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MOLECULAR MECHANISMS OF NEURAL CREST FORMATION

Carole LaBonne and Marianne Bronner-Fraser
Division of Biology 139-74, California Institute of Technology, Pasadena, California 91125; e-mail: Clabonne@caltech.edu; MBronner@caltech.edu

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■ **Abstract** The neural crest is a transient population of multipotent precursor cells named for its site of origin at the crest of the closing neural folds in vertebrate embryos. Following neural tube closure, these cells become migratory and populate diverse regions throughout the embryo where they give rise to most of the neurons and support cells of the peripheral nervous system (PNS), pigment cells, smooth muscle, craniofacial cartilage, and bone. Because of its remarkable ability to generate such diverse derivatives, the neural crest has fascinated developmental biologists for over one hundred years. A great deal has been learned about the migratory pathways neural crest cells follow and the signals that may trigger their differentiation, but until recently comparatively little was known about earlier steps in neural crest development. In the past few years progress has been made in understanding these earlier events, including how the precursors of these multipotent cells are specified in the early embryo and the mechanisms by which they become migratory. In this review, we first examine the mechanisms underlying neural crest induction, paying particular attention to a number of growth factor and transcription factor families that have been implicated in this process. We also discuss when and how the fate of neural crest precursors may diverge from those of nearby neural and epidermal populations. Finally, we review recent advances in our understanding of how neural crest cells become migratory and address the process of neural crest diversification.

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INDUCTION OF THE NEURAL CREST

The population of cells that will ultimately give rise to the neural crest is induced to form in the ectoderm at the junction between the neural plate and the prospective epidermis, a region often referred to as the neural plate border. Regions of the neural plate border caudal to the diencephelon give rise to neural crest, whereas more rostral regions give rise to placodes and, in *Xenopus*, the cement gland. An exception to this is mouse neural crest, which also arises from more rostral axial levels. In *Xenopus* and zebrafish, localized expression of neural crest markers such as *Snail* or *Slug* can be detected at the lateral edges of the neural plate by mid- to late-gastrula stages (Essex et al 1993, Thisse et al 1993, Hammerschmidt & Nusslein-Volhard 1993, Mayor et al 1995, Thisse et al 1995). Expression of these markers in *Xenopus* can be detected around or soon after the time markers of regional identity within the neural plate begin to be expressed, including the expression of *Pax-3* at the neural plate border (Bang et al 1997). In contrast, although expression of *Pax-3* also becomes restricted to the border of the open neural plate in the chick (Goulding et al 1991, Bang et al 1997), *Slug* is not expressed in this region until later stages, when neural folds are closing (Nieto et al 1994). In avians, single-cell lineage analysis has indicated that cells in neural crest-forming regions of the neural plate border are not committed to neural crest fates (Selleck & Bronner-Fraser 1995), and this appears to be the case in amphibians as well (C LaBonne & A Collazo, unpublished observation). Interestingly, this is true even after cells in these regions begin to express neural crest markers (Selleck & Bronner-Fraser 1995; C LaBonne & A Collazo, unpublished observation). Thus the expression of neural crest markers at these early stages denotes cells with the potential to give rise to neural crest, not cells that are uniquely fated to do so.

The expression of early neural crest markers at gastrula stages in *Xenopus* and zebrafish suggests that in these embryos, if neural crest induction can be considered a discrete phenomenon, it is tightly linked both temporally and spatially to the process of neural induction and formation of the neural plate border. Any understanding of the molecular mechanisms underlying the specification of the neural crest must therefore take into account the early events that induce and pattern the neural plate.

Neural Induction

Neural induction is initiated when the ectoderm is exposed to signals emanating from Spemann's organizer in amphibians, or its functional equivalents, the dorsal shield in teleosts and the node in amniotes. In recent years, researchers have

identified a number of secreted molecules that are expressed in the organizer. These factors, which have potent neural-inducing activities in *Xenopus* ectodermal explants and mimic the ability of the organizer to induce and pattern a secondary neural axis, include *noggin* (Lamb et al 1993), *chordin* (Sasai et al 1994), and *follistatin* (Hemmati-Brivanlou et al 1994). Interestingly, these unrelated molecules have been found to mediate neural induction via similar mechanisms: by binding to and inhibiting the activity of a subset of bone morphogenetic proteins (BMP2, 4, and 7), members of the transforming growth factor-β (TGF-β) superfamily (reviewed in Sasai & De Robertis 1997, Weinstein & Hemmati-Brivanlou 1997).

In *Xenopus*, *BMP-4* is expressed throughout the ectoderm prior to neural induction (Dale et al 1992, Fainsod et al 1994, Hemmati-Brivanlou & Thomsen 1995). When this ectoderm is explanted and cultured in isolation, it gives rise to epidermis. In response to organizer signals, however, *BMP-4* expression is lost from the portion of the ectoderm fated to give rise to the neural plate (Fainsod et al 1994, Hemmati-Brivanlou & Thomsen 1995). Inhibition of BMP-2/-4/-7 signaling in the ectoderm suppresses epidermal fate and induces the expression of neural-specific markers (Lamb et al 1993, Sasai et al 1994, Hemmati-Brivanlou et al 1994, Fainsod et al 1994, Xu et al 1995, Hawley et al 1995). These findings have led to a default model for neural induction (Weinstein & Hemmati-Brivanlou 1997, Wilson & Hemmati-Brivanlou 1997). According to this model, gastrula ectodermal cells adopt a default neural state when removed from extracellular signals. In *Xenopus* embryos, high levels of BMP4 signaling in the ventral ectoderm suppress its neural tendencies and cause it to adopt an epidermal fate (Wilson & Hemmati-Brivanlou 1995). BMP antagonists expressed in the organizer repress the epidermalizing activity of BMP-4, and this release from inhibition is sufficient to specify neural fates. In addition, BMP-4 has been found to have dose-dependent effects on *Xenopus* ectoderm, inducing epidermis at high doses and neural plate border fates, such as cement gland, at lower doses (Wilson et al 1997; Figure 1*a*). It should be noted that, although signaling by BMP-2/-4/-7 is often referred to for simplicity as BMP signaling, not all related BMPs have been tested for similar activities and, BMP-1 is not a TGF-β-related growth factor (Wozney 1989).

The neural default model nicely fits most of the experimental data obtained to date in amphibians, and BMPs seem to play a similar role in patterning the ectoderm of zebrafish, a teleost (Hammerschmidt et al 1996, Neave et al 1997). Difficulties arise, however, when attempting to extrapolate this model to amniotes such as the mouse and chicken. The amniote node, like the *Xenopus* organizer, expresses BMP antagonists, including noggin and chordin (Connolly et al 1997, Streit et al 1998, McMahon et al 1998). In amniotes, however, expression of BMP-2 and -4 is not detected in ectodermal regions prior to neural induction (Winnier et al 1995, Schultheiss et al 1997, Streit et al 1998). Therefore, in these organisms there is no need for BMP antagonists to suppress BMP-mediated epidermalizing influences in order to reveal the underlying neural potential of the neural plate. Accordingly, ectopic chordin expression cannot induce expression

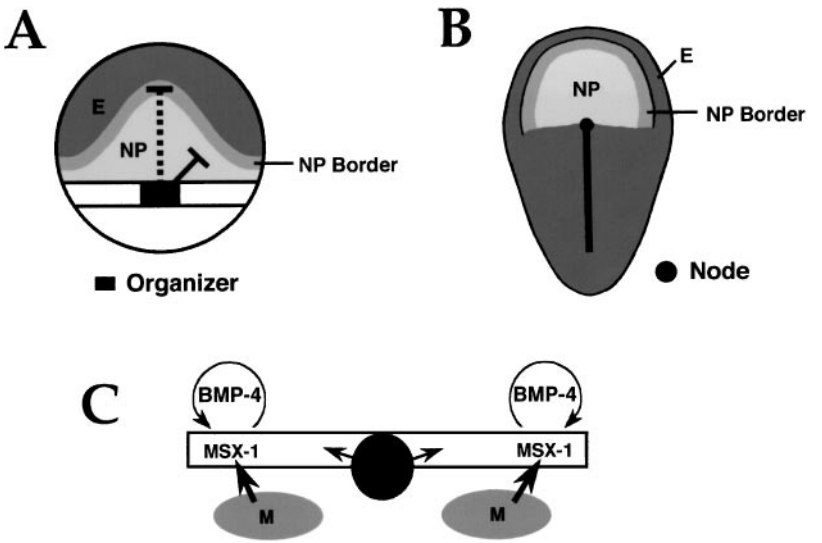


Figure 1 Role of BMP signaling in neural induction and formation of the neural plate border. (*A*) The neural default model. In *Xenopus* gastrulae, BMP antagonists expressed in the organizer inhibit BMP signaling in the ectoderm. Regions close to the organizer are strongly inhibited (solid line) and form neural plate (NP). Regions farther from the organizer are more weakly inhibited (broken line) and form the neural plate border (NP border). In the absence of inhibition, ectoderm gives rise to epidermis (E). (*B*) Experiments on avian gastrulae do not support the default model. Misexpression of BMPs within neural plate-forming regions or BMP antagonists within epidermis-forming regions does not affect ectodermal patterning, while misexpressing these factors within the neural plate border does. (*C*) Model for induction/maintenance of neural plate border fates in the chick. Signals from the node (thin arrows) and the non-axial mesoderm (thick arrows) establish a domain at the lateral edges of the neural plate that expresses *Msx-1*. Msx-1 activates *BMP-4* expression, whereas BMP-4 signaling maintains *Msx-1* expression. Signals from the non-axial mesoderm may include FGF. (Adapted from Streit & Stern 1999.)

of neural markers in chick extra-embryonic or non-neural ectoderm, even though these tissues can form an ectopic neural plate in response to a grafted node (Streit et al 1998). This suggests that the node produces additional neural inducers that are not BMP antagonists. Similarly, misexpression of BMP-4 or BMP-7 within the neural plate does not induce epidermis (Streit et al 1998; see Figure 1*b*). Consistent with these findings, mouse knockouts of *noggin*, *follistatin*, *BMP-2*, *BMP-4*, or *BMP-7* show no obvious defects in early ectodermal patterning (Winnier et al 1995, Zhang & Bradley 1996, Dudley & Robertson 1997), although this could be attributed to redundancy. In addition, recent experiments in avian embryos suggest that neural induction requires signals originating in the underlying mesendoderm

in addition to those from the organizer (Pera et al 1999). Thus the data from amniotes seem to indicate that neural induction is more complex than the neural default model initially suggested. Other signals appear to be required in addition to BMP inhibition, and these signals may also affect the formation of border cell types such as the neural crest.

When studying a developmental process, some degree of variability between organisms is to be expected. Some variation may be the result of the obvious differences in embryonic morphogenesis between amphibians, which at the onset of gastrulation consist of a hollow ball of cells, and avians, which develop from a flat epiblast. Moreover, unlike amniotes, early *Xenopus* development relies on localized maternal stores of mRNA and protein, since zygotic transcription does not commence until mid-blastula stages. A consequence of the early localization of cell fate determinants is that some cells may initially be exposed to signals that actually interfere with subsequent fate decisions, and mechanisms would then be necessary to counteract these signals. In addition, cell proliferation is significantly greater in amniotes, and increased cell number may allow for a greater separation of patterning events, both spatially and with respect to cell generation. Despite all these differences, however, it would seem rather unlikely that amniotes and anamniotes have evolved fundamentally different mechanisms for specifying neural fate at the molecular level. This idea is supported by the observation that the avian node can substitute for the amphibian organizer in neural induction assays (Kintner & Dodd 1991).

