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Developmental Biology 275 (2004) 1–11

DEVELOPMENTAL BIOLOGY

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Review

Induction of the neural crest and the opportunities of life on the edge

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Received for publication 8 March 2004, revised 21 July 2004, accepted 23 July 2004
Available online 26 August 2004

Abstract

The neural crest is a multipotent population of migratory cells unique to the vertebrate embryo. Neural crest arises at the lateral edge of the neural plate and migrates throughout the embryo to give rise to a wide variety of cell types including peripheral and enteric neurons and glia, craniofacial cartilage and bone, smooth muscle, and pigment cells. Here we review recent studies that have addressed the role of several signaling pathways in the induction of the neural crest. Work in the mouse, chick, *Xenopus*, and zebrafish have shown that a complex network of genes is activated at the neural plate border in response to neural crest-inducing signals. We also summarize some of these findings and discuss how the differential activation of these genes may contribute to the establishment of neural crest diversity.

Keywords: Neural crest; Induction; Bmp; Wnt; Fgf; Notch/Delta; Transcriptional regulators

Introduction

The neural crest was first described in chick embryos by Wilhelm His in 1868 as "the cord in between" (Zwischenstrang), a group of cells giving rise to spinal and cranial ganglia and located between the developing neural plate and the future epidermal ectoderm (Horstadius, 1950). Since then, the neural crest has fascinated generations of developmental and evolutionary biologists.

The neural crest is a population of multipotent cells unique to the vertebrate embryo. At the end of gastrulation, the neural crest arises at the border of the non-neural ectoderm and the neural plate (Fig. 1). This border, also known as the neural fold, flanks the neural plate bilaterally. In most vertebrates, neural crest arises from the entire length of the neuraxis starting at a level caudal to the prospective diencephalon. During neurulation, the neural plate closes bringing together the neural folds at the dorsal midline. Subsequently, neural crest cells go through an epithelial-to-mesenchymal transition, allowing them to delaminate from the neuroepithelium and to migrate throughout the embryo in a rostrocaudal wave. The ability to segregate and migrate away from the neuroepithelium is one of the unique features of the developing neural crest, and for many years, the neural crest has been a preferred model to analyze the molecular basis of cell migration in normal and pathological situations (reviewed by Thiery, 2003).

While the overall pattern of neural crest migration appears to be similar between species, there are also some differences. For example, in chick, fish, and frog, neural crest migration is initiated at neural tube closure, while in the mouse neural crest migration commences before the neural tube is fully closed (reviewed by Kulesa et al., 2004). Species-specific differences in the mechanism of neurulation (primary vs. secondary) impact not only the timing of emergence of the neural crest but also its mode of induction (reviewed by Colas and Schoenwolf, 2001; Lowery and Sive, in press).

Besides its migratory capabilities, one of the most fascinating characteristics of the neural crest is its ability to generate multiple cell lineages. Once neural crest cells reach their final location in the embryo they will contribute to cartilage, bone, and connective tissue of the face, neurons, and glial cells of the peripheral nervous system, pigment cells of the skin, and mesenchyme and smooth

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doi:10.1016/j.ydbio.2004.07.033

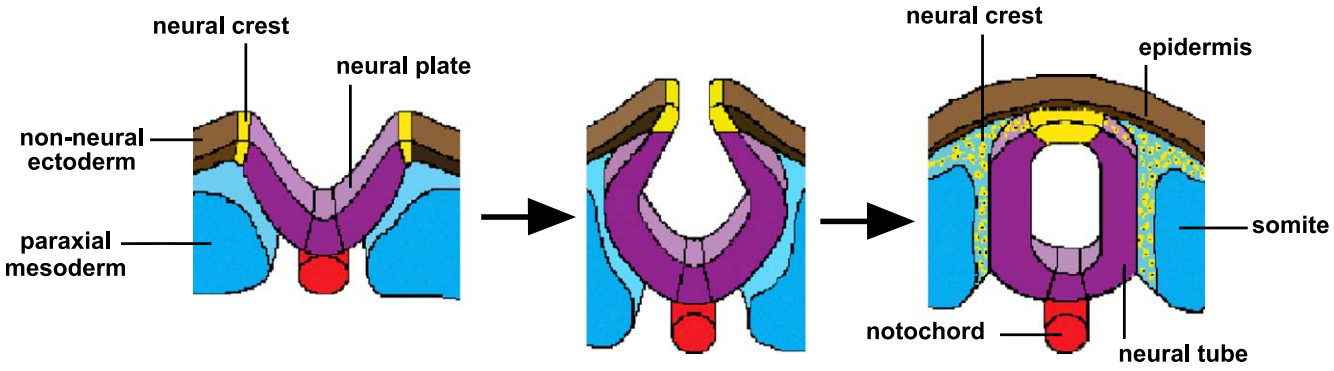

Fig. 1. The neural crest is induced at the neural plate border. At the beginning of neurulation, the neural crest is positioned at the boundary between the neural plate and the non-neural ectoderm. As the neuroectoderm folds, the neural crest is positioned at the dorsal aspect of the neural tube. In most vertebrates, neural crest delamination and migration start upon neural tube closure.

muscle cells in restricted regions of the cardiovascular system (Fig. 2).

Neural crest cells originating from different levels along the anteroposterior axis form distinct sets of derivatives. For example, only cranial neural crest gives rise to craniofacial cartilage, while sensory neurons and glia are trunk-specific neural crest derivatives. However, in avian embryos, heterotopic transplantations of segments of the neural crest from different axial levels indicate that these premigratory cells are highly plastic. In most instances, the neural crest cells derived from the ectopically grafted tissues migrate into positions characteristic for their new location on the neuraxis and subsequently adopt the appropriate fate for this position (reviewed by LeDouarin and Kalcheim, 1999; Trainor and Krumlauf, 2001). The developmental potential of neural crest cells along the anteroposterior axis is therefore much greater than the fate they will normally assume.

Because of its contribution to multiple lineages, abnormal development of the neural crest and its derivatives can have dramatic consequences for many different organ systems. These pathologies, known as neurocristopathies (Bolande, 1997), include conditions such as Waardenburg–Shah syndrome (hypopigmentation and aganglionic megacolon), frontonasal dysplasia (multiple craniofacial defects), and DiGeorge syndrome (craniofacial and heart defects). Therefore, defining the mechanisms of neural crest formation and diversification represents an important step in understanding the basis of these pathologies.

While in the past two decades much has been learned about the migration of the neural crest (reviewed by LeDouarin and Kalcheim, 1999), still a great deal remains to be known about the specification of the neural crest at the neural plate border and about the processes involved in defining the fates of individual neural crest progenitors.

Induction of the neural crest

The induction of neural crest is a multistep process, starting at the early gastrula stage and continuing until neural tube closure. Neural crest induction can be monitored by the differentiation of pigment cells, which can be easily identified macroscopically, or by the expression of neural crest-specific genes. These genes include several families of transcriptional regulators (reviewed by Gammill and Bronner-fraser, 2003), among which the zinc finger transcription factor *Slug*, has become one of the most reliable markers for premigratory neural crest cells in chick and amphibian embryos (Mayor et al., 1995; Nieto et al., 1994).

At the time of its induction the neural crest is flanked by the neural plate on one side and the non-neural ectoderm on the other side and is overlaying the paraxial mesoderm (Fig. 1). Because of their position relative to the neural crest each one of these tissues has been proposed as a source of inducer of neural crest fate.

Transplantation experiments in amphibian embryos have established that interactions between the neural plate and the surrounding non-neural ectoderm are involved in neural crest formation (Moury and Jacobson, 1989). In chick and *Xenopus* embryos, grafts of neural plate explants into the adjacent non-neural ectoderm induce expression of *Slug* at the boundary, and lineage tracing studies indicate that *Slug*-positive cells derive from both the graft and host tissues (Mancilla and Mayor, 1996; Selleck and Bronner-Fraser, 1995).

