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Development of Neural Crest in *Xenopus*


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The neural crest is a unique cell population among embryonic cell types, displaying properties of both ectodermal and mesodermal lineages. Most of the recent studies examining the neural crest have been performed in avian embryos. Only in the first half of this century were amphibians extensively used. We first summarize this important older source of information, reviewing studies made since the turn of the century. Due to the increasingly detailed in cellular and molecular knowledge of the early development of *Xenopus laevis*, the remainder of the review focuses on this species. We describe the route of migration and fate of the neural crest and propose a new model of neural crest induction in which prospective cells are induced independently of the neural plate by a double gradient of a morphogen that patterns the entire ectoderm. This model is also discussed in a more general context in connection with the dorsoventral patterning of the neural tube. Finally, we discuss some ideas concerning neural crest evolution and propose a novel hypothesis about its phylogenetic origin. Copyright © 1999 by Academic Press.

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1. Introduction

The neural crest comprises a unique set of neuroepithelial cells. One of the most dramatic morphogenetic events during early embryogenesis is the segregation of neural crest cells from the dorsal portion of the neural tube and their subsequent migration throughout the embryo to generate a prodigious array of cell types (Le Douarin, 1982). The developmental potency of the neural crest as a tissue is intermediate between classically defined ectoderm and mesoderm. It produces ectodermal-type derivatives such as neurons and glia of the peripheral nervous system. It also produces mesodermal-type derivatives such as bone, dermis, and cartilage-forming cells of the face.

Although the neural crest was discovered in the chick more than 100 years ago (His, 1868) and studied in different organisms including amphibia, only in the past 20 years have important aspects of neural crest development been elucidated. These are related to the migration pathways and the fate of the neural crest cells during their developmental history. The major problem in the study of the neural crest is that the cells, after migration, become indistinguishable from neighboring cells. The quail-chick chimera technique of Le Douarin (1969) and its wide application by many laboratories made it possible to study the migration and differentiation of the crest in the chick. Recent cell-labeling techniques also offer insights into important questions of cell migration and lineage (Serbedzija *et al.*, 1989). Although most of these techniques have been developed in avian systems, allowing us to analyze interesting aspects of the neural crest in the chick, earlier experiments utilized amphibian embryos. Because of their extensive use in embryological techniques and recently in genetic experiments, in which overexpression of genes and manipulation of their activities are easy to perform, amphibians offer some distinct advantages for studying questions of neural crest development.

In this review we focus on a number of different aspects of neural crest development in amphibia, particularly in *Xenopus* because of the detailed knowledge of the cellular and molecular aspects of its early development. We summarize the knowledge generated in amphibia since the turn of the century, following the methods used by experimental embryologists in their attempts not only to establish which structures originate from the neural crest but also to study its properties. We compare these data and the analysis of migration pathways with the most recent reports using the chick and *Xenopus*.

Xenopus embryos have been very useful in understanding the inductive process in terms of cell interactions and molecules involved. We analyze what is known about the specification of the neural crest at the border of the neural plate and how all the data can be organized into a new model of neural crest induction. An increasing number of genes expressed in the crest have been identified. We summarize some of these data and propose a possible cascade of gene regulation

within the crest cells. Finally, we discuss and propose a novel hypothesis concerning the evolution of the neural crest cells.

II. Early Studies on Neural Crest Development in Amphibia

Extensive studies on neural crest development were made in the past using amphibian embryos. However, because of the lack of appropriate cell-labeling techniques and limitations in the grafting experiments, not all this information can be considered as equally reliable. Very important observations were made in the first half of this century that have not been confirmed with modern techniques of cell and molecular biology. We review some of these early reports.

During the past century, it was observed that the material from which the primary organs arise in embryonic development is arranged in layers. This observation led von Baer to propose in 1828 the germ-layer theory, in which homologous structures in different animals are formed of material from corresponding layers. Ectoderm forms skin and nerves, endoderm forms respiratory and digestive tubes, and mesoderm forms connective tissue, blood cells, the heart, the urogenital system, and part of most internal organs. This concept was soon considered a "law," although it was never tested experimentally.

The discovery of the neural crest and of some of its properties generated a violent controversy that reflected the power of the germ-layer theory. His (1868) gave the first description of the neural crest in the chick as a band of material lying between the presumptive epidermis and the neural plate. From the description of the neural crest made by Brachet (1908) in *Rana* and by Baker and Graves (1939) in *Ambystoma*, it was concluded that the neural crest material was already present in the open neural plate stage. At the time, this was interpreted as evidence that the neural crest and the neural tube had a different origin. It is interesting to point out that this old discussion is still relevant today, as the multipotentiality of the cells lying at the border of the neural plate has been demonstrated: neural crest cells can originate from prospective neural plate, neural folds, or even epidermal cells (Selleck and Bronner-Fraser, 1995). In addition, the expression of many genes is shared between the neural crest cells and the dorsal part of the neural tube (Section VI).

A. Fate of the Neural Crest

The fate of the neural crest in amphibia has been investigated by marking the crest cells, either by vital staining or by exchanging them for corresponding cells from embryos of another species with nuclei of different appearance. Another method is to excise neural crest cells and to study the resulting deficiencies. A

third method consists of transplanting the neural crest to another part of the body to see what it can give rise to in the new surroundings.

1. Neural Derivatives of the Neural Crest

The origin of the spinal ganglia was analyzed by Harrison in *Rana* (1924), who eliminated the dorsal part of the trunk immediately after the closure of the neural folds and found that the resulting larva lacked spinal ganglia. On the other hand, Detwiler and Kehoe (1939) performed vital staining of the neural crest by pressing small blocks of agar impregnated with Nile-blue sulfate or neutral red against the neural ridge or plate. They concluded that most of the cells in the ganglia derived from the neural crest. However, it should be mentioned that with such kind of staining it is impossible to judge how far the dye has diffused into the tissue. Raven (1937) introduced a new method of experimentation by grafting neural crest cells between animals from different genera. Neural crest cells from *Ambystoma* were transplanted into the same region in *Triturus*, and the grafted cells were distinguished because of the difference in size and color of the nuclei. However, as Raven made unilateral transplantations, his data did not allow the conclusion that the spinal ganglia were of mixed origin, because cells from the unoperated side could migrate to the other side of the embryo. In fact, it was found that in the trunk 45% of the cells migrate down the opposite side. Interestingly, the removal of the trunk neural folds on both sides of an *Ambystoma* larva led to the absence of spinal ganglia, suggesting that the neural crest was involved in its development.

The origin of cranial ganglia was also analyzed by staining methods, and it was concluded that neural crest and placodal cells contribute to it (Yntema, 1943).