The conflicting experimental findings that have been reported in these systems are likely due, at least in part, to the nature of the assays employed and the responding tissues utilized. For example, although late blastula stage *Xenopus* ectodermal explants (animal caps) are routinely treated experimentally as naive ectoderm; they appear to have already received weak mesoderm-inducing signals (Sokol 1993, Lamb et al 1993) and have been prepatterned such that the dorsal ectoderm is biased toward non-epidermal fates (Savage & Phillips 1989, Sokol & Melton 1991). This raises the possibility that neural induction in *Xenopus* animal caps may require additional signals that have already been received by this ectoderm prior to its isolation. It should be noted, however, that the dorsal prepattern of the animal cap does not itself seem to be required for neural induction in response to noggin (Wilson & Hemmati-Brivanlou 1995, Knecht et al 1995).

Despite some important differences with anamniotes, it seems likely that BMP inhibition also plays a role in patterning the early ectoderm of amniotes. It has been suggested that during neural induction, BMP antagonists act downstream of, or in conjunction with, other signals from the organizer. For example in the chick, BMP inhibition can stabilize the expression of neural plate markers in ectoderm that has been briefly exposed to signals from the node (Streit et al 1998). In addition, it has been shown that at primitive streak (gastrula) stages, the border of the newly induced neural plate is sensitive to changes in the level of BMP signaling (Pera et al 1999, Streit & Stern 1999). This suggests that BMP signaling may be an important determinant of the border between neural plate and epidermis.

Specification of Neural Crest-Forming Regions at the Neural Plate Border

Many models have been proposed to explain the induction of the neural crest at the neural plate border (reviewed in Baker & Bronner-Fraser 1997a). The prevailing view is that interactions between the neural plate and the surrounding non-neural ectoderm specify the neural crest. Grafting experiments in urodele embryos demonstrate that when axolotl neural plates contact prospective epidermis, neural crest is generated at the border between the two cell types (Moury & Jacobson 1990). More recently, these experiments have been repeated using chick or *Xenopus* embryos, with similar findings (Selleck & Bronner-Fraser 1995, Mancilla & Mayor 1996). Moreover, neural crest is also induced when explants of neural plate and non-neural ectoderm are juxtaposed in vitro (Selleck & Bronner-Fraser 1995, Dickinson et al 1995, Mancilla & Mayor 1996). Interestingly, in such experiments the induced neural crest arises from both the neural plate and the prospective epidermis. Although these findings suggest that interactions between the neural plate and epidermis are sufficient to generate neural crest, the necessity of this interaction for neural crest formation in vivo has not been demonstrated. There is also evidence that signals from the non-axial mesoderm are required for neural crest formation. Non-axial mesoderm from both chick and *Xenopus* has been shown to be a potent inducer of neural plate border/neural crest markers (Bang et al 1997, Bonstein et al 1998, Marchant et al 1998), whereas *Xenopus* embryos from which presumptive non-axial mesoderm has been removed fail to express neural crest markers (Bonstein et al 1998, Marchant et al 1998). Much recent work has focused on identifying the molecular nature of the signals that induce neural crest. Growth factors from the BMP, FGF, and Wnt families have all been implicated in this process, and the evidence supporting a role for each of these factors is discussed below.

BMPs There is evidence in both amniotes and anamniotes that BMP signaling plays a role in positioning the neural plate border during gastrulation. In avians, injecting BMP-2- or BMP-4-expressing cells, or BMP-coated beads, at the neural plate border of primitive streak stage embryos, narrows the neural plate (Pera et al 1999, Streit & Stern 1999). In contrast, injecting cells expressing chordin, a BMP antagonist, expands the neural plate at the expense of epidermis (Streit & Stern 1999). In both cases, however, the effects are modest and the size of the border region as assayed by one marker of this region, *Msx-1*, remains largely unchanged (Streit & Stern 1999). These findings appear to contrast somewhat with those in *Xenopus*, where misexpression of BMP antagonists does expand the expression of neural crest markers at the neural plate border (LaBonne & Bronner-Fraser 1998a, Marchant et al 1998). In *Xenopus*, it has been proposed that levels of BMP signaling intermediate to those that specify neural plate and epidermis play a role in establishing neural plate border fates, including neural crest (Morgan & Sargent 1997, Wilson et al 1997, LaBonne & Bronner-Fraser 1998a, Marchant

et al 1998). There is also evidence that this may be true in zebrafish (Neave et al 1997, Nguyen et al 1998a). However, if intermediate levels of BMP signaling are required for border formation, this requirement is likely to be transitory. By late gastrula stages in *Xenopus*, chick, and mouse, the expression levels of both *BMP-4* and the BMP-responsive gene *Msx-1* are highest at the neural plate border (Hemmati-Brivanlou & Thomsen 1995, Suzuki et al 1997).

In avian embryos, *BMP-4* and *-7* are expressed in the non-neural ectoderm that abuts the open neural plate (Liem et al 1995, Schultheiss et al 1997). As the neural tube closes, *BMP-4* expression is downregulated in the prospective epidermis, although the dorsal neural tube continues to express several TGF-β family members including *BMP-4* (Watanabe & Le Douarin 1996, Liem et al 1995, 1997). This pattern of BMP expression is both temporally and spatially consistent with a role in neural crest induction. Moreover, Liem et al (1995) have shown that the ability of epidermis to induce the expression of neural crest markers in trunk neural plate explants can be mimicked by exogenously added BMP proteins. This led them to suggest a model in which BMP proteins, secreted by the epidermis, act upon the neural plate to induce neural crest in vivo. It is important to realize, however, that there is no direct evidence demonstrating that the epidermis-derived inducing signal is BMP mediated. Exogenously added BMP proteins could induce neural crest by bypassing the need for an epidermal signal and mimicking the later action of endogenous BMPs within the dorsal neural tube. Similarly, although BMP antagonists can block the expression of neural crest markers in neural plate/epidermal conjugates (Liem et al 1997), they could do so by inhibiting BMP signaling within the responding tissue rather than by blocking an inducing signal from the epidermis. Resolving this point will require experiments in which the timing of BMP inhibition is carefully controlled.

A recent study by Selleck et al (1998) attempted to determine when BMP signaling was required for neural crest formation in the trunk of avian embryos. In these experiments, cells secreting the BMP antagonist noggin were injected adjacent to the neural folds at various times during neurulation. Noggin-secreting cells could not prevent neural crest formation when located next to the open neural plate, when a signal from the prospective epidermis is thought to be required. However, when these cells were present adjacent to the closing tube, at a time when *BMP-4* is expressed in the neural folds but not in the prospective epidermis, they were able to prevent neural crest formation. Noggin-secreting cells could also inhibit the expression of some but not all dorsal neural tube markers when injected at this later stage. The experiments of Selleck et al (1998) seem to indicate that epidermally derived BMPs are not required for the induction of the neural crest at open neural plate stages in the trunk. However, it cannot be ruled out that BMPs produced by the non-neural ectoderm are required at stages prior to those examined in this study, or indeed at later stages, when *BMP-7* continues to be expressed in the epidermis. In addition, since BMP-7 is less efficiently inhibited by noggin than is BMP-4 (Zimmerman et al 1996, Liem et al 1997), the block to BMP signaling at open neural plate stages could be incomplete. A conclusion that can be drawn

from this study is that inhibiting BMP signaling later, in the closing neural folds, does prevent neural crest formation (Selleck et al 1998). As *BMP-4* expression in the closing neural folds precedes expression of the neural crest marker *Slug*, it is possible that neural crest specification does not commence until these stages and that the initial steps of this process are BMP dependent. Alternatively, neural crest induction may in fact begin at or before open neural plate stages. The requirement for BMP signaling within the neural folds/dorsal neural tube may then reflect a later step in this process, perhaps the maintenance of neural crest precursor fate.

These findings highlights our current lack of understanding of precisely when the process of neural crest induction commences in the embryo, whether it is distinct from the induction of other dorsal neural tube cell types and, indeed, at what point a dorsal neural tube cell should be considered a neural crest precursor. Explant experiments have indicated that when pieces of prospective dorsal neural tube are isolated at open neural plate stages and cultured in isolation, they can give rise to neural crest (Yamada et al 1993, Selleck & Bronner-Fraser 1995). However, such explants also contain prospective epidermis (Selleck & Bronner-Fraser 1995), a source of inducing signals, and thus it cannot be concluded that neural crest has been specified by these stages. If signals from the prospective epidermis are required for neural crest formation in vivo, the experiments of Selleck et al (1998) suggest that they are mediated by factors other than BMPs (or at least BMP-4). For example, non-BMP signals from the prospective epidermis may induce the expression of *BMP-4* within the neural folds. BMP-4 signaling within the neural folds/dorsal neural tube could then induce and/or maintain neural crest precursors. It is also possible that the non-neural ectoderm is not required for neural crest induction in vivo but can induce neural crest in vitro because it is a source of BMPs. In an additional level of complexity, there may also be some variation in the mechanisms underlying neural crest induction at different axial levels. Following ablation of the dorsal neural tube in caudal hindbrain regions, neural crest regeneration is accompanied by re-expression of *Slug*, but not *BMP-4* (Buxton et al 1997). This finding suggests that BMP-4 signaling within the neural epithelium is not required for neural crest formation at this level of the neuraxis. Overall, while the weight of the evidence suggests BMP signaling is a necessary component of neural crest formation, more work is clearly needed to determine when and where this signaling is required.

FGFs FGF signaling can, in conjunction with BMP inhibition, induce the expression of neural crest markers in *Xenopus* ectodermal explants (Kengaku & Okamoto 1993, Mayor et al 1997, LaBonne & Bronner-Fraser 1998a). Moreover, overexpression of mRNA encoding a dominant inhibitory FGF receptor1 (FGFR1) has been found to inhibit the expression of at least one neural crest marker, *XSlug*, in whole embryos (Mayor et al 1997). Similar overexpression experiments have also implicated FGF signaling in the establishment of posterior CNS fates (Kengaku & Okamoto 1993, Cox & Hemmati-Brivanlou 1995, Lamb & Harland 1995, Launay et al 1996, Xu et al 1997), and several members of the FGF family

are expressed at a time and place consistent with a role in both of these processes (Tannahill et al 1992, Isaacs et al 1992, Mahmood et al 1995, Riese et al 1995, Bueno et al 1996, Storey et al 1998). These experiments suggest that FGF signals may play a role in the generation of both posterior CNS (caudal to forebrain) and posteriolateral (neural crest) neural fates. However, conflicting data have emerged from studies of transgenic *Xenopus* embryos overexpressing a dominant inhibitory FGFR1 (Kroll & Amaya 1996). In these embryos, posterior neural tissue develops normally, and the expression of *Pax-3*, an early marker of neural crest-forming regions at the lateral edges of the neural plate, is unaffected, although a full range of neural and neural crest markers was not examined in these experiments. It is also possible that if FGF signals are required for neural crest formation, their role may not be an instructive one. Despite suggestions that FGFs may mediate the ability of epidermis to induce neural crest in neural plate explants, FGF treatment of *Xenopus* neural plate explants does not result in neural crest induction (Mayor et al 1997). Moreover, the ability of FGF to induce neural crest markers in ectoderm that has been neuralized by BMP antagonists may be secondary to its ability to induce the expression of at least one member of the Wnt family (LaBonne & Bronner-Fraser 1998a).

In avian embryos, posterior non-axial mesoderm can "posteriorize" anterior neural tissue, rendering it competent to express neural crest markers in response to BMP-4 (Muhr et al 1997). Although FGFs cannot mimic this posteriorizing activity, they appear to play a role in conferring this ability on the mesoderm (Muhr et al 1997). FGF misexpression can also induce the expression of some early neural markers in chick embryos (Alvarez et al 1998, Storey et al 1998), although no experiments have addressed its necessity for neural patterning in this system. Similarly, FGF misexpression has been found to induce expression of the neural plate border marker *Msx-1,* and it has been suggested that FGFs may establish the initial expression of *Msx-1* at the neural plate border (Streit & Stern 1999). According to this model, following the FGF-mediated onset of its expression, *Msx-1* would establish a positive feedback loop with BMP4, thereby maintaining the expression of both of these genes at the border (Figure 1*c*). Such an Msx-1/BMP feedback loop has been described during tooth development (Bei & Maas 1998, Tucker et al 1998). Whether FGF signaling is required for *Msx-1* expression at the neural plate border has yet to be established.

