inner ear. *Nat. Genet.* 20, 374–376.
- Hulander, M., Kiernan, A.E., Blomqvist, S.R., Carlsson, P., Samuelsson, E.J., Johansson, B.R., Steel, K.P., Enerback, S., 2003. Lack of pendrin expression leads to deafness and expansion of the endolymphatic compartment in inner ears of Foxi1 null mutant mice. *Development* 130, 2013–2025.
- Hutcheson, D.A., Vetter, M.L., 2001. The bHLH factors Xath5 and XNeuroD can upregulate the expression of XBrn3d, a POU-homeodomain transcription factor. *Dev. Biol.* 232, 327–338.
- Ikeda, Y., 1937. Beiträge zur entwicklungsmechanischen Stütze der Kupferschen Theorie der Sinnesplakoden. *Roux's Arch. Entwicklungsmech.* 136, 672–675.
- Ikeda, Y., 1938. Über die wechselseitigen Beziehungen der Sinnesorgane untereinander in ihrer normalen und experimentell bedingten Entwicklung. *Arch. Anat. Inst. Sendai* 21, 1–44.
- Ikeda, K., Watanabe, Y., Ohto, H., Kawakami, K., 2002. Molecular interaction and synergistic activation of a promoter by Six, Eya, and Dach proteins mediated through CREB binding protein. *Mol. Cell. Biol.* 22, 6759–6766.
- Irvine, K.D., Rauskolb, C., 2001. Boundaries in development: formation and function. *Annu. Rev. Cell Dev. Biol.* 17, 189–214.
- Ishibashi, S., Yasuda, K., 2001. Distinct roles of maf genes during *Xenopus* lens development. *Mech. Dev.* 101, 155–166.
- Ishii, Y., Abu-Elmagd, M., Scotting, P.J., 2001. Sox3 expression defines a common primordium for the epibranchial placodes in chick. *Dev. Biol.* 236, 344–353.
- Itoh, M., Kudoh, T., Dedekian, M., Kim, C.H., Chitnis, A.B., 2002. A role for iro1 and iro7 in the establishment of an anteroposterior compartment of the ectoderm adjacent to the midbrain–hindbrain boundary. *Development* 129, 2317–2327.
- Jacobson, C.-O., 1959. The localization of the presumptive cerebral regions in the neural plate of the axolotl larva. *J. Embryol. Exp. Morphol.* 7, 1–21.
- Jacobson, A.G., 1963a. The determination and positioning of the nose, lens and ear: III. Effects of reversing the antero-posterior axis of epidermis, neural plate and neural fold. *J. Exp. Zool.* 154, 293–303.
- Jacobson, A.G., 1963b. The determination and positioning of the nose, lens and ear: I. Interactions within the ectoderm and between the ectoderm and underlying tissues. *J. Exp. Zool.* 154, 273–283.
- Jacobson, A.G., 1963c. The determination and positioning of the nose, lens and ear: II. The role of the endoderm. *J. Exp. Zool.* 154, 285–291.
- Jacobson, A.G., 1966. Inductive processes in embryonic development. *Science* 152, 25–34.
- Jean, D., Bernier, G., Gruss, P., 1999. Six6 (Optx2) is a novel murine Six3-related homeobox gene that demarcates the presumptive pituitary/hypothalamic axis and the ventral optic stalk. *Mech. Dev.* 84, 31–40.
- Jen, Y., Manova, K., Benezra, R., 1997. Each member of the Id gene family exhibits a unique expression pattern in mouse gastrulation and neurogenesis. *Dev. Dyn.* 208, 92–106.
- Jerome, L.A., Papaioannou, V.E., 2001. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat. Genet.* 27, 286–291.
- Johnson, K.R., Cook, S.A., Erway, L.C., Matthews, A.N., Sanford, L.P., Paradies, N.E., Friedman, R.A., 1999. Inner ear and kidney anomalies caused by IAP insertion in an intron of the Eya1 gene in a mouse model of BOR syndrome. *Hum. Mol. Genet.* 8, 643–645.
- Kablar, B., Vignali, R., Menotti, L., Pannese, M., Andreazzoli, M., Polo, C., Gribaldi, M.G., Boncinelli, E., Barsacchi, G., 1996. Xotx genes in the developing brain of *Xenopus laevis*. *Mech. Dev.* 55, 145–158.
- Kaji, T., Artinger, K.B., 2004. dlx3b and dlx4b function in the development of Rohon–Beard sensory neurons and trigeminal placode in the zebrafish neurula. *Dev. Biol.* 276, 523–540.
- Kalatzis, V., Sahly, I., El-Amraoui, A., Petit, C., 1998. Eya1 expression in the developing ear and kidney: towards the understanding of the pathogenesis of Branchio–Oto–Renal (BOR) syndrome. *Dev. Dyn.* 213, 486–499.
- Kalchauer, C., 2000. Mechanisms of early neural crest development: from cell specification to migration. *Int. Rev. Cytol.* 200, 143–196.
- Kamachi, Y., Uchikawa, M., Collignon, J., Lovellbadge, R., Kondoh, H., 1998. Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction. *Development* 125, 2521–2532.
- Kamachi, Y., Uchikawa, M., Tanouchi, A., Sekido, R., Kondoh, H., 2001. Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Gene Dev.* 15, 1272–1286.
- Kan, L., Israsena, N., Zhang, Z., Hu, M., Zhao, L.R., Jalali, A., Sahni, V., Kessler, J.A., 2004. Sox1 acts through multiple independent pathways to promote neurogenesis. *Dev. Biol.* 269, 580–594.
- Kanekar, S., Perron, M., Dorsky, R., Harris, W.A., Jan, L.Y., Jan, Y.N., Vetter, M.L., 1997. Xath5 participates in a network of bhlh genes in the developing *Xenopus* retina. *Neuron* 19, 981–994.
- Kardon, G., Heanue, T.A., Tabin, C.J., 2004. The Pax/Six/Eya/Dach network in

- development and evolution. In: Schlosser, G., Wagner, G.P. (Eds.), *Modularity in Development and Evolution*. University of Chicago Press, Chicago.
- Karis, A., Pata, I., van Doorninck, J.H., Grosveld, F., de Zeeuw, C.I., de Caprona, D., Fritzsche, B., 2001. Transcription factor GATA-3 alters pathway selection of olivocochlear neurons and affects morphogenesis of the ear. *J. Comp. Neurol.* 429, 615–630.
- Karlstrom, R.O., Talbot, W.S., Schier, A.F., 1999. Comparative synteny cloning of zebrafish you-too: mutations in the Hedgehog target *gli2* affect ventral forebrain patterning. *Genes Dev.* 13, 388–393.
- Katahira, T., Sato, T., Sugiyama, S., Okafuji, T., Araki, I., Funahashi, J., Nakamura, H., 2000. Interaction between *Otx2* and *Gbx2* defines the organizing center for the optic tectum. *Mech. Dev.* 91, 43–52.
- Kaufman, C.K., Zhou, P., Pasolli, H.A., Rendl, M., Bolotin, D., Lim, K.C., Dai, X., Alegre, M.L., Fuchs, E., 2003. GATA-3: an unexpected regulator of cell lineage determination in skin. *Genes Dev.* 17, 2108–2122.
- Kawahara, A., Dawid, I.B., 2002. Developmental expression of zebrafish *emx1* during early embryogenesis. *Gene Expression Pattern* 2, 201–206.
- Kawakami, K., Sato, S., Ozaki, H., Ikeda, K., 2000. Six family genes—Structure and function as transcription factors and their roles in development. *Bioessays* 22, 616–626.
- Kawamura, K., Kikuyama, S., 1992. Evidence that hypophysis and hypothalamus constitute a single entity from the primary stage of histogenesis. *Development* 115, 1–9.
- Kawamura, K., Kikuyama, S., 1998. Morphogenesis of the hypothalamus and hypophysis: their association, dissociation and reassociation before and after “Rathke”. *Arch. Histol. Cytol.* 61, 189–198.
- Kawauchi, S., Beites, C.L., Crocker, C.E., Wu, H.H., Binnin, A., Murray, R., Calof, A.L., 2004. Molecular signals regulating proliferation of stem and progenitor cells in mouse olfactory epithelium. *Dev. Neurosci.* 26, 166–180.
- Kazanskaya, O.V., Severtzova, E.A., Barth, K.A., Ermakova, G.V., Lukyanov, S.A., Benyumov, A.O., Pannese, M., Boncinelli, E., Wilson, S.W., Zaraisky, A.G., 1997. *Anf*: a novel class of vertebrate homeobox genes expressed at the anterior end of the main embryonic axis. *Gene* 200, 25–34.
- Kee, Y., Bronner-Fraser, M., 2001a. Temporally and spatially restricted expression of the helix–loop–helix transcriptional regulator *Id1* during avian embryogenesis. *Mech. Dev.* 109, 331–335.
- Kee, Y., Bronner-Fraser, M., 2001b. The transcriptional regulator *Id3* is expressed in cranial sensory placodes during early avian embryonic development. *Mech. Dev.* 109, 337–340.
- Kee, Y., Bronner-Fraser, M., 2005. To proliferate or to die: role of *Id3* in cell cycle progression and survival of neural crest progenitors. *Genes Dev.* 19, 744–755.
- Keller, R.E., 1976. Vital dye mapping of the gastrula and neurula of *Xenopus laevis*: II. Prospective areas and morphogenetic movements of the deep layer. *Dev. Biol.* 51, 118–137.
- Kelley, M.W., 2002. Determination and commitment of mechanosensory hair cells. *Sci. World J.* 2, 1079–1094.
- Kelley, C., Yee, K., Harland, R., Zon, L.I., 1994. Ventral expression of GATA-1 and GATA-2 in the *Xenopus* embryo defines induction of hematopoietic mesoderm. *Dev. Biol.* 165, 193–205.
- Kemperman, M.H., Koch, S.M., Joosten, F.B., Kumar, S., Huygen, P.L., Cremers, C.W., 2002. Inner ear anomalies are frequent but nonobligatory features of the branchio–oto–renal syndrome. *Arch. Otolaryngol., Head Neck Surg.* 128, 1033–1038.
- Kenyon, K.L., Moody, S.A., Jamrich, M., 1999. A novel fork head gene mediates early steps during *Xenopus* lens formation. *Development* 126, 5107–5116.
- Kiecker, C., Lumsden, A., 2005. Compartments and their boundaries in vertebrate brain development. *Nat. Rev., Neurosci.* 6, 553–564.
- Kiernan, A.E., Pelling, A.L., Leung, K.K., Tang, A.S., Bell, D.M., Tease, C., Lovell-Badge, R., Steel, K.P., Cheah, K.S., 2005. *Sox2* is required for sensory organ development in the mammalian inner ear. *Nature* 434, 1031–1035.
- Kil, S.H., Collazo, A., 2001. Origins of inner ear sensory organs revealed by fate map and time-lapse analyses. *Dev. Biol.* 233, 365–379.
- Kim, P., Helms, A.W., Johnson, J.E., Zimmerman, K., 1997. XATH-1, a vertebrate homolog of *Drosophila* *atonal*, induces neuronal differentiation within ectodermal progenitors. *Dev. Biol.* 187, 1–12.
- Kim, S.S., Zhang, R.G., Braunstein, S.E., Joachimiak, A., Cvekl, A., Hegde, R.S., 2002. Structure of the retinal determination protein Dachshund reveals a DNA binding motif. *Structure (Cambridge)* 10, 787–795.
- Kim, M., Choi, J., Carlson, B.A., Han, J.K., Rhee, K., Sargent, T., Hatfield, D. L., Lee, B.J., 2003. A novel TBP-interacting zinc finger protein functions in early development of *Xenopus laevis*. *Biochem. Biophys. Res. Commun.* 306, 1106–1111.
- Kintner, C.R., Dodd, J., 1991. Hensen’s node induces neural tissue in *Xenopus* ectoderm—Implications for the action of the organizer in neural induction. *Development* 113, 1495–1505.
- Kious, B.M., Baker, C.V., Bronner-Fraser, M., Knecht, A.K., 2002. Identification and characterization of a calcium channel gamma subunit expressed in differentiating neurons and myoblasts. *Dev. Biol.* 243, 249–259.
- Kioussi, C., Carrière, C., Rosenfeld, M.G., 1999a. A model for the development of the hypothalamic–pituitary axis: transcribing the hypophysis. *Mech. Dev.* 81, 23–35.
- Kioussi, C., O’Connell, S., St Onge, L., Treier, M., Gleiberman, A.S., Gruss, P., Rosenfeld, M.G., 1999b. *Pax6* is essential for establishing ventral–dorsal cell boundaries in pituitary gland development. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14378–14382.
- Kioussi, C., Briata, P., Baek, S.H., Rose, D.W., Hamblet, N.S., Herman, T., Ohgi, K.A., Lin, C., Gleiberman, A., Wang, J., Brault, V., Ruiz-Lozano, P., Nguyen, H.D., Kemler, R., Glass, C.K., Wynshaw-Boris, A., Rosenfeld, M. G., 2002. Identification of a *Wnt/Dvl/beta-Catenin* → *Pitx2* pathway mediating cell-type-specific proliferation during development. *Cell* 111, 673–685.
- Kishi, M., Mizuseki, K., Sasai, N., Yamazaki, H., Shiota, K., Nakanishi, S., Sasai, Y., 2000. Requirement of *Sox2*-mediated signaling for differentiation of early *Xenopus* neuroectoderm. *Development* 127, 791–800.
- Klein, S.L., Graziadei, P.P.C., 1983. The differentiation of the olfactory placode in *Xenopus laevis*: a light and electron microscope study. *J. Comp. Neurol.* 217, 7–30.
- Klesert, T.R., Cho, D.H., Clark, J.I., Maylie, J., Adelman, J., Snider, L., Yuen, E.C., Soriano, P., Tapscott, S.J., 2000. Mice deficient in *Six5* develop cataracts: implications for myotonic dystrophy. *Nat. Genet.* 25, 105–109.