2. Mesodermal Derivatives of the Neural Crest

Landacre stated in 1921 (Landacre, 1921) that in Urodeles the mesenchyme in the ventral part of the head came from the neural crest, and Stone (1922) came to a similar conclusion, adding that the mesenchyme of gills also arises from crest material. This mesenchyme aggregates in the head to form a precartilaginous cell mass which differentiates into the cartilaginous neural arches. Although grafting experiments (Raven, 1935) showed that the trunk neural crest was not able to differentiate into cartilage, it was quite clear that most of the head crest was used in the formation of the chondrocranium. Landacre (1921) was able to follow the ventral migration of the neural crest cells along the lateral side of the visceral arches, where they give rise to cartilage. These morphological observations were corroborated by Stone (1922) in *Ambystoma* and *Rana* and by Raven (1935) and de Beer (1947) in *Ambystoma*. This conclusion was further proved by Harrison (1935) using heteroplastic grafts.

All these experiments showed the ability of the cephalic crest to differentiate

into cartilage; however, when these cells were grafted into the trunk, no cartilage was observed. In a series of transplant experiments, Hörstadius (1950) analyzed this problem. He grafted cephalic neural crest into the trunk region of an embryo under different conditions. He found that the cephalic crest would only produce cartilage in the trunk if some other factor was present. He showed that the other factor could be pharynx endoderm or a wound in the somites near the graft. These results can be explained by the assumption that either activating substances had emerged from the damaged tissue or the graft had come into contact with activating tissues. It seemed to suggest that notochord and intestine promoted chondrification.

3. Pigment Cells Derived from the Neural Crest

Originally, pigment cells were considered to be modified connective cells. The first suggestion that the neural crest might be their source was due to observations by Borcea (1909) in teleosts and by Harrison (1910) in tissue culture. However, these observations did not receive much attention until DuShane analyzed the problem in a series of deficiency, transplantation, and explanation experiments (DuShane, 1935, 1936, 1938). The bilateral removal of the trunk neural crest produced an absence of pigment in the larva, whereas pieces of neural folds produced pigment cells when grafted to the ventral side of the embryo or in tissue culture. The production of melanoblasts is not the same in all of the neural crest. Niu (1947) found that cranial pieces gave fewer pigment cells than trunk pieces. Removal of the cranial crest can result in a subsequent excess of pigment cells, which was interpreted by Niu as evidence that they have the potential to develop as pigment cells and that this outcome is normally inhibited by the influence of the intact cranial neural folds. However, the possibility that the new pigmented cells could arise from the trunk neural crest was not ruled out. Analysis of this old literature shows us not only how fascinating it is to follow the lines of thought and methods that embryologists have used but also due to "shows" that many interesting problems of fate and differentiation of the neural crest are still open (e.g., what is the molecular nature of the signals that inhibit the production of melanocytes from the cephalic crest or the signals that control chondrogenesis in the cephalic neural crest cells?).

III. Migration Pathway and Fate of Neural Crest Cells in *Xenopus*

During the past years *Xenopus laevis* has been extensively used to study many aspects of early development, and a large amount of information has been generated about the molecular mechanisms involved in the first steps of differentiation. Amphibian embryos have been used in a wide range of embryological experi-

ments for a long time, and the advent of new techniques of molecular biology in recent years has made *Xenopus* the amphibian of choice. However, there are few reports studying the migration and fate of the neural crest cells in *Xenopus*. Here, we summarize the available information.

Various methods have been used so far in *Xenopus* to follow the different steps of crest cell migration. They are based on intra- and interspecific grafts of tissues and labeling of cells with fluorescent components (Sadaghiani and Thiébaud, 1987; Krotoski *et al.*, 1988; Collazo *et al.*, 1993). They show the neural crest migration progresses as a wave along the embryo, starting at stage 19 in the cephalic crest and finishing by stage 46 caudally. We summarize the reports concerning neural crest migration in an anterior–posterior order (Fig. 1).

A. Migration of the Cephalic Neural Crest

At the early neurula stage (15–16) three swellings of neural crest cells can be observed on each side of the neural primordium (mandibular, hyoid, and bran-

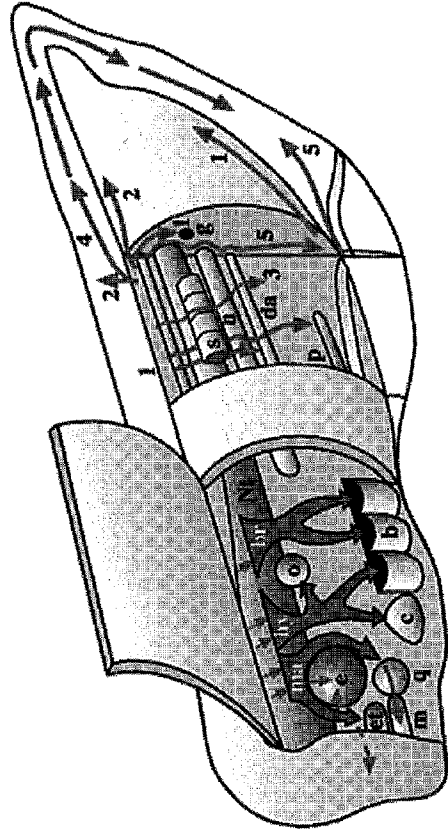


Fig. 1 Summary of the migration pathways of neural crest cells in *Xenopus laevis*. An advanced embryo is shown although not all the cell migrations are simultaneous. Cephalic crest migration: ma, mandibular crest segment; hy, hyoid crest segment; br, branchial crest segment; Nt, neural tube. The small arrows above the anterior neural tube represent cell delaminations from the tube that contribute to the cephalic crest. e, eye tissue (sclera, choroid, cornea); et, ethmoid-trabecular cartilage; q, quadrate; m, Meckel's cartilage; o, otic tissue; c, ceratohyal; b, branchial arches. Trunk crest migration: 1, ventral pathway; s, somite; n, notochord; g, spinal ganglia; p, pronephric tubules; da, dorsal aorta. 2, dorsal pathway. 3, lateral pathway. 4, pathway around the tail. 5, enteric pathway into the ventral fin. See text for more details.

chial crest segments; abbreviated ma, hy, and ba, respectively). These crest cell masses occupy the lateral half of the elevating neural folds and become continuous with the placodal thickening. However, not all cephalic crest material originates from these lateral masses. There is also a progressive cell delamination, which is accompanied by morphogenetic movement of the neural folds, from the superficial layer of the future neural tube wall (Fig. 1, small arrows in the anterior neural tube; Sadaghiani and Thiébaud, 1987).

1. Mandibular Crest Segment

From stage 19 onward, the first signs of migration appear on the border of the neural crest segments. By stage 21 the first segment (ma), which originates from the mesencephalon, moves and curves ventrally to the optic vesicle; some cells from its dorsal part migrate rostrally over the eye. These pioneer cells move toward the frontal region of the head around the prosencephalon. Some are located on the olfactory vesicle on the tip of the future ethmoid cartilage and the adjacent ectoderm (Fig. 1, et). Subsequently, they can be found at the edge of the ethmoid-trabecular plate, in the junction of this cartilage with the quadrate cartilage (Fig. 1, q) and behind the eye in the arcus subocularis. Crest-derived cells are numerous in the region where the optic nerve penetrates into the eye and where the muscles are attached to the mesenchymal coat of the eye. The mesenchymal coat, also originating from the crest, invades the space created after detachment of the lens from the epidermis and it forms the inner cornea. Some crest cells from the exterior mesenchymal coat migrate into the pigmented layer of the retina. The ma invades the mandibular arch and takes part in the formation of the quadrate and Meckel's cartilage (Fig. 1, m). These crest cells contribute to the profundus, the gasserian, and the geniculate ganglion, where they differentiate into the ganglionic cell body and the sheath Schwann cells of the related nerves. However, the mixed origin of these ganglia should be mentioned (Sadaghiani and Thiébaud, 1987).