Wnts Members of the Wnt family of growth factors have also been implicated in the establishment of regional identity along the anterioposterior and mediolateral axes of the developing nervous system. For example, while over-expression of BMP antagonists induces anterior neural tissue in *Xenopus* ectodermal explants (Lamb et al 1993, Knecht et al 1995), co-expression of *XWnt3A* and BMP antagonists reduces the expression of more anterior markers and induces the expression of more posterior markers such as *En-2,* a marker of the midbrain/hindbrain boundary, and *Krox-20,* a marker for rhombomeres 3 and 5 (McGrew et al 1995). Wnts can also induce the expression of neural crest markers in ectodermal explants in

conjunction with BMP inhibition (Saint-Jeannet et al 1997, Chang & Hemmati-Brivanlou 1998, LaBonne & Bronner-Fraser 1998a). Conversely, over-expression of a dominant inhibitory Wnt ligand in *Xenopus* embryos reduces the expression of *En-2* and *Krox-20* (McGrew et al 1997) and prevents the expression of early neural crest markers (LaBonne & Bronner-Fraser 1998a).

Because neural crest is not produced from the anterior-most regions of the neural plate in *Xenopus* (Sadaghiani & Thiebaud 1987), one possibility is that the effects of Wnts and FGFs on neural crest formation are directly tied to their ability to posteriorize anterior neural tissue. At least for Wnts, however, several lines of evidence suggest that this is not the case. First, misexpression of Wnt signals in early embryos results in the ectopic expression of neural crest markers in cells normally fated to become epidermis, not neural tissue (Saint-Jeannet et al 1997, LaBonne & Bronner-Fraser 1998a). In addition, Wnts can induce the expression of neural crest markers in ectoderm over-expressing *XSlug* (LaBonne & Bronner-Fraser 1998a), a transcription factor normally expressed in the neural crest but not the neural plate (Mayor et al 1995), which cannot itself induce neural tissue (LaBonne & Bronner-Fraser 1998a). Finally, by over-expressing *GSK-3* it has been shown that perturbing the Wnt signaling pathway can inhibit the production of *Krox-20*-expressing neural crest cells without the loss of *Krox-20* expression in rhombomere 5 itself (Saint-Jeannet et al 1997). Thus, in this case, inhibition of Wnt signaling results in a loss of neural crest fates at a level of the anterioposterior axis where positional identity has not been altered. Together, these findings argue strongly that the requirement for Wnt signals during neural crest formation is independent of any role they may play in the establishment of posterior neural fates. However, Wnts do appear to play multiple distinct roles in the patterning of the nervous system and the neural crest. For example, there is evidence in zebrafish that Wnt signals may play a role in promoting neural crest progenitors to form pigment cells at the expense of neurons and glia (Dorsky et al 1998).

Are Wnts expressed at a time and place consistent with an early role in neural crest induction? In *Xenopus*, *XWnt-8* is expressed in the ventrolateral mesoderm during gastrulation (Christian et al 1991), when the initial expression of neural crest markers is induced at the lateral edges of the neural plate (Essex et al 1993). Similarly, *CWnt-8C* is expressed in the non-axial mesoderm of chick gastrulae (Hume & Dodd 1993). In *Xenopus*, *XWnt-3A* is expressed at the lateral edges of the neural plate at early neurula stages (McGrew et al 1997), while *XWnt-1* is expressed in the dorsal neural tube (Wolda et al 1993). Thus both factors could play a role in neural crest formation. However, in chick embryos, expression of *Wnt-1* and *Wnt-3A* occurs later than the initial expression of neural crest markers, and neural crest can be induced in conjugates of neural plate and non-neural ectoderm under conditions where neither *Wnt-1* nor *Wnt-3A* is expressed (Dickinson et al 1995). These results would seem to preclude a role for these Wnts in the earliest aspects of neural crest induction. However, a murine *Wnt-1/Wnt-3A* double knockout displays a marked deficiency in neural crest derivatives (Ikeya et al 1997), suggesting

that these Wnts are required during later stages of neural crest formation, perhaps for the maintenance and/or proliferation of neural crest precursors. In *Xenopus*, *XWnt-7B* is expressed throughout the ectoderm at early stages (Chang & Hemmati-Brivanlou 1998), raising the possibility that the early requirement for Wnt signals during neural crest formation is permissive rather than instructive.

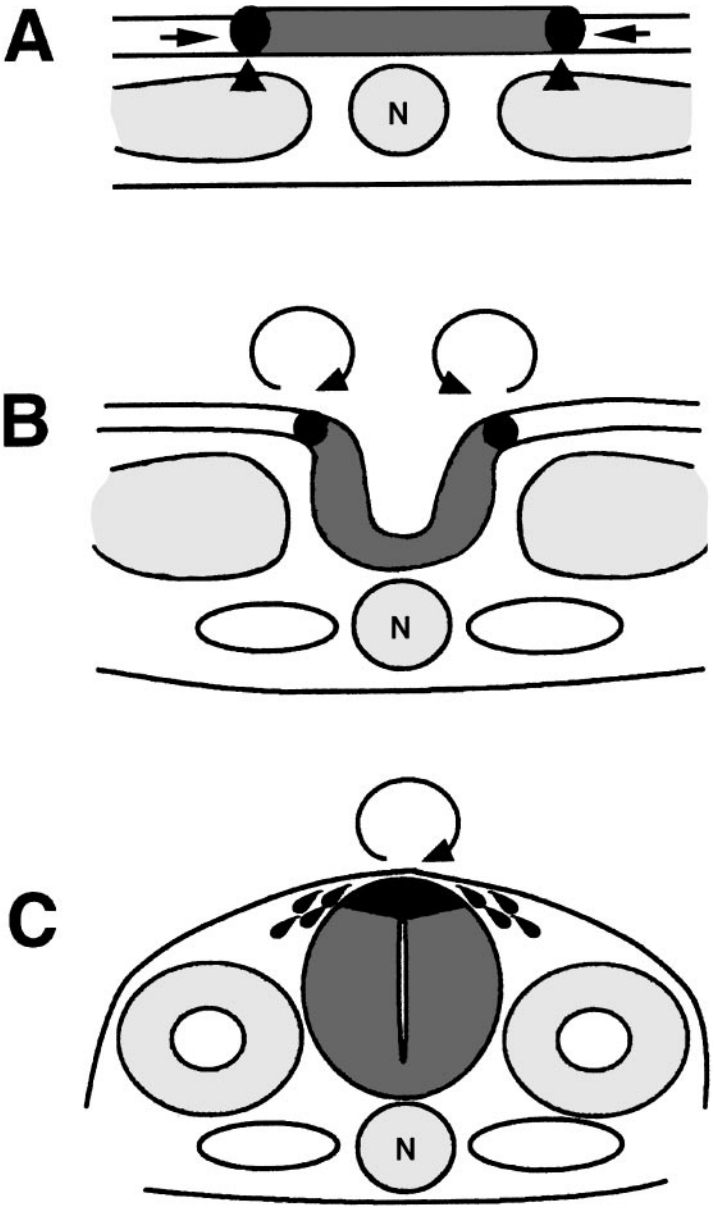
Neural Crest Formation Requires Multiple Signals Taken together, the evidence suggests that Wnt-, FGF- and BMP- generated signals all participate in some aspect of neural crest formation. Currently, experiments carried out in different organisms seem to implicate different growth factor families in this process. Despite this, it seems likely that it will ultimately be found that the underlying molecular mechanisms are conserved across vertebrates. How then can the apparent differences be explained? In particular, is it paradoxical that inhibition of BMP signals may be a component of neural crest induction in *Xenopus*, while in the chick, BMP signals induce neural crest in neural plate explants. When analyzing such findings, it is helpful to keep in mind a number of points. First, misexpression experiments in *Xenopus* affect the earliest aspects of neural crest formation, including neural induction itself, whereas experiments in avians have generally been carried out later in development, on neurula-stage embryos. This is an important distinction, as it has become increasingly clear that neural crest formation is a multistep process during which BMPs, Wnts, and possibly FGFs, each play multiple distinct roles. The competence of the responding tissue in each experiment must therefore be carefully considered because the response evoked by a particular growth factor is likely to change as a function of time. In addition, BMPs are initially expressed throughout the prospective neural plate in *Xenopus* and zebrafish, but not in amniotes. Thus the need to antagonize them represents a necessary additional step unique to anamniotes. Experiments in *Xenopus* that interfere with this early step may therefore yield findings that cannot be duplicated in amniote embryos. Finally, it is worth stressing that, to date, experiments in *Xenopus* have focused primarily upon cranial neural crest precursors, whereas most experiments in the chick have been carried out on spinal cord explants, and different mechanisms could operate at different axial levels. With these caveats in mind, is it possible to assemble the results of experiments in multiple systems into one view of neural crest induction?

The evidence from chick, frog, and zebrafish suggests that BMPs are involved in specifying the neural plate border, although experimentally their effects may be more dramatic in anamniotes where inhibition of BMP signals in the dorsal ectoderm appears to play a more central role in neural specification. Interestingly, several *Xenopus* genes, expression of which is ultimately restricted to the neural plate border, are initially expressed throughout the mediolateral aspect of the neural plate, including *Pax-3* (Bang et al 1997) and several Zic family members (Kuo et al 1998, Mizuseki et al 1998, Nakata et al 1998). This observation suggests the possibility that in frog gastrulae, the entire neural plate transiently acquires a

border identity in response to planar signals from the organizer. Midline signals from the involuting mesoderm may subsequently restrict the expression of these markers to the lateral edges of the neural plate.

Data from avians and amphibians suggest that signals from the ectoderm and/or underlying mesoderm specify neural crest-forming regions at the lateral edges of the neural plate. Experiments in *Xenopus* implicate Wnt and FGF signals in this process. Although the necessity of FGF signals during early aspects of neural crest induction has yet to be examined in amniotes, data from our laboratory suggest that Wnt signals are required for this process in avian embryos (M Garcia-Castro & M Bronner-Fraser, manuscript in preparation). Moreover, it has been suggested that neural crest-inducing signals from the ectoderm may not be BMP mediated (Selleck et al 1998). It is therefore possible that the ectodermal signal and/or those from the non-axial mesoderm are mediated by Wnts or FGFs, although at the moment there is no direct evidence for this. Although it has been found that treatment of avian neural plate explants with exogenous BMP protein is sufficient to generate neural crest (Liem et al 1995) and that BMP signals clearly play an important role in this process, there are several distinct times when they could be exerting their effects in vivo. First, it is possible that in these explant experiments, BMP signals act by respecifying neural plate border fates, a role they may play in vivo at gastrula stages. Second, as has been shown by Selleck et al (1998), neural crest formation requires BMP signaling within the closing neural folds/dorsal neural tube. Thus treatment of neural plate explants with BMP proteins may bypass the need for earlier inducing signals and directly mimic the action of BMP signals within the neural epithelium. Alternatively, the earlier action of BMPs secreted by the non-neural ectoderm (most likely BMP-7) may be required in addition to those expressed within the closing neural folds. Ultimately however, in response to some combination of the above signals, neural crest markers become restricted to the lateral neural plate/dorsal neural tube. *Wnt-1* and *Wnt3A* are also expressed within the dorsal neural tube and appear to be required for the maintenance and/or proliferation of neural crest precursors (Ikeya et al 1997). *BMP-4* and other TGF-β-related growth factors expressed within the dorsal neural tube could also play a similar role. These steps are summarized in Figure 2.

Figure 2 Multiple signals are implicated in neural crest formation in the avian spinal cord. (*A*) At open neural plate stages, signals from the adjacent non-neural ectoderm (arrows) and the underlying non-axial mesoderm (arrowheads) are thought to induce neural crest-forming regions at the neural plate border (black circles). These signals may include Wnts, FGFs, and BMPs. (*B*) In the closing neural tube, neural crest-forming regions express BMPs, and BMP signaling at these stages is required for neural crest formation (circular arrows). (*C*) After neural folds approximate, neural crest cells migrate out from the dorsal neural tube. This region of the neural tube expresses a number of BMPs and Wnts, and these factors may continue to be required for maintenance and/or proliferation of neural crest precursors (circular arrows).