- Knecht, A.K., Bronner-Fraser, M., 2002. Induction of the neural crest: a multigene process. *Nat. Rev., Genet.* 3, 453–461.
- Knecht, A.K., Harland, R.M., 1997. Mechanisms of dorsal–ventral patterning in *noggin*-induced neural tissue. *Development* 124, 2477–2488.
- Knouff, R.A., 1927. The origin of the cranial ganglia of *Rana*. *J. Comp. Neurol.* 44, 259–361.
- Knouff, R.A., 1935. The developmental pattern of ectodermal placodes in *Rana pipiens*. *J. Comp. Neurol.* 62, 17–71.
- Kobayashi, M., Toyama, R., Takeda, H., Dawid, I.B., Kawakami, K., 1998. Overexpression of the forebrain-specific homeobox gene *six3* induces rostral forebrain enlargement in zebrafish. *Development* 125, 2973–2982.
- Kobayashi, M., Osanai, H., Kawakami, K., Yamamoto, M., 2000. Expression of three zebrafish *Six4* genes in the cranial sensory placodes and the developing somites. *Mech. Dev.* 98, 151–155.
- Kobayashi, M., Nishikawa, K., Suzuki, T., Yamamoto, M., 2001. The homeobox protein *Six3* interacts with the Groucho corepressor and acts as a transcriptional repressor in eye and forebrain formation. *Dev. Biol.* 232, 315–326.
- Kobayashi, D., Kobayashi, M., Matsumoto, K., Ogura, T., Nakafuku, M., Shimamura, K., 2002. Early subdivisions in the neural plate define distinct competence for inductive signals. *Development* 129, 83–93.
- Koebernick, K., Hollemann, T., Pieler, T., 2003. A restrictive role for Hedgehog signalling during otic specification in *Xenopus*. *Dev. Biol.* 260, 325–338.
- Kohan, R., 1944. Chordamesoderm as an inductor of the ear vesicle. *C. R. Acad. Sci. URSS* 45, 39–41.
- Kondoh, H., Uchikawa, M., Yoda, H., Takeda, H., Furutani-Seiki, M., Karlstrom, R.O., 2000. Zebrafish mutations in *Gli*-mediated hedgehog signaling lead to lens transdifferentiation from the adeno-hypophysis anlage. *Mech. Dev.* 96, 165–174.
- Kondoh, H., Uchikawa, M., Kamachi, Y., 2004. Interplay of *Pax6* and *SOX2* in

- lens development as a paradigm of genetic switch mechanisms for cell differentiation. *Int. J. Dev. Biol.* 48, 819–827.
- Korzh, V., Strähle, U., 2002. Proneural, prosensory, antilial: the many faces of neurogenins. *Trends Neurosci.* 25, 603–605.
- Korzh, V., Edlund, T., Thor, S., 1993. Zebrafish primary neurons initiate expression of the LIM homeodomain protein *Isl-1* at the end of gastrulation. *Development* 118, 417–425.
- Köster, R.W., Kühnlein, R.P., Wittbrodt, J., 2000. Ectopic Sox3 activity elicits sensory placode formation. *Mech. Dev.* 95, 175–187.
- Kouki, T., Imai, H., Aoto, K., Eto, K., Shioda, S., Kawamura, K., Kikuyama, S., 2001. Developmental origin of the rat adenohypophysis prior to the formation of Rathke's pouch. *Development* 128, 959–963.
- Koyano-Nakagawa, N., Kim, J., Anderson, D., Kintner, C., 2000. *Hes6* acts in a positive feedback loop with the neurogenins to promote neuronal differentiation. *Development* 127, 4203–4216.
- Kozlowski, D.J., Murakami, T., Ho, R.K., Weinberg, E.S., 1997. Regional cell movement and tissue patterning in the zebrafish embryo revealed by fate mapping with caged fluorescein. *Biochem. Cell. Biol.* 75, 551–562.
- Kozlowski, D.J., Whitfield, T.T., Hukriede, N.A., Lam, W.K., Weinberg, E.S., 2005. The zebrafish dog-eared mutation disrupts *eya1*, a gene required for cell survival and differentiation in the inner ear and lateral line. *Dev. Biol.* 277, 27–41.
- Kumar, J.P., Moses, K., 2001a. Eye specification in *Drosophila*: perspectives and implications. *Semin. Cell Dev. Biol.* 12, 469–474.
- Kumar, J.P., Moses, K., 2001b. Expression of evolutionarily conserved eye specification genes during *Drosophila* embryogenesis. *Dev. Genes Evol.* 211, 406–414.
- La Bonne, C., Bronner-Fraser, M., 1998. Neural crest induction in *Xenopus*: evidence for a two-signal model. *Development* 125, 2403–2414.
- La Mantia, A.S., Bhasin, N., Rhodes, K., Heemskerk, J., 2000. Mesenchymal/epithelial induction mediates olfactory pathway formation. *Neuron* 28, 411–425.
- Laclef, C., Souil, E., Demignon, J., Maire, P., 2003a. Thymus, kidney and craniofacial abnormalities in *Six1* deficient mice. *Mech. Dev.* 120, 669–679.
- Laclef, C., Hamard, G., Demignon, J., Souil, E., Houbbron, C., Maire, P., 2003b. Altered myogenesis in *Six1*-deficient mice. *Development* 130, 2239–2252.
- Ladher, R.K., Anakwe, K.U., Gurney, A.L., Schoenwolf, G.C., Francis-West, P.H., 2000a. Identification of synergistic signals initiating inner ear development. *Science* 290, 1965–1967.
- Ladher, R.K., Church, V.L., Allen, S., Robson, L., Abdelfattah, A., Brown, N.A., Hattersley, G., Rosen, V., Luyten, F.P., Dale, L., Francis-West, P.H., 2000b. Cloning and expression of the Wnt antagonists *Sfrp-2* and *Frzb* during chick development. *Dev. Biol.* 218, 183–198.
- Ladher, R.K., Wright, T.J., Moon, A.M., Mansour, S.L., Schoenwolf, G.C., 2005. *FGF8* initiates inner ear induction in chick and mouse. *Genes Dev.* 19, 603–613.
- Lagutin, O., Zhu, C.Q.C., Furuta, Y., Rowitch, D.H., McMahon, A.P., Oliver, G., 2001. *Six3* promotes the formation of ectopic optic vesicle-like structures in mouse embryos. *Dev. Dyn.* 221, 342–349.
- Lagutin, O.V., Zhu, C.C., Kobayashi, D., Topczewski, J., Shimamura, K., Puelles, L., Russell, H.R., McKinnon, P.J., Solnica-Krezel, L., Oliver, G., 2003. *Six3* repression of Wnt signaling in the anterior neuroectoderm is essential for vertebrate forebrain development. *Genes Dev.* 17, 368–379.
- Lamb, T.M., Knecht, A.K., Smith, W.C., Stackel, S.E., Economides, A.N., Stahl, N., Yancopoulos, G.D., Harland, R.M., 1993. Neural induction by the secreted polypeptide *noggin*. *Science* 262, 713–718.
- Lamonerie, T., Tremblay, J.J., Lanctot, C., Therrien, M., Gauthier, Y., Drouin, J., 1996. *Ptx1*, a bicoid-related homeobox transcription factor involved in transcription of the pro-opiomelanocortin gene. *Genes Dev.* 10, 1284–1295.
- Lanctot, C., Lamolet, B., Drouin, J., 1997. The bicoid-related homeoprotein *Ptx1* defines the most anterior domain of the embryo and differentiates posterior from anterior lateral mesoderm. *Development* 124, 2807–2817.
- Landacre, F.L., 1910. The origin of the cranial ganglia in *Ameiurus*. *J. Comp. Neurol.* 20, 309–411.
- Landacre, F.L., 1912. The epibranchial placodes of *Lepidosteus osseus* and their relation to the cerebral ganglia. *J. Comp. Neurol.* 22, 1–69.
- Landacre, F.L., 1916. The cerebral ganglia and early nerves of *Squalus acanthias*. *J. Comp. Neurol.* 27, 19–67.
- Landacre, F.L., 1926. The primitive lines of *Amblystoma jeffersonianum*. *J. Comp. Neurol.* 40, 471–495.
- Landacre, F.L., 1927. The differentiation of preauditory and postauditory primitive lines into preauditory and postauditory placodes, lateralis ganglia and migratory lateral-line placodes in *Amblystoma jeffersonianum*. *J. Comp. Neurol.* 44, 29–59.
- Landacre, F.L., Conger, A.C., 1913. The origin of the lateral line primordia in *Lepidosteus osseus*. *J. Comp. Neurol.* 23, 575–633.
- Lang, R.A., 2004. Pathways regulating lens induction in the mouse. *Int. J. Dev. Biol.* 48, 783–791.
- Latchman, D.S., 1999. POU family transcription factors in the nervous system. *J. Cell. Physiol.* 179, 126–133.
- Lawoko-Kerali, G., Rivolta, M.N., Holley, M., 2002. Expression of the transcription factors *GATA3* and *Pax2* during development of the mammalian inner ear. *J. Comp. Neurol.* 442, 378–391.
- Lawoko-Kerali, G., Rivolta, M.N., Lawlor, P., Cacciabue-Rivolta, D.I., Langton-Hewer, C., van Doorninck, J.H., Holley, M.C., 2004. *GATA3* and *NeuroD* distinguish auditory and vestibular neurons during development of the mammalian inner ear. *Mech. Dev.* 121, 287–299.
- Le Douarin, N.M., Dupin, E., 2003. Multipotentiality of the neural crest. *Curr. Opin. Genet. Dev.* 13, 529–536.
- Le Douarin, N.M., Kalcheim, C., 1999. *The Neural Crest*. Cambridge Univ. Press, Cambridge.
- Lecaudey, V., Anselme, I., Rosa, F., Schneider-Maunoury, S., 2004. The zebrafish *Iroquois* gene *iro7* positions the *r4/r5* boundary and controls neurogenesis in the rostral hindbrain. *Development* 131, 3121–3131.
- Lee, J.E., 1997. *NeuroD* and neurogenesis. *Dev. Neurosci.* 19, 27–32.
- Lee, J.E., Hollenberg, S.M., Snider, L., Turner, D.L., Lipnick, N., Weintraub, H., 1995. Conversion of *Xenopus* ectoderm into neurons by *NeuroD*, a basic helix–loop–helix protein. *Science* 268, 836–844.
- Lee, S.A., Shen, E.L., Fiser, A., Sali, A., Guo, S., 2003. The zebrafish forkhead transcription factor *Foxi1* specifies epibranchial placode-derived sensory neurons. *Development* 130, 2669–2679.
- Lef, J., Clement, J.H., Oswald, R., Koster, M., Knochel, W., 1994. Spatial and temporal transcription patterns of the forkhead related *XFD-2/XFD-2'* genes in *Xenopus laevis* embryos. *Mech. Dev.* 45, 117–126.
- Léger, S., Brand, M., 2002. *Fgf8* and *Fgf3* are required for zebrafish ear placode induction, maintenance and inner ear patterning. *Mech. Dev.* 119, 91–108.
- Lehmann, O.J., Sowden, J.C., Carlsson, P., Jordan, T., Bhattacharya, S.S., 2003. *Fox*'s in development and disease. *Trends Genet.* 19, 339–344.
- Levi, G., Crossin, K.L., Edelman, G.M., 1987. Expression sequences and distribution of two primary cell adhesion molecules during embryonic development of *Xenopus laevis*. *J. Cell Biol.* 105, 2359–2372.
- Levi, G., Puche, A.C., Mantero, S., Barbieri, O., Trombino, S., Paleari, L., Egeo, A., Merlo, G.R., 2003. The *Dlx5* homeodomain gene is essential for olfactory development and connectivity in the mouse. *Mol. Cell. Neurosci.* 22, 530–543.
- Lewis, J.L., Bonner, J., Modrell, M., Ragland, J.W., Moon, R.T., Dorsky, R.I., Raible, D.W., 2004. Reiterated Wnt signaling during zebrafish neural crest development. *Development* 131, 1299–1308.
- Leyns, L., Bouwmeester, T., Kim, S.H., Piccolo, S., De Robertis, E.M., 1997. *Frzb-1* is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88, 747–756.
- Li, H.S., Yang, J.M., Jacobson, R.D., Pasko, D., Sundin, O., 1994. *Pax-6* is first expressed in a region of ectoderm anterior to the early neural plate: implications for stepwise determination of the lens. *Dev. Biol.* 162, 181–194.
- Li, X., Perissi, V., Liu, F., Rose, D.W., Rosenfeld, M.G., 2002. Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science* 297, 1180–1183.
- Li, X., Oghi, K.A., Zhang, J., Krones, A., Bush, K.T., Glass, C.K., Nigam, S.K., Aggarwal, A.K., Maas, R., Rose, D.W., Rosenfeld, M.G., 2003. Eya protein phosphatase activity regulates *Six1*–*Dach*–*Eya* transcriptional effects in mammalian organogenesis. *Nature* 426, 247–254.
- Li, H., Liu, H., Corrales, C.E., Mutai, H., Heller, S., 2004a. Correlation of *Pax-2* expression with cell proliferation in the developing chicken inner ear. *J. Neurobiol.* 60, 61–70.

- Li, H., Liu, H., Sage, C., Huang, M., Chen, Z.Y., Heller, S., 2004b. Islet-1 expression in the developing chicken inner ear. *J. Comp. Neurol.* 477, 1–10.
- Liberg, D., Sigvardsson, M., Akerblad, P., 2002. The EBF/Olf/Collier family of transcription factors: regulators of differentiation in cells originating from all three embryonic germ layers. *Mol. Cell. Biol.* 22, 8389–8397.
- Liem Jr., K.F., Tremml, G., Roelink, H., Jessell, T.M., 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* 82, 969–979.
- Light, W., Vernon, A.E., Lasorella, A., Iavarone, A., LaBonne, C., 2005. *Xenopus* Id3 is required downstream of Myc for the formation of multipotent neural crest progenitor cells. *Development* 132, 1831–1841.