2. Hyoid and Branchial Crest Segment

The hy, which arises from the anterior part of the rhombencephalon, descends underneath the ectoderm until the hyoid arch. These cells then migrate ventrally to the bottom of the pharynx, where they differentiate into the ceratohyal cartilage (Fig. 1, c). The hy also contributes to the orbitohyal-oides (Fig. 1, o) and to the muscle that connects some of these cartilages. In the preotic region, some cells colonize the acusticofacial ganglionic complex (Sadaghiani and Thiébaud, 1987).

The ba, which originates from the posterior part of the rhombencephalon, is destined to contribute to the formation of the branchial arches (Fig. 1, b). These

cells can be found in the cartilages of the gill in the loose connective tissues under the pharyngeal epithelium and in the mesenchyme of the gills (Sadaghiani and Thiébaud, 1987).

B. Migration of the Trunk Neural Crest

The trunk neural crest follows five distinct pathways (Krotoski *et al.*, 1988; Collazo *et al.*, 1993). These include the ventral pathway between the somites and the neural tube and the notochord (Fig. 1, 1), the dorsal pathway into the dorsal fin (Fig. 1, 2), the lateral pathway between the epidermis and the somites (Fig. 1, 3), the pathway around the tail (Fig. 1, 4), and the enteric pathway into the ventral fin (Fig. 1, 5).

The ventral pathway is followed by neural crest cells which are distributed along the neural tube and the notochord in the narrow space between these structures and the somites. Some neural crest cells following this route continue to migrate along the dorsal mesentery, around the pronephric tubules, and ventrally along the flank mesoderm where they reach the ventral midline by stage 44. Cells migrating along the ventral pathway have been shown to contribute to the formation of the spinal ganglia, the cromaffin cells of the adrenal medulla, supporting cells of the peripheral neurons, the enteric ganglia, pigment cells of the gut, the pronephric duct, and the posterior portion of the dorsal aorta.

The dorsal pathway is characterized by a population of cells appearing in the dorsal fin, which begins to expand after stage 32. In addition to cells occupying the dorsal fin, a number of neural crest cells remain on the surface of the neural tube where they differentiated into melanocytes.

The lateral pathway is characterized by cells located between the somites and the epidermis. This route has been shown to be a major pathway for pigment cells in other amphibia (Hörstadius, 1950; Keller and Spieth, 1984). However, in *Xenopus* only a small number of cells use this pathway (Krotoski *et al.*, 1988; Collazo *et al.*, 1993).

In the pathway around the tail, cells migrate caudally within the dorsal fin to circumnavigate the tail tip and return rostrally along the ventral fin.

Crest cells following the enteric pathway move ventrally toward the anus, directly down the presumptive enteric region and into the ventral fin.

There are many differences between the migration of crest in *Xenopus* and in the chick. Dye-labeled clones in the chick migrate as closely associated sheets of numerous cells (Bronner-Fraser, 1986) and are restricted to 2–3 segments; whereas those in *Xenopus* are fewer in number, migrate as sparse individual cells, and often are spread over 8 or more myotomal segment lengths. Within each somite, chick neural crests move through the rostral part of the sclerotome (Rickmann *et al.*, 1985; Bronner-Fraser, 1986). In contrast, *Xenopus* neural crest

of the ventral pathway migrates between the neural tube and caudal portion of each somite.

IV. Induction of the Neural Crest versus Early Patterning of the Ectoderm

It has been accepted that the induction of the neural crest corresponds to a transformation of the previously induced neural plate by signals that are involved in the establishment of the dorsoventral axis of the neural tube. We review here the mechanisms that control dorsoventral patterning.

A. Dorsoventral Patterning of the Neural Tube versus Mediolateral Specification of the Neural Plate

1. Current View of Dorsoventral Patterning of the Neural Tube

Patterning of the nervous system has been extensively studied with respect to its two main axes. One is the anterior–posterior axis which has a critical role in establishing the subdivisions of the neural tube that prefigure the formation of the forebrain, midbrain, and spinal chord. A second signaling system patterns the neural tube along the dorsoventral axis, initially the mediolateral axis. We focus our discussion on the midbrain to spinal chord region, because it is here that the neural crest is induced.

At early stages in the development of the spinal chord, three major classes of cells are generated in the ventral neural tube: floor plate cells at the ventral midline, motor neurons at a ventrolateral position, and ventral interneurons at a more dorsal location. Cells in the dorsal neural tube give rise initially to neural crest cells and subsequently to roof plate cells at the dorsal midline and to several classes of dorsal sensory relay interneurons (Fig. 2C). The generation of ventral cell types is controlled by signals from the notochord; in contrast, dorsal cell types are generated in response to signals derived from the overlying epidermal ectoderm. Tissue grafting assays in the chick and *Xenopus* (van Straaten *et al.*, 1988; Placzek *et al.*, 1990; Yamada, 1990; Clarke *et al.*, 1991) and several experiments in the mouse, zebrafish embryos, and neural plate cells grown *in vitro* (Tanabe *et al.*, 1995) have shown that the notochord is the source of the ventral signals, controlled by Sonic Hedgehog (Shh), a member of a family of secreted proteins.

The differentiation of the dorsal neural tube appears to be initiated by a signal from the adjacent epidermis (Selleck and Bronner-Fraser, 1995; Mancilla and Mayor, 1996). Members of the TGF- β family, notably BMPs, are likely mediators of this signal (Liem *et al.*, 1995; Wilson *et al.*, 1997; Marchant *et al.*, 1998).