Early experiments of Raven and Kloos (1945) demonstrated that lateral plate mesoderm transplanted into the blastocoel had the ability to induce ectopic neural crest in amphibians. Since then, a number of groups have confirmed a potential role for paraxial mesoderm in neural crest induction in chick (Selleck and Bronner-Fraser, 1995) and *Xenopus* (Bonstein et al., 1998; Marchant et al., 1998; Monsoro-Burq et al., 2003). For instance, paraxial mesoderm can induce formation of melanocytes in chick neural plate explants (Selleck and Bronner-Fraser, 1995). Removal of the presumptive paraxial mesoderm in *Xenopus* embryos results in reduced *Slug* expression and recombination of ectoderm explants with paraxial mesoderm can activate *Slug* expression and induce melanocyte formation (Bonstein et al., 1998). However, so far there is no evidence that paraxial

mesoderm plays a role in neural crest induction in mice or zebrafish. Mouse embryos carrying a targeted deletion of the T-box gene *Tbx6* (Chapman and Papaioannou, 1998) or of the signaling molecule Wnt-3a (Yoshikawa et al., 1997) lack some paraxial mesoderm, and these embryos appear to form normal neural crest derivatives, suggesting that paraxial mesoderm may not be as critical in mammals in generating neural crest.

In the last 10 years, a great deal of effort has been put into the identification of the signaling molecules involved in the induction of the neural crest. At least four distinct signaling pathways have been implicated in this process in different species. These include the bone morphogenetic proteins (Bmp), the Wnts, the fibroblast growth factors (Fgf), and more recently the Notch/Delta signaling pathways (Fig. 3).

Bmp signaling

The role of Bmp signaling in neural crest induction is tightly linked to the induction of the neural plate. During gastrulation, signals derived from the dorsal mesoderm induce the neural plate, making it distinct from the adjacent non-neural ectoderm. Experiments performed primarily in *Xenopus* embryos have shown that these inductive signals, including noggin, chordin, and follistatin, share the same molecular property, the ability to block Bmp4 signaling in the ectoderm, thereby eliciting a neural differentiation program (reviewed by Sasai and De Robertis, 1997; Weinstein and Hemmati-Brivanlou, 1999; Wilson and Edlund, 2001). Therefore, a model for neural induction suggests that neural fate is acquired in response to the activity of dorsal mesoderm-specific Bmp antagonists. In zebrafish, a mutation in the chordin gene (known as *dino* or *chordino*) causes embryos to develop a reduced neural plate (Hammerschmidt et al., 1996), providing genetic evidence that BMP signaling inhibition in the ectoderm is necessary to promote neural development.

A requirement for Bmp antagonists to generate neural fate is not as firmly established in chick and mouse embryos. For example, chordin/noggin double mutant mouse embryos form a normal neural plate (Bachiller et al., 2000), and in the chick the timing and pattern of expression of noggin, chordin, and follistatin indicate that BMP antagonists are neither sufficient nor needed to generate neural fate (Levin, 1997; Storey et al., 1992; Streit et al., 1998). Bmp inhibition in the chick epiblast appears to be mediated indirectly by Fgf signaling rather than Bmp antagonists (Streit et al., 2000; Wilson et al., 2000). Bmp transcripts are absent from epiblast explants specified as neural plate. Exposure of the epiblast to an Fgf receptor antagonist prevents down-regulation of Bmp transcripts and converts the epiblast to an epidermal fate (Wilson et al., 2000). While inhibition of Bmp signaling appears to be required in both frogs and birds to generate neural tissue, the molecular effectors involved in this process are different.

A current model of neural crest induction suggests that diffusion of Bmp antagonists in the ectoderm creates a gradient of Bmp activity, such that neural crest forms at levels of Bmp signaling intermediate to those required for formation of the neural plate (low Bmp signaling) and non-

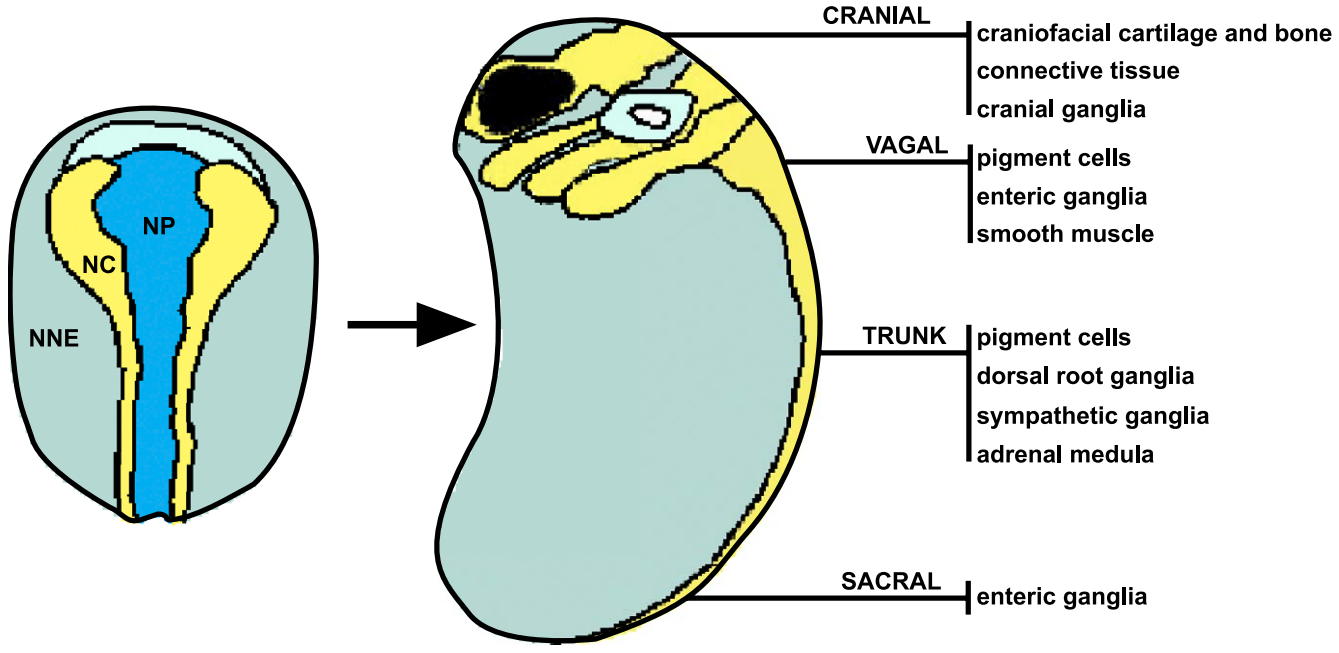

Fig. 2. The neural crest differentiates into a wide variety of cell types. Schematic representation of a *Xenopus* neurula stage embryo viewed from the dorsal side (approximately 17 h postfertilization). Anterior is to the top. Neural crest (NC) arises at the boundary between the neural plate (NP) and the non-neural ectoderm (NNE). On a lateral view of a frog embryo at the tailbud stage (approximately 30 h post fertilization), the major subdivisions of the neuraxis are shown and examples of some of the neural crest derivatives produced from different axial levels are listed. Anterior is to the top.

neural ectoderm (high Bmp signaling). This is primarily based on work performed in frog and zebrafish. In *Xenopus*, modulation of Bmp signaling in the ectoderm by over-expression of Bmp4 leads to a reduction of *Slug* expression (Mayor et al., 1995; Morgan and Sargent, 1997), while injection of Bmp antagonists or a dominant-negative Bmp receptor causes an expansion of the *Slug* expression domain (LaBonne and Bronner-Fraser, 1998; Marchant et al., 1998). Zebrafish embryos with mutations in distinct components of the Bmp signaling pathway, leading to graded Bmp signaling levels, express variable levels of neural crest markers (Nguyen et al., 1998). Although it is clear that Bmp levels are critical to specify the neural crest in these species, a gradient of Bmp proteins in the ectoderm has yet to be demonstrated.

Earlier work in the chick suggested that Bmps expressed in the neural folds and the dorsal neural tube might function as neural crest inducers. Conditioned medium from Bmp4- or Bmp7-transfected cells can induce neural crest markers in neural plate explants, mimicking the neural crest-inducing activity of the non-neural ectoderm (Liem et al., 1995, 1997). However, Bmps are only weakly and transiently expressed in the non-neural ectoderm at the time of neural crest induction, suggesting that the situation might be more complicated. Recent work has now shown that Bmp-mediated neural crest induction in these neural plate explants was highly dependent on the type of culture medium used (Garcia-Castro et al., 2002). While Bmp4 is a potent neural crest inducer in a medium containing additives (F12-N2), in a chemically defined medium (DMEM), the same signal failed to induce neural crest markers. However, under the same experimental conditions (defined medium), a Wnt signal is capable of inducing neural crest markers (Garcia-Castro et al., 2002; see below). These observations indicate that Bmp signaling is not sufficient to induce neural crest markers in chick neural plate explants and suggest that the importance of this signaling pathway for neural crest induction differs in amniotes and anamniotes (Labonne, 2002; Trainor and Krumlauf, 2002).