- Lillevali, K., Matilainen, T., Karis, A., Salminen, M., 2004. Partially overlapping expression of Gata2 and Gata3 during inner ear development. *Dev. Dyn.* 231, 775–781.
- Lin, C.R., Kiousi, C., O'Connell, S., Briata, P., Szeto, D., Liu, F., Izpisua-Belmonte, J.C., Rosenfeld, M.G., 1999. Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphogenesis. *Nature* 401, 279–282.
- Litsiou, A., Hanson, S., Streit, A., 2005. A balance of FGF, BMP and WNT signalling positions the future placode territory in the head. *Development* 132, 4051–4062.
- Liu, K.J., Harland, R.M., 2003. Cloning and characterization of *Xenopus* Id4 reveals differing roles for Id genes. *Dev. Biol.* 264, 339–351.
- Liu, W., Li, G., Chien, J.S., Raft, S., Zhang, H., Chiang, C., Frenz, D.A., 2002. Sonic hedgehog regulates otic capsule chondrogenesis and inner ear development in the mouse embryo. *Dev. Biol.* 248, 240–250.
- Liu, D., Chu, H., Maves, L., Yan, Y.L., Morcos, P.A., Postlethwait, J.H., Westerfield, M., 2003. Fgf3 and Fgf8 dependent and independent transcription factors are required for otic placode specification. *Development* 130, 2213–2224.
- Liu, Y., Helms, A.W., Johnson, J.E., 2004. Distinct activities of Msx1 and Msx3 in dorsal neural tube development. *Development* 131, 1017–1028.
- Lombardo, A., Slack, J.M.W., 1998. Postgastrulation effects of fibroblast growth factor on *Xenopus* development. *Dev. Dyn.* 212, 75–85.
- Long, J.E., Garel, S., Depew, M.J., Tobet, S., Rubenstein, J.L., 2003. DLX5 regulates development of peripheral and central components of the olfactory system. *J. Neurosci.* 23, 568–578.
- Loosli, F., Köster, R.W., Carl, M., Krone, A., Wittbrodt, J., 1998. Six3, a medaka homologue of the *Drosophila* homeobox gene sine oculis is expressed in the anterior embryonic shield and the developing eye. *Mech. Dev.* 74, 159–164.
- Loosli, F., Winkler, S., Wittbrodt, J., 1999. Six3 overexpression initiates the formation of ectopic retina. *Genes Dev.* 13, 649–654.
- López-Ríos, J., Gallardo, M.E., de Córdoba, S.R., Bovolenta, P., 1999. Six9 (Optx2), a new member of the Six gene family of transcription factors, is expressed at early stages of vertebrate ocular and pituitary development. *Mech. Dev.* 83, 155–159.
- López-Ríos, J., Tessmar, K., Loosli, F., Wittbrodt, J., Bovolenta, P., 2003. Six3 and Six6 activity is modulated by members of the groucho family. *Development* 130, 185–195.
- Lovicu, F.J., McAvoy, J.W., 2005. Growth factor regulation of lens development. *Dev. Biol.* 280, 1–14.
- Luo, T., Matsuo-Takasaka, M., Lim, J.H., Sargent, T.D., 2001a. Differential regulation of Dlx gene expression by a BMP morphogenetic gradient. *Int. J. Dev. Biol.* 45, 681–684.
- Luo, T., Matsuo-Takasaka, M., Sargent, T.D., 2001b. Distinct roles for distal-less genes Dlx3 and Dlx5 in regulating ectodermal development in *Xenopus*. *Mol. Reprod. Dev.* 60, 331–337.
- Ma, Q.F., Kintner, C., Anderson, D.J., 1996. Identification of neurogenin, a vertebrate neuronal determination gene. *Cell* 87, 43–52.
- Ma, Q.F., Chen, Z.F., Barrantes, I.D., de la Pompa, J.L., Anderson, D.J., 1998. Neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron* 20, 469–482.
- Ma, Q., Anderson, D.J., Fritsch, B., 2000. Neurogenin1 null mutant ears develop fewer, morphologically normal hair cells in smaller sensory epithelia devoid of innervation. *J. Assoc. Res. Otolaryngol.* 1, 129–143.
- Mackenzie, A., Ferguson, M.W.J., Sharpe, P.T., 1991. Hox-7 expression during murine craniofacial development. *Development* 113, 601–611.
- Mackereth, M.D., Kwak, S.J., Fritz, A., Riley, B.B., 2005. Zebrafish pax8 is required for otic placode induction and plays a redundant role with Pax2 genes in the maintenance of the otic placode. *Development* 132, 371–382.
- Maeda, R., Kobayashi, A., Sekine, R., Lin, J.J., Kung, H.F., Maeno, M., 1997. Xmsx-1 modifies mesodermal tissue pattern along dorsoventral axis in *Xenopus laevis* embryo. *Development* 124, 2553–2560.
- Maeno, M., Mead, P.E., Kelley, C., Xu, R.H., Kung, H.F., Suzuki, A., Ueno, N., Zon, L.I., 1996. The role of BMP-4 and GATA-2 in the induction and differentiation of hematopoietic mesoderm in *Xenopus laevis*. *Blood* 88, 1965–1972.
- Mancilla, H., Mayor, R., 1996. Neural crest formation in *Xenopus laevis*: mechanisms of Xslug induction. *Dev. Biol.* 177, 580–589.
- Mansouri, A., 1998. The role of Pax3 and Pax7 in development and cancer. *Crit. Rev. Oncogen.* 9, 141–149.
- Mansouri, A., Stoykova, A., Torres, M., Gruss, P., 1996. Dysgenesis of cephalic neural crest derivatives in pax7(−/−) mutant mice. *Development* 122, 831–838.
- Mansouri, A., Chowdhury, K., Gruss, P., 1998. Follicular cells of the thyroid gland require Pax8 gene function. *Nat. Genet.* 19, 87–90.
- Marazzi, G., Wang, Y., Sassoon, D., 1997. Msx2 is a transcriptional regulator in the BMP4-mediated programmed cell death pathway. *Dev. Biol.* 186, 127–138.
- Marchant, L., Linker, C., Ruiz, P., Guerrero, N., Mayor, R., 1998. The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. *Dev. Biol.* 198, 319–329.
- Mardon, G., Solomon, N.M., Rubin, G.M., 1994. dachshund encodes a nuclear protein required for normal eye and leg development in *Drosophila*. *Development* 120, 3473–3486.
- Maroon, H., Walshe, J., Mahmood, R., Kiefer, P., Dickson, C., Mason, I., 2002. Fgf3 and Fgf8 are required together for formation of the otic placode and vesicle. *Development* 129, 2099–2108.
- Martinez-Barbera, J.P., Signore, M., Boyle, P.P., Puelles, E., Acampora, D., Gogoi, R., Schubert, F., Lumsden, A., Simeone, A., 2002. Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between OTX2 and GBX2. *Development* 128, 4789–4800.
- Martynoga, B., Morrison, H., Price, D.J., Mason, J.O., 2005. Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. *Dev. Biol.* 283, 113–127.
- Mathers, P.H., Miller, A., Doniach, T., Dirksen, M.L., Jamrich, M., 1995. Initiation of anterior head specific gene expression in uncommitted ectoderm of *Xenopus laevis* by ammonium chloride. *Dev. Biol.* 171, 641–654.
- Matsunaga, E., Araki, I., Nakamura, H., 2000. Pax6 defines the di-mesencephalic boundary by repressing En1 and Pax2. *Development* 127, 2357–2365.
- Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., Aizawa, S., 1995. Mouse Otx2 functions in the formation and patterning of rostral head. *Gene Dev.* 9, 2646–2658.
- Mayor, R., Aybar, M.J., 2001. Induction and development of neural crest in *Xenopus laevis*. *Cell Tissue Res.* 305, 203–209.
- Mayor, R., Morgan, R., Sargent, M.G., 1995. Induction of the prospective neural crest of *Xenopus*. *Development* 121, 767–777.
- Mayor, R., Guerrero, N., Martínez, C., 1997. Role of FGF and Noggin in neural crest induction. *Dev. Biol.* 189, 1–12.
- Mayor, R., Young, R., Vargas, A., 1999. Development of neural crest in *Xenopus*. *Curr. Top. Dev. Biol.* 43, 85–113.
- Mazet, F., Hutt, J.A., Milloz, J., Millard, J., Graham, A., Shimeld, S.M., 2005. Molecular evidence from *Ciona intestinalis* for the evolutionary origin of vertebrate sensory placodes. *Dev. Biol.* 282, 494–508.
- McAvoy, J.W., 1980. Induction of the eye lens. *Differentiation* 17, 137–149.
- McAvoy, J.W., Chamberlain, C.G., de Jongh, R.U., Hales, A.M., Lovicu, F.J., 1999. Lens development. *Eye* 13, 425–437.
- McCabe, K.L., Manzo, A., Gammill, L.S., Bronner-Fraser, M., 2004. Discovery of genes implicated in placode formation. *Dev. Biol.* 274, 462–477.
- McCauley, D.W., Bronner-Fraser, M., 2002. Conservation of Pax gene expression in ectodermal placodes of the lamprey. *Gene* 287, 129–139.
- McEvilly, R.J., Erkmann, L., Luo, L., Sawchenko, P.E., Ryan, A.F., Rosenfeld, M.G., 1996. Requirement for brn-3.0 in differentiation and survival of sensory and motor neurons. *Nature* 384, 574–577.

- McGrew, L.L., Hoppler, S., Moon, R.T., 1997. Wnt and fgf pathways cooperatively pattern anteroposterior neural ectoderm in *Xenopus*. *Mech. Dev.* 69, 105–114.
- McKeown, S.J., Newgreen, D.F., Farlie, P.G., 2005. Dlx2 over-expression regulates cell adhesion and mesenchymal condensation in ectomesenchyme. *Dev. Biol.* 281, 22–37.
- McLarren, K.W., Litsiou, A., Streit, A., 2003. DLX5 positions the neural crest and preplacode region at the border of the neural plate. *Dev. Biol.* 259, 34–47.
- Meech, R., Kallunki, P., Edelman, G.M., Jones, F.S., 1999. A binding site for homeodomain and Pax proteins is necessary for L1 cell adhesion molecule gene expression by Pax-6 and bone morphogenetic proteins. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2420–2425.
- Mendonsa, E.S., Riley, B.B., 1999. Genetic analysis of tissue interactions required for otic placode induction in the zebrafish. *Dev. Biol.* 206, 100–112.
- Mendoza, A.S., Breipohl, W., Miragall, F., 1982. Cell migration from the chick olfactory placode: a light and electron microscopic study. *J. Embryol. Exp. Morphol.* 69, 47–59.
- Merlo, G.R., Zerega, B., Paleari, L., Trombino, S., Mantero, S., Levi, G., 2000. Multiple functions of Dlx genes. *Int. J. Dev. Biol.* 44, 619–626.
- Merlo, G.R., Paleari, L., Mantero, S., Zerega, B., Adamska, M., Rinkwitz, S., Bober, E., Levi, G., 2002. The Dlx5 homeobox gene is essential for vestibular morphogenesis in the mouse embryo through a BMP4-mediated pathway. *Dev. Biol.* 248, 157–169.
- Meulemans, D., Bronner-Fraser, M., 2004. Gene–regulatory interactions in neural crest evolution and development. *Dev. Cell* 7, 291–299.
- Meulemans, D., McCauley, D., Bronner-Fraser, M., 2003. Id expression in amphioxus and lamprey highlights the role of gene cooption during neural crest evolution. *Dev. Biol.* 264, 430–442.
- Miller, D.J., Hayward, D.C., ReeceHoyes, J.S., Scholten, I., Catmull, J., Gehring, W.J., Callaerts, P., Larsen, J.E., Ball, E.E., 2000. Pax gene diversity in the basal cnidarian *Acropora millepora* (Cnidaria, Anthozoa): implications for the evolution of the Pax gene family. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4475–4480.
- Millet, S., Campbell, K., Epstein, D.J., Losos, K., Harris, E., Joyner, A.L., 1999. A role for Gbx2 in repression of Otx-2 and positioning the mid/hindbrain organizer. *Nature* 401, 161–164.
- Miragall, F., Kadmon, G., Schachner, M., 1989. Expression of L1 and N-CAM cell adhesion molecules during development of the mouse olfactory system. *Dev. Biol.* 135, 272–286.
- Mishima, N., Tomarev, S., 1998. Chicken Eyes absent 2 gene: isolation and expression pattern during development. *Int. J. Dev. Biol.* 42, 1109–1115.
- Mizuseki, K., Kishi, M., Matsui, M., Nakanishi, S., Sasai, Y., 1998. *Xenopus* zinc-related-1 and sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. *Development* 125, 579–587.
- Molenaar, M., Brian, E., Roose, J., Clevers, H., Destree, O., 2000. Differential expression of the Groucho-related genes 4 and 5 during early development of *Xenopus laevis*. *Mech. Dev.* 91, 311–315.
- Monsoro-Burq, A.H., Fletcher, R.B., Harland, R.M., 2003. Neural crest induction by paraxial mesoderm in *Xenopus* embryos requires FGF signals. *Development* 130, 3111–3124.
- Monsoro-Burq, A.H., Wang, E., Harland, R., 2005. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during *Xenopus* neural crest induction. *Dev. Cell* 8, 167–178.
- Moraes, F., Novoa, A., Jerome-Majewska, L.A., Papaioannou, V.E., Mallo, M., 2005. Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear development. *Mech. Dev.* 122, 199–212.
- Morasso, M.I., Markova, N.G., Sargent, T.D., 1996. Regulation of epidermal differentiation by a Distal-less homeodomain gene. *J. Cell Biol.* 135, 1879–1887.
- Morgan, R., Sargent, M.G., 1997. The role in neural patterning of translation initiation factor eIF4AII; induction of neural fold genes. *Development* 124, 2751–2760.
- Morita, T., Nitta, H., Kiyama, Y., Mori, H., Mishina, M., 1995. Differential expression of two zebrafish *emx* homeoprotein mRNAs in the developing brain. *Neurosci. Lett.* 198, 131–134.