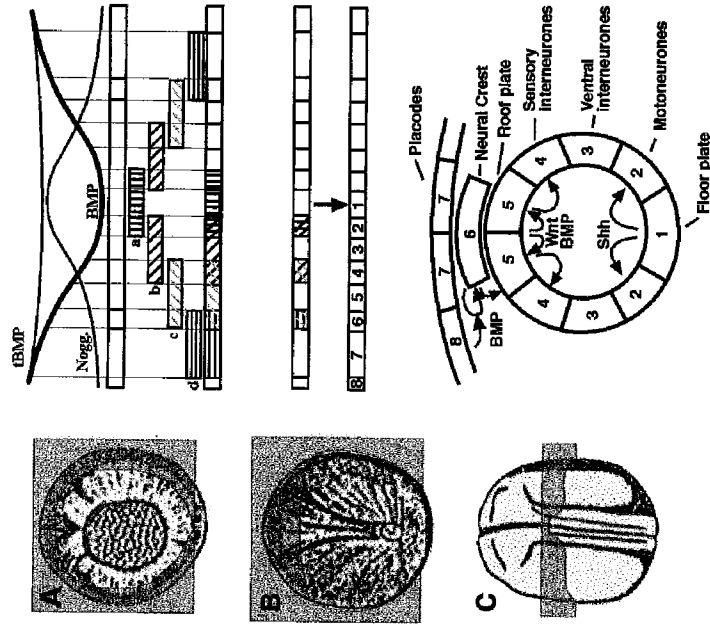


Fig. 2. Patterning the ectoderm. (A) Early gastrula stage where a gradient of noggin (Noggin) is established in the ectoderm, with the highest level in the dorsal ectoderm. Initially the total BMP (BMP) is distributed homogeneously, but by the interaction with noggin and other molecules, an opposite gradient of BMP is generated (BMP). This gradient of BMP induces the expression of different prepattern genes at different threshold concentrations (genes a, b, c, and d are shown). These prepattern genes are expressed in broad and overlapping domains. (B) In a later stage, the overlapping of these prepattern genes produces the activation of a new set of genes (1–8), which subdivide the ectoderm into different regions. (C) At the neural tube stage, the ectoderm is already subdivided, but the maintenance of the different regions requires the production of new signals (BMP, Wnt, and Shh).

The foregoing experiments suggest that neural crests are induced in the following sequence. First the neural plate is induced. Then, probably during neurulation, signals arising from the epidermis transform the lateral border of the neural plate into neural crest cells. Therefore, the induction of the neural crest cells requires (i) a previous induction of neural plate cells and (ii) an interaction of these neural plate cells with the surrounding epidermis, which would be sufficient to induce the neural crest cells in the embryo. We will review evidence against these conclusions.

2. A Novel Model of Patterning the Ectoderm

a. Neural Plate and Neural Crest Induction Are Two Independent Processes. It has been proposed by Nieuwkoop and collaborators (Nieuwkoop and Albers, 1990) that induction of the neural plate could be an earlier event than the induction of the neural crest and placodal tissue. Raven and Kloos (1945) have shown that the mesoderm underlying the neural folds from an early neurula is able to induce neural crest derivatives without the neural plate. Hofreiter and Hamburger (1955) have also claimed that in many induction experiments the neural crest is a more frequent outcome than the neural plate, again suggesting that these two inductive processes could be independent. Recent experiments indicate that the lateral mesoderm is a strong neural crest inducer but is a poor inducer of the neural plate, whereas the dorsal mesoderm induces the neural plate more strongly than the neural crest (Mayor *et al.*, 1995; Marchant *et al.*, 1998). These examples lend support to the idea that the formation of the neural plate and the neural crest are independent events. Many recent molecular manipulations also bear on this issue. Manipulations of different proneural and neurogenic genes (*X-ngr-1*, *Xash-3*, *ATH-3*, *Notch*, etc.) produce an increase in the size of the neural plate and a decrease of the neural crest cells (Coffman *et al.*, 1993; Zimmerman *et al.*, 1993; Turner and Weintraub, 1994; Ma *et al.*, 1996; Takebayashi *et al.*, 1997). If neural crest cells are generated by a neural plate–epidermis interaction exclusively, we would expect the same number of neural crest cells to be generated in these cases, but a more lateral position in the embryo.

All these results argue against a simple transformation of the neural plate into the neural crest by epidermal signals. A different model of ectodermal patterning could account for these experiments and the induction of neural crest cells.

b. A Model of Ectodermal Patterning. Three pieces of information have been used here to propose our model:

- (i) The neural induction process. The past few years have witnessed a significant change in our understanding of the molecular mechanisms responsible for neural induction. In 1924 Spemann and Mangold grafted the blastopore lip of an early salamander gastrula, which consists of dorsal mesoderm and endoderm, into the ventral side of another early gastrula. Remarkably, a second nervous system was induced. Spemann named the blastopore lip the organizer and proposed that in normal development this region induces and organizes a correctly patterned nervous system in neighboring dorsal ectoderm. Recent studies indicate that the secreted growth factor BMPs play a pivotal role in this process. Whereas the default fate for intact ectodermal tissue is epidermal, the default for isolated cells is neural. This discrepancy is explained by the idea that BMP4 is expressed in all the ectodermal cells and “self-induces” the tissue-default epider-

mal fate. The neural fate is produced by reducing BMP4 activity, either through the antagonistic effects of neural inducers such as noggin, chordin, and follistatin or by cell dissociation, which is thought to cause dilution of the BMP protein (reviewed in Hemmati-Brivanlou and Melton, 1997).

(ii) The patterning of the ectoderm. Recent reports show that the ectodermal cells of the early gastrula choose among at least three fates—epidermis, neural plate border (cement gland and neural crest), and neural plate—according to the strength of local BMP signaling (Morgan and Sargent, 1997; Wilson *et al.*, 1997; Marchant *et al.*, 1998).

(iii) The patterning of the neuroectoderm of *Drosophila*. The patterning of the peripheral nervous system, which includes the epidermal sensory organs (SOs) of the adult fly, is established by the expression of the proneural genes, among the most important of which are the *achaete* (*ac*) and *scute* (*sc*) genes. *ac* and *sc* are coexpressed in clusters of cells, the proneural clusters, from which the SO mother cells arise (reviewed in Campuzano and Modollet, 1992). The expression of *ac-sc* in each cluster is due to the activation of enhancers of the *ac-sc* genes, which are presumed to respond to local combinations of factors (Gómez-Skarmeta *et al.*, 1995). The combination of these factors, each of which is distributed in domains larger than the proneural clusters, constitutes a prepatterning (Stern, 1954) that subdivides the tissue and thus creates positional information. Recently, two of these prepatterning components have been characterized in *Drosophila* (Gómez-Skarmeta *et al.*, 1996) and three *Xenopus* homologs have been identified (Bellefroid *et al.*, 1998; Gómez-Skarmeta *et al.*, 1998). The *Xenopus* homologs, called *Xiro-1*, -2, and -3, are expressed at the early gastrula stage in a broad domain of the prospective neural plate. They are induced by the neural inducers and they seem to control the expression of the proneural genes *Xash3*, *X-ngnr-1*, and *ATH-3*.