Wnt signaling

The involvement of Wnt signaling in neural crest induction has been well documented in several species. A large number of studies in the mouse (Brault et al., 2001; Dunn et al., 2000; Ikeya et al., 1997), chick (Garcia-Castro et al., 2002), frog (Bang et al., 1999; Chang and Hemmati-Brivanlou, 1998; Deardorff et al., 2001; LaBonne and Bronner-Fraser, 1998; Luo et al., 2003; Saint-Jeannet et al., 1997), and zebrafish (Dorsky et al., 1998, 2000; Lewis et al., 2004) have implicated Wnt signaling in neural crest formation (reviewed by Wu et al., 2003; Yanfeng et al., 2003). In *Xenopus*, ectopic expression of some Wnt family members, as well as downstream components of the pathway, enhances production of neural crest progenitors (Bang et al., 1999; Chang and Hemmati-Brivanlou, 1998; Deardorff et al., 2001; LaBonne and Bronner-Fraser, 1998; Saint-Jeannet et al., 1997), whereas inhibition of Wnt signaling blocks neural crest formation (Deardorff et al., 2001; LaBonne and Bronner-Fraser, 1998; Luo et al., 2003; Saint-Jeannet et al., 1997).

Mouse embryos with targeted inactivation of the *β-catenin* gene (a downstream component of Wnt signaling pathway) in the dorsal neural tube have severe defects in the formation of cranial and dorsal root ganglia and in several craniofacial skeletal elements of neural crest origin (Brault et al., 2001). In these embryos, it is believed that signaling through β-catenin is required for survival and/or differentiation of cranial neural crest cells (Brault et al., 2001). Compound mutant embryos lacking both Wnt-1 and Wnt-3a (two Wnts expressed in the dorsal neural tube) show defects in a broad range of neural crest derivatives and it has been proposed that Wnt signaling in the dorsal neural tube is primarily required for the expansion of neural crest progenitors (Ikeya et al., 1997). More recently, it has been shown that sustained Wnt activity in mouse neural crest progenitors had little effect on the population size and instead regulated fate decisions (Lee et al., 2004). In *Xenopus*, the increase in neural crest progenitors generated upon XWnt-3a overexpression has been shown to occur independently of cell proliferation (Saint-Jeannet et al.,

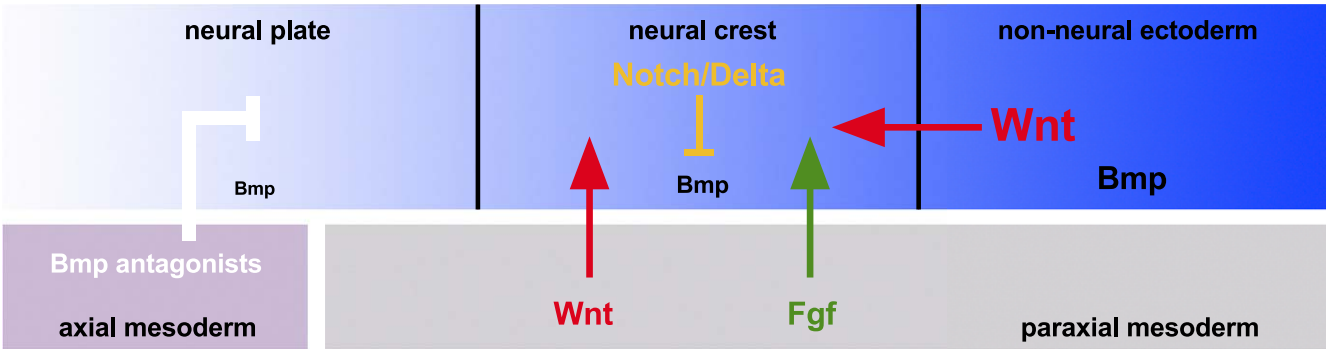

Fig. 3. Signals involved in neural crest induction. Schematic representation of the signals involved in neural crest induction at the neural plate border. Interactions between the neural plate and the surrounding epidermis (Bmp, Wnt, and Notch/Delta) as well as signals derived from the underlying paraxial mesoderm (Wnt and Fgf) are believed to be involved in neural crest induction (see text for details).

1997), suggesting that Wnt signaling may also be involved in the specification of neural crest fate. Consistent with this result, the *Slug Xenopus* promoter possesses a functional Lef/Tcf binding site (transcription factor involved in the activation of Wnt-dependent target genes), suggesting that the neural crest-specific gene *Slug* is a direct target of Wnt signaling (Vallin et al., 2001).

In chick embryos, gain- and loss-of-function experiments designed to interfere with the canonical Wnt signaling pathway indicate that Wnt ligands can function as inducers of neural crest fate (Garcia-Castro et al., 2002). Wnt-6 is expressed in the non-neural ectoderm at the time of neural crest specification and is therefore a good candidate to mediate this activity (Garcia-Castro et al., 2002; Schubert et al., 2002). However, the requirement for this specific Wnt family member during neural crest specification in the chick epiblast has not been tested directly.

While *Wnt-1* and *Wnt-3a* are expressed in the dorsal neural tube in mouse, chick, frog, and zebrafish (Hollyday et al., 1995; Molven et al., 1991; Roelink and Nusse, 1991; Wolda et al., 1993), this relatively late expression suggests that they may have a later function in neural crest development rather than in its induction. In *Xenopus*, the source of Wnt-mediated neural crest-inducing signal has been proposed to lie in the ectoderm (Wnt-7b; Chang and Hemmati-Brivanlou, 1998) and/or the paraxial mesoderm (Wnt-8; Bang et al., 1999), as the temporal expression of both ligands appears to be compatible with this function. However, recently the existence of such a paraxial mesoderm-derived Wnt signal in neural crest induction has been challenged (Monsoro-Burq et al., 2003). In this study, the authors argue that interfering with Wnt signaling in the extracellular space, using Wnt antagonists such as dominant-negative Wnt-8 (Bang et al., 1999) or Nfz8, a truncated and diffusible form of the Wnt receptor, Fz8 (Monsoro-Burq et al., 2003), prevents neural crest formation not by blocking the activity of a paraxial–mesoderm-derived Wnt signal but rather by disrupting the specification and signaling properties of the paraxial mesoderm. This view is supported by the observation that blocking the response of the ectoderm to Wnt signaling by intracellular Wnt antagonists, such as GSK3 and dominant-negative TCF3, did not inhibit the induction of neural crest markers by the paraxial mesoderm (Monsoro-Burq et al., 2003). However, since the activity of these antagonists was not directly tested in these explants, it remains possible that these two intracellular components of the Wnt pathway were not fully active at blocking Wnt function.

In zebrafish, *Wnt-8* is expressed at the appropriate time and place to have a role in neural crest induction (Lekven et al., 2001; Lewis et al., 2004). *Wnt8* morpholino-mediated knock down of Wnt-8 protein prevents expression of early neural crest markers (Lewis et al., 2004). Importantly, this process appears to occur independently from the posteriorizing activity of Wnt signaling (McGrew et al., 1995; Villanueva et al., 2002), as development of the Rohon–Beard sensory neurons, which share a common progenitor with neural crest cells, is unaffected. This activity of Wnt signaling in neural crest induction, together with its later role in promoting pigment cell fate (Dorsky et al., 1998), suggests that Wnt signaling may have multiple functions during development of the neural crest (Lewis et al., 2004).

Fgf signaling

Fgfs have also been implicated in neural crest induction in *Xenopus*. In two distinct experimental systems, dissociated cells (Kengaku and Okamoto, 1993) or intact ectoderm explants (Mayor et al., 1995), basic Fgf (bFgf) in combination with attenuation of Bmp signaling was reported to induce pigment cells and *Slug* expression, respectively. However, bFgf induces mesoderm in these explants; therefore, the emergence of neural crest cells could be the result of a mesoderm-derived crest-inducing signal (LaBonne and Bronner-Fraser, 1998; Mayor et al., 1995). More definitive evidence for the role of Fgf signaling in neural crest induction came from the overexpression of a dominant-negative Fgf receptor, which blocks expression of *Slug* in intact *Xenopus* embryos without affecting the neural plate (Mayor et al., 1997). Moreover, recombination experiments between neural plate and non-neural ectoderm indicated that activation of downstream components of the Fgf signaling pathway was required in the neural plate to induce *Slug* expression (Mayor et al., 1997), suggesting that in this experimental setting, the non-neural ectoderm was the source of the Fgf-mediated *Slug* induction.