Gene Expression at the Neural Plate Border

The combined effects of the signals discussed in the previous section result in the establishment of a domain at the lateral edges of the neural plate and/or the dorsal aspect of the developing neural tube, which has the potential to give rise to neural crest. The expression of a large number of genes within this region has been described. Some of these are likely to contribute to dorsal neural tube fates whereas others may be required for neural crest formation. Recent advances in our understanding of how a number of key families of transcription factors may influence neural crest fate are described below.

Snail *Family* The zinc finger transcription factor Snail was first described in *Drosophila*, where it is required for the invagination of mesodermal cells during gastrulation (Ip et al 1994). *Drosophila Snail* has been shown to function as a transcriptional repressor (Gray et al 1994) and inhibits the expression of neurectodermal genes in the mesoderm (Leptin 1991). Two *Snail*-related transcription factors have been described in vertebrates, *Snail* and *Slug*. Mouse, chick, and *Xenopus* embryos appear to possess one *Snail* and one *Slug* gene; zebrafish appear to have two *Snail* genes (Nieto et al 1992, 1994; Hammerschmidt & Nusslein-Volhard 1993; Essex et al 1993; Thisse et al 1993, 1995; Mayor et al 1995; Sefton et al 1998; Jiang et al 1998). Evidence from *Xenopus* suggests that *Slug*, like *Snail*, is a transcriptional repressor (C LaBonne & M Bronner-Fraser, manuscript in preparation).

In addition to being expressed in the mesoderm, vertebrate *Snail* family members are also expressed in the neural crest. In *Xenopus*, *Snail* and *Slug* are expressed in both premigratory and migratory neural crest cells (Essex et al 1993, Mayor et al 1995), as are chick *Slug* (*CSlug*), mouse *Snail* (*MSnail*), and zebrafish *Snail-2* (*ZSnail-2*) (Nieto et al 1994, Thisse et al 1995, Sefton et al 1998). In contrast, chick *Snail* (*CSnail*), mouse *Slug* (*MSlug*), and zebrafish *Snail-1* (*ZSnail-1*) are expressed in the neural crest only after the onset of migration (Thisse et al 1993, Sefton et al 1998, Jiang et al 1998). Nonvertebrate chordates such as amphioxus and ascidians also have a single *Snail* homologue that is expressed at the lateral edge of the neural plate and then subsequently in the dorsal neural tube (Corbo et al 1997, Langeland et al 1998). This is of particular interest given that the neural crest is a cell type unique to vertebrates. It suggests that neural crest may have originated as a population of *Snail*-expressing cells within the neural tube of the common ancestor of modern vertebrates (see Baker & Bronner-Fraser 1997b). These cells may then have acquired migratory ability, allowing them to disperse throughout the embryo and ultimately give rise to new derivatives, including those that form the "new head" of vertebrates (Northcutt & Gans 1983).

Inhibition of *CSlug* expression using antisense oligonucleotides has been reported to prevent neural crest emigration from the neural tube (Nieto et al 1994), while over-expression of *MSlug* in bladder carcinoma cells promotes desmosome

dissociation (Savagner et al 1997), a key step in epithelial-to-mesenchymal transitions. Although mice homozygous for null mutations in *MSlug* display no obvious neural crest defects (Jiang et al 1998), this may be because it is *MSnail* and not *MSlug* that is expressed in premigratory neural crest cells in this system (Sefton et al 1998, Jiang et al 1998). In addition, functional redundancy with *MSnail* could mask any role for *MSlug* after the onset of migration. Experiments in *Xenopus* suggest that *XSlug* may play a role in regulating its own expression and that it is involved in establishing or maintaining the neural crest-forming regions at the lateral edges of the neural plate (LaBonne & Bronner-Fraser 1998a). Overexpression of *XSlug* results in the expansion of neural crest-forming regions in vivo as well as the formation of supernumerary melanocytes, a neural crest derivative. Although over-expression of *XSlug* alone does not induce expression of neural crest markers in ectodermal explants, it can do so in conjunction with Wnt- or FGF-generated signals (LaBonne & Bronner-Fraser 1998a).

It is not known whether *CSlug* plays a role in early aspects of neural crest formation in the chick, where it is first expressed when neural folds are closing, several hours prior to the onset of neural crest migration (Nieto et al 1994). In contrast, expression of *XSnail* and *XSlug* can be detected at the neural plate border by mid-late gastrula stages (Essex et al 1993, Mayor et al 1995), long before the start of neural crest migration. The earlier onset of *XSlug/XSnail* expression occurs in cranial regions where, in amphibians, the prospective neural crest segregates from the neural tube prior to its closure (Sadaghiani & Thiebaud 1987). In contrast, trunk neural crest does not emigrate from the neural tube until after tube closure. It is possible that early *XSlug* expression regulates the condensation of the cranial neural crest masses, although a role for *XSlug* in this process, or in the epithelial-to-mesenchymal transformation and subsequent migration of the neural crest, has yet to be addressed in *Xenopus*.

Pax *Family* Pax genes encode transcription factors containing a paired-box DNA-binding domain. At least two members of this family, *Pax-3* and *Pax-7*, are expressed in the developing neural crest (Goulding et al 1991, 1993; Tremblay et al 1995; Liem et al 1995; Ericson et al 1996; Kawakami et al 1997; Bang et al 1997). Indeed, in both chick and frog, the restriction of *Pax-3* expression to the lateral edge of the neural plate is one of the earliest known responses to the signals that pattern the nervous system along the dorsoventral axis (Goulding et al 1991, Bang et al 1997). Expression of *Pax-3* is thought to be regulated by inductive signals from the non-axial mesoderm and repressive signals from the notochord (Goulding et al 1993, Bang et al 1997). The early *Pax-3* expression domain includes presumptive neural crest-forming regions, although this may not be true at all axial levels in the chick (Buxton et al 1997). *Pax-3* can act as both a transcriptional activator and a transcriptional repressor over a narrow range of protein concentration (Chalepakis et al 1994), and over-expression of Pax genes can transform fibroblasts into tumors in nude mice (Maulbecker & Gruss 1993). Conversely, when expression of *Pax-3*

is inhibited in neuronal cell lines it leads to the onset of differentiation and a loss of proliferation (Reeves et al 1998), suggesting that *Pax-3* may function to maintain cells in an undifferentiated state.

Splotch mice, which carry loss-of-function mutations in the *Pax-3* gene, have deficiencies in neural crest derivatives (Franz 1990, Epstein et al 1991), as do mice carrying a *Pax-7* knockout (Mansouri et al 1996). However, expression of *Pax-3* does not appear to be required for neural crest induction. Conjugates of early neural plate and epidermis, which express *Slug* and can form neural crest derivatives (Selleck & Bronner-Fraser 1995, Dickinson et al 1995), do not express *Pax-3* (Bang et al 1997). Moreover, expression of *Pax-3* in the dorsal neural tube does not appear to be sufficient to direct neural crest formation (Selleck et al 1998). The expression of *Pax-7* was not examined in these experiments, however, and the functional redundancy of these two genes during at least some aspects of development has been postulated (Mansouri et al 1996). In *Splotch* embryos, neural crest cells fail to exit the neural tube in caudal regions (Auerbach 1954, Serbedzija & McMahon 1997). Recently, experiments in which dorsal neural tubes from *Splotch* mice were grafted into chick host embryos showed that the defect in these mice was not intrinsic to the neural crest cells themselves (Serbedzija & McMahon 1997). When caudal neural tubes from *Splotch* embryos were grafted into non-Splotch hosts, normal neural crest migration was observed. This suggests that neural crest generation requires interaction with neighboring tissues and that these interactions are defective in *Splotch* mice. It remains possible that additional neural crest deficiencies intrinsic to the neural crest cells themselves will be found in *Pax-3/7* double knockouts.

Zic *Family* *Zic* genes, the vertebrate homologues of the *Drosophila* pair-rule gene *odd-paired* (*opa*), encode zinc finger-containing DNA-binding proteins. Four vertebrate *Zic* genes have been identified to date, *Zic-1* (also called *Zicr-1* and *Opl*), *Zic-2*, *-3*, and *-4* (Aruga et al 1996a, b; Nakata et al 1997, 1998; Brewster et al 1998; Kuo et al 1998; Mizuseki et al 1998). In mice, *Zic-1* is expressed in the presumptive dorsal neurectoderm from very early stages, and by neural tube closure expression of *Zic-1*, *-2*, and *-3* is restricted to the dorsal neural tube in overlapping but non-identical domains along the anterioposterior axis (Nagai et al 1997). The restricted expression of these genes appears to be regulated, in part, by signals from the notochord. More recently, *Zic-1*, *-2*, and *-3* have been cloned in *Xenopus* (Nakata et al 1997, 1998; Kuo et al 1998; Mizuseki et al 1998; Brewster et al 1998). At late blastula stages all three *Xenopus Zic* genes are expressed throughout the ectoderm, but become progressively restricted first to the presumptive neural plate and then to the lateral edges of the induced neural plate. As the neural tube closes, *Zic* genes continue to be expressed in the dorsal neural tube and cranial neural crest. *Zic-1* (*Opl*) is also expressed in the migrating neural crest in spinal cord regions (Kuo et al 1998), although this has not been examined for *Zic-2* and *-3*. At neurula stages, *Zic-2* and *-3* are expressed in four longitudinal lines in the trunk neural plate that are alternating and non-overlapping with stripes of primary neurons expressing

n-tubulin (Nakata et al 1997, 1998; Brewster et al 1998). While Zic-1 (Opl) has been shown to function as a transcriptional activator and contains a C-terminal domain that represses this activity (Kuo et al 1998), it has been suggested that Zic-2 may act as a transcriptional repressor (Brewster et al 1998). In *Xenopus*, expression of all three *Zic* genes is a very early response to neural inducing signals (Nakata et al 1997, 1998; Kuo et al 1998; Mizuseki et al 1998). It has been postulated that Zic proteins, which can bind to the same DNA binding sites as Gli proteins (Aruga et al 1994), may act together with Gli proteins to pattern the neural plate along the dorsovental axis (Brewster et al 1998).

Functional studies of *Zic* family members in *Xenopus* ectodermal explants have yielded both overlapping and conflicting results. When over-expressed in early embryos, all three *Xenopus Zic* genes have been found to induce ectopic expression of neural crest markers (Nakata et al 1997, 1998; Kuo et al 1998; Mizuseki et al 1998; Brewster et al 1998). The results of explant experiments are more confusing, however. For example, Nakata and colleagues have found that overexpression of *Zic-1*, *Zic-2*, or *Zic-3* induces expression of both neural and neural crest markers in ectodermal explants, including the neuronal differentiation marker *n-tubulin*. (Nakata et al 1997, 1998). Mizuseki et al (1998) have reported similar finding for *Zic-1*. In contrast, Brewster et al (1998) have found that *Zic-2* represses neuronal differentiation in whole embryos, although they did not examine its function in explants. Moreover, Kuo et al (1998) have studied the function of full length *Zic-1* (*Opl*) and found that full-length *Zic-1* does not induce either neural or neural crest markers in isolated ectoderm. Interestingly, the same investigators found that an activated form of *Zic-1* (*Opl*) carrying a C-terminal truncation can induce neural crest markers in the absence of neural markers. The different results obtained for *Zic-1* in explant assays are likely due to differences in assay conditions. For example, Kuo et al (1998) have found that *Zic-1* (*Opl*) can induce expression of neural and neural crest markers in neuralized ectoderm. Although clearly much remains to be learned about this interesting new gene family, Zic proteins seems likely to play an important role in neural crest formation.