On the basis of this information we propose the following model of ectodermal patterning (Fig. 2). At the early gastrula stage (Fig. 2A) a dorsoventral gradient of neural inducers (noggin, chordin, and follistatin) is established by diffusion within the ectoderm. These molecules bind to BMPs, which are initially homogeneously distributed in the ectoderm, generating a ventrodorsal gradient of free BMP (Fig. 2A). This BMP gradient is able to induce, at different threshold concentrations, the activation of a set of prepatterning genes, which are transcription factors expressed in broad and overlapping domains (Fig. 2A, genes a–d). In a second step (Fig. 2B), the overlapping prepatterning factors interact with each other, and possibly with a new value of the dynamic BMP gradient, to control the expression of genes in more restricted domains, which reflect the progressive subdivision of the ectoderm as it develops, including the neural crest cells (Fig. 2B,C). Examples of the prepatterning genes could be the *Xiro* genes (Bellefroid *et al.*, 1998; Gómez-Skarmeta *et al.*, 1998) and the Pax genes (Bang *et al.*, 1997). Some of the genes activated by the prepatterning factors could correspond to se-

creted molecules with inducer activity and could be required to maintain or modify the differentiated stage of the cells. Examples of these molecules could be *Shh*, expressed in the floor plate, which have a later ventralizing activity of the neural tube, and BMPs, expressed in the neural crest, which have a later dorsalizing activity of the neural tube (Fig. 2C; Liem *et al.*, 1995).

In this model the neural crest is specified by a threshold value in the BMP gradient through a genetic cascade of progressive spatial restriction. This gradient model is discussed in more detail in the next section.

V. A Model of Neural Crest Induction

A. Models Proposed to Explain the Origin of the Neural Crest

Albers (Albers, 1987; reviewed in Nieuwkoop and Albers, 1990) performed a series of experiments in which *Triturus* ectodermal implants were grafted into *Ambystoma* embryos. She analyzed the differentiation of the neural plate in the graft under many different conditions, such as the position of the graft in the host, the age of the host, and the age of the graft. Albers found that young pieces of ectoderm grafted outside the neural plate were able to differentiate as neural tissue and that this capacity was lost with the age of the graft but was not affected by the age of the host or the position of the graft. Albers concluded that the boundary of the neural plate, or the neural crest cell, was not determined by a threshold value in a diffusion gradient but depended solely upon the loss of competence of the reacting ectoderm during the mediolateral spreading of the induction process. Although there is evidence that supports the idea of a neural inductive signal spreading outside the neural plate (Albers, 1987; Servetnick and Grainger, 1991; Mancilla and Mayor, 1996), when this model was tested for the induction of the neural crest, no experimental support was found (Mancilla and Mayor, 1996). In addition, although Albers (1987) did not find any evidence for a gradient of neural inducer being important in determining the size of the neural plate, we suggest that her grafts were too large to be able to discriminate small changes in the value of the gradient. The main criticism of Nieuwkoop and Albers (1990) to the gradient theory is the doubt that “the reacting cells ought to be able to recognize minute concentration differences.” Although the molecular mechanisms of how a cell is able to discriminate different thresholds are still unknown, there are several examples that show this can be the case (e.g., dorsoventral differentiation of the mesoderm (Green *et al.*, 1992; Marchant *et al.*, 1998)).

Many recent experiments favor a second hypothesis of neural crest induction in which cells are induced by the interaction of signals coming from the neural plate and from the epidermis. Transplantation experiments in the chicken and

Xenopus show that juxtaposing neural plate and epidermis induces formation of neural crest cells (Selleck and Bronner-Fraser, 1995; Mancilla and Mayor, 1996). In addition, treatment of chick neural plate with BMPs produces an induction of dorsal markers of the neural tube, including neural crest markers (Liem *et al.*, 1995), and overexpression of noggin in *Xenopus* embryos produces ectopic expression of neural crest markers on the ventral side of the embryo (Mayor *et al.*, 1995). These results have been interpreted as evidence that once the neural plate is induced, signals originating from the epidermis, presumably BMPs, can dorsalize the neural tube, inducing the formation of the neural crest and roof plate (reviewed in Tanabe and Jessell, 1996).

A third model of neural crest induction has recently been proposed by Morgan and Sargent (1997). They suggest that in developing embryos the neuralizing signals emanating from the organizer first induce the RNA-helicase translation initiation factor (eIF4AII) and the prospective neural crest in a low arc close to a region that includes the prospective neural plate and neural crest domains. As the neuralizing signal increases in intensity close to the organizer region, the neural crest there is transformed into the neural plate. Although interesting results are reported by Morgan and Sargent (1997), additional experiments are required to support this induction-transformation model. At the same time, they provide valuable results which can be used as evidence for a fourth model, the gradient model.

B. A Novel Gradient Model of Neural Crest Induction

In this review we favor the gradient model as we think it is the simplest explanation that accounts for all experimental results.

Pioneering work by Raven and Kloos (1945) showed that whereas notochord tended to induce the neural tube without the accompanying neural crest, lateral mesoderm induced neural tissue and neural crest derivatives. They suggested the existence of a combined neural tube-neural crest inducer molecule that is produced at different concentrations across the archenteron roof. Medially, a high concentration of inducer leads to neural tube development, whereas lower concentrations laterally induce the formation of the neural crest cells. However, as Holfreter and Hamburger (1955) claimed, the differences found in the induction of neural plate and neural crest cells could be qualitative, so that a specific inducer exists for each tissue. They also stated that the only way to distinguish between these two hypotheses is to identify the inductive molecules. Another limitation in the studies of Raven and Kloos (1945) is that they did not use any cell lineage labeling to distinguish between the induced and the inducer tissues, and induction was recognized only by morphological criteria.

Recently several neural crest markers have been identified and reliable cell

lineage markers exist. We (Marchant *et al.*, 1998) have analyzed the role of mesoderm in the induction of the neural crest in *Xenopus*. We used the expression of neural plate and neural crest genes as markers to study this induction. Conjugation experiments with different kinds of mesoderm and embryo dissection experiments suggested that the dorsolateral mesoderm has an activity capable of inducing neural crest cell specifically. We found that neural crest markers can be induced in competent ectoderm at varying distances from the mesoderm-inducing tissue, depending on the dorsolateral origin of the mesoderm: dorsal tissue induces crest at a distance whereas dorsolateral tissue induces crest directly adjacent to itself. These results can be interpreted as different mesodermal tissues having different amounts of an inducer, with high levels in dorsal mesoderm and lower levels in dorsolateral mesoderm. The inducer diffuses from the mesoderm to generate a gradient and, at a distance from the source of inducer, a neural crest threshold is reached. Marchant *et al.* (1998) explored the possible role of BMPs and noggin in the generation of this hypothetical gradient. They found the following: (1) Progressively higher levels of BMP activity are sufficient for specification of neural plate, neural crest, and nonneural cells, in that order. (2) In directional conjugates, progressively higher levels of noggin are able to induce neural crest at greater distances from the source of inducer. (3) By modifying the level of BMP activity, we were able to induce neural crest in the absence of neural plate, suggesting these tissues are induced independently. These results suggest a model in which a gradient of BMP activity is established in the ectoderm (Fig. 3A). Neural crest is induced when BMP activity in the ectoderm is between two threshold levels. This gradient is established by interactions between BMP from the ectoderm and BMP-binding molecules arising from the mesoderm. The dorsolateral mesoderm has an important role in inducing neural crest either by providing the right amount of anti-BMP signals or by producing a specific neural crest inducer. Low BMP activity induces neural plate whereas high levels of BMP induce epidermis. Therefore BMP can be considered as an ectoderm-patterning morphogen. Some of the experiments performed by Morgan and Sargent (1997) provide direct support for this model. They could separate the induction of neural plate and neural crest by changing the concentration of three different neural inducer regimes. In addition, an interesting model of ectodermal patterning has been proposed by Wilson *et al.* (1997), in which a gradient of BMP activity is able to differentiate the induction of neural plate from cement gland and epidermis. The cement gland is located very close to the anterior neural fold and it can be considered as a marker for the anterior border of the neural plate. Although we (Marchant *et al.*, 1998) did not analyze the induction of the cement gland and Wilson *et al.* (1997) did not show results concerning neural crest induction, both models are coherent and they support each other's data.