- Moury, J.D., Jacobson, A.G., 1989. Neural fold formation at newly created boundaries between neural plate and epidermis in the axolotl. *Dev. Biol.* 133, 44–57.
- Moury, J.D., Jacobson, A.G., 1990. The origins of neural crest cells in the axolotl. *Dev. Biol.* 141, 243–253.
- Müller, U., Littlewood-Evans, A., 2001. Mechanisms that regulate mechanosensory hair cell differentiation. *Trends Cell Biol.* 11, 334–342.
- Murakami, S., Arai, Y., 1994. Direct evidence for the migration of LHRH neurons from the nasal region to the forebrain in the chick embryo: a carbocyanine dye analysis. *Neurosci. Res.* 19, 331–338.
- Murakami, Y., Ogasawara, M., Sugahara, F., Hirano, S., Satoh, N., Kuratani, S., 2001. Identification and expression of the lamprey Pax6 gene: evolutionary origin of the segmented brain of vertebrates. *Development* 128, 3521–3531.
- Murray, R.C., Navi, D., Fesenko, J., Lander, A.D., Calof, A.L., 2003. Widespread defects in the primary olfactory pathway caused by loss of Mash1 function. *J. Neurosci.* 23, 1769–1780.
- Muske, L.E., 1993. Evolution of gonadotropin-releasing hormone (GnRH) neuronal systems. *Brain Behav. Evol.* 42, 215–230.
- Muske, L.E., Moore, F.L., 1988. The nervus terminalis in amphibians: anatomy, chemistry and relationship with the hypothalamic gonadotropin-releasing hormone system. *Brain Behav. Evol.* 32, 141–150.
- Muta, M., Kamachi, Y., Yoshimoto, A., Higashi, Y., Kondoh, H., 2002. Distinct roles of SOX2, Pax6 and Maf transcription factors in the regulation of lens-specific delta1-crystallin enhancer. *Genes Cells* 7, 791–805.
- Muzio, L., Mallamaci, A., 2005. Foxg1 confines Cajal–Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *J. Neurosci.* 25, 4435–4441.
- Naiche, L.A., Harrelson, Z., Kelly, R.G., Papaioannou, V.E., 2005. T-box genes in vertebrate development. *Annu. Rev. Genet.* 39, 219–239.
- Namba, A., Abe, S., Shinkawa, H., Kimberling, W.J., Usami, S., 2001. Genetic features of hearing loss associated with ear anomalies: PDS and EYA1 mutation analysis. *J. Hum. Genet.* 46, 518–521.
- Narayanan, C.H., Narayanan, Y., 1980. Neural crest and placodal contributions in the development of the glossopharyngeal–vagal complex in the chick. *Anat. Rec.* 196, 71–82.
- Neave, B., Rodaway, A., Wilson, S.W., Patient, R., Holder, N., 1995. Expression of zebrafish GATA 3 (*gta3*) during gastrulation and neurulation suggests a role in the specification of cell fate. *Mech. Dev.* 51, 169–182.
- Neave, B., Holder, N., Patient, R., 1997. A graded response to BMP-4 spatially coordinates patterning of the mesoderm and ectoderm in the zebrafish. *Mech. Dev.* 62, 183–195.
- Nechiporuk, A., Linbo, T., Raible, D.W., 2005. Endoderm-derived Fgf3 is necessary and sufficient for inducing neurogenesis in the epibranchial placodes in zebrafish. *Development* 132, 3717–3730.
- Nedelec, S., Foucher, I., Brunet, I., Bouillot, C., Prochiantz, A., Trembleau, A., 2004. Emx2 homeodomain transcription factor interacts with eukaryotic translation initiation factor 4E (eIF4E) in the axons of olfactory sensory neurons. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10815–10820.
- Nguyen, V.H., Schmid, B., Trout, J., Connors, S.A., Ekker, M., Mullins, M.C., 1998. Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a *bmp2b/swirl* pathway of genes. *Dev. Biol.* 199, 93–110.
- Nguyen, V.H., Trout, J., Connors, S.A., Andermann, P., Weinberg, E., Mullins, M.C., 2000. Dorsal and intermediate neuronal cell types of the spinal cord are established by a BMP signaling pathway. *Development* 127, 1209–1220.
- Niehrs, C., 2004. Regionally specific induction by the Spemann–Mangold organizer. *Nat. Rev., Genet.* 5, 425–434.
- Nieuwkoop, P.D., 1963. Pattern formation in artificially activated ectoderm (*Rana pipiens* and *Ambystoma punctatum*). *Dev. Biol.* 7, 255–279.
- Nieuwkoop, P.D., 1985. Inductive interactions in early amphibian development and their general nature. *J. Embryol. Exp. Morphol.* 89, 333–347.
- Nieuwkoop, P.D., Faber, J., 1967. Normal Table of *Xenopus laevis* (Daudin). North-Holland, Amsterdam.
- Nieuwkoop, P.D., Johnen, A.G., Albers, B., 1985. The Inductive Nature of Early Chordate Development. Cambridge Univ. Press, Cambridge.
- Niimi, T., Seimiya, M., Kloter, U., Flister, S., Gehring, W.J., 1999. Direct regulatory interaction of the eyeless protein with an eye-specific enhancer in

- the sine oculis gene during eye induction in *Drosophila*. *Development* 126, 2253–2260.
- Nissen, R.M., Yan, J., Amsterdam, A., Hopkins, N., Burgess, S.M., 2003. Zebrafish foxi one modulates cellular responses to Fgf signaling required for the integrity of ear and jaw patterning. *Development* 130, 2543–2554.
- Noden, D.M., 1980a. Somatotopic and functional organization of the avian trigeminal ganglion: an HRP analysis in the hatchling chick. *J. Comp. Neurol.* 190, 405–428.
- Noden, D.M., 1980b. Somatotopic organization of the embryonic chick trigeminal ganglion. *J. Comp. Neurol.* 190, 429–444.
- Noden, D.M., 1991. Cell movements and control of patterned tissue assembly during craniofacial development. *J. Craniofac. Genet. Dev. Biol.* 11, 192–213.
- Noramlly, S., Grainger, R.M., 2002. Determination of the embryonic inner ear. *J. Neurobiol.* 53, 100–128.
- Norgren Jr., R.B., Ratner, N., Brackenbury, R., 1992. Development of olfactory nerve glia defined by a monoclonal antibody specific for Schwann cells. *Dev. Dyn.* 194, 231–238.
- Norris, H.W., 1925. Observations upon the peripheral distribution of the cranial nerves of certain ganoid fishes (*Amia*, *Lepidosteus*, *Polyodon*, *Scapirhynchus* and *Acipenser*). *J. Comp. Neurol.* 39, 345–432.
- Norris, H.W., Hughes, S.P., 1920. The cranial, occipital, and anterior spinal nerves of the dogfish, *Squalus acanthias*. *J. Comp. Neurol.* 31, 293–402.
- Northcutt, R.G., 1992. The phylogeny of octavolateralis ontogenies: a reaffirmation of Garstang's hypothesis. In: Webster, D.B., Fay, R.R., Popper, A.N. (Eds.), *The Evolutionary Biology of Hearing*. Springer, New York, pp. 21–47.
- Northcutt, R.G., 1993a. A reassessment of Goodrich's model of cranial nerve phylogeny. *Acta Anat.* 148, 71–80.
- Northcutt, R.G., 1993b. The primitive pattern of development of lateral line organs. *J. Comp. Physiol., A Sens. Neural Behav. Physiol.* 173, 717–718.
- Northcutt, R.G., 1996. The origin of craniates: neural crest, neurogenic placodes, and homeobox genes. *Isr. J. Zool.* 42, 273–313.
- Northcutt, R.G., 1997. Evolution of gnathostome lateral line ontogenies. *Brain Behav. Evol.* 50, 25–37.
- Northcutt, R.G., 2004. Taste buds: development and evolution. *Brain Behav. Evol.* 64, 198–206.
- Northcutt, R.G., 2005. The New Head hypothesis revisited. *J. Exp. Zool. Part B Mol. Dev. Evol.* 304, 274–297.
- Northcutt, R.G., Bemis, W.E., 1993. Cranial nerves of the coelacanth, *Latimeria chalumnae* [Osteichthyes: Sarcopterygii: Actinistia], and comparisons with other craniata. *Brain Behav. Evol.* 42, 1–76.
- Northcutt, R.G., Brändle, K., 1995. Development of branchiomic and lateral line nerves in the axolotl. *J. Comp. Neurol.* 355, 427–454.
- Northcutt, R.G., Gans, C., 1983. The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Q. Rev. Biol.* 58, 1–28.
- Northcutt, R.G., Muske, L.E., 1994. Multiple embryonic origins of gonadotropin-releasing hormone (GnRH) immunoreactive neurons. *Brain Res. Dev. Brain Res.* 78, 279–290.
- Northcutt, R.G., Catania, K.C., Criley, B.B., 1994. Development of lateral line organs in the axolotl. *J. Comp. Neurol.* 340, 480–514.
- Novince, Z.M., Azodi, E., Marrs, J.A., Raymond, P.A., Liu, Q., 2003. Cadherin expression in the inner ear of developing zebrafish. *Gene Expression Pattern* 3, 337–339.
- Novoselov, V.V., Alexandrova, E.M., Ermakova, G.V., Zaraisky, A.G., 2003. Expression zones of three novel genes about the developing anterior neural plate of *Xenopus* embryo. *Gene Expr. Patt.* 3, 225–230.
- Ogino, H., Yasuda, K., 2000. Sequential activation of transcription factors in lens induction. *Dev. Growth Differ.* 42, 437–448.
- Ohto, H., Takizawa, T., Saito, T., Kobayashi, M., Ikeda, K., Kawakami, K., 1998. Tissue and developmental distribution of Six family gene products. *Int. J. Dev. Biol.* 42, 141–148.
- Ohto, H., Kamada, S., Tago, K., Tominaga, S., Ozaki, H., Sato, S., Kawakami, K., 1999. Cooperation of Six and Eya in activation of their target genes through nuclear translocation of Eya. *Mol. Cell. Biol.* 19, 6815–6824.
- Ohshima, T., Groves, A.K., 2004. Expression of mouse Foxi class genes in early craniofacial development. *Dev. Dyn.* 231, 640–646.
- Oliver, G., Wehr, R., Jenkins, A., Copeland, N.G., Cheyette, B.N.R., Hartenstein, V., Zipursky, S.L., Gruss, P., 1995a. Homeobox genes and connective tissue patterning. *Development* 121, 693–705.
- Oliver, G., Mailhos, A., Wehr, R., Copeland, N.G., Jenkins, N.A., Gruss, P., 1995b. Six3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. *Development* 121, 4045–4055.
- Oliver, G., Loosli, F., Köster, R., Wittbrodt, J., Gruss, P., 1996. Ectopic lens induction in fish in response to the murine homeobox gene Six3. *Mech. Dev.* 60, 233–239.
- Onitsuka, I., Takeda, M., Maeno, M., 2000. Expression and function of Xmsx-2B in dorso-ventral axis formation in gastrula embryos. *Zool. Sci.* 17, 1107–1113.
- Ooi, G.T., Tawadros, N., Escalona, R.M., 2004. Pituitary cell lines and their endocrine applications. *Mol. Cell. Endocrinol.* 228, 1–21.
- Orts-LLorca, F., Jimenez-Collado, J., 1971. Regulation of the embryo after the extirpation of Hensen's node. Consequences on the differentiation of the otic placode. *Arch. Anat. Histol. Embryol.* 54, 1–11.
- Ozaki, H., Watanabe, Y., Takahashi, K., Kitamura, K., Tanaka, A., Urase, K., Momoi, T., Sudo, K., Sakagami, J., Asano, M., Iwakura, Y., Kawakami, K., 2001. Six4, a putative myogenin gene regulator, is not essential for mouse embryonal development. *Mol. Cell. Biol.* 21, 3343–3350.
- Ozaki, H., Nakamura, K., Funahashi, J., Ikeda, K., Yamada, G., Tokano, H., Okamura, H.O., Kitamura, K., Muto, S., Kotaki, H., Sudo, K., Horai, R., Iwakura, Y., Kawakami, K., 2004. Six1 controls patterning of the mouse otic vesicle. *Development* 131, 551–562.
- Pandur, P.D., Moody, S.A., 2000. *Xenopus* Six1 gene is expressed in neurogenic cranial placodes and maintained in differentiating lateral lines. *Mech. Dev.* 96, 253–257.
- Panganiban, G., Rubenstein, J.L., 2002. Developmental functions of the Distal-less/Dlx homeobox genes. *Development* 129, 4371–4386.
- Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G., Boncinelli, E., 1995. The *Xenopus* homologue of Otx2 is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* 121, 707–720.
- Pannese, M., Lupo, G., Kablar, B., Boncinelli, E., Barsacchi, G., Vignali, R., 1998. The *Xenopus* Emx genes identify presumptive dorsal telencephalon and are induced by head organizer signals. *Mech. Dev.* 73, 73–83.
- Papaioannou, V.E., 2001. T-box genes in development: from hydra to humans. *Int. Rev. Cytol.* 207, 1–70.
- Papalopolu, N., Kintner, C., 1993. *Xenopus*-Distal-less related homeobox genes are expressed in the developing forebrain and are induced by planar signals. *Development* 117, 961–975.
- Pappu, K.S., Mardon, G., 2004. Genetic control of retinal specification and determination in *Drosophila*. *Int. J. Dev. Biol.* 48, 913–924.
- Pappu, K.S., Ostrin, E.J., Middlebrooks, B.W., Sili, B.T., Chen, R., Atkins, M. R., Gibbs, R., Mardon, G., 2005. Dual regulation and redundant function of two eye-specific enhancers of the *Drosophila* retinal determination gene dachshund. *Development* 132, 2895–2905.
- Parhar, I.S., 2002. Cell migration and evolutionary significance of GnRH subtypes. *Prog. Brain Res.* 141, 3–17.
- Park, D., Eisthen, H.L., 2003. Gonadotropin releasing hormone (GnRH) modulates odorant responses in the peripheral olfactory system of axolotls. *J. Neurophysiol.* 90, 731–738.