Additional Factors Many other gene families are also likely to play important roles in early aspects of neural crest formation. For example, several members of the winged helix family of transcription factors are expressed in premigratory and migratory neural crest cells (Dirksen & Jamrich 1995, Scheucher et al 1995, Koster et al 1998, Labosky & Kaestner 1998, Odenthal & Nusslein-Volhard 1998). Interestingly, one member of this family, *FAST-1*, has been shown to act as a transcriptional co-factor for some Smad proteins, which transduce BMP/TGF-β signals (reviewed in Whitman 1998). Indeed *Xenopus Smad-1*, a BMP-restricted smad, is expressed in both premigratory and migratory neural crest cells (Thomsen 1996). MSH class homeobox genes, including *Msx-1* and *-2*, are expressed in cells that undergo epithelial-to-mesenchymal transformations, including neural crest precursors (Watanabe & Le Douarin 1996, Suzuki et al 1997, Foerst-Potts & Sadler 1997), and *Msx-1* knockout mice have deficiencies in neural crest derivatives

(Foerst-Potts & Sadler 1997). Helix-loop-helix genes are also likely to play pivotal roles in neural crest formation. *Id2*, an inhibitor of helix-loop-helix function, is expressed in the cranial neural folds in the chick, where it has been shown to influence cell lineage decisions (Martinsen & Bronner-Fraser 1998). Moreover, another helix-loop-helix factor, *XTwist*, is expressed in the neural crest in *Xenopus* from early stages (Hopwood et al 1989).

ESTABLISHMENT OF NEURAL CREST FATE

The combined action of neural crest-inducing factors, together with the early transcriptional responses to these factors, establishes a domain at the lateral edges of the neural plate with the potential to give rise to neural crest cells. Not all cells in this region will ultimately contribute to the neural crest, however. Following neural tube closure, neural crest precursors undergo an epithelial-to-mesenchymal transition and migrate to diverse locations throughout the embryo.

Segregation of the Neural Crest Lineage

Classical studies assumed that the fates of neural crest cells are segregated from other cells within the dorsal neural tube from early stages of development. However, no marker has been discovered that unequivocally marks neural crest precursors prior to the onset of migration. Indeed, when single dorsal neural tube cells are labeled with fluorescent dextrans prior to neural crest emigration, their progeny have been shown to contribute to both neural tube and neural crest derivatives (Bronner-Fraser & Fraser 1988, 1989; Collazo et al 1993; Serbedzija et al 1994). Moreover, when similar studies are carried out prior to neural tube closure, single-neural fold cells can give rise to neural tube, neural crest, and epidermal derivatives (Selleck & Bronner-Fraser 1995). These findings indicate that through the end of neurulation there are no firm boundaries for three of the four ectodermal cell types (placodal fates were not examined in these studies), a fact that is important to keep in mind when interpreting neural induction experiments. In addition, these data suggest that not all of the progeny of cells that express early neural crest markers such as *Slug* will ultimately contribute to the neural crest, and this has recently been confirmed in *Xenopus* using DiI labeling (C LaBonne & A Collazo, unpublished observation). Taken together, these studies indicate that the expression of genes such as *Slug* marks a population of cells with the potential to give rise to neural crest, but that neural and neural crest cell fates do not segregate until around the time of neural crest emigration. Consistent with this, *Snail*-expressing cells in cephalochordates, likely to represent the ancestors of the vertebrate neural crest, remain within the dorsal neural tube (Langeland et al 1998).

Given the above findings, it is possible that the epithelial-to-mesenchymal transition of the neural crest, together with accompanying changes in cell adhesion, is what ultimately establishes neural crest fate. However, even if the fates of neural

crest and neural tube cells become physically segregated by neural crest emigration, their developmental potential may remain largely unchanged. When early migrating neural crest cells are injected back into the neural tube of chick embryos, they appear to contribute to the floorplate, a ventral structure (Ruffins et al 1998). Similarly, cells taken from either the dorsal or ventral neural tube after neural crest production has ceased can, if transplanted into neural crest migratory pathways, migrate along these pathways and contribute to neural crest derivatives (Korade & Frank 1996).

Evidence against the idea that detachment from the neural tube specifies the neural crest lineage comes from in vitro studies of neuroepithelial cells. When rat E10.5 spinal cord cells are dissociated and cultured, they can give rise to neurons, astrocytes, and oligodendrocytes typical of the central nervous system (CNS) (Kalyani et al 1997). They can also give rise to neural crest derivatives such as Schwann cells and smooth muscle when cultured under conditions that promote neural crest differentiation (Mujtaba et al 1998). It is likely that these are the cells that contribute to neural crest in the transplantation experiments of Korade & Frank (1996). Clonal analysis has demonstrated that individual neuroepithelial cells are multipotent and can give rise to both CNS and neural crest derivatives (Mujtaba et al 1998). In these experiments, the generation of neural crest derivatives appears to proceed via the establishment of neural crest precursor cells that no longer give rise to CNS derivatives, at least in culture. Interestingly, treatment of neuroepithelial cells with BMP-4 was found to promote the formation of these restricted neural crest progenitors (Figure 3). The experiments of Mujtaba et al (1998) indicate that signals localized within the dorsal neural tube can restrict the ability of neural crest precursors to contribute to the CNS. Thus the establishment of the neural crest may not be linked to its egress from the neural tube. Lineage analyses have not demonstrated the presence of such a restricted progenitor population prior to the onset of neural crest migration, however.

Onset of Neural Crest Migration

A great deal is known about the pathways along which neural crest cells migrate, and molecules have been identified that may play a role in guiding them (for a review see Le Douarin 1982, Bronner-Fraser 1993, 1994, Perris 1997). However, much less is known about the events that promote the epithelial-to-mesenchymal transition and subsequent migration of the neural crest. Although it remains unclear what causes a subset of dorsal neural tube cells to emigrate from the neural tube and what prevents the remaining cells from following suite, recent evidence has implicated both cell adhesion molecules and Rho-family GTPases in this process.

The delamination of neural crest cells from the dorsal neural tube is accompanied by cytoskeletal reorganization, as well as changes in the expression of adhesion molecules at the cell surface, particularly cadherins (Duband et al 1995).

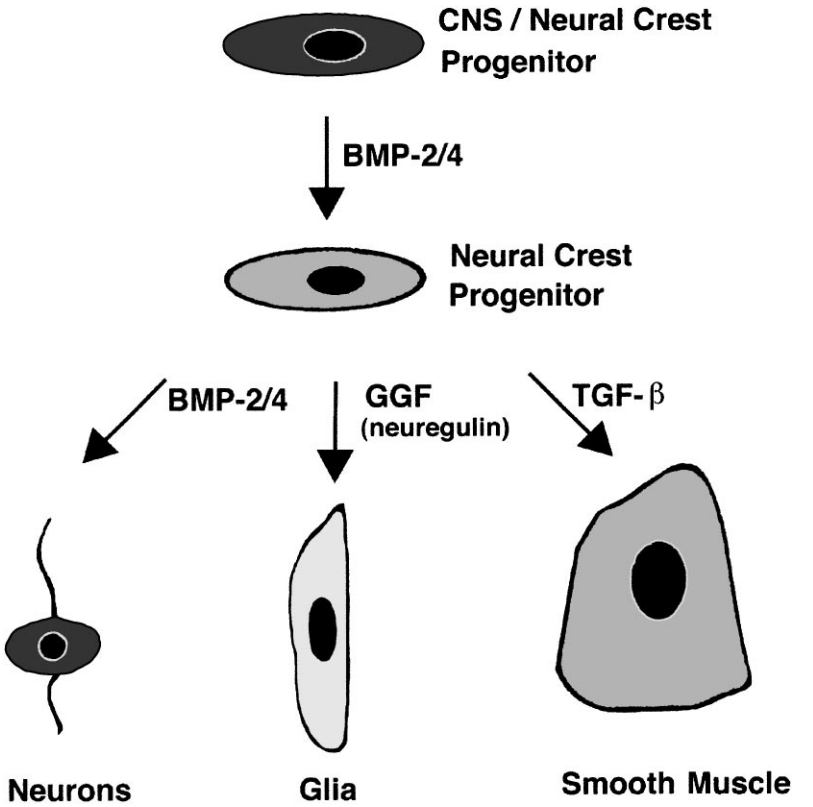


Figure 3 Differentiation of neural crest progenitors in vitro. Neuroepithelial cells isolated from rat spinal cord can differentiate as CNS or neural crest derivatives. BMP-2/-4 treatment promotes the formation of restricted neural crest progenitors that have lost the ability to differentiate as CNS derivatives. These neural crest progenitors can subsequently be instructed to differentiate as specific neural crest derivatives in culture. Note that BMP-2/-4 treatment instructs these restricted progenitors to differentiate as autonomic neurons (after Mujtaba et al 1998, Shah et al 1996).

These calcium-dependent homophilic glycoproteins are differentially expressed during development in patterns that correlate with specific morphogenetic events such as tissue segregation and cell migration (Takeichi 1995). Prior to delamination, cells in the dorsal neural tube exhibit localized expression of cadherins throughout sites of cell-cell contact. Specifically, two members of this family, *N-cadherin* and *cadherin-6B*, are expressed in the closing neural folds and dorsal neural tube of avian embryos prior to neural crest emigration (Nakagawa & Takeichi 1995). Interestingly, once neural crest cells have exited the neural tube, expression of these cadherins is downregulated or lost. Migrating neural crest

cells express other cadherins, however, including *cadherin 7* and *cadherin 11* (Nakagawa & Takeichi 1995, Kimura et al 1995, Hadeball et al 1998). It has been suggested that this cadherin type switching plays a role in neural crest emigration (Nakagawa & Takeichi 1995, 1998). Over-expression of cadherins in the dorsal neural tube prevents neural crest emigration without disrupting cell motility (Nakagawa & Takeichi 1998). It is unclear whether this loss of emigration is meaningful, however, in light of the general increase in cell adhesion following cadherin over-expression. In similar experiments, a putative dominant inhibitory cadherin had no effect on neural crest emigration (Nakagawa & Takeichi 1998). This suggests that inhibition of cadherin function alone is insufficient to direct neural crest emigration. It remains possible, however, that the levels of the inhibitory protein achieved in these experiments were insufficient to disrupt cadherin function.

It has been postulated that regulation of *N-cadherin* function, perhaps by phosphorylation of junctional proteins, may help drive neural crest emigration (Monier-Gavelle & Duband 1995, Newgreen & Minichello 1996). Moreover, it has recently been reported that the transcription factor *Msx-1*, which is co-expressed with cadherins in the dorsal neural tube, can modulate calcium-dependent cell adhesion in vitro, perhaps by modulating cadherin function (Lincecum et al 1998). The intracellular domain of cadherins is associated with several proteins that may regulate their adhesive activities. One such protein, β-catenin, is also a mediator of Wnt signals (Willert & Nusse 1998), and it has been suggested that Wnts play a role in regulating cadherin function (Hinck et al 1994). This is particularly interesting in light of the overlapping expression domains of Wnts and cadherins in the dorsal neural tube prior to the onset of neural crest emigration. Wnts may also regulate cadherin expression in the dorsal neural tube. Another transducer of Wnt signals, Lef-1, has been found to bind to the *E-cadherin* promoter (Huber et al 1996), and *E-cadherin* expression is increased in Wnt1/3A mutant mice (Ikeya et al 1997).

The preceding experiments suggest that changes in cadherin-mediated cell adhesion play a role in the process by which neural crest cells escape from the neural tube, but the precise nature of that involvement remains unclear. Regardless of the mechanisms that initiate neural crest emigration, it has been demonstrated that this process is dependent on Rho family GTPases (Liu & Jessell 1998). These small GTP-binding proteins have been implicated in cytoskeletal rearrangements that drive changes in cell shape and behavior in a number of systems, often in response to extracellular signals (Van Aelst & D'Souza-Schorey 1997, Hall 1998). Treatment of neural tube explants with C3-exotoxin, which inhibits the activity of Rho-family members but not that of other closely related small GTPases (Ridley & Hall 1992), prevents the delamination of neural crest cells (Liu & Jessell 1998). In contrast, inhibition of Rho function has no effect on the BMP-mediated induction of *Slug* in intermediate neural tube explants, or on the subsequent migration of neural crest cells in vitro. At least one Rho family member, RhoB, is expressed in the dorsal neural tube and is a good candidate for the target of C3-exotoxin in these experiments. Expression of RhoB is upregulated in response to BMP treatment and is lost following the onset of neural crest migration (Liu & Jessell 1998).