It is interesting to note that this gradient model allows us both to reinterpret

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 experimental results that supported previous models and also to explain other results that did not have a proper explanation.

First, the overexpression of neural inducers or the blocking of BMP receptors leads to an expansion of the neural plate and neural crest region (Fig. 3B; Mayor *et al.*, 1995; Marchant *et al.*, 1998). This can be explained as a change in the gradient of BMPs in the treated side of the embryo, with a decrease in BMP activity; as the threshold values for neural plate and neural crest specification are not changed, these are now reached at a larger distance from the dorsal midline and, as a consequence, the neural plate and crest domains are expanded.

Second, grafts of anterior neural plate or ectoderm injected with noggin mRNA into the epidermis are sufficient to induce neural crest cells (Fig. 3C; Mancilla and Mayor, 1996; Marchant *et al.*, 1998). The anterior neural plate graft could contain chordin or noggin produced by the underlying dorsal mesoderm (Smith *et al.*, 1993; Sasai *et al.*, 1994) or from the anterior neural plate (Knecht and Harland, 1997). When this tissue is grafted into an epidermal region containing a precise amount of BMPs, which specify epidermal differentiation, noggin diffuses from the graft into the epidermis and changes the gradient of BMP to a lower value which specifies neural crest cells.

Third, changing the activity of proneural or neurogenic genes produces an expansion of the neural plate which is always accompanied by a disappearance of neural crest cells (Coffman *et al.*, 1993; Zimmerman *et al.*, 1993; Turner and Weintraub, 1994; Gómez-Skarmeta *et al.*, 1998). In a gradient model (Fig. 3D), specification of neural plate and neural crest cells can be independent events, such that an expanded neural plate domain, due to an effect downstream of BMP, which changes the specification of those cells by the proneural activity, could completely eclipse the region of ectoderm containing the right concentration of BMP for neural crest specification. This would result in the complete absence of neural crest cells, which would not be the case if neural crest induction depended simply on an interaction between neural plate and epidermis.

Finally, we stress that this model is proposed for the early specification of the neural crest, but once this region is initially specified (by a gradient of BMP activity, which could activate prepatterning genes required for neural crest specification), the induced cells could start to receive and produce additional signals which are required for the final determination of these cells or to maintain the differentiated state. Some of these signals could include other members of the BMP family or other TGF β factors, as has been shown in the chicken (Liem *et al.*, 1995). Other factors are also involved in the specification of the crest cells. FGF activity is required to induce neural crest cells and, in combination with noggin, is sufficient to induce the expression of neural crest markers (Mayor *et al.*, 1995, 1997). However, the role of FGF could be related to its posteriorizing activity of the ectoderm (Cox and Hemmati-Brivanlou, 1995) or could be involved in controlling the activity of BMP in the ectoderm (Wang and Sassoon,

1995). Other factors such as Wnt family members (Saint-Jeannet *et al.*, 1997), the product of the *Zic3* gene (Nakata *et al.*, 1997), and the already mentioned eIF4AII (Morgan and Sargent, 1997), together with other genes expressed in the neural crest, including some that encode for proteins involved in the signal transduction pathway of BMP, are discussed in the next section.

VI. Genes Expressed in the Neural Crest

A number of genes have recently been identified in *Xenopus* that are expressed in the prospective, migratory, or differentiating neural crest cells. Many of them code for transcription factors and it is assumed they play roles in neural crest development. However, very little is known about the possible functions of these genes and no targets for the putative transcription factors have been identified. Based on the domains and timing of expression and on overexpression experiments involving some of these genes, we will propose a possible scenario of gene interactions which could describe the roles of these genes in the process of cell specification and differentiation of the neural crest.

A possible cascade of gene regulation is illustrated in Fig. 4, and the expression pattern of many of these genes is summarized in Fig. 5. Initially, until the midgastrula stage, BMP-4 is expressed in the entire ectoderm (Schmid *et al.*, 1995), where, possibly through the action of Smad1 protein, the expression of neural plate genes is inhibited (Wilson *et al.*, 1997). Other signal transduction systems are probably also involved in this inhibitory process, including other members of the Smad family. Smad1 is expressed in the entire ectoderm and BMPs probably control its activity but not its transcription. During gastrulation the neural inducers secreted from the dorsal mesoderm bind to BMPs, generating a ventrodorsal gradient of free BMP (as described in Section IV.A.2). The highest concentration of BMP, stimulating the highest activity of Smad1, activates the expression of *Msx1* in the ventrolateral ectoderm (Suzuki *et al.*, 1997). This expression is required for the activation of epidermal genes in the ventral ectoderm and probably at lower levels, near the neural plate, for the activation of genes at the neural plate border. When the concentration of BMP starts to decrease at the dorsal ectoderm, some neural plate genes are activated there, such as *Zic3* (Nakata *et al.*, 1997) and *Pax3* (Bang *et al.*, 1997). The activation of *Zic3* within the neural plate seems to be sufficient to activate a series of proneural genes in that region, which has been analyzed by the overexpression of *Zic3* in animal caps (Nakata *et al.*, 1997). As it has been shown that the proneural genes are activated by the prepatterning genes *Xiro-1*, -2, and -3 and that these *Xiro* genes are activated by the gli-related proteins (Gómez-Skarmeta *et al.*, 1998), we propose the following sequence of activation: *Zic3*-Gli-*Xiro*-proneural genes. The activation of the proneural genes leads to the specification of those cells as

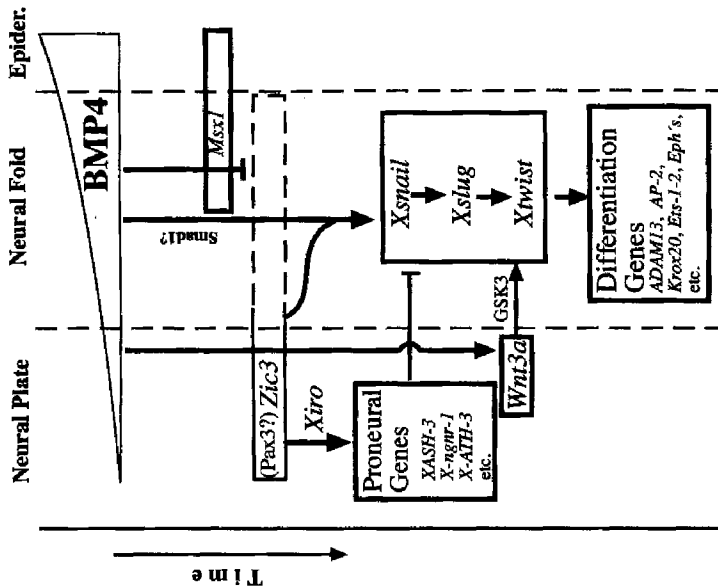


Fig. 4 Cascade of gene regulation in the neural crest. See text for more details.