In contrast to these observations, recent studies have defined the paraxial mesoderm as the source of Fgf-neural crest-inducing signal in *Xenopus* (Monsoro-Burq et al., 2003). This group demonstrated that paraxial mesoderm-mediated neural crest induction is blocked by expression of a dominant-negative Fgf receptor in ectoderm explants recombined with paraxial mesoderm. Furthermore, Fgf-mediated neural crest induction can occur independently from its posteriorizing activity (Cox and Hemmati-Brivanlou, 1995; Holowacz and Sokol, 1999; Lamb and Harland, 1995) arguing for a direct role of Fgf in neural crest induction (Monsoro-Burq et al., 2003).

These studies also propose that Fgf8, which is detected in the paraxial mesoderm around the time of neural crest induction, is a potential candidate to mediate the neural crest-inducing activity of the paraxial mesoderm. However, expression of Fgf8 in ectoderm is not able to recapitulate the full range of neural crest markers induced by the paraxial mesoderm and it activates only a transient expression of some of these markers (Monsoro-Burq et al., 2003). This indicates that other factors produced by the mesoderm are also required in conjunction with an Fgf signal. Since the paraxial mesoderm can induce neural plate markers (Bonstein et al., 1998), the stronger neural crest-inducing activity of the paraxial mesoderm could be explained by attenuation

of Bmp signaling in these explants. To test this possibility, it would be of interest to determine whether Fgf8 becomes a more potent neural crest inducer in the context of explants neuralized by injection of Bmp antagonists. While Fgf8 is an attractive candidate to mediate some of the paraxial mesoderm activity, its requirement for neural crest induction has not been directly tested.

Notch/Delta signaling

Signaling by the membrane-bound protein Delta and its receptor Notch has also been implicated in neural crest formation in zebrafish, frog, and chick embryos. In the zebrafish, Delta has been reported to be required for trunk neural crest formation, as Delta-deficient embryos form supernumerary Rohon–Beard sensory neurons at the expense of neural crest cells (Cornell and Eisen, 2000). However, the cranial neural crest cells are unaffected in these embryos, suggesting that trunk and cranial neural crest development are regulated by different mechanisms (Cornell and Eisen, 2000).

In *Xenopus*, constitutive activation of Notch signaling by expression of a Notch construct lacking the ligand-binding domain resulted in a dramatic expansion of neural tissues and prevented expression of epidermal and neural crest markers (Coffman et al., 1993). However, in these experiments, the underlying mesoderm tissue was also severely affected, suggesting that the effect of Notch activation on neural crest progenitors may not be direct. Recently, using a hormone-inducible version of activated Notch, Glavic et al. (2004) demonstrated that the timing of Notch activation was critical for neural crest formation. Activation of Notch during gastrulation had no effect on neural plate or mesoderm tissues while neural crest, as monitored by expression of *Slug*, was dramatically expanded. Consistent with this observation, expression of dominant-negative Delta blocked the expression of neural crest markers (Glavic et al., 2004), suggesting that the Notch pathway plays an active role in neural crest formation. Notch-mediated expansion of neural crest progenitors is believed to occur through down-regulation of Bmp4 transcripts in the ectoderm (Glavic et al., 2004).

In birds, Delta is involved in maintaining Bmp4 expression in the ectoderm and as such is believed to be indirectly required for neural crest induction. However, as activation or inhibition of Notch signaling reduced Bmp4 expression, it has been proposed that a modulation of Notch signaling might be required to generate appropriate levels of Bmp signaling in the ectoderm, thereby allowing neural crest formation (Endo et al., 2002).

Other signaling pathways

The secreted glycoprotein Noelin-1 is expressed in the open neural plate and in the neural folds of the chick embryo (Barembaum et al., 2000). Noelin-1 overexpression causes an overproduction of cranial neural crest, presumably by acting as a competence factor in the neural tube rather than a direct inducer of neural crest fate (Barembaum et al., 2000). In *Xenopus*, Noelin-1 appears to be involved in promoting neurogenesis (Moreno and Bronner-Fraser, 2001).

Retinoic acid has been shown to induce *Slug* expression in anterior neural plate explants in *Xenopus* (Villanueva et al., 2002). However, since retinoic acid functions as a posteriorizing signal in the neural tube (Papalopulu and Kintner, 1996), it is believed that *Slug* induction in these explants reflects a posteriorization of the anterior neural plate.

Speculation

The most recent advances in the neural crest field do not permit to draw a universal model of neural crest induction in fishes, frogs, chicks, and mice. Species-specific differences in the role of these signaling pathways may in fact reflect mechanistic variations in morphogenetic and inductive processes. Moreover, the relative importance of these signaling pathways during the different phases of neural crest development (specification vs. proliferation vs. maintenance vs. migration vs. diversification) is still not fully understood.

Attenuation of Bmp signaling in the ectoderm, while not sufficient, appears to be key to specify the neural crest at least in fish and frogs. Interestingly, one of the consequences of activating Notch/Delta signaling in the ectoderm is the down-regulation of Bmp4 transcription (Endo et al., 2002; Glavic et al., 2004). Similarly, Wnt and Fgf signaling can regulate the levels of Bmp transcripts in *Xenopus* and Chick ectoderm, respectively (Baker et al., 1999; Streit et al., 2000; Wilson et al., 2000). These observations suggest that during neural crest induction one of the functions of Wnt, Fgf and Notch/Delta signaling could be to act in concert with Bmp antagonists to maintain and reinforce intermediate level of Bmp activity in this narrow region of the ectoderm fated to form the neural crest. While this model may apply to the generation of neural crest in anamniotes, it is not compatible with the current view of neural crest induction in amniotes, in which the importance of Bmp signaling remains unclear.

Are these overlapping mechanisms of neural crest induction purely redundant or do they reflect a genuine requirement for distinct signaling pathways in this process? This multiplicity of signals is likely to represent a possible mechanism to establish heterogeneity within the neural crest. In this model, signal diversity may help contribute to the specification of different subsets of neural crest progenitors.

Gene activation at the neural plate border

The number of transcription factors expressed at the neural plate border in response to neural crest-inducing signals has been growing very rapidly in the last few years (reviewed by Gammill and Bronner-fraser, 2003; Fig. 4). These factors belong to several classes of transcriptional