DIVERSIFICATION OF NEURAL CREST FATES

Cell lineage studies indicate that once a cell has emigrated from the neural tube it is specified as a neural crest cell. However, experiments in which migratory neural crest cells are injected back into the neural tube indicate that at least some such cells are not committed to neural crest fates. What then does it mean to be specified as a neural crest cell?

Neural Crest Cells Are Multipotent

As a population, neural crest cells give rise to an amazingly diverse set of derivatives, ranging from neurons and glia to smooth muscle and cartilage (Le Douarin 1982). Fate-mapping studies have indicated that neural crest cells from any one position along the neuraxis normally give rise to only a subset of these cell types. However, when the potential of these cells has been challenged by grafting them to different axial levels, it has generally been found that they possess the potential to give rise to a wider range of derivatives than they realize during normal development (Le Douarin 1982, 1986). For example, although trunk neural crest cells do not normally produce enteric neurons, they can do so when transplanted to vagal positions (Le Douarin et al 1975). A major exception to this pluripotency is the ability to give rise to cartilage, which appears to be possessed only by cranial neural crest cells originating from midbrain and hindbrain regions (Le Douarin 1982, Le Douarin & Smith 1988).

Although the neural crest is clearly multipotent as a population, such a population could be composed of multiple lineage restricted precursors, individual multipotent precursor cells, or some combination of these cell types. By studying cultures of neural crest cells grown at clonal density, it has been demonstrated that at least some individual neural crest cells can give rise to multiple types of derivatives (Stemple & Anderson 1992). These findings confirmed in vivo studies in which individual premigratory or migratory neural crest cells were labeled and the fates of their descendants followed (Bronner-Fraser & Fraser 1988, 1989; Fraser & Bronner-Fraser 1991). In both types of studies, however, some individual neural crest cells gave rise to only one type of derivative. Although it has been inferred from the latter findings that the potential of some neural crest cells is restricted from early stages, this need not be the case. True restrictions in potential can be demonstrated only by challenging cells with conditions instructive of alternative fates, a criterion that has seldom, if ever, been met in such studies. Although migrating neural crest cells are antigenically diverse (see Groves & Bronner-Fraser 1999 and references therein), differences in marker expression have not, for the most part, been found to correlate with restrictions in fate. Indeed, it has been suggested that at the onset of migration the neural crest is a stem cell-like population analogous to hematopoietic precursors (Stemple & Anderson 1992). In support of this idea, multipotent neural crest cells can be isolated from target sites

after migration has ceased (Sieber-Blum et al 1993, Ito & Sieber-Blum 1993, Lo & Anderson 1995, Lo et al 1997). This implies either that they migrated there in a quiescent state, or that their divisions en route were self-renewing. Moreover, it has been demonstrated that multipotent neural crest precursors are capable of at least limited self-renewal in cell culture (Stemple & Anderson 1992). However, a hierarchy of neural crest progenitor populations exhibiting progressively restricted potentials, analogous to those described during hematopoiesis, has yet to be demonstrated for the neural crest. In contrast to what has been observed in other organisms, most zebrafish neural crest cells give rise to only one type of derivative (Schilling & Kimmel 1994, Raible & Eisen 1994). This may be explained by the observations that zebrafish produce relatively few neural crest cells and these cells undergo few divisions prior to differentiating. In experiments in which the fate of zebrafish neural crest cells has been challenged, they have been found to possess greater potential than fate maps would indicate (Raible & Eisen 1996).

Mechanisms of Neural Crest Diversification

If many neural crest cells are multipotent, at least at the onset of migration, what then causes individual neural crest cells to adopt specific fates? This is a complex question that has yet to be fully answered. For a more complete discussion of this topic, the reader is referred to a number of recent reviews (LaBonne & Bronner-Fraser 1998b, Sieber-Blum et al 1998, Groves & Bronner-Fraser 1999). In general, it is thought that neural crest cells are directed toward particular fates by signals localized along migratory pathways and at target sites. Many such factors appear to act selectively, affecting the proliferation or survival of specific neural crest derivatives. A few factors have been identified, however, that appear to instruct multipotent neural crest progenitors to form specific derivatives. Interestingly, among these factors are BMPs, demonstrating again that BMP signaling is involved at multiple stages of neural crest development.

When cultures of primary neural crest cells are treated with BMP-2 or -4, they differentiate mainly as autonomic neurons (Shah et al 1996). In contrast, treatment of these cultures with glial growth factor (neuregulin) promotes glial development (Shah et al 1994), whereas smooth muscle is generated in response to TGF-β (Figure 3) (Shah et al 1996). Interestingly, each of these factors is expressed in the embryo at sites appropriate to the type of derivative they promote. For example, *BMP-2* and *-4* are expressed in the walls of the dorsal aorta, near sites of autonomic neurogenesis in adjacent sympathetic ganglia (Bitgood & McMahon 1995, Lyons et al 1995, Shah et al 1996). Neural crest cultures are thus a promising system for investigating the cell-intrinsic and -extrinsic factors that regulate neural crest cell-fate decisions.

There is also evidence that the time at which a neural crest cell exits the neural tube may also influence its subsequent fate decisions, although not necessarily its

potential. In the trunk, late migrating neural crest cells invade the dorsolateral space between the somite and ectoderm and give rise to melanocytes. Recent evidence has suggested that these cells may already be specified as melanoblasts prior to entering the dorsolateral migration pathway (Reedy et al 1998). Indeed, acquiring melanoblast potential may be a requirement for dorsolateral migration, since when other neural crest cells are grafted onto this pathway they fail to migrate (Erickson & Goins 1995). It is possible that prolonged exposure to signals within the dorsal neural tube may bias cells toward a melanocyte fate, and recent work in zebrafish has suggested that Wnts could mediate this effect (Dorsky et al 1998). It should be noted, however, that acquiring melanoblast potential does not mean that a cell's potential to give rise to other neural crest derivatives has been restricted. Late migrating neural crest cells can adopt ventral fates when grafted into younger embryos (Weston & Butler 1966, Baker et al 1997) and neural crest cells isolated from skin can give rise to neurons in culture (Richardson & Sieber-Blum 1993).

CONCLUSION

In the past few years investigators have uncovered a substantial number of new pieces in the puzzle of neural crest formation, although these pieces have yet to be assembled into a coherent picture of this process. While progress has been made in identifying signals that may initiate the earliest steps of neural crest induction, apparent differences between amniotes and anamniotes remain to be resolved. This will require carefully designed parallel experiments that control for differences in timing, axial level, and tissue morphogenesis. Dissecting this process is complicated by the observation that several of the implicated factors, including BMPs and Wnts, appear to play multiple distinct roles during neural crest development. Similarly, the precise mechanisms by which a subset of cells in the dorsal neural tube are selected to undergo an epithelial-to-mesenchymal transition and emigrate remain to be elucidated. Additional challenges include understanding how neural crest cells maintain their undifferentiated stem cell-like state, what factors confer competence to respond to lineage restricting signals, and whether the differentiation of neural crest cells proceeds via a process of progressive restriction. What is most clear is that more than a century after the neural crest was first described, much work remains before the generation of this fascinating cell type is fully understood.

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LITERATURE CITED

Alvarez IS, Araujo M, Nieto MA. 1998. Neural induction in whole chick embryo cultures by FGF. *Dev. Biol.* 199:42–54

Aruga J, Nagai T, Tokuyama T, Hayashizaki Y, Okazaki Y, et al. 1996a. The mouse zic gene family. Homologues of the *Drosophila* pair-rule gene odd-paired. *J. Biol. Chem.* 271: 1043–47

Aruga J, Yokota N, Hashimoto M, Furuichi T, Fukuda M, Mikoshiba K. 1994. A novel zinc finger protein, zic, is involved in neurogenesis, especially in the cell lineage of cerebellar granule cells. *J. Neurochem.* 63:1880–90

Aruga J, Yozu A, Hayashizaki Y, Okazaki Y, Chapman VM, Mikoshiba K. 1996b. Identification and characterization of *Zic4*, a new member of the mouse Zic gene family. *Gene* 172:291–94

Auerbach R. 1954. Analysis of the developmental effects of the lethal mutation in the house mouse. *J. Exp. Zool.* 127:305–29

Baker CV, Bronner-Fraser M. 1997a. The origins of the neural crest. Part I: embryonic induction. *Mech. Dev.* 69:3–11

Baker CV, Bronner-Fraser M. 1997b. The origins of the neural crest. Part II: an evolutionary perspective. *Mech. Dev.* 69:13–29

Baker CVH, Bronner-Fraser M, Le Douarin NM, Teillet M. 1997. Early- and late-migrating cranial neural crest cell populations have equivalent developmental potential in vivo. *Development* 124:3077–87

Bang AG, Papalopulu N, Kintner C, Goulding MD. 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* 124:2075–85

Bei M, Maas, R. 1998. FGFs and BMP4 induce both Msx1–independent and Msx1–dependent signaling pathways in early tooth development. *Development* 125:4325–33

Bitgood MJ, McMahon AP. 1995. Hedgehog and BMP genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev. Biol.* 172:126–38

Bonstein L, Elias S, Frank D. 1998. Paraxial-fated mesoderm is required for neural crest induction in *Xenopus* embryos. *Dev. Biol.* 193:56–68

Brewster R, Lee J, Ruiz i Altaba A. 1998. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. *Nature* 393:579–83

Bronner-Fraser M. 1993. Mechanisms of neural crest cell migration. *BioEssays* 15:221–30

Bronner-Fraser M. 1994. Neural crest cell formation and migration in the developing embryo. *FASEB J.* 8:699–706

Bronner-Fraser M, Fraser SE. 1988. Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature* 335:161–64

Bronner-Fraser M, Fraser SE. 1989. Developmental potential of avian neural crest cells in situ. *Neuron* 3:755–66

Bueno D, Skinner J, Abud H, Heath JK. 1996. Spatial and temporal relationships between *Shh*, *Fgf4*, and *Fgf8* gene expression at diverse signalling centers during mouse development. *Dev. Dyn.* 207:291–99

Buxton P, Hunt P, Ferretti P, Thorogood P. 1997. A role for midline closure in the reestablishment of dorsoventral pattern following dorsal hindbrain ablation. *Dev. Biol.* 183:150–65

Chalepakis G, Jones FS, Edelma GM, Gruss P. 1994. Pax-3 contains domains for transcription activation and transcription inhibition. *Proc. Natl. Acad. Sci. USA* 91:12745–49

Chang C, Hemmati-Brivanlou A. 1998. Neural crest induction by Xwnt7B in *Xenopus*. *Dev. Biol.* 194:29–34

Christian JL, McMahon JA, McMahon AP, Moon RT. 1991. Xwnt-8, a *Xenopus* Wnt-1/int-1–related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning dur-

ing embryogenesis. *Development* 111:1045–55

Collazo A, Bronner-Fraser M, Fraser SE. 1993. Vital dye labeling of *Xenopus laevis* trunk neural crest reveals multipotency and novel pathways of migration. *Development* 118:363–76

Connolly DJ, Patel K, Cooke J. 1997. Chick *noggin* is expressed in the organizer and neural plate during axial development, but offers no evidence of involvement in primary axis formation. *Int. J. Dev. Biol.* 41:389–96

Corbo JC, Erives A, Di Gregorio A, Chang A, Levine M. 1997. Dorsoventral patterning of the vertebrate neural tube is conserved in a protochordate. *Development* 124:2335–44

Cox WG, Hemmati-Brivanlou A. 1995. Caudalization of neural fate by tissue recombination and bFGF. *Development* 121:4349–58