- Patient, R.K., McGhee, J.D., 2002. The GATA family (vertebrates and invertebrates). *Curr. Opin. Genet. Dev.* 12, 416–422.
- Pattyn, A., Morin, X., Cremer, H., Goridis, C., Brunet, J.F., 1997. Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. *Development* 124, 4065–4075.
- Pellegrini, M., Mansouri, A., Simeone, A., Boncinelli, E., Gruss, P., 1996. Dentate gyrus formation requires Emx2. *Development* 122, 3893–3898.
- Penzel, R., Oswald, R., Chen, Y., Tacke, L., Grunz, H., 1997. Characterization and early embryonic expression of a neural specific transcription factor xSOX3 in *Xenopus laevis*. *Int. J. Dev. Biol.* 41, 667–677.
- Pera, E.M., De Robertis, E.M., 2000. A direct screen for secreted proteins in *Xenopus* embryos identifies distinct activities for the Wnt antagonists Crescent and Frzb-1. *Mech. Dev.* 96, 183–195.
- Pera, E., Stein, S., Kessel, M., 1999. Ectodermal patterning in the avian embryo: epidermis versus neural plate. *Development* 126, 63–73.

- Pevny, L., Placzek, M., 2005. SOX genes and neural progenitor identity. *Curr. Opin. Neurobiol.* 15, 7–13.
- Pevny, L.H., Sockanathan, S., Placzek, M., Lovell-Badge, R., 1998. A role for SOX1 in neural determination. *Development* 125, 1967–1978.
- Pfeffer, P.L., Gerster, T., Lun, K., Brand, M., Busslinger, M., 1998. Characterization of three novel members of the zebrafish Pax2/5/8 family: dependency of Pax5 and Pax8 expression on the Pax2.1 (noi) function. *Development* 125, 3063–3074.
- Phillips, B.T., Bolding, K., Riley, B.B., 2001. Zebrafish *fgf3* and *fgf8* encode redundant functions required for otic placode induction. *Dev. Biol.* 235, 351–365.
- Phillips, B.T., Storch, E.M., Lekven, A.C., Riley, B.B., 2004. A direct role for Fgf but not Wnt in otic placode induction. *Development* 131, 923–931.
- Piatigorsky, J., 1981. Lens differentiation in vertebrates. A review of cellular and molecular features. *Differentiation* 19, 134–153.
- Piatigorsky, J., 1998. Multifunctional lens crystallins and corneal enzymes. More than meets the eye. *Ann. N. Y. Acad. Sci.* 842, 7–15.
- Piccolo, S., Agius, E., Leyns, L., Bhattacharya, S., Grunz, H., Bouwmeester, T., De Robertis, E.M., 1999. The head inducer Cerberus is a multifunctional antagonist of nodal, BMP and Wnt signals. *Nature* 397, 707–710.
- Pichon, B., Taelman, V., Kricha, S., Christophe, D., Bellefroid, E.J., 2002. XHRT-1, a hairy and Enhancer of split related gene with expression in floor plate and hypochord during early *Xenopus* embryogenesis. *Dev. Genes Evol.* 212, 491–495.
- Pignoni, F., Hu, B., Zavitz, K.H., Xiao, J., Garrity, P.A., Zipursky, S.L., 1997. The eye-specification proteins So and Eya form a complex and regulate multiple steps in *Drosophila* eye development. *Cell* 91, 881–891.
- Piotrowski, T., Northcutt, R.G., 1996. The cranial nerves of the *Senegal bichir*, *Polypterus senegalus* [Osteichthyes: Actinopterygii: Cladistia]. *Brain Behav. Evol.* 47, 55–102.
- Piotrowski, T., Ahn, D.G., Schilling, T.F., Nair, S., Ruvinsky, I., Geisler, R., Rauch, G.J., Haffter, P., Zon, L.I., Zhou, Y., Foote, H., Dawid, I.B., Ho, R.K., 2003. The zebrafish *van gogh* mutation disrupts *tbx1*, which is involved in the DiGeorge deletion syndrome in humans. *Development* 130, 5043–5052.
- Pispa, J., Thesleff, I., 2003. Mechanisms of ectodermal organogenesis. *Dev. Biol.* 262, 195–205.
- Platt, J.B., 1894. Ontogenetische Differenzierung des Ektoderms in *Necturus*. *Archiv. Mikrosk. Anat.* 43, 911–966.
- Plendl, J., Stierstorfer, B., Sinowatz, F., 1999. Growth factors and their receptors in the olfactory system. *Anat. Histol. Embryol.* 28, 73–79.
- Pohl, B.S., Knöchel, W., 2004. Isolation and developmental expression of *Xenopus* FoxJ1 and FoxK1. *Dev. Genes Evol.* 214, 200–205.
- Pohl, B.S., Knöchel, W., 2005. Of Fox and Frogs: Fox (fork head/winged helix) transcription factors in *Xenopus* development. *Gene* 344, 21–32.
- Pohl, B.S., Knöchel, S., Dillinger, K., Knöchel, W., 2002. Sequence and expression of FoxB2 (XFD-5) and FoxI1c (XFD-10) in *Xenopus* embryogenesis. *Mech. Dev.* 117, 283–287.
- Pohl, B.S., Rossner, A., Knöchel, W., 2005. The Fox gene family in *Xenopus laevis*: FoxI2, FoxM1 and FoxP1 in early development. *Int. J. Dev. Biol.* 49, 53–58.
- Pommereit, D., Pieler, T., Hollemann, T., 2001. Xpitx3: a member of the Rieg/Pitx gene family expressed during pituitary and lens formation in *Xenopus laevis*. *Mech. Dev.* 102, 255–257.
- Pozzoli, O., Bosetti, A., Croci, L., Consalez, G.G., Vetter, M.L., 2001. Xebf3 is a regulator of neuronal differentiation during primary neurogenesis in *Xenopus*. *Dev. Biol.* 233, 495–512.
- Prouty, S.M., Levitt, P., 1993. Immunocytochemical analysis of a novel carbohydrate differentiation antigen (CDA-3C2) associated with olfactory and otic systems during embryogenesis in the rat. *J. Comp. Neurol.* 332, 444–470.
- Punzo, C., Seimiya, M., Flister, S., Gehring, W.J., Plaza, S., 2002. Differential interactions of eyeless and twin of eyeless with the sine oculis enhancer. *Development* 129, 625–634.
- Purcell, P., Oliver, G., Mardon, G., Donner, A.L., Maas, R.L., in press. Pax6-dependence of Six3, Eya1 and Dach1 expression during lens and nasal placode induction. *Gene Expression Pattern*.
- Püschel, A.W., Gruss, P., Westerfield, M., 1992a. Sequence and expression pattern of pax-6 are highly conserved between zebrafish and mice. *Development* 114, 643–651.
- Püschel, A.W., Westerfield, M., Dressler, G.R., 1992b. Comparative analysis of Pax-2 protein distributions during neurulation in mice and zebrafish. *Mech. Dev.* 38, 197–208.
- Quint, E., Zerucha, T., Ekker, M., 2000. Differential expression of orthologous Dlx genes in zebrafish and mice: implications for the evolution of the Dlx homeobox gene family. *J. Exp. Zool. (Mol. Dev. Evol.)* 288, 235–241.
- Radde-Gallwitz, K., Pan, L., Gan, L., Lin, X., Segil, N., Chen, P., 2004. Expression of *Islet1* marks the sensory and neuronal lineages in the mammalian inner ear. *J. Comp. Neurol.* 477, 412–421.
- Raft, S., Nowotschin, S., Liao, J., Morrow, B.E., 2004. Suppression of neural fate and control of inner ear morphogenesis by *Tbx1*. *Development* 131, 1801–1812.
- Ramón-Cueto, A., Avila, J., 1998. Olfactory ensheathing glia: properties and function. *Brain Res. Bull.* 46, 175–187.
- Raven, C.P., Kloos, J., 1945. Induction by medial and lateral pieces of the archenteron roof, with special reference to the determination of the neural crest. *Acta Neerl. Morphol.* 5, 348–362.
- Rayapureddi, J.P., Kattamuri, C., Steinmetz, B.D., Frankfort, B.J., Ostrin, E.J., Mardon, G., Hegde, R.S., 2003. Eyes absent represents a class of protein tyrosine phosphatases. *Nature* 426, 295–298.
- Read, E.M., Rodaway, A.R.F., Neave, B., Brandon, N., Holder, N., Patient, R.K., Walmsley, M.E., 1998. Evidence for non-axial A/P patterning in the nonneural ectoderm of *Xenopus* and zebrafish pregastrula embryos. *Int. J. Dev. Biol.* 42, 763–774.
- Rebay, I., Silver, S.J., Tootle, T.L., 2005. New vision from Eyes absent: transcription factors as enzymes. *Trends Genet.* 21, 163–171.
- Redies, C., Puelles, L., 2001. Modularity in vertebrate brain development and evolution. *Bioessays* 23, 1100–1111.
- Redkar, A., Montgomery, M., Litvin, J., 2001. Fate map of early avian cardiac progenitor cells. *Development* 128, 2269–2279.
- Reiss, J.O., Burd, G.D., 1997. Cellular and molecular interactions in the development of the *Xenopus* olfactory system. *Semin. Cell Dev. Biol.* 8, 171–179.
- Relaix, F., Buckingham, M., 1999. From insect eye to vertebrate muscle: redeployment of a regulatory network. *Genes Dev.* 13, 3171–3178.
- Represa, J., Leon, Y., Miner, C., Giraldez, F., 1991. The int-2 proto-oncogene is responsible for induction of the inner ear. *Nature* 353, 561–563.
- Rex, M., Orme, A., Uwanogo, D., Tointon, K., Wigmore, P.M., Sharpe, P.T., Scotting, P.J., 1997. Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissues. *Dev. Dyn.* 209, 323–332.
- Reyer, R.W., 1958. Studies on lens induction in *Amblystoma punctatum* and *Triturus viridescens viridescens*: I. Transplants of prospective belly ectoderm. *J. Exp. Zool.* 138, 505–555.
- Reza, H.M., Yasuda, K., 2004. Roles of Maf family proteins in lens development. *Dev. Dyn.* 229, 440–448.
- Riccomagno, M.M., Martinu, L., Mulheisen, M., Wu, D.K., Epstein, D.J., 2002. Specification of the mammalian cochlea is dependent on Sonic hedgehog. *Genes Dev.* 16, 2365–2378.
- Riccomagno, M.M., Takada, S., Epstein, D.J., 2005. Wnt-dependent regulation of inner ear morphogenesis is balanced by the opposing and supporting roles of Shh. *Genes Dev.* 19, 1612–1623.
- Richardson, G.P., Crossin, K.L., Chuong, C.M., Edelman, G.M., 1987. Expression of cell adhesion molecules during embryonic induction: III. Development of the otic placode. *Dev. Biol.* 119, 217–230.
- Riley, B.B., Phillips, B.T., 2003. Ringing in the new ear: resolution of cell interactions in otic development. *Dev. Biol.* 261, 289–312.
- Riley, B.B., Chiang, M.Y., Farmer, L., Heck, R., 1999. The deltaA gene of zebrafish mediates lateral inhibition of hair cells in the inner ear and is regulated by pax2.1. *Development* 126, 5669–5678.
- Rinkwitz-Brandt, S., Arnold, H.H., Bober, E., 1996. Regionalized expression of Nkx5-1, Nkx5-2, Pax2 and sek genes during mouse inner ear development. *Hear. Res.* 99, 129–138.
- Rizzotti, K., Lovell-Badge, R., 2005. Early development of the pituitary gland: induction and shaping of Rathke's pouch. *Rev. Endocr. Metab. Disord.* 6, 161–172.
- Robert, B., Sassoon, D., Jacq, B., Gehring, W., Buckingham, M., 1989. Hox-7, a

- mouse homeobox gene with a novel pattern of expression during embryogenesis. *EMBO J.* 8, 91–100.
- Röhlich, K., 1931. Gestaltungsbewegungen der präsumptiven Epidermis während der Neurulation und Kopfbildung bei Triton taeniatus. *Roux' Arch. Entwickl.mech.* 124, 66–81.
- Rollhäuser-ter-Horst, J., 1979. Artificial neural crest formation in amphibia. *Anat. Embryol.* 157, 113–120.
- Romanoff, A.L., 1960. *The Avian Embryo*. Macmillan, New York.
- Rosenquist, G.C., 1970. Location and movements of cardiogenic cells in the chick embryo: the heart-forming portion of the primitive streak. *Dev. Biol.* 22, 461–475.
- Rouzankina, I., Abate-Shen, C., Niswander, L., 2004. Dlx genes integrate positive and negative signals during feather bud development. *Dev. Biol.* 265, 219–233.
- Ruf, R.G., Xu, P.X., Silvius, D., Otto, E.A., Beekmann, F., Muerb, U.T., Kumar, S., Neuhaus, T.J., Kemper, M.J., Raymond Jr., R.M., Brophy, P.D., Berkman, J., Gattas, M., Hyland, V., Ruf, E.M., Schwartz, C., Chang, E.H., Smith, R.J., Stratakis, C.A., Weil, D., Petit, C., Hildebrandt, F., 2004. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1–SIX1–DNA complexes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8090–8095.
- Russell, I.J., 1976. Amphibian lateral line receptors. In: Llinás, R., Precht, W. (Eds.), *Frog Neurobiology*. Springer, Berlin, pp. 513–550.
- Ruvinsky, I., Oates, A.C., Silver, L.M., Ho, R.K., 2000. The evolution of paired appendages in vertebrates: T-box genes in the zebrafish. *Dev. Genes Evol.* 210, 82–91.
- Ruzinova, M.B., Benezra, R., 2003. Id proteins in development, cell cycle and cancer. *Trends Cell Biol.* 13, 410–418.
- Ryan, K., Butler, K., Bellefroid, E., Gurdon, J.B., 1998. *Xenopus* Eomesodermin is expressed in neural differentiation. *Mech. Dev.* 75, 155–158.