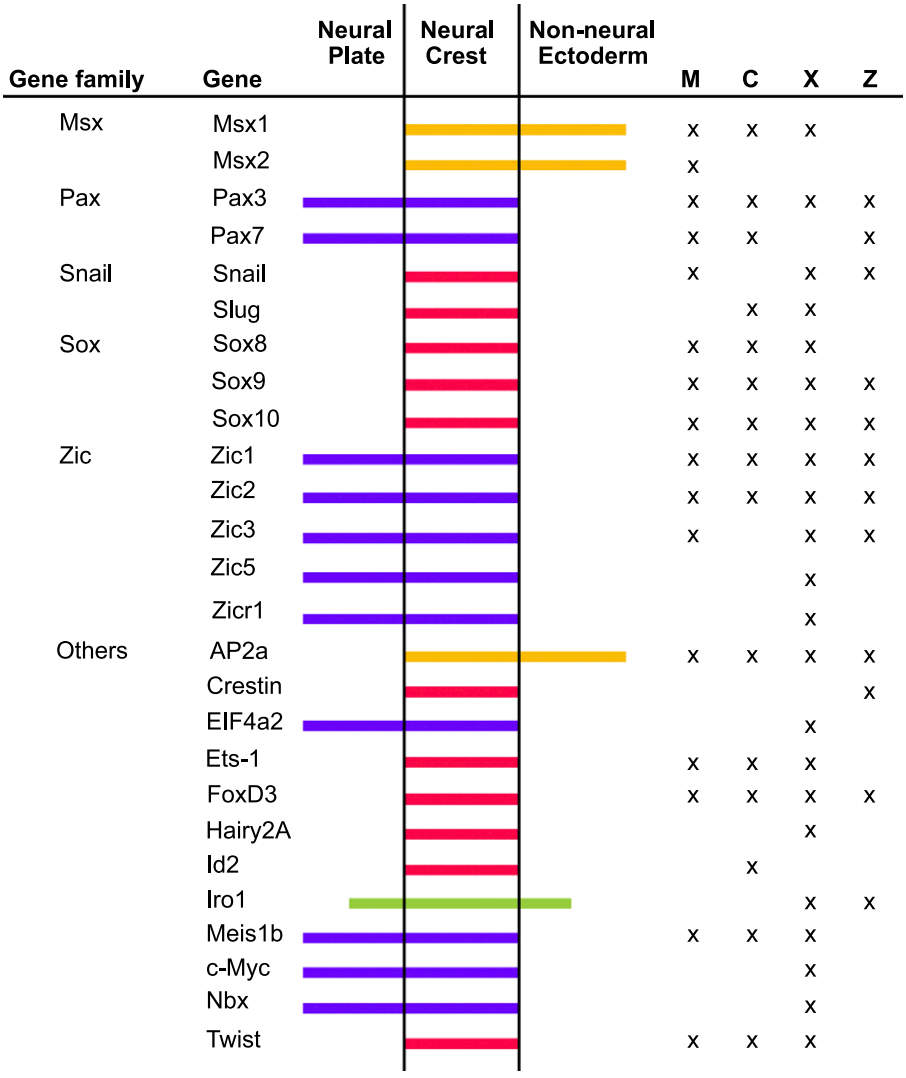

Fig. 4. Several families of transcription factors are expressed in overlapping domains in the neural crest. Shortly after neural crest induction, these factors are either limited to the neural crest tissue (red), or their domain of expression extend into the non-neural ectoderm (orange), or into the neural pate (purple), or span all three regions of the ectoderm (green). When available, the neural crest expression of these genes in mouse (M), Chick (C), *Xenopus* (X), or zebrafish (Z) embryos is indicated (see text for references).

regulators that have been shown to play important function during embryogenesis.

So far, these transcription factors include two members of the Msx family, Msx1 (Catron et al., 1996; Streit and Stern, 1999; Suzuki et al., 1997) and Msx2 (Catron et al., 1996); two paired box-containing gene, *Pax3* (Bang et al., 1997; Liem et al., 1995; Seo et al., 1998) and *Pax7* (Ericson et al., 1996; Mansouri et al., 1996; Seo et al., 1998); the snail-related genes, *Slug* (Linker et al., 2000; Mayor et al., 1995; Nieto et al., 1994) and *Snail* (Essex et al., 1993; Locascio et al., 2002); three HMG Box-containing genes, *Sox8* (Bell et al., 2000; Cheung and Briscoe, 2003; M. O'Donnell, X.H. and J.-P. S.-J., unpublished results), *Sox9* (Cheung and Briscoe, 2003; Li et al., 2002; Mori-Akiyama et al., 2003; Spokony et al., 2002), and *Sox10* (Aoki et al., 2003; Britsch et al., 2001; Cheng et al., 2000; Dutton et al., 2001; Honore et al., 2003; Southard-Smith et al., 1998); five members of the Zic family of zinc finger proteins (Nagai et al., 1997; Nakata et al., 1997, 1998, 2000; Aruga et al., 2002; Brewster et al., 1998; Grinblat and Sive, 2001; Kuo et al., 1998; Mizuseki et al., 1998; Warner et al., 2003); one member of the Ap2 family, *Ap2α* (Knight et al., 2003; Luo et al., 2003; Mitchell et al., 1991; Shen et al., 1997); a zebrafish gene, *Crestin* (Rubinstein et al., 2000); the *Xenopus* translation initiation factor EIf4a2 (Morgan and Sargent, 1997); one member of the Ets family, *Ets-1* (Maroulakou et al., 1994; Meyer et al., 1997; Tahtakran and Selleck, 2003); the winged-helix/forkhead gene, *Foxd3* (Dottori et al., 2001; Kos et al., 2001; Labosky and Kaestner, 1998; Odenthal and Nusslein-Volhard, 1998; Sasai et al., 2001); *Xenopus* Hairy2A (Glavic et al., 2004); chick *Id2* (Martinsen and Bronner-Fraser, 1998); the Iroquois gene, *Iro1* (Gomez-Skarmeta et al., 1998; Itoh et al., 2002); three unrelated *Xenopus* genes, *Meis1b*

(Maeda et al., 2001), *cMyc* (Bellmeyer et al., 2003), and *Nbx* (Kurata and Ueno, 2003); and finally the bHLH protein, Twist (Hopwood et al., 1989; Soo et al., 2002; Tavares et al., 2001).

Based on their expression pattern at the neural plate border, these factors can be classified into four groups (Fig. 4): either their expression domain is restricted to the presumptive neural crest tissue (such as *Foxd3*, *Slug*, *Ets-1*, *Twist*, and *Sox* genes), extends into the non-neural ectoderm (Ap2α and Msx genes), extends into the neural pate (such as *c-Myc*, *Nbx*, *Pax*, and *Zic* genes), or spans all three regions of the ectoderm (*Iro1*).

The function of some of these molecules has been studied in several species (reviewed by Gammill and Bronner-fraser, 2003). For example, the transcriptional repressors Slug and Snail have been implicated in the control of neural crest specification and migration in *Xenopus* (Aybar et al., 2003; Carl et al., 1999; LaBonne and Bronner-Fraser, 2000). Another transcriptional repressor, Foxd3, is involved in establishing neural crest fate in frog and chick embryos (Dottori et al., 2001; Kos et al., 2001; Sasai et al., 2001), and gain-of-function studies in chick indicate that Foxd3 may also function in promoting delamination and migration of neural crest cell from the dorsal neural tube (Dottori et al., 2001; Kos et al., 2001).

In the mouse, gene targeting of *Ap2α* caused severe reduction of craniofacial bones of neural crest origin among other defects, suggesting a requirement for Ap2α in the development of the cranial neural crest (Schorle et al., 1996; Zhang et al., 1996). The zebrafish mutant *lockjaw*, carrying a mutation in *Ap2α*, has impaired neural crest specification and migration, leading to defects in subsets of neural crest derivatives (Knight et al., 2003). The early requirement of Ap2α for neural crest induction was also demonstrated in *Xenopus* using antisense morpholino oligonucleotides (Luo et al., 2003).

Among the Sox family of transcription factors, Sox9 and Sox10 are required for specification of neural crest progenitors in *Xenopus* (Honore et al., 2003; Spokony et al., 2002) and in several species have been reported to be implicated in the development of cranial (Mori-Akiyama et al., 2003; Spokony et al., 2002) and trunk (Aoki et al., 2003; Dutton et al., 2001; Honore et al., 2003; Southard-Smith et al., 1998) neural crest derivates, respectively.

However, the function of most of these transcription factors is not fully understood and an important challenge for the years to come will be to define the organization of this regulatory network of genes at the neural plate border.

Neural crest cells diversity

Because of the diversity of its derivatives (Fig. 2), the neural crest has been a preferred model to study the mechanisms by which embryonic cells undergo cell fate restrictions. In the last few years, much effort has been directed toward understanding when the fates of individual neural crest cells become distinct from one another. This issue is still a matter of debate.

There is strong evidence that the developmental potential of neural crest cells becomes progressively restricted as they migrate into the periphery and reach their final position in the embryo. In vitro studies in chick and rat have identified several families of cytokines that can promote the differentiation of neural crest precursors along specific lineages (reviewed by Anderson, 1997; Dorsky et al., 2000; Groves and Bronner-Fraser, 1999). However, it is not clear whether the neural crest already consists of a heterogeneous population with some lineage-restricted precursor cells before the onset of migration. The multitude of transcriptional regulators expressed in the neural crest forming region shortly after its induction (Fig. 4) is quite intriguing in that respect. These factors have overlapping expression domains within the neural crest but it is not known whether all cells at the neural plate border express the same repertoire of genes or whether discrete subpopulations of neural crest progenitors can be identified based on the differential expression of some of these transcription factors. The level of resolution of in situ hybridization and the lack of specific antibodies has limited attempts to address this issue directly.