Dale L, Howes G, Price BMJ, Smith JC. 1992. Bone morphogenetic protein 4: a ventralizing factor in *Xenopus* development. *Development* 115:573–85

Dickinson ME, Selleck MAJ, McMahon AP, Bronner-Fraser M. 1995. Dorsalization of the neural tube by the non-neural ectoderm. *Development* 121:2099–2106

Dirksen ML, Jamrich M. 1995. Differential expression of *fork head* genes during early *Xenopus* and zebrafish development. *Dev. Genet.* 17:107–16

Dorsky R I, Moon RT, Raible DW. 1998. Control of neural crest cell fate by the Wnt signalling pathway. *Nature* 396:370–73

Duband J, Monier F, Delannet M, Newgreen D. 1995. Epithelium-mesenchyme transition during neural crest development. *Acta Anat.* 154:63–78

Dudley AT, Robertson EJ. 1997. Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7-deficient embryos. *Dev. Dyn.* 208:349–62

Epstein DJ, 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* 67:767–74

Erickson CA, Goins TL. 1995. Avian neural crest cells can migrate in the dorsolateral path only if they are specified as melanocytes. *Development* 121:915–24

Ericson J, Morton S, Kawakami A, Roelink H, Jessell TM. 1996. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell* 87:661–73

Essex LJ, Mayor R, Sargent MG. 1993. Expression of *Xenopus snail* in mesoderm and prospective neural fold ectoderm. *Dev. Dyn.* 198:08–22

Fainsod A, Steinbeisser H, DeRobertis E. 1994. On the function of BMP4 in patterning the ventral marginal zone of the *Xenopus* embro. *EMBO J.* 13:5015–25

Foerst-Potts L, Sadler TW. 1997. Disruption of *Msx-1* and *Msx-2* reveals roles for these genes in craniofacial, eye, and axial development. *Dev. Dyn.* 209:70–84

Franz T. 1990. Defective ensheathment of motoric nerves in the *Splotch* mutant mouse. *Acta Anat.* 138:246–53

Fraser SE, Bronner-Fraser M. 1991. Migrating neural crest cells in the trunk of the avian embryo are multipotent. *Development* 112:913–20

Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P. 1991. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* 10:1135–47

Goulding MD, Lumsden A, Gruss P. 1993. Signals from the notochord and floor plate regulate the region-specific expression of two Pax genes in the developing spinal cord. *Development* 117:1001–16

Gray S, Szymanksi P, Levine M. 1994. Short-range repression permits multiple enhancers to function autonomously within a complex promoter. *Genes Dev.* 8:1829–38

Groves A, Bronner-Fraser M. 1999. Neural crest diversification. *Curr. Top. Dev. Biol.* 43:221–58

Hadeball B, Borchers A, Wedlich D. 1998. *Xenopus* cadherin-11 (Xcadherin-11) expression requires the Wg/Wnt signal. *Mech. Dev.* 72:101–13

Hall A. 1998. Rho GTPases and the actin cytoskeleton. *Science* 279:509–14

Hammerschmidt M, Serbedzija GN, McMahon AP. 1996. Genetic analysis of dorsoventral pattern formation in the zebrafish: requirement of a BMP-like ventralizing activity and its dorsal repressor. *Genes Dev.* 10:2452–61

Hammerschmidt M, Nusslein-Volhard C. 1993. The expression of a zebrafish gene homologous to *Drosophila snail* suggests a conserved function in invertebrate and vertebrate gastrulation. *Development* 119:1107–18

Hawley SH, Wunnenberg-Stapleton K, Hashimoto C, Laurent MN, Watabe T, et al. 1995. Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. *Genes Dev.* 9:2923–35

Hemmati-Brivanlou A, Kelly OG, Melton DA. 1994. *Follistatin*, an antagonist of activin is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell* 77:283–95

Hemmati-Brivanlou A, Thomsen G. 1995. Ventral mesodermal patterning in *Xenopus* embryos: expression patterns and activities of BMP-2 and BMP-4. *Dev. Genet.* 17(1):78–89

Hinck L, Nelson WJ, Papkof J. 1994. Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. *J. Cell Biol.* 124:729–41

Hopwood ND, Pluck A, Gurdon JB. 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

Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R. 1996. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* 59:3–10

Hume CR, Dodd J. 1993. *Cwnt-8C*: a novel Wnt gene with a potential role in primitive streak formation and hindbrain organization. *Development* 119:1147–60

Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. 1997. Wnt signaling required for expansion of neural crest and CNS progenitors. *Nature* 389:966–70

Ip YT, Maggert K, Levine M. 1994. Uncoupling gastrulation and mesoderm differentiation in the *Drosophila* embryo. *EMBO J.* 13:5826–34

Isaacs HV, Tannahill D, Slack JMW. 1992. Expression of a novel FGF in the *Xenopus* embryo. A new candidate inducing factor for mesoderm formation and anteroposterior specification. *Development* 114:711–20

Ito K, Sieber-Blum M. 1993. Pluripotent and developmentally restricted neural-crest-derived cells in posterior visceral arches. *Dev. Biol.* 156:191–200

Jiang R, Lan Y, Norton CR, Sundberg JP, Gridley T. 1998. The *Slug* gene is not essential for mesoderm or neural crest development in mice. *Dev. Biol.* 198:77–85

Kalyani A, Hobson K, Rao MS. 1997. Neuroepithelial stem cells from the embryonic spinal cord: isolation, characterization, and clonal analysis. *Dev. Biol.* 186:202–23

Kawakami A, Kimura-Kawakami M, Nomura T, Fujisawa H. 1997. Distributions of PAX6 and PAX7 proteins suggest their involvement in both early and late phases of chick brain development. *Mech. Dev.* 66:119–30

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* 119:1067–78

Kimura Y, Matsunami H, Inoue T, Shimamura K, Uchida N, et al. 1995. *Cadherin-11* expressed in association with mesenchymal morphogenesis in the head, somite, and limb bud of early mouse embryos. *Dev. Biol.* 169: 347–58

Kintner CR, 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

Knecht AK, Good PG, Dawid IB, Harland RM. 1995. Dorsal-ventral patterning and

differentiation of noggin-induced neural tissue in the absence of mesoderm. *Development* 121:1927–36

Korade Z, Frank E. 1996. Restriction in cell fates of developing spinal cord cells transplanted to neural crest pathways. *J. Neurosci.* 16:7638–48

Koster M, Dillinger K, Knochel W. 1998. Expression pattern of the winged helix factor *XFD-11* during *Xenopus* embryogenesis. *Mech. Dev.* 76:169–73

Kroll KL, Amaya, E. 1996. Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development* 122:3173–83

Kuo JS, Patel M, Gamse J, Merzdorf C, Liu X, et al. 1998. Opl: a zinc finger protein that regulates neural determination and patterning in *Xenopus*. *Development* 125:2867–82

LaBonne C, Bronner-Fraser M. 1998a. Neural crest induction in *Xenopus*: evidence for a two signal model. *Development* 125:2403–14

LaBonne C, Bronner-Fraser M. 1998b. Induction and patterning of the neural crest, a stem cell-like precursor population. *Neurobiology* 36:175–89

Labosky PA, Kaestner KH. 1998. The winged helix transcription factor *Hfh2* is expressed in neural crest and spinal cord during mouse development. *Mech. Dev.* 76:185–90

Lamb TM, Harland RM. 1995. Fibroblast growth factor is a direct neural inducer, which combined with noggin, generates anterior-posterior neural pattern. *Development* 121: 3627–36

Lamb TM, Knecht AK, Smith WC, Stachel SE, Economides AN, et al. 1993. Neural induction by the secreted peptide noggin. *Science* 262:713–18

Langeland JA, Tomsa J, Jackman WR, Kimmel CB. 1998. An amphioxus *snail* gene: expression in paraxial mesoderm and neural plate suggests a conserved role in patterning the chordate embryo. *Dev. Genes Evol.* 208:569–77

Launay CV, Fromentoux V, Shi DL, Boucaut JC. 1996. A truncated FGF receptor blocks neural induction by endogenous *Xenopus* inducers. *Development* 122:869–80

Le Douarin N. 1982. *The Neural Crest* Cambridge, UK: Cambridge Univ. Press

Le Douarin NM. 1986. Cell lineage segregation during peripheral nervous system ontogeny. *Science* 231:1515–22

Le Douarin NM, Renaud D, Teillet MA, Le Douarin GH. 1975. Cholinergic differentiation of presumptive adrenergic neuroblasts in interspecific chimeras after heterotopic transplantations. *Proc. Natl. Acad. Sci. USA* 72:728–32

Le Douarin NM, Smith J. 1988. Development of the peripheral nervous system from the neural crest. *Annu. Rev. Cell Biol.* 4:375–404

Leptin M. 1991. *twist* and *snail* as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev.* 5:1568–76

Liem KF, Tremmi G, Jessell TM. 1997. A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91:127–38

Liem KF, Tremmi G, Roelink H, Jessell TM. 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* 82:969–79

Lincecum JM, Fannon A, Song K, Wang Y, Sassoon DA. 1998. Msh homeobox genes regulate cadherin-mediated cell adhesion and cell-cell sorting. *J. Cell Biochem.* 70:22–28

Liu JP, Jessell TM. 1998. A role for rhoB in the delamination of neural crest cells from the dorsal neural tube. *Development* 125:5055–67

Lo L, Anderson DJ. 1995. Postmigratory neural crest cells expressing *c-Ret* display restricted developmental and proliferative capacities. *Neuron* 15:527–39

Lo L, Sommer L, Anderson DA. 1997. MASH1 maintains competence for BMP2–induced neuronal differentiation in post-migratory neural crest cells. *Curr. Biol.* 7:440–50

Lyons KM, Hogan BLM, Robertson EJ. 1995. Colocalization of *BMP2* and *BMP7* RNAs suggests that these factors cooperatively

mediate tissue interaction during murine development. *Mech. Dev.* 50:71–83

Mahmood R, Kiefer P, Guthrie S, Dickson C, Mason I. 1995. Multiple roles for FGF-3 during cranial neural development in the chicken. *Development* 121:1399–410

Mancilla A, Mayor R. 1996. Neural crest formation in *Xenopus laevis*: mechanisms of *Xslug* induction. *Dev. Biol.* 177:580–89

Mansouri A, Stoykova A, Torres M, Gruss P. 1996. Dysgenesis of cephalic neural crest derivatives in Pax7$^{-/-}$ mutant mice. *Development* 122:831–38

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.* 198:19–29

Martinsen BJ, Bronner-Fraser M. 1998. Neural crest specification regulated by the helix-loop-helix repressor Id2. *Science* 281:988–91

Maulbecker CC, Gruss P. 1993. The oncogenic potential of Pax genes. *EMBO J.* 12:2361–67

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

Mayor R, Morgan R, Sargent MG. 1995. Induction of the prospective neural crest of *Xenopus*. *Development* 121:767–77

McGrew LL, Hoppler S, Moon RT. 1997. Wnt and FGF pathways cooperatively pattern anteroposterior neural ectoderm in *Xenopus*. *Mech. Dev.* 69:105–14

McGrew LL, Lai CJ, Moon RT. 1995. Specification of the anterioposterior neural axis through synergistic interaction of the Wnt signaling cascade with noggin and follistatin. *Dev. Biol.* 172:337–42

McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP. 1998. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes Dev.* 12:1438–52

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* 125:579–87

Monier-Gavelle F, Duband JL. 1995. Control of N-cadherin-mediated intercellular adhesion in migrating neural crest cells in vitro. *J. Cell Sci.* 108:3839–53

Morgan R, Sargent MG. 1997. The role in neural patterning of translation initiation factor eIF4AII; induction of neural fold genes. *Development* 124:2751–60

Moury JD, Jacobson AG. 1990. The origins of neural crest cells in the axolotl. *Dev. Biol.* 141:243–53

Muhr J, Jessell TM, Edlund T. 1997. Assignment of early caudal identity to neural plate cells by a signal from caudal paraxial mesoderm. *Neuron* 19:487–502