neural plate cells and, in some cases, as neuronal cells, which in turn inhibits the expression of neural crest genes (Ferreiro *et al.*, 1994; Turner and Weintraub, 1994; Ma *et al.*, 1996; Takebayashi *et al.*, 1997). At the end of gastrulation, the expression of *Zic3* disappears from the central part of the neural plate. A possible explanation for this is that activation of a neural program in the neural plate leads to the inhibition of *Zic3* in this region. This inhibitory function of the proneural genes could be mediated by the *Notch* interaction as has been shown for the suppression of neural differentiation by these factors (Chitnis and Kintner, 1996; Ma *et al.*, 1996). However, a residual *Zic3* expression is left at the border of the neural plate, which now by itself or in combination with another factor, probably BMP, present in that region, could specify the induction of the neural crest cells, as has recently been suggested (Nakata *et al.*, 1997). Animal caps injected with *Zic3* mRNA exhibited expression of neural plate and neural crest markers, but

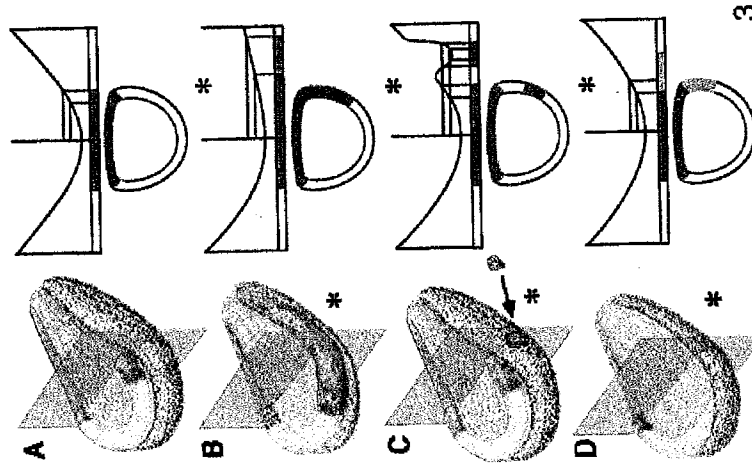


Fig. 3 (Left panel) A model of neural crest induction. (A) Control embryo. A threshold concentration of a BMP gradient specifies the neural crest cell at the border of the neural plate. (B) When neural inducers or a mutated receptor of BMP is expressed in one side of the embryo, the BMP gradient is affected. This produces the expansion of the neural plate and neural crest cells. (C) When a piece of ectoderm containing noggin is grafted into the lateral epidermis, the BMP concentration is decreased in that region, reaching the neural crest threshold at the border of the graft. (D) When the proneural genes are expressed in one side of the embryo, the BMP gradient is not affected but the cells that are at the neural crest threshold of BMP cannot differentiate as crest cells because they are specified as neural plate cells by the proneural genes. See text for more details.

Fig. 5 (Right panel) Pattern of expression of genes expressed in the neural crest. (A) Neural plate stage embryo. NP, neural plate; NF, neural fold. (B) Neural tube stage embryo. NC, neural crest; RP, roof plate; NT, neural tube; FP, floor plate; S, somite; n, notochord.

when these animal caps were dissociated, only neural plate markers were observed.

The Wnt gene family encodes a group of cysteine-rich secreted glycoproteins involved in a wide range of activities during embryogenesis. It has recently been reported that Wnt-3A and possibly Wnt-1 have a role in patterning the neural tube along its dorsoventral axis and function in the differentiation of the neural crest (Saint-Jeannet *et al.*, 1997).

Overexpression of Wnt-3A or Wnt-1 in animal caps previously neuralized with noggin or chordin leads to the expression of neural crest markers through the inhibition of GSK-3 (Saint-Jeannet *et al.*, 1997). Although all these experiments suggest very strongly a role for Wnt-3A in neural crest induction, it should be remembered that expression of this gene is detected after the neural crest is specified (Wolda *et al.*, 1993; Mancilla and Mayor, 1996). This suggests that the Wnt factors are involved in the maintenance of the neural crest differentiation program rather than in its initial specification. We do not have information concerning the role of Pax3 in neural crest specification in *Xenopus*, although certainly it has an important role in mammalian neural crest development. The Pax3 mouse mutant (*split*) exhibits a phenotype that has been explained as a failure in the closure of the neural tube and in the proper development of some neural crest derivatives (Epstein *et al.*, 1991). Based on its timing and pattern of expression, it is possible to propose that Pax3 could have an early role in the specification of the neural fold–neural crest region. If these three genes, *Zic3*, *Msx1*, and *Pax3*, are expressed in broad domains during early neural development and by overlapping initiate patterning of the ectoderm, then they could be considered as prepattern genes (discussed in Section IV.A.2). We should mention that although these three genes could be expressed in the prospective neural crest region at an early stage of development (Fig. 5A), it is clear that at a later stage (postgastrulation) their expression is excluded from the neural crest region (Fig. 5B). They can therefore only be involved in the early patterning of the ectoderm, including the specification of the neural crest, but not in more advanced steps in the development of the crest cells.

The earliest genes expressed in the prospective crest region and whose expression continues during the migratory phase of the crest cells are the Zinc-finger gene *Xsnail* (Essex *et al.*, 1993; Mayor *et al.*, 1993), the *Xsnail* related gene *Xslug* (Mayor *et al.*, 1995), and a helix–loop–helix gene, called *Xrwtist* (Hopwood *et al.*, 1989). Their expression suggests that these genes could be the direct targets of the specification genes, *Zic3* or *Pax3*, and that their expression is required to confer the properties typical for neural crest cells. One of these properties is the ability to migrate, and it has been shown in the chick by the use of oligo antisense technology that the *slug* gene is required for the proper migration of the crest (Nieto *et al.*, 1994). We have analyzed the role of *Xslug* in neural crest development by expressing in the embryos *Xslug* antisense RNA or a