The development of new technologies to analyze the profile of gene expression in single cells (Bhattacherjee et al., 2004; Chiang and Melton, 2003; Tietjen et al., 2003) may help provide some answers to this controversial question. For example, this type of approach has proven to be quite powerful in analyzing the level of heterogeneity of the mammalian olfactory system at a single-cell level (Tietjen et al., 2003). Profiling the transcriptome of individual neural crest cells may help define the level of heterogeneity of the neural crest shortly after its induction and may provide critical information on some of the molecular determinisms underlying neural crest diversification.

Acknowledgments

We thank Peter Klein, Trish Labosky, and Christine Credidio for critical comments on the manuscript. We apologize to colleagues whose work is not cited here due to space limitations. Work in J.-P. S.-J.'s lab is supported by grants from the March of Dimes Birth Defects Foundation and National Institutes of Health (RO1-DE14212).

References

Anderson, D.J., 1997. Cellular and molecular biology of neural crest cell lineage determination. Trends Genet. 13, 337–345.

Aoki, Y., Saint-Germain, N., Gyda, M., Magner-Fink, E.K., Lee, Y.-H., Credidio, C., Saint-Jeannet, J.-P., 2003. Sox10 regulates the develop-

ment of the neural crest-derived melanocytes in *Xenopus*. Dev. Biol. 259, 19–33.

Aruga, J., Tohmonda, T., Homma, S., Mikoshiba, K., 2002. Zic1 promotes the expansion of dorsal neural progenitors in spinal cord by inhibiting neuronal differentiation. Dev. Biol. 244, 329–341.

Aybar, M.J., Nieto, A.A., Mayor, R., 2003. Snail precedes *Slug* in the genetic cascade required for the specification and migration of the *Xenopus* neural crest. Development 130, 483–494.

Bachiller, D., Klingensmith, J., Kemp, C., Belo, J.A., Anderson, R.M., May, S.R., McMahon, J.A., McMahon, A.P., Harland, R.M., Rossant, J., De Robertis, E.M., 2000. The organizer factors, Chordin and Noggin are required for mouse forebrain development. Nature 403, 658–661.

Baker, J.C., Beddington, R.S.P., Harland, R.M., 1999. Wnt signaling in *Xenopus* embryos inhibits Bmp4 expression and activates neural development. Genes Dev. 13, 3149–3159.

Bang, A.G., Papalopulu, N., Kintner, C., Goulding, M.D., 1997. Expression of Pax3 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 the posterior non-axial mesoderm. Dev. Biol. 212, 366–380.

Barembaum, M., Moreno, T.A., LaBonne, C., Sechrist, J., Bronner-Fraser, M., 2000. Noelin-1 is a secreted glycoprotein involved in generation of the neural crest. Nat. Cell Biol. 2, 219–225.

Bell, K.M., Western, P.S., Sinclair, A.H., 2000. Sox8 expression during chick embryogenesis. Mech. Dev. 94, 257–260.

Bellmeyer, A., Krase, J., Lindgren, J., LaBonne, C., 2003. The proto-oncogene c-*myc* is an essential regulator of neural crest formation in *Xenopus*. Dev. Cell 4, 827–839.

Bhattacherjee, V., Mukhopadhyay, P., Singh, S., Roberts, E.A., Hackmiller, R.C., Greene, R.M., Pisano, M.M., 2004. Laser capture microdissection of fluorescently labeled embryonic cranial neural crest cells. Genesis 39, 58–64.

Bolande, R.P., 1997. Neurocristopathy: its growth and development in 20 years. Pediatr. Pathol. Lab. Med. 17, 1–25.

Bonstein, L., Elias, S., Frank, D., 1998. Paraxial-fated mesoderm is required for neural crest induction in *Xenopus* embryos. Dev. Biol. 193, 156–168.

Brault, V., Moore, R., Kutsch, S., Ishibashi, M., Rowitch, D.S., McMahon, A.P., Sommer, L., Boussadia, O., Kemler, R., 2001. Inactivation of the β-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of cranifacial development. Development 128, 1253–1264.

Brewster, R., Lee, J., Ruiz I Altaba, A., 1998. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. Nature 393, 579–583.

Britsch, S., Goerich, D.E., Riethmacher, D., Peirano, R.I., Rossner, M., Nave, K.-A., Birchmeier, C., Wegner, M., 2001. The transcription factor Sox10 is a key regulator of peripheral gial development. Genes Dev. 15, 66–78.

Carl, T.F., Dufton, C., Hanken, J., Klymkowsky, M.W., 1999. Inhibition of neural crest migration in *Xenopus* using antisense *Slug* RNA. Dev. Biol. 213, 101–115.

Catron, K.M., Wang, H., Hu, G., Shen, M.M., Abate-Shen, C., 1996. Comparison of MSX-1 and MSX-2 suggests a molecular basis for functional redundancy. Mech. Dev. 55, 185–199.

Chang, C., Hemmati-Brivanlou, A., 1998. Neural crest induction by XWnt7B in *Xenopus*. Dev. Biol. 194, 129–134.

Chapman, D.L., Papaioannou, V.E., 1998. Three neural tubes in mouse embryos with mutations in the T-box gene *Tbx6*. Nature 391, 695–697.

Cheng, Y., Cheung, M., Abu-Elmagd, M.M., Orme, A., Scotting, P.J., 2000. Chick Sox10, a transcription factor expressed in both early neural crest cells and central nervous system. Dev. Brain Res. 121, 233–241.

Cheung, M., Briscoe, J., 2003. Neural crest development is regulated by the transcription factor Sox9. Development 130, 5681–5693.

Chiang, M.-K., Melton, D.A., 2003. Single cell transcript analysis of pancreas development. Dev. Cell 4, 383–393.

Coffman, C.R., Skoglund, P., Harris, W.A., Kintner, C.R., 1993. Expression of an extracellular deletion of Xotch diverts cell fate in *Xenopus* embryos. Cell 73, 659–671.

Colas, J.-F., Schoenwolf, G.C., 2001. Towards a cellular and molecular understanding of neurulation. Dev. Dyn. 221, 117–145.

Cornell, R.A., Eisen, J.S., 2000. Delta signaling mediates segregation of neural crest and spinal sensory neurons from zebrafish lateral neural plate. Development 127, 2873–2882.

Cox, W.G., Hemmati-Brivanlou, A., 1995. Caudalization of neural fate by tissue recombination and bFGF. Development 121, 4349–4358.

Deardorff, M.A., Tan, C., Saint-Jeannet, J.-P., Klein, P.S., 2001. A role for frizzled-3 in neural crest development. Development 128, 3655–3663.

Dorsky, R.I., Moon, R.T., Raible, D.W., 1998. Control of neural crest cell fate by the Wnt signalling pathway. Nature 396, 370–373.

Dorsky, R.I., Moon, R.T., Raible, D.W., 2000. Environmental signals and cell fate specification in premigratory neural crest. BioEssays 22, 708–716.

Dottori, M., Gross, M.K., Labosky, P., Goulding, M., 2001. The winged-helix transcription factor *Foxd3* suppresses interneuron differentiation and promotes neural crest cell fate. Development 128, 4127–4138.

Dunn, K.J., Williams, B.O., Li, Y., Pavan, W.J., 2000. Neural crest-directed gene transfer demonstrates Wnt1 role in melanocyte expansion and differentiation during mouse development. Proc. Natl. Acad. Sci. U. S. A. 97, 10050–10055.

Dutton, K.A., Pauliny, A., Lopes, S.S., Elworthy, S., Carney, T.J., Rauch, J., Geisler, R., Haffter, P., Kelsh, R.N., 2001. Zebrafish colourless encodes sox10 and specifies non-ectomesenchymal neural crest fates. Development 128, 4113–4125.

Endo, Y., Osumi, N., Wakamatsu, Y., 2002. Bimodal functions of Notch-mediated signaling are involved in neural crest formation during avian ectoderm development. Development 129, 863–873.

Ericson, J., Morton, S., Kawakami, A., Roelink, H., Jessell, T.M., 1996. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. Cell 87, 661–673.

Essex, L.J., Mayor, R., Sargent, M.G., 1993. Expression of *Xenopus* snail in mesoderm and prospective neural fold ectoderm. Dev. Dyn. 198, 108–122.

Gammill, L.S., Bronner-fraser, M., 2003. Neural crest specification: migrating into genomics. Nat. Rev., Neurosci. 4, 795–805.