- Sahly, I., Andermann, P., Petit, C., 1999. The zebrafish *eyal* gene and its expression pattern during embryogenesis. *Dev. Genes Evol.* 209, 399–410.
- Saint-Germain, N., Lee, Y.H., Zhang, Y., Sargent, T.D., Saint-Jeannet, J.P., 2004. Specification of the otic placode depends on Sox9 function in *Xenopus*. *Development* 131, 1755–1763.
- Saka, Y., Smith, J.C., 2001. Spatial and temporal patterns of cell division during early *Xenopus* embryogenesis. *Dev. Biol.* 229, 307–318.
- Santagati, F., Rijli, F.M., 2003. Cranial neural crest and the building of the vertebrate head. *Nat. Rev. Neurosci.* 4, 806–818.
- Sasai, Y., 2001. Roles of Sox factors in neural determination: conserved signaling in evolution? *Int. J. Dev. Biol.* 45, 321–326.
- Sasai, Y., De Robertis, E.M., 1997. Ectodermal patterning in vertebrate embryos. *Dev. Biol.* 182, 5–20.
- Sasai, Y., Lu, B., Steinbeisser, H., De Robertis, E.M., 1995. Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in *Xenopus*. *Nature* 376, 333–336.
- Sasai, N., Mizuseki, K., Sasai, Y., 2001. Requirement of FoxD3-class signaling for neural crest determination in *Xenopus*. *Development* 128, 2525–2536.
- Sasaki, F., Doshita, A., Matsumoto, Y., Kuwahara, S., Tsukamoto, Y., Ogawa, K., 2003. Embryonic development of the pituitary gland in the chick. *Cells Tissues Organs* 173, 65–74.
- Sato, T., Sasai, N., Sasai, Y., 2005. Neural crest determination by co-activation of Pax3 and Zic1 genes in *Xenopus* ectoderm. *Development* 132, 2355–2363.
- Satokata, I., Maas, R., 1994. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat. Genet.* 6, 348–356.
- Satokata, I., Ma, L., Ohshima, H., Bei, M., Woo, I., Nishizawa, K., Maeda, T., Takano, Y., Uchiyama, M., Heaney, S., Peters, H., Tang, Z., Maxson, R., Maas, R., 2000. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat. Genet.* 24, 391–395.
- Sauka-Spengler, T., Le Mentec, C., Lepage, M., Mazan, S., 2002. Embryonic expression of Tbx1, a DiGeorge syndrome candidate gene, in the lamprey *Lampetra fluviatilis*. *Gene Expression Pattern* 2, 99–103.
- Savage, J.J., Yaden, B.C., Kiratipranon, P., Rhodes, S.J., 2003. Transcriptional control during mammalian anterior pituitary development. *Gene* 319, 1–19.
- Saxod, R., 1996. Ontogeny of the cutaneous sensory organs. *Microsc. Res. Tech.* 34, 313–333.
- Sbrogna, J.L., Barresi, M.J., Karlstrom, R.O., 2003. Multiple roles for Hedgehog signaling in zebrafish pituitary development. *Dev. Biol.* 254, 19–35.
- Schlosser, G., 2002a. Development and evolution of lateral line placodes in amphibians: I. Development. *Zoology* 105, 119–146.
- Schlosser, G., 2002b. Development and evolution of lateral line placodes in amphibians: II. Evolutionary diversification. *Zoology* 105, 177–193.
- Schlosser, G., 2003. Hypobranchial placodes in *Xenopus laevis* give rise to hypobranchial ganglia, a novel type of cranial ganglia. *Cell Tissue Res.* 312, 21–29.
- Schlosser, G., 2005. Evolutionary origins of vertebrate placodes: insights from developmental studies and from comparisons with other deuterostomes. *J. Exp. Zool. B Mol. Dev. Evol.* 304B, 347–399.
- Schlosser, G., Ahrens, K., 2004. Molecular anatomy of placode development in *Xenopus laevis*. *Dev. Biol.* 271, 439–466.
- Schlosser, G., Northcutt, R.G., 2000. Development of neurogenic placodes in *Xenopus laevis*. *J. Comp. Neurol.* 418, 121–146.
- Schlosser, G., Northcutt, R.G., 2001. Lateral line placodes are induced during neurulation in the axolotl. *Dev. Biol.* 234, 55–71.
- Schlosser, G., Roth, G., 1997. Evolution of nerve development in frogs: I. The development of the peripheral nervous system in *Discoglossus pictus* (Discoglossidae). *Brain Behav. Evol.* 50, 61–93.
- Schlosser, G., Kintner, C., Northcutt, R.G., 1999. Loss of ectodermal competence for lateral line placode formation in the direct developing frog *Eleutherodactylus coqui*. *Dev. Biol.* 213, 354–369.
- Schmidt, J.E., Suzuki, A., Ueno, N., Kimelman, D., 1995. Localized BMP-4 mediates dorsal ventral patterning in the early *Xenopus* embryo. *Dev. Biol.* 169, 37–50.
- Schubert, F.R., Mootosamy, R.C., Walters, E.H., Graham, A., Tumiotto, L., Munsterberg, A.E., Lumsden, A., Dietrich, S., 2002. Wnt6 marks sites of epithelial transformations in the chick embryo. *Mech. Dev.* 114, 143–148.
- Schwanzel-Fukuda, M., Pfaff, D.W., 1989. Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338, 161–164.
- Schwarz, M., Alvarez-Bolado, G., Dressler, G., Urbanek, P., Busslinger, M., Gruss, P., 1999. Pax2/5 and Pax6 subdivide the early neural tube into three domains. *Mech. Dev.* 82, 29–39.
- Schwarz, M., Cecconi, F., Bernier, G., Andrejewski, N., Kammandel, B., Wagner, M., Gruss, P., 2000. Spatial specification of mammalian eye territories by reciprocal transcriptional repression of pax2 and pax6. *Development* 127, 4325–4334.
- Schweickert, A., Steinbeisser, H., Blum, M., 2001. Differential gene expression of *Xenopus* Pitx1, Pitx2b and Pitx2c during cement gland, stomodeum and pituitary development. *Mech. Dev.* 107, 191–194.
- Schwob, J.E., 2002. Neural regeneration and the peripheral olfactory system. *Anat. Rec.* 269, 33–49.
- Scully, K.M., Rosenfeld, M.G., 2002. Pituitary development: regulatory codes in mammalian organogenesis. *Science* 295, 2231–2235.
- Selleck, M.A.J., Bronner-Fraser, M., 1995. Origins of the avian neural crest: the role of neural plate–epidermal interactions. *Development* 121, 525–538.
- Selleck, M.A.J., Garcia-Castro, M.I., Artinger, K.B., Bronner-Fraser, M., 1998. Effects of Shh and Noggin on neural crest formation demonstrate that BMP is required in the neural tube but not ectoderm. *Development* 125, 4919–4930.
- Semina, E.V., Reiter, R., Leysens, N.J., Alward, W.L., Small, K.W., Datson, N.A., Siegel-Bartelt, J., Bierke-Nelson, D., Bitoun, P., Zabel, B.U., Carey, J.C., Murray, J.C., 1996. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat. Genet.* 14, 392–399.
- Seo, H.-C., Drivenes, O., Fjose, A., 1998a. A zebrafish Six4 homologue with early expression in head mesoderm. *Biochim. Biophys. Acta* 1442, 427–431.
- Seo, H.-C., Saetre, B.O., Havik, B., Ellingsen, S., Fjose, A., 1998b. The zebrafish Pax3 and Pax7 homologues are highly conserved, encode multiple isoforms and show dynamic segment-like expression in the developing brain. *Mech. Dev.* 70, 49–63.
- Serikaku, M.A., O'Tousa, J.E., 1994. sine oculis is a homeobox gene required for *Drosophila* visual system development. *Genetics* 138, 1137–1150.
- Servetnick, M., Grainger, R.M., 1991. Changes in neural and lens competence in

- Xenopus* ectoderm: evidence for an autonomous developmental timer. *Development* 112, 177–188.
- Shamim, H., Mason, I., 1999. Expression of Fgf4 during early development of the chick embryo. *Mech. Dev.* 85, 189–192.
- Shanmugalingam, S., Houart, C., Picker, A., Reifers, F., Macdonald, R., Barth, A., Griffin, K., Brand, M., Wilson, S.W., 2000. Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon. *Development* 127, 2549–2561.
- Shen, W., Mardon, G., 1997. Ectopic eye development in *Drosophila* induced by directed dachshund expression. *Development* 124, 45–52.
- Sheng, G., Stern, C.D., 1999. Gata2 and Gata3: novel markers for early embryonic polarity and for non-neural ectoderm in the chick embryo. *Mech. Dev.* 87, 213–216.
- Sheng, H.Z., Westphal, H., 1999. Early steps in pituitary organogenesis. *Trends Genet.* 15, 236–240.
- Shi, D.L., Goisset, C., Boucaut, J.C., 1998. Expression of xfb3, a *Xenopus* frizzled family member, is restricted to the early nervous system. *Mech. Dev.* 70, 35–47.
- Shi, X., Bosenko, D.V., Zinkevich, N.S., Foley, S., Hyde, D.R., Semina, E.V., Vihtelic, T.S., 2005. Zebrafish pitx3 is necessary for normal lens and retinal development. *Mech. Dev.* 122, 513–527.
- Shibata, K., Ishimura, A., Maeno, M., 1998. GATA-1 inhibits the formation of notochord and neural tissue in *Xenopus* embryo. *Biochem. Biophys. Res. Commun.* 252, 241–248.
- Shimada, N., Aya-Murata, T., Reza, H.M., Yasuda, K., 2003. Cooperative action between L-Maf and Sox2 on delta-crystallin gene expression during chick lens development. *Mech. Dev.* 120, 455–465.
- Shirasaki, R., Pfaff, S.L., 2002. Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* 25, 251–281.
- Showell, C., Binder, O., Conlon, F.L., 2004. T-box genes in early embryogenesis. *Dev. Dyn.* 229, 201–218.
- Silver, S.J., Davies, E.L., Doyon, L., Rebay, I., 2003. Functional dissection of eyes absent reveals new modes of regulation within the retinal determination gene network. *Mol. Cell. Biol.* 23, 5989–5999.
- Simeone, A., 2000. Positioning the isthmus organizer where Otx2 and Gbx2 meet. *Trends Genet.* 16, 237–240.
- Simeone, A., Acampora, D., 2001. The role of Otx2 in organizing the anterior patterning in mouse. *Int. J. Dev. Biol.* 45, 337–345.
- Simeone, A., Gulisano, M., Acampora, D., Stornaiuolo, A., Rambaldi, M., Boncinelli, E., 1992. 2 vertebrate homeobox genes related to the *Drosophila*-empty spiracles gene are expressed in the embryonic cerebral cortex. *EMBO J.* 11, 2541–2550.
- Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M.R., Nigro, V., Boncinelli, E., 1993. A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. *EMBO J.* 12, 2735–2747.
- Simeone, A., Puelles, E., Acampora, D., 2002. The Otx family. *Curr. Opin. Genet. Dev.* 12, 409–415.
- Simonneau, L., Broders, F., Thiery, J.P., 1992. N-cadherin transcripts in *Xenopus laevis* from early tailbud to tadpole. *Dev. Dyn.* 194, 247–260.
- Simpson, T.I., Price, D.J., 2002. Pax6; a pleiotropic player in development. *Bioessays* 24, 1041–1051.
- Sive, H., Bradley, L., 1996. A sticky problem—The *Xenopus* cement gland as a paradigm for anteroposterior patterning. *Dev. Dyn.* 205, 265–280.
- Smith, S.C., 1996. Pattern formation in the urodele mechanoreceptive lateral line—What features can be exploited for the study of development and evolution. *Int. J. Dev. Biol.* 40, 727–733.
- Smith, W.C., Harland, R.M., 1992. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell* 70, 829–840.
- Solomon, K.S., Fritz, A., 2002. Concerted action of two dlx paralogs in sensory placode formation. *Development* 129, 3127–3136.
- Solomon, K.S., Kudoh, T., Dawid, I.B., Fritz, A., 2003a. Zebrafish foxl1 mediates otic placode formation and jaw development. *Development* 130, 929–940.
- Solomon, K.S., Logsdon Jr., J.M., Fritz, A., 2003b. Expression and phylogenetic analyses of three zebrafish Foxl class genes. *Dev. Dyn.* 228, 301–307.
- Solomon, K.S., Kwak, S.J., Fritz, A., 2004. Genetic interactions underlying otic placode induction and formation. *Dev. Dyn.* 230, 419–433.
- Somoza, G.M., Miranda, L.A., Strobl-Mazzulla, P., Guilgur, L.G., 2002. Gonadotropin-releasing hormone (GnRH): from fish to mammalian brains. *Cell. Mol. Neurobiol.* 22, 589–609.
- Song, J., Northcutt, R.G., 1991. Morphology, distribution and innervation of the lateral-line receptors of the Florida gar, *Lepisosteus platyrhynchus*. *Brain Behav. Evol.* 37, 10–37.
- Spokony, R.F., Aoki, Y., Saint-Germain, N., Magner-Fink, E., Saint-Jeannet, J.P., 2002. The transcription factor Sox9 is required for cranial neural crest development in *Xenopus*. *Development* 129, 421–432.
- St. Amand, A.L., Klymkowsky, M.W., 2001. Cadherins and catenins, Wnts and SOXs: embryonic patterning in *Xenopus*. *Int. Rev. Cytol.* 291–355.
- Stark, M.R., Sechrist, J., Bronner-Fraser, M., Marcelle, C., 1997. Neural tube–ectoderm interactions are required for trigeminal placode formation. *Development* 124, 4287–4295.
- Steger, D.J., Hecht, J.H., Mellon, P.L., 1994. GATA-binding proteins regulate the human gonadotropin alpha-subunit gene in the placenta and pituitary gland. *Mol. Cell. Biol.* 14, 5592–5602.
- Stern, C.D., 2005. Neural induction: old problem, new findings, yet more questions. *Development* 132, 2007–2021.