Mujtaba T, Mayer-Proschel M, Rao MS. 1998. A common neural progenitor for the CNS and PNS. *Dev. Biol.* 200:1–15

Nagai T, Aruga J, Takada S, Gunther T, Sporle R, et al. 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

Nakagawa S, Takeichi M. 1995. Neural crest cell-cell adhesion controlled by sequential and subpopulation-specific expression of novel cadherins. *Development* 121:1321–32

Nakagawa S, Takeichi M. 1998. Neural crest emigration from the neural tube depends on regulated cadherin expression. *Development* 125:2963–71

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. USA* 94:11980–85

Nakata K, Nagai T, Aruga J, Mikoshiba K. 1998. *Xenopus* Zic family and its role in neural and neural crest development. *Mech. Dev.* 75:43–51

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.* 62:183–95

Newgreen DF, Minichiello J. 1996. Control

of epitheliomesenchymal transformation. II. Cross-modulation of cell adhesion and cytoskeletal systems in embryonic neural cells. *Dev. Biol.* 176:300–12

Nguyen VH, Schmid B, Trout J, Connors SA, Ekker M, Mullins MC. 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.* 199:3–110

Nieto MA, Bennett MF, Sargent M, Wilkinson DG. 1992. Cloning and developmental expression of *Sna*, a murine homologue of the *Drosophila snail* gene. *Development* 116:227–37

Nieto MA, Sargent MG, Wilkinson DG, Cooke J. 1994. Control of cell behaviour during vertebrate development by *Slug*, a zinc finger gene. *Science* 264:835–39

Northcutt RG, Gans C. 1983. The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Q. Rev. Biol.* 58:1–28

Odenthal J, Nusslein-Volhard C. 1998. *fork head* domain genes in zebrafish. *Dev. Genes Evol.* 208:245–58

Papalopulu N, Kintner C. 1996. A posteriorising factor, retinoic acid, reveals that anteroposterior patterning controls the timing of neuronal differentiation in *Xenopus* neuroectoderm. *Development* 122:3409–18

Pera E, Stein S, Kessel M. 1999. Ectodermal patterning in the avian embryo: epidermis versus neural plate. *Development* 126:63–73

Perris R. 1997. The extracellular matrix in neural crest-cell migration. *Trends Neurosci.* 20:23–31

Raible DW, Eisen JS. 1994. Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. *Development* 120:495–503

Raible DW, Eisen JS. 1996. Regulative interactions in zebrafish neural crest. *Development* 122:501–7

Reedy MV, Faraco CD, Erickson CA. 1998. The delayed entry of thoracic neural crest cells into the dorsolateral path is a consequence of the late emigration of melanogenic neural crest cells from the neural tube. *Dev. Biol.* 200:234–46

Reeves FC, Burdge GC, Fredericks WJ, Rauscher FJ, Lillycrop K. 1998. Induction of antisense *Pax-3* expression leads to the rapid morphological differentiation of neuronal cells and an altered response to the mitogenic growth factor bFGF. *J. Cell Sci.* 112:253–61

Richardson MK, Sieber-Blum M. 1993. Pluripotent neural crest cells in the developing skin of the quail embryo. *Dev. Biol.* 157:348–58

Ridley AJ, Hall A. 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and X actin stress fibers in response to growth factors. *Cell* 70:389–99

Riese J, Zeller R, Dono R. 1995. Nucleocytoplasmic translocation and secretion of fibroblast growth factor-2 during avian gastrulation. *Mech. Dev.* 49:13–22

Ruffins S, Artinger K, Bronner-Fraser M. 1998. Early migrating neural crest cells can form ventral neural tube derivatives when challenged by transplantation. *Dev. Biol.* 203:295–304

Sadaghiani B, Thiebaud CH. 1987. Neural crest development in the *Xenopus laevis* embryo, studied by interspecific transplantation and scanning electron microscopy. *Dev. Biol.* 124:91–110

Saint-Jeannet J, He X, Varmus HE, Dawid IB. 1997. Regulation of dorsal fate in the neural axis by Wnt-1 and Wnt-3a. *Proc. Natl. Acad. Sci. USA* 94:13713–18

Sasai Y, De Robertis EM. 1997. Ectodermal patterning in vertebrate embryos. *Dev. Biol.* 182:5–20

Sasai Y, Lu B, Steinbesser H, Geissert D, Gont LK, De Robertis, EM. 1994. *Xenopus chordin*: a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* 79:779–90

Savage R, Phillips CR. 1989. Signals from the dorsal blastopore lip region during gastrulation bias the ectoderm toward a nonepidermal

pathway of differentiation in *Xenopus laevis*. *Dev. Biol.* 133:157–68

Savagner P, Yamada KM, Thiery JP. 1997. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition. *J. Cell Biol.* 137:1403–19

Scheucher M, Dege P, Lef J, Hille S, Knochel W. 1995. Transcription patterns of four different fork head/HNF-3 related genes (*XFD-4, 6, 9* and *10*) in *Xenopus laevis* embryos. *Roux's Arch. Dev. Biol.* 204:203–11

Schilling TF, Kimmel CB. 1994. Segment and cell type lineage restrictions during pharyngeal arch development in the zebrafish embryo. *Development* 120:483–94

Schultheiss TM, Burch JB, Lassar AB. 1997. A role for bone morphogenetic proteins in the induction of cardiac myogenesis. *Genes Dev.* 11:451–62

Sefton M, Sanchez S, Nieto MA. 1998. Conserved and divergent roles for members of the Snail family of transcription factors in the chick and mouse embryo. *Development* 125:3111–21

Selleck MAJ, Bronner-Fraser M. 1995. Origins of the avian neural crest: the role of neural plate-epidermal interactions. *Development* 121:525–38

Selleck MAJ, Garcia-Castro MI, Artinger KB, 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* 125:4919–30

Serbedzija GN, Bronner-Fraser M, Fraser SE. 1994. Developmental potential of trunk neural crest cells in the mouse. *Development* 120:1709–18

Serbedzija GN, McMahon AP. 1997. Analysis of neural crest cell migration in Splotch mice using a neural crest-specific LacZ reporter. *Dev. Biol.* 185:139–47

Shah NM, Groves AK, Anderson DJ. 1996. Alternative neural crest cell fates are instructively promoted by TGF superfamily members. *Cell* 85:331–43

Shah NM, Marchionni MA, Isaacs I, Stroobant PW, Anderson DJ. 1994. Glial growth factor restricts mammalian neural crest stem cells to a glial fate. *Cell* 77:349–60

Sieber-Blum M, Ito K, Richardson MK, Langtimm CJ, Duff RS. 1993. Distribution of pluripotent neural crest cells in the embryo and the role of brain-derived neurotrophic factor in the commitment to the primary sensory neuron lineage. *J. Neurobiol.* 24:173–84

Sokol S, Melton DA. 1991. Pre-existent pattern in *Xenopus* animal pole cells revealed by induction with activin. *Nature* 351:409–11

Sokol SY. 1993. Mesoderm formation in *Xenopus* ectodermal explants overexpressing *Xwnt8*: evidence for a cooperating signal reaching the animal pole by gastrulation. *Development* 118:1335–42

Stemple DL, Anderson DJ. 1992. Isolation of a stem cell for neurons and glia derived from the mamalian neural crest. *Cell* 71:973–85

Storey KG, Goriely A, Sargent CM, Brown JM, Burns HD, et al. 1998. Early posterior neural tissue is induced by FGF in the chick embryo. *Development* 125:473–84

Streit A, Lee KJ, Woo I, Roberts C, Jessell TM, Stern CD. 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* 125:507–19

Streit A, Stern CD. 1999. Establishment and maintenance of the border of the neural plate in the chick: involvement of FGF and BMP activity. *Mech. Dev.* In press

Suzuki A, Ueno N, Hemmati-Brivanlou A. 1997. *Xenopus msx1* mediates epidermal induction and neural inhibition by BMP4. *Development* 124:3037–44

Takeichi M. 1995. Morphogenetic roles of classic cadherins. *Curr. Opin. Cell Biol.* 7:619–27

Tannahill D, Isaacs HV, Close MJ, Peters G, Slack JMW. 1992. Developmental expression of the *Xenopus int-2* (FGF-3) gene: activation by mesodermal and neural induction. *Development* 115:695–702

Thisse C, Thisse B, Postlethwait. 1995. Expression of *snail2*, a second member of the zebrafish Snail family, in cephalic mesendoderm and presumptive neural crest of wild-type and *spadetail* mutant embryos. *Dev. Biol.* 172:86–99

Thisse C, Thisse B, Schilling TH, Postlethwait JH. 1993. Structure of the zebrafish snail gene and its expression in wild-type, *spadetail* and *no tail* mutant embryos. *Development* 119:1203–15

Thomsen GH. 1996. *Xenopus mothers against decapentaplegic* is an embryonic ventralizing agent that acts downstream of the BMP2/4 receptor. *Development* 122:2359–66

Tremblay P, Kessel M, Gruss P. 1995. A transgenic neuroanatomical marker identifies cranial neural crest deficiencies associated with the Pax3 mutant Splotch. *Dev. Biol.* 171:317–29

Tucker AS, Al Khamis A, Sharpe PT. 1998. Interactions between Bmp-4 and Msx-1 act to restrict gene expression to odontogenic mesenchyme. *Dev. Dyn.* 212:533–39

Van Aelst L, D'Souza-Schorey C. 1997. Rho GTPases and signaling networks. *Genes Dev.* 11:2295–22

Watanabe Y, Le Douarin NM. 1996. A role for BMP-4 in the development of subcutaneous cartilage. *Mech. Dev.* 57:69–78

Weinstein DC, Hemmati-Brivanlou A. 1997. Neural induction in X*enopus laevis*: evidence for the default model. *Curr. Opin. Neurobiol.* 7:7–12

Weston JA, Butler SL. 1996. Temporal factors affecting localization of neural crest cells in the chicken embryo. *Dev. Biol.* 14:246–66

Whitman M. 1998. Smads and early developmental signaling by the TGFbeta superfamily. *Genes Dev.* 2445–62

Willert K, Nusse R. 1998. Beta-catenin: a key mediator of Wnt signaling. *Curr. Opin. Genet. Dev.* 8:95–102

Wilson PA, Hemmati-Brivanlou A. 1995. Induction of epidermis and inhibition of neural fate by Bmp-4. *Nature* 376:331–33

Wilson PA, Hemmati-Brivanlou A. 1997. Vertebrate neural induction: inducers, inhibitors, and a new synthesis. *Neuron* 18:699–710

Wilson PA, Lagna G, Suzuki A, Hemmati-Brivanlou A. 1997. Concentration-dependent patterning of the *Xenopus* ectoderm by BMP4 and its signal transducer Smad1. *Development* 124:3177–84

Winnier G, Blessing M, Labosky PA, Hogan BL. 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* 9:2105–16

Wolda SL, Moody CJ, Moon RT. 1993. Overlapping expression of *Xwnt-3a* and *Xwnt-1* in neural tissue of *Xenopus laevis* embryos. *Dev. Biol.* 155:46–57

Wozney JM. 1989. Bone morphogenetic proteins. *Prog. Growth Factor Res.* 1:267–80

Xu RH, Kim J, Taira M, Sredni D, Kung H. 1997. Studies on the role of fibroblast growth factor signaling in neurogenesis using conjugated/aged animal caps and dorsal ectoderm-grafted embryos. *J. Neurosci.* 17:6892–98

Xu RH, Kim J, Taira M, Zhan S, Sredni D, Kung HF. 1995. A dominant negative bone morphogenetic protein 4 receptor causes neuralization in *Xenopus* ectoderm. *Biochem. Biophys. Res. Commun.* 212:212–19

Yamada T, Pfaff SL, Edlund T, Jessell TM. 1993. Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate. *Cell* 73:673–86

Zhang H, Bradley A. 1996. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* 122:2977–86

Zimmerman LB, De Jeso es-Escobar JM, Harland RM. 1996. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* 86:599–606

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