Garcia-Castro, M.I., Marcelle, C., Bronner-Fraser, M., 2002. Ectodermal Wnt function as a neural crest inducer. Science 297, 848–851.

Glavic, A., Silva, F., Aybar, M.J., Bastidas, F., Mayor, R., 2004. Interplay between Notch signaling and the homeoprotein Xiro1 is required for neural crest induction in *Xenopus* embryos. Development 131, 347–359.

Gomez-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.

Grinblat, Y., Sive, H., 2001. zic gene expression marks anteroposterior pattern in the presumptive neurectoderm of the zebrafish gastrula. Dev. Dyn. 222, 688–693.

Groves, A.K., Bronner-Fraser, M., 1999. Neural crest diversification. Curr. Top. Dev. Biol. 43, 221–258.

Hammerschmidt, M., Pelegri, F., Mullins, M.C., Kane, D.A., van Eeden, F.J., Granato, M., Brand, M., Furutani-Seiki, M., Haffter, P., Heisenberg, C.P., Jiang, Y.J., Kelsh, R.N., Odenthal, J., Warga, R.M., Nusslein-Volhard, C., 1996. dino and mercedes, two genes regulating dorsal development in the zebrafish embryo. Development 123, 95–102.

Hollyday, M., McMahon, J.A., McMahon, A.P., 1995. Wnt expression patterns in chick embryo nervous system. Mech. Dev. 52, 9–25.

Holowacz, T., Sokol, S., 1999. FGF is required for posterior neural patterning but not for neural induction. Dev. Biol. 205, 296–308.

Honore, 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.

Hopwood, N.D., Pluck, A., Gurdon, J.B., 1989. A *Xenopus* mRNA related to Drosophila twist is expressed in response to induction in the mesoderm and the neural crest. Cell 59, 893–903.

Horstadius, S., 1950. The Neural Crest. Oxford Univ. Press, Oxford.

Ikeya, M., Lee, S.M.K., Johnson, J.E., McMahon, A.P., Takada, S., 1997. Wnt signaling is required for expansion of neural crest and CNS progenitors. Nature 389, 966–970.

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.

Kengaku, M., Okamoto, H., 1993. Basic fibroblast growth factor induces differentiation of neural tube and neural crest lineages of cultured ectoderm cells from *Xenopus* gastrula. Development 119, 1067–1078.

Knight, R.D., Nair, S., Nelson, S.S., Afshar, A., Javidan, Y., Geisler, R., Rauch, G.J., Schilling, T.F., 2003. Lockjaw encodes a zebrafish tfap2a required for early neural crest development. Development 130, 5755–5768.

Kos, R., Reedy, M.V., Johnson, R.L., Erickson, C.A., 2001. The winged-helix transcription factor *Foxd3* is important for establishing the neural crest lineage and repressing melanogenesis in avian embryos. Development 128, 1467–1479.

Kulesa, P., Ellies, D.L., Trainor, P.A., 2004. Comparative analysis of neural crest cell death, migration and function during vertebrate embryogenesis. Dev. Dyn. 229, 14–29.

Kuo, J., Patel, M., Gamse, J., Merzdorf, C., Liu, X., Apekin, V., Sive, H., 1998. opl: a zinc finger protein that regulates neural determination and patterning in *Xenopus*. Development 125, 2867–2882.

Kurata, T., Ueno, N., 2003. *Xenopus* NBX, a novel NK-1 related gene essential for neural crest formation. Dev. Biol. 257, 30–40.

LaBonne, C., 2002. Vertebrate development: Wnt signals at the crest. Curr. Biol. 12, R743–R744.

LaBonne, C., Bronner-Fraser, M., 1998. Neural crest induction in *Xenopus*: evidence for a two-signals model. Development 125, 2403–2414.

LaBonne, C., Bronner-Fraser, M., 2000. Snail-related transcriptional repressors are required in *Xenopus* for both the induction of the neural crest and its subsequent migration. Dev. Biol. 221, 195–205.

Labosky, P.A., Kaestner, K.H., 1998. The winged helix transcription factor Hfh2 is expressed in neural crest and spinal cord during mouse development. Mech. Dev. 76, 185–190.

Lamb, T.M., Harland, R.M., 1995. Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. Development 121, 3627–3636.

LeDouarin, N.M., Kalcheim, C., 1999. The Neural Crest, second ed. Cambridge Univ. Press, London.

Lee, H.-Y., Kleber, M., Hari, L., Brault, V., Suter, U., Taketo, M.M., Kemler, R., Sommer, L., 2004. Instructive role of Wnt/β-catenin in sensory fate specification in neural crest stem cells. Science 303, 1020–1023.

Lekven, A.C., Thorpe, C.J., Waxman, J.S., Moon, R.T., 2001. Zebrafish Wnt8 encodes two Wnt8 proteins on a bicistronic transcript and is required for mesoderm and neurectoderm patterning. Dev. Cell 1, 103–114.

Levin, M., 1997. The roles of activin and follistatin signaling in chick gastrulation. Int. J. Dev. Biol. 42, 553–559.

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.

Li, M., Zhao, C., Wang, Y., Zhao, Z., Meng, A., 2002. Zebrafish sox9b is an early neural crest marker. Dev. Genes Evol. 212, 203–206.

Liem, 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.

Liem, K.F., Tremml, G., Jessell, T.M., 1997. A role for roof plate and its resident TGF-β-related proteins in neuronal patterning in the dorsal spinal cord. Cell 91, 127–138.

Linker, C., Bronner-Fraser, M., Mayor, R., 2000. Relationship between gene expression domains of Xsnail, Xslug and Twist and cell movement in the prospective neural crest of *Xenopus*. Dev. Biol. 224, 215–225.

Locascio, A., Manzanares, M., Blanco, M.J., Nieto, M.A., 2002. Modularity and reshuffling of Snail and *Slug* expression during vertebrate evolution. Proc. Natl. Acad. Sci. U. S. A. 99, 16841–16846.

Lowery, L.A., Sive, H., 2004. Strategies of vertebrate neurulation and a re-evaluation of teleost neural tube formation. Mech. Dev. (in press).

Luo, T., Lee, Y.-H., Saint-Jeannet, J.-P., Sargent, T.D., 2003. Induction of neural crest in *Xenopus* by transcription factor AP2α. Proc. Natl. Acad. Sci. U. S. A. 100, 532–537.

Maeda, R., Mood, K., Jones, T.L., Aruga, J., Buchberg, A.M., Daar, I.O., 2001. Xmeis1, a proto-oncogene involved in specifying neural crest cell fate in *Xenopus* embryos. Oncogene 20, 1329–1342.

Mancilla, A., Mayor, R., 1996. Neural crest formation in *Xenopus laevis*: mechanism of Xslug induction. Dev. Biol. 177, 580–589.

Mansouri, A., Stoykova, A., Torres, M., Gruss, P., 1996. Dysgenesis of cephalic neural crest derivatives in Pax7−/− mutant mice. Development 122, 831–838.

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.

Maroulakou, I.G., Papas, T.S., Green, J.E., 1994. Differential expression of ets-1 and ets-2 proto-oncogenes during murine embryogenesis. Oncogene 9, 551–1565.

Martinsen, B., Bronner-Fraser, M., 1998. Neural crest specification regulated by the helix–loop–helix repressor, Id2. Science 281, 988–991.

Mayor, R., Morgan, R., Sargent, M., 1995. Induction of the prospective neural crest of *Xenopus*. Development 121, 767–777.

Mayor, R., Guerrero, N., Martinez, C., 1997. Role of FGF and noggin in neural crest induction. Dev. Biol. 189, 1–12.

McGrew, L.L., Lai, C.J., Moon, R.T., 1995. Specification of the anteroposterior neural axis through synergistic interaction of the Wnt signaling cascade with noggin and follistatin. Dev. Biol. 172, 337–342.

Meyer, D., Durliat, M., Senan, F., Wolff, M., Andre, M., Hourdry, J., Remy, P., 1997. Ets-1 and Ets-2 proto-oncogenes exhibit differential and restricted expression patterns during *Xenopus laevis* oogenesis and embryogenesis. Int. J. Dev. Biol. 41, 607–620.

Mitchell, P., Timmons, P., Herbert, J., Rigby, P., Tijan, R., 1991. Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. Genes Dev. 5, 105–119.