- Stone, L.S., 1922. Experiments on the development of the cranial ganglia and the lateral line sense organs in *Amblystoma punctatum*. *J. Exp. Zool.* 35, 421–496.
- Stoykova, A., Gotz, M., Gruss, P., Price, J., 1997. Pax6-dependent regulation of adhesive patterning, R-cadherin expression and boundary formation in developing forebrain. *Development* 124, 3765–3777.
- Street, S.F., 1937. The differentiation of the nasal area of the chick embryo in grafts. *J. Exp. Zool.* 77, 49–80.
- Streit, A., 2002. Extensive cell movements accompany formation of the otic placode. *Dev. Biol.* 249, 237–254.
- Streit, A., 2004. Early development of the cranial sensory nervous system: from a common field to individual placodes. *Dev. Biol.* 276, 1–15.
- Streit, A., Stern, C.D., 1999a. Establishment and maintenance of the border of the neural plate in the chick: involvement of FGF and BMP activity. *Mech. Dev.* 82, 51–66.
- Streit, A., Stern, C.D., 1999b. Neural induction—A bird's eye view. *Trends Genet.* 15, 20–24.
- Streit, A., Lee, K.J., Woo, I., Roberts, C., Jessell, T.M., Stern, C.D., 1998. Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. *Development* 125, 507–519.
- Suh, H., Gage, P.J., Drouin, J., Camper, S.A., 2002. Pitx2 is required at multiple stages of pituitary organogenesis: pituitary primordium formation and cell specification. *Development* 129, 329–337.
- Suzuki, T., Saigo, K., 2000. Transcriptional regulation of atonal required for *Drosophila* larval eye development by concerted action of eyes absent, sine oculis and hedgehog signaling independent of fused kinase and cubitus interruptus. *Development* 127, 1531–1540.
- Suzuki, A., Ueno, N., Hemmati-Brivanlou, A., 1997. *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. *Development* 124, 3037–3044.
- Szeto, D.P., Ryan, A.K., O'Connell, S.M., Rosenfeld, M.G., 1996. P-OTX: a PIT-1-interacting homeodomain factor expressed during anterior pituitary gland development. *Proc. Natl. Acad. Sci. U. S. A.* 93, 7706–7710.
- Szeto, D.P., Rodriguez-Esteban, C., Ryan, A.K., O'Connell, S.M., Liu, F., Kiuoussi, C., Gleiberman, A.S., Izpisua-Belmonte, J.C., Rosenfeld, M.G., 1999. Role of the Bicoid-related homeodomain factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. *Genes Dev.* 13, 484–494.
- Taira, M., Hayes, W.P., Otani, H., Dawid, I.B., 1993. Expression of LIM class homeobox gene Xlim-3 in *Xenopus* development is limited to neural and neuroendocrine tissues. *Dev. Biol.* 159, 245–256.
- Takabatake, Y., Takabatake, T., Takeshima, K., 2000. Conserved and divergent expression of T-box genes Tbx2–Tbx5 in *Xenopus*. *Mech. Dev.* 91, 433–437.
- Takabatake, Y., Takabatake, T., Sasagawa, S., Takeshima, K., 2002. Conserved expression control and shared activity between cognate T-box genes Tbx2

- and Tbx3 in connection with Sonic hedgehog signaling during *Xenopus* eye development. *Dev. Growth Differ.* 44, 257–271.
- Takebayashi, K., Yakahashi, S., Yokota, C., Tsuda, H., Nakanishi, S., Asashima, M., Kageyama, R., 1997. Conversion of ectoderm into a neural fate by ATH-3, a vertebrate basic helix–loop–helix gene homologous to *Drosophila* proneural gene atonal. *EMBO J.* 16, 384–395.
- Takuma, N., Sheng, H.Z., Furuta, Y., Ward, J.M., Sharma, K., Hogan, B.L., Pfaff, S.L., Westphal, H., Kimura, S., Mahon, K.A., 1998. Formation of Rathke's pouch requires dual induction from the diencephalon. *Development* 125, 4835–4840.
- Talikka, M., Stefani, G., Brivanlou, A.H., Zimmerman, K., 2004. Characterization of *Xenopus* Phox2a and Phox2b defines expression domains within the embryonic nervous system and early heart field. *Gene Expression Pattern* 4, 601–607.
- Tao, W., Lai, E., 1992. Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. *Neuron* 8, 957–966.
- Tarozzo, G., Peretto, P., Fasolo, A., 1995. Cell migration from the olfactory placode and the ontogeny of the neuroendocrine compartments. *Zool. Sci.* 12, 367–383.
- Thiery, J.P., Duband, J.L., Rutishauser, U., Edelman, G.M., 1982. Cell adhesion molecules in early chicken embryogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 79, 6737–6741.
- Thomas, P., Beddington, R., 1996. Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol.* 6, 1487–1496.
- Tiveron, M.C., Hirsch, M.R., Brunet, J.F., 1996. The expression pattern of the transcription factor Phox2 delineates synaptic pathways of the autonomic nervous system. *J. Neurosci.* 16, 7649–7660.
- Tootle, T.L., Silver, S.J., Davies, E.L., Newman, V., Latek, R.R., Mills, I.A., Selengut, J.D., Parlikar, B.E., Rebay, I., 2003. The transcription factor eyes absent is a protein tyrosine phosphatase. *Nature* 426, 299–302.
- Toresson, H., Martinez-Barbera, J.P., Bardsley, A., Caubit, X., Krauss, S., 1998. Conservation of BF-1 expression in amphioxus and zebrafish suggests evolutionary ancestry of anterior cell types that contribute to the vertebrate forebrain. *Dev. Genes Evol.* 208, 431–439.
- Torres, M., Giraldez, F., 1998. The development of the vertebrate inner ear. *Mech. Dev.* 71, 5–21.
- Torres, M., Gómez-Pardo, E., Gruss, P., 1996. Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development* 122, 3381–3391.
- Tour, E., Pillemer, G., Gruenbaum, Y., Fainsod, A., 2001. The two *Xenopus* Gbx2 genes exhibit similar, but not identical expression patterns and can affect head formation. *FEBS Lett.* 507, 205–209.
- Tour, E., Pillemer, G., Gruenbaum, Y., Fainsod, A., 2002. Gbx2 interacts with Otx2 and patterns the anterior–posterior axis during gastrulation in *Xenopus*. *Mech. Dev.* 112, 141–151.
- Treier, M., Gleiberman, A.S., O'Connell, S.M., Szeto, D.P., McMahon, J.A., McMahon, A.P., Rosenfeld, M.G., 1998. Multistep signaling requirements for pituitary organogenesis in vivo. *Genes Dev.* 12, 1691–1704.
- Tremblay, P., Kessel, M., Gruss, P., 1995. A transgenic neuroanatomical marker identifies cranial neural crest deficiencies associated with the Pax3 mutant *Spotch*. *Dev. Biol.* 171, 317–329.
- Tribulo, C., Aybar, M.J., Nguyen, V.H., Mullins, M.C., Mayor, R., 2003. Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. *Development* 130, 6441–6452.
- Tribulo, C., Aybar, M.J., Sanchez, S.S., Mayor, R., 2004. A balance between the anti-apoptotic activity of Slug and the apoptotic activity of msx1 is required for the proper development of the neural crest. *Dev. Biol.* 275, 325–342.
- Tsuiji, S., Cho, K.W., Hashimoto, C., 2003. Expression pattern of a basic helix–loop–helix transcription factor Xhair2b during *Xenopus laevis* development. *Dev. Genes Evol.* 213, 407–411.
- Turner, D.L., Weintraub, H., 1994. Expression of achaete–scute homolog 3 in *Xenopus* embryos converts ectodermal cells to a neural fate. *Genes Dev.* 8, 1434–1447.
- Tyas, D.A., Pearson, H., Rashbass, P., Price, D.J., 2003. Pax6 regulates cell adhesion during cortical development. *Cereb. Cortex* 13, 612–619.
- Tzeng, S.F., 2003. Inhibitors of DNA binding in neural cell proliferation and differentiation. *Neurochem. Res.* 28, 45–52.
- Uchida, K., Murakami, Y., Kuraku, S., Hirano, S., Kuratani, S., 2003. Development of the adenohypophysis in the lamprey: evolution of epigenetic patterning programs in organogenesis. *J. Exp. Zool. Part B Mol. Dev. Evol.* 300, 32–47.
- Underhill, D.A., 2000. Genetic and biochemical diversity in the Pax gene family. *Biochem. Cell. Biol.* 78, 629–638.
- van Heyningen, V., Williamson, K.A., 2002. PAX6 in sensory development. *Hum. Mol. Genet.* 11, 1161–1167.
- van Raamsdonk, C.D., Tilghman, S.M., 2000. Dosage requirement and allelic expression of PAX6 during lens placode formation. *Development* 127, 5439–5448.
- van Wijhe, J.W., 1883. Über die Mesodermsegmente und die Entwicklung der Nerven des Selachierkopfes. *Verhandl. Konink. Akad. Wetensch.* 22, 1–50.
- Varga, Z.M., Amores, A., Lewis, K.E., Yan, Y.L., Postlethwait, J.H., Eisen, J. S., Westerfield, M., 2001. Zebrafish smoothened functions in ventral neural tube specification and axon tract formation. *Development* 128, 3497–3509.
- Vasiliauskas, D., Stern, C.D., 2000. Expression of mouse HES-6, a new member of the Hair/Enhancer of split family of bHLH transcription factors. *Mech. Dev.* 98, 133–137.
- Vendrell, V., Carnicero, E., Giraldez, F., Alonso, M.T., Schimmang, T., 2000. Induction of inner ear fate by FGF3. *Development* 127, 2011–2019.
- Verwoerd, C.D.A., van Oostrom, C.G., 1979. Cephalic neural crest and placodes. *Adv. Anat., Embryol. Cell Biol.* 58, 1–75.
- Vignali, R., Colombetti, S., Lupo, G., Zhang, W., Stachel, S., Harland, R.M., Barsacchi, G., 2000. Xotx5b, a new member of the Otx gene family, may be involved in anterior and eye development in *Xenopus laevis*. *Mech. Dev.* 96, 3–13.
- Villanueva, S., Glavic, A., Ruiz, P., Mayor, R., 2002. Posteriorization by FGF, Wnt, and retinoic acid is required for neural crest induction. *Dev. Biol.* 241, 289–301.
- Vincent, C., Kalatzis, V., Abdelhak, S., Chaib, H., Compain, S., Helias, J., Vaneecloo, F.M., Petit, C., 1997. BOR and BO syndromes are allelic defects of EYA1. *Eur. J. Hum. Genet.* 5, 242–246.
- Vitelli, F., Viola, A., Morishima, M., Pramparo, T., Baldini, A., Lindsay, E., 2003. TBX1 is required for inner ear morphogenesis. *Hum. Mol. Genet.* 12, 2041–2048.
- Vogt, W., 1929. Gestaltungsanalyse am amphibienkeim mit örtlicher vitalfärbung: II. Gastrulation und mesodermbildung bei urodelen und anuren. *Wilhelm Roux's Arch. Dev. Biol.* 120, 384–706.
- Vokes, S.A., Krieg, P.A., 2000. Gdf16, a novel member of the growth/differentiation factor subgroup of the TGF-beta superfamily, is expressed in the hindbrain and epibranchial placodes. *Mech. Dev.* 95, 279–282.
- Von Bartheld, C.S., 2004. The terminal nerve and its relation with extrabulbar “olfactory” projections: lessons from lampreys and lungfishes. *Microsc. Res. Tech.* 65, 13–24.
- von Bubnoff, A., Schmidt, J.E., Kimelman, D., 1996. The *Xenopus laevis* homeobox gene Xgbx-2 is an early marker of anteroposterior patterning in the ectoderm. *Mech. Dev.* 54, 149–160.
- von Kupffer, C., 1891. The development of the cranial nerves of vertebrates. *J. Comp. Neurol.* 1, 246–264, 315–332.
- von Kupffer, C., 1895. Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten: III. Die Entwicklung der Kopfnerven von *Ammocoetes planeri*. J.F. Lehmann, Munich.
- von Kupffer, C., 1900. Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten: IV. Zur Kopfentwicklung von *Bdellostoma*. J.F. Lehmann, Munich.
- Walmsley, M.E., Guille, M.J., Bertwistle, D., Smith, J.C., Pizzey, J.A., Patient, R.K., 1994. Negative control of *Xenopus* GATA-2 by activin and noggin with eventual expression in precursors of the ventral blood islands. *Development* 120, 2519–2529.
- Wang, S.S., Tsai, R.Y., Reed, R.R., 1997. The characterization of the Olf-1/EBF-like HLH transcription factor family: implications in olfactory gene regulation and neuronal development. *J. Neurosci.* 17, 4149–4158.
- Wang, X.K., Emelyanov, A., SleptsovaFriedrich, I., Korzh, V., Gong, Z.Y., 2001. Expression of two novel zebrafish iroquois homologues (Zirol and

- ziro5) during early development of axial structures and central nervous system. *Mech. Dev.* 105, 191–195.
- Washausen, S., Obermayer, B., Brunnett, G., Kuhn, H.J., Knabe, W., 2005. Apoptosis and proliferation in developing, mature, and regressing epibranchial placodes. *Dev. Biol.* 278, 86–102.
- Watkins-Chow, D., Camper, S.A., 1998. How many homeobox genes does it take to make a pituitary gland? *Trends Genet.* 14, 284–290.
- Wawersik, S., Maas, R.L., 2000. Vertebrate eye development as modeled in *Drosophila*. *Hum. Mol. Genet.* 9, 917–925.
- Wawersik, S., Purcell, P., Rauchman, M., Dudley, A.T., Robertson, E.J., Maas, R., 1999. BMP7 acts in murine lens placode development. *Dev. Biol.* 207, 176–188.
- Wayne, S., Robertson, N.G., DeClau, F., Chen, N., Verhoeven, K., Prasad, S., Tranebjarg, L., Morton, C.C., Ryan, A.F., Van Camp, G., Smith, R.J., 2001. Mutations in the transcriptional activator EYA4 cause late-onset deafness at the DFNA10 locus. *Hum. Mol. Genet.* 10, 195–200.