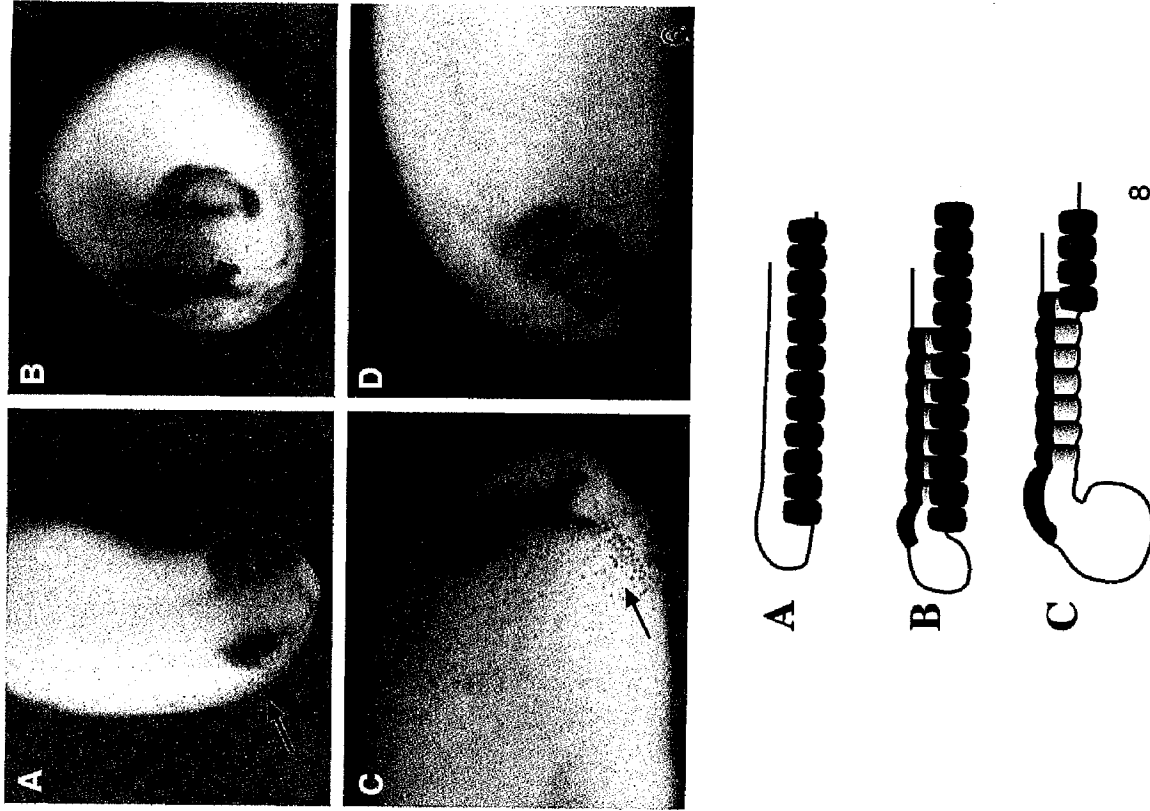


Fig. 6 (Top panel) Role of *Xslug* in crest migration. A dominant negative of the *Xslug* gene was expressed at one side of the embryo (arrow) together with β -galactosidase (blue dots). The expression of *Xrwtist* was analyzed (purple). A slight decreasing in *Xrwtist* expression is seen at the injected side but a more dramatic effect is on the inhibition of crest migration. (A–D) Four different views of the same embryo: (A) dorsal view; (B) anterior view; (C) right side; (D) left side.

Fig. 8 (Bottom panel) The "transference to ectoderm" hypothesis. A mesodermal developmental system is transferred to the ectoderm, bringing along sclerotomal properties of the cephalic crest. (A) Situation in cephalochordates previous to the transference. (B) Cyclostomes retain cephalic enterocelic somites. (C) Cephalic mesoderm segmentation is greatly reduced (nearly lost) in amniotes, which show somitomeres. Rostrocaudally, somites begin where rhombomeres end.

dominant negative form of it. We observed a complete blocking of neural crest migration with these two treatments (Fig. 6, unpublished results). These observations support the idea that *Xslug*, and perhaps *Xsnail* and *Xtwist*, could directly activate some of the cellular properties characteristic of the neural crest cells. We do not know what the relationship is between these three transcription factors, and based only on their order of expression in the prospective crest, we propose the following sequence of gene regulation: *Xsnail* (expressed from stage 11)–*Xslug* (expressed from stage 12)–*Xtwist* (expressed from stage 12.5).

There are a number of genes also expressed in the neural crest that are probably not involved in the early specification of these cells but which probably play roles in its later differentiation, as they are expressed at later stages or they do not encode for transcription factors. We will mention some of these genes.

The fork head/HNF-3 related genes, particularly XFD-6 and -10, are expressed in the neural crest cells at the neurula stage and after the migration of the cells, respectively (Scheucher *et al.*, 1995). Another group of transcription factors that are expressed in the migrating neural crest cells are the Ets-1 and Ets-2 and the related *Xl-ffi* gene (Wolff *et al.*, 1990; Meyer *et al.*, 1993, 1997). It is interesting to note that the Ets genes are also expressed in other migratory cells or migration pathways, which suggests that they play a role in cell migration. *Krox-20*, another transcription factor gene, is expressed only in the subpopulation of neural crest cells that correspond to rhombomere 5 (Bradley *et al.*, 1992). This restricted expression could suggest a role in the specification of this specific segment, as it is also expressed in rhombomere 5, or it could be involved in controlling some specific properties of the crest cell derived from this segment. A cell adhesion molecule expressed in the cephalic neural crest is ADAM-13 (Alfandari *et al.*, 1997). Its expression starts almost immediately after *Xslug* and *Xtwist* expression and includes not only the cephalic neural crest but also the anterior neural fold, which is fated to differentiate as forebrain. Another important family of proteins implied in axon guidance and in controlling the pathway of neural crest migration in the chicken are the Eph-related receptors and ligands (Krull *et al.*, 1997). In *Xenopus* some members of this family are expressed in subpopulations of the neural crest and in the neural crest migration pathway (Winning and Sargent, 1994; Xu *et al.*, 1995). It has been shown in *Xenopus*, by expressing a mutated form of EphA4 and overexpressing the ligand ephrin B-2, that these molecules are involved in restricting the intermingling of adjacent streams of the migrating neural crest in the arches (Smith *et al.*, 1997).

Finally, a series of other factors are expressed in the differentiating neural crest, and they are probably involved in the differentiation of specific cell types derived from the neural crest. It is interesting to note that many of the factors that are involved in the very early specification of the crest may also play a role in its later differentiation of the cells. Among these factors are the BMP (Schmidt *et al.*, 1995) and Smad proteins (Thomsen, 1996), the Pax and Wnt genes, the retinoic acid receptors (Sharpe, 1992), and a number of secreted factors such as

fibroblast growth factor, neurotrophin-3, ciliary neurotrophic factor, leukemia inhibitory factor, and steel factor (reviewed in Murphy and Bartlett, 1993).

VII. Events in the Evolution of the Neural Crest

Although this review has been focused on the development of the neural crest in *Xenopus*, the conservation of genes involved in its development together with other observations allows us to suggest some novel hypotheses concerning early events in its evolution.

The origin of the neural crest is considered an important event in chordate evolution since most structures found exclusively in vertebrates, such as the skull, develop from this tissue. Northcutt and Gans (1983) suggested chordates lacking complex heads such as cephalochordates (e.g., amphioxus) and tunicates also lack a neural crest and therefore situated the origin of a crest as an event subsequent to their divergence (Fig. 7A