Mizuseki, K., Kishi, M., Matsui, M., Nakanishi, S., Sasai, Y., 1998. *Xenopus* Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. Development 125, 579–587.

Molven, A., Njolstad, P.R., Fjose, A., 1991. Genomic structure and restricted neural expression of the zebrafish Wnt-1 (int-1) gene. EMBO J. 10, 799–807.

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.

Moreno, T.A., Bronner-Fraser, M., 2001. The secreted glycoprotein Noelin-1 promotes neurogenesis in *Xenopus*. Dev. Biol. 240, 340–360.

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.

Mori-Akiyama, Y., Akiyama, H., Rowitch, D.H., de Crombrugghe, B., 2003. Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. Proc. Natl. Acad. Sci. U. S. A. 100, 9360–9365.

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.

Nagai, T., Aruga, J., Takada, S., Gunther, T., Sporle, R., Schughart, K.,

Flavell, R.A., 1997. The expression of the mouse *ZIC1*, *ZIC2*, and *ZIC3* gene suggests an essential role for zic genes in body pattern formation. Dev. Biol. 182, 299–313.

Nakata, K., Nagai, T., Aruga, J., Mikoshiba, K., 1997. *Xenopus* Zic3, a primary regulator both in neural and neural crest development. Proc. Natl. Acad. Sci. U. S. A. 94, 11980–11985.

Nakata, K., Koyabu, Y., Aruga, J., Mikoshiba, K., 2000. A novel member of the Xenopus Zic family, Zic5, mediates neural crest development. Mech. Dev. 99, 83–91.

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.

Nieto, M., Sargent, M., Wilkinson, D., Cooke, J., 1994. Control of cell behavior during vertebrate development by *Slug*, a zinc finger gene. Science 264, 835–839.

Odenthal, J., Nusslein-Volhard, C., 1998. Fork head domain genes in zebrafish. Dev. Genes Evol. 208, 245–258.

Papalopulu, N., Kintner, C., 1996. A posteriorising factor, retinoic acid, reveals that anteroposterior patterning controls the timing of neuronal differentiation in *Xenopus* neuroectoderm. Development 122, 3409–3418.

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 Néerl. Morphol. 5, 348–362.

Roelink, H., Nusse, R., 1991. Expression of two members of the Wnt family during mouse development-restricted temporal and spatial patterns in the developing neural tube. Genes Dev. 5, 381–388.

Rubinstein, A.L., Lee, D., Luo, R., Henion, P.D., Halpern, M.E., 2000. Genes dependent on zebrafish cyclops function identified by AFLP differential gene expression screen. Genesis 26, 86–97.

Saint-Jeannet, J.-P., He, X., Varmus, H.E., Dawid, I.B., 1997. Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. Proc. Natl. Acad. Sci. U. S. A. 94, 13713–13718.

Sasai, Y., De Robertis, E.M., 1997. Ectodermal patterning in vertebrate embryos. Dev. Biol. 182, 5–20.

Sasai, N., Mizuseki, K., Sasai, Y., 2001. Requirement of *FoxD3*-class signaling for neural crest determination in *Xenopus*. Development 128, 2525–2536.

Schorle, H., Meier, P., Buchert, M., Jaenisch, R., Mitchell, P.J., 1996. Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 381, 235–238.

Schubert, F., Mootoosamy, R., Walters, E., Graham, A., Tumiotto, L., Munsterberg, A., Lumsden, A., Dietrich, S., 2002. Wnt6 marks sites of epithelial transformations in the chick embryo. Mech. Dev. 114, 143–148.

Selleck, M.A., Bronner-Fraser, M., 1995. Origins of the avian neural crest: the role of neural plate-epidermal interactions. Development 121, 525–538.

Seo, H.C., Saetre, B.O., Havik, B., Ellingsen, S., Fjose, A., 1998. 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.

Shen, H., Wilke, T., Ashique, A.M., Narvey, M., Zerucha, T., Savino, E., Williams, T., Richman, J.M., 1997. Chicken transcription factor AP-2: cloning, expression and its role in outgrowth of facial prominances and limb buds. Dev. Biol. 188, 248–266.

Soo, K., O'Rourke, M.P., Khoo, P.L., Steiner, K.A., Wong, N., Behringer, R.R., Tam, P.P., 2002. Twist function is required for the morphogenesis of the cephalic neural tube and the differentiation of the cranial neural crest cells in the mouse embryo. Dev. Biol. 247, 251–270.

Southard-Smith, E.M., Kos, L., Pavan, W.J., 1998. Sox10 mutation disrupts neural crest development in Dom Hirschprung mouse model. Nat. Genet. 18, 60–64.

Spokony, R.F., Aoki, Y., Saint-Germain, N., Magner-Fink, E.K., Saint-Jeannet, J.-P., 2002. The transcription factor Sox9 is required for cranial neural crest development in *Xenopus*. Development 129, 421–432.

Storey, K.G., Crossley, J.M., De Robertis, E.M., Norris, W.E., Stern, C.D., 1992. Neural induction and regionalization in the chick embryo. Development 114, 729–741.

Streit, A., Stern, C., 1999. 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., 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.

Streit, A., Berliner, A.J., Papanayotou, C., Sirulnik, A., Stern, C.D., 2000. Initiation of neural induction by FGF signalling before gastrulation. Nature 406, 74–78.

Suzuki, A., Ueno, N., Hemmati-Brivanlou, A., 1997. *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. Development 124, 3037–3044.

Tahtakran, S.A., Selleck, M.A., 2003. Ets-1 expression is associated with cranial neural crest migration and vasculogenesis in the chick embryo. Gene Expression Patterns 3, 455–458.

Tavares, A.T., Izpisuja-Belmonte, J.C., Rodriguez-Leon, J., 2001. Developmental expression of chick twist and its regulation during limb patterning. Int. J. Dev. Biol. 45, 707–713.

Thiery, J.-P., 2003. Epithelial-mesenchymal transitions in development and pathologies. Curr. Opin. Cell Biol. 15, 740–746.

Tietjen, I., Rihel, J.M., Cao, Y., Koentges, G., Zakhary, L., Dulac, C., 2003. Single-cell transcriptional analysis of neuronal progenitors. Neuron 38, 161–175.

Trainor, P.A., Krumlauf, R., 2001. Hox genes, neural crest cells and branchial arch patterning. Curr. Opin. Cell Biol. 13, 698–705.

Trainor, P.A., Krumlauf, R., 2002. Riding the crest of the Wnt signaling wave. Science 297, 781–783.

Vallin, J., Thuret, R., Giacomello, E., Faraldo, M.M., Thiery, J.P., Broders, F., 2001. Cloning and characterization of three *Xenopus Slug* promoters reveal direct regulation by Lef/beta-catenin signaling. J. Biol. Chem. 276, 30350–30358.

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.

Warner, S.J., Hutson, M.R., Oh, S.H., Gerlach-Bank, L.M., Lomax, M.I., Barald, K.F., 2003. Expression of ZIC genes in the development of the chick inner ear and nervous system. Dev. Dyn. 226, 702–712.

Weinstein, D.C., Hemmati-Brivanlou, A., 1999. Neural induction. Annu. Rev. Cell Dev. Biol. 15, 411–433.

Wilson, S.I., Edlund, T., 2001. Neural induction: toward a unifying mechanism. Nat. Neurosci., Supp. 4, 1161–1168.

Wilson, S.I., Graziano, E., Harland, R.M., Jessell, T.M., Edlund, T., 2000. An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. Curr. Biol. 10, 421–429.

Wolda, S.L., Moody, C.J., Moon, R.T., 1993. Overlapping expression of XWnt-3A and XWnt-1 in neural tissue of *Xenopus laevis* embryos. Dev. Biol. 155, 46–57.

Wu, J., Saint-Jeannet, J.-P., Klein, P.S., 2003. Wnt-frizzled signaling in neural crest formation. Trends Neurosci. 26, 40–45.

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.

Yoshikawa, Y., Fujimori, T., McMahon, A.P., Takada, S., 1997. Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm in the mouse. Dev. Biol. 183, 234–242.

Zhang, J., Hagopian-Donaldson, S., Serbedzija, G., Elsemore, J., Plehn-Dujowich, D., McMahon, A.P., Flavell, R.A., Williams, T., 1996. Neural tube, skeletal and body wall defects in mice lacking transcription factor AP-2. Nature 381, 238–241.