- Webb, J.F., Noden, D.M., 1993. Ectodermal placodes—Contributions to the development of the vertebrate head. *Am. Zool.* 33, 434–447.
- Wegner, M., 1999. From head to toes: the multiple facets of Sox proteins. *Nucleic Acids Res.* 27, 1409–1420.
- Weinstein, D.C., Hemmati-Brivanlou, A., 1999. Neural induction. *Annu. Rev. Cell Dev. Biol.* 15, 411–433.
- Whitfield, T.T., Granato, M., van Eeden, F.J., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Nüsslein-Volhard, C., 1996. Mutations affecting development of the zebrafish inner ear and lateral line. *Development* 123, 241–254.
- Whitfield, T.T., Riley, D.B., Chiang, M.Y., Phillips, B., 2002. Development of the zebrafish inner ear. *Dev. Dyn.* 223, 427–458.
- Whitlock, K.E., 2005. Origin and development of GnRH neurons. *Trends Endocrinol. Metab.* 16, 145–151.
- Whitlock, K.E., Westerfield, M., 2000. The olfactory placodes of the zebrafish form by convergence of cellular fields at the edge of the neural plate. *Development* 127, 3645–3653.
- Whitlock, K.E., Wolf, C.D., Boyce, M.L., 2003. Gonadotropin-releasing hormone (GnRH) cells arise from cranial neural crest and adenohypophyseal regions of the neural plate in the zebrafish, *Danio rerio*. *Dev. Biol.* 257, 140–152.
- Wilson, V., Conlon, F.L., 2002. The T-box family. *Genome Biol.* 3, 3008.1–3008.7.
- Wilson, S.I., Edlund, T., 2001. Neural induction: toward a unifying mechanism. *Nat. Neurosci.* 4, 1161–1168 (Suppl.).
- Wilson, R., Mohun, T., 1995. Xlhx, a dominant negative regulator of bHLH function in early *Xenopus* embryos. *Mech. Dev.* 49, 211–222.
- Winklbauer, R., 1989. Development of the lateral line system in *Xenopus*. *Prog. Neurobiol.* 32, 181–206.
- Woda, J.M., Pastagia, J., Mercola, M., Artinger, K.B., 2003. Dlx proteins position the neural plate border and determine adjacent cell fates. *Development* 130, 331–342.
- Wood, H.B., Episkopou, V., 1999. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mech. Dev.* 86, 197–201.
- Woods, C., Montcouquiol, M., Kelley, M.W., 2004. Math1 regulates development of the sensory epithelium in the mammalian cochlea. *Nat. Neurosci.* 7, 1310–1318.
- Wray, S., 2002. Development of gonadotropin-releasing hormone-1 neurons. *Front. Neuroendocrinol.* 23, 292–316.
- Wray, S., Grant, P., Gainer, H., 1989. Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proc. Natl. Acad. Sci. U. S. A.* 86, 8132–8136.
- Wright, T.J., Mansour, S.L., 2003. Fgf3 and Fgf10 are required for mouse otic placode induction. *Development* 130, 3379–3390.
- Wright, T.J., Ladher, R., McWhirter, J., Murre, C., Schoenwolf, G.C., Mansour, S.L., 2004. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev. Biol.* 269, 264–275.
- Wu, J., Saint-Jeannet, J.P., Klein, P.S., 2003. Wnt-frizzled signaling in neural crest formation. *Trends Neurosci.* 26, 40–45.
- Wu, J., Yang, J., Klein, P.S., 2005. Neural crest induction by the canonical Wnt pathway can be dissociated from anterior–posterior neural patterning in *Xenopus*. *Dev. Biol.* 279, 220–232.
- Wurst, W., Bally-Cuif, L., 2001. Neural plate patterning: upstream and downstream of the isthmus organizer. *Nat. Rev., Neurosci.* 2, 99–108.
- Xiang, M., Gan, L., Li, D., Chen, Z.Y., Zhou, L., O'Malley Jr., B.W., Klein, W., Nathans, J., 1997. Essential role of POU-domain factor Brn-3c in auditory and vestibular hair cell development. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9445–9450.
- Xiang, M., Gao, W.Q., Hasson, T., Shin, J.J., 1998. Requirement for Brn-3c in maturation and survival, but not in fate determination of inner ear hair cells. *Development* 125, 3935–3946.
- Xu, P.-X., Woo, I., Her, H., Beier, D.R., Maas, R.L., 1997a. Mouse Eya homologues of the *Drosophila* eyes absent gene require Pax6 for expression in lens and nasal placode. *Development* 124, 219–231.
- Xu, R.H., Kim, J., Taira, M., Lin, J.J., Zhang, C.H., Sredni, D., Evans, T., Kung, H.F., 1997b. Differential regulation of neurogenesis by the two *Xenopus* GATA-1 genes. *Mol. Cell. Biol.* 17, 436–443.
- Xu, P.X., Adams, J., Peters, H., Brown, M.C., Heaney, S., Maas, R., 1999. Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. *Nat. Genet.* 23, 113–117.
- Xu, P.X., Zheng, W., Laclef, C., Maire, P., Maas, R.L., Peters, H., Xu, X., 2002a. Eya1 is required for the morphogenesis of mammalian thymus, parathyroid and thyroid. *Development* 129, 3033–3044.
- Xu, L., Overbeek, P.A., Reneker, L.W., 2002b. Systematic analysis of E-, N- and P-cadherin expression in mouse eye development. *Exp. Eye Res.* 74, 753–760.
- Xu, P.X., Zheng, W., Huang, L., Maire, P., Laclef, C., Silvius, D., 2003. Six1 is required for the early organogenesis of mammalian kidney. *Development* 130, 3085–3094.
- Xuan, S., Baptista, C.A., Balas, G., Tao, W., Soares, V.C., Lai, E., 1995. Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. *Neuron* 14, 1141–1152.
- Yamamoto, N., Uchiyama, H., Ohki-Hamazaki, H., Tanaka, H., Ito, H., 1996. Migration of GnRH-immunoreactive neurons from the olfactory placode to the brain: a study using avian embryonic chimeras. *Brain Res. Dev. Brain Res.* 95, 234–244.
- Yamamoto, T.S., Takagi, C., Hyodo, A.C., Ueno, N., 2001. Suppression of head formation by Xmsx-1 through the inhibition of intracellular nodal signaling. *Development* 128, 2769–2779.
- Yanfeng, W., Saint-Jeannet, J.P., Klein, P.S., 2003. Wnt-frizzled signaling in the induction and differentiation of the neural crest. *Bioessays* 25, 317–325.
- Yang, L., Zhang, H.L., Hu, G.Z., Wang, H.Y., Abateshen, C., Shen, M.M., 1998. An early phase of embryonic Dlx5 expression defines the rostral boundary of the neural plate. *J. Neurosci.* 18, 8322–8330.
- Yntema, C.L., 1933. Experiments on the determination of the ear ectoderm in the embryo of *Amblystoma punctatum*. *J. Exp. Zool.* 65, 317–357.
- Yntema, C.L., 1937. An experimental study of the origin of the cells which constitute the VIIth and VIIIth cranial ganglia and nerves in the embryo of *Amblystoma punctatum*. *J. Exp. Zool.* 75, 75–101.
- Yntema, C.L., 1939. Self-differentiation of heterotopic ear ectoderm in the embryo of *Amblystoma punctatum*. *J. Exp. Zool.* 80, 1–17.
- Yntema, C.L., 1943. An experimental study on the origin of the sensory neurones and sheath cells of the IXth and Xth cranial nerves in *Amblystoma punctatum*. *J. Exp. Zool.* 92, 93–119.
- Yntema, C.L., 1944. Experiments on the origin of the sensory ganglia of the facial nerve in the chick. *J. Comp. Neurol.* 81, 147–167.
- Yntema, C.L., 1950. An analysis of induction of the ear from foreign ectoderm in the salamander embryo. *J. Exp. Zool.* 113, 211–244.
- Yokota, Y., 2001. Id and development. *Oncogene* 20, 8290–8298.
- Yoshida, M., Suda, Y., Matsuo, I., Miyamoto, N., Takeda, N., Kuratani, S., Aizawa, S., 1997. Emx1 and Emx2 functions in development of dorsal telencephalon. *Development* 124, 101–111.
- Yu, Y., Khan, J., Khanna, C., Helman, L., Meltzer, P.S., Merlino, G., 2004. Expression profiling identifies the cytoskeletal organizer ezrin and the developmental homeoprotein Six-1 as key metastatic regulators. *Nat. Med.* 10, 175–181.

- Zaraisky, A.G., Lukyanov, S.A., Vasiliev, O.L., Smirnov, Y.V., Belyavsky, A.V., Kazanskaya, O.V., 1992. A novel homeobox gene expressed in the anterior neural plate of the *Xenopus* embryo. *Dev. Biol.* 152, 373–382.
- Zaraisky, A.G., Ecochard, V., Kazanskaya, O.V., Lukyanov, S.A., Fesenko, I.V., Duprat, A.M., 1995. The homeobox-containing gene XANF-1 may control development of the Spemann organizer. *Development* 121, 3839–3847.
- Zeiske, E., Kasumyan, A., Bartsch, P., Hansen, A., 2003. Early development of the olfactory organ in sturgeons of the genus *Acipenser*: a comparative and electron microscopic study. *Anat. Embryol.* 206, 357–372.
- Zhang, C., Evans, T., 1996. BMP-like signals are required after the midblastula transition for blood cell development. *Dev. Genet.* 18, 267–278.
- Zhang, H., Reynaud, S., Kloc, M., Etkin, L.D., Spohr, G., 1995. Id gene activity during *Xenopus* embryogenesis. *Mech. Dev.* 50, 119–130.
- Zhang, Y., Knosp, B.M., Maconochie, M., Friedman, R.A., Smith, R.J., 2004. A comparative study of Eya1 and Eya4 protein function and its implication in branchio-oto-renal syndrome and DFNA10. *J. Assoc. Res. Otolaryngol.* 5, 295–304.
- Zhang, L., Yang, N., Huang, J., Buckanovich, R.J., Liang, S., Barchetti, A., Vezzani, C., O'Brien-Jenkins, A., Wang, J., Ward, M.R., Courreges, M.C., Fracchioli, S., Medina, A., Katsaros, D., Weber, B.L., Coukos, G., 2005. Transcriptional coactivator *Drosophila* eyes absent homologue 2 is up-regulated in epithelial ovarian cancer and promotes tumor growth. *Cancer Res.* 65, 925–932.
- Zhao, S., Nichols, J., Smith, A.G., Li, M., 2004. SoxB transcription factors specify neuroectodermal lineage choice in ES cells. *Mol. Cell. Neurosci.* 27, 332–342.
- Zheng, J.L., Shou, J.Y., Guillemot, F., Kageyama, R., Gao, W.Q., 2000. Hes1 is a negative regulator of inner ear hair cell differentiation. *Development* 127, 4551–4560.
- Zheng, W., Huang, L., Wei, Z.B., Silvius, D., Tang, B., Xu, P.X., 2003. The role of Six1 in mammalian auditory system development. *Development* 130, 3989–4000.
- Zhou, X.L., Hollemann, T., Pieler, T., Gruss, P., 2000. Cloning and expression of xSix3, the *Xenopus* homologue of murine Six3. *Mech. Dev.* 91, 327–330.
- Zhu, X., Rosenfeld, M.G., 2004. Transcriptional control of precursor proliferation in the early phases of pituitary development. *Curr. Opin. Genet. Dev.* 14, 567–574.
- Zhu, C.C., Dyer, M.A., Uchikawa, M., Kondoh, H., Lagutin, O.V., Oliver, G., 2002. Six3-mediated auto repression and eye development requires its interaction with members of the Groucho-related family of co-repressors. *Development* 129, 2835–2849.
- Zilinski, C., Brownell, I., Hashimoto, R., Medina-Martinez, O., Swindell, E.C., Jamrich, M., 2004. Expression of FoxE4 and Rx visualizes the timing and dynamics of critical processes taking place during initial stages of vertebrate eye development. *Dev. Neurosci.* 26, 294–307.
- Zilinski, C.A., Shah, R., Lane, M.E., Jamrich, M., 2005. Modulation of zebrafish pitx3 expression in the primordia of the pituitary, lens, olfactory epithelium and cranial ganglia by hedgehog and nodal signaling. *Genesis* 41, 33–40.
- Zimmerman, J.E., Bui, Q.T., Steingrimsson, E., Nagle, D.L., Fu, W., Genin, A., Spinner, N.B., Copeland, N.G., Jenkins, N.A., Bucan, M., Bonini, N.M., 1997. Cloning and characterization of two vertebrate homologs of the *Drosophila* eyes absent gene. *Genome Res.* 7, 128–141.
- Zine, A., Aubert, A., Qiu, J.P., Therianos, S., Guillemot, F., Kageyama, R., deRibaupierre, F., 2001. Hes1 and Hes5 activities are required for the normal development of the hair cells in the mammalian inner ear. *J. Neurosci.* 21, 4712–4720.
- Zou, D., Silvius, D., Fritzsche, B., Xu, P.X., 2004. Eya1 and Six1 are essential for early steps of sensory neurogenesis in mammalian cranial placodes. *Development* 131, 5561–5572.
- Zuber, M.E., Perron, M., Philpott, A., Bang, A., Harris, W.A., 1999. Giant eyes in *Xenopus laevis* by overexpression of XOptx2. *Cell* 98, 341–352.
- Zwilling, E., 1940. An experimental analysis of the development of the anuran olfactory organ. *J. Exp. Zool.* 84, 291–323.
- Zwilling, E., 1941. The determination of the otic vesicle in *Rana pipiens*. *J. Exp. Zool.* 86, 333–342.
- Zygar, C.A., Cook, T.L., Grainger, R.M., 1998. Gene activation during early stages of lens induction in *Xenopus*. *Development* 125, 3509–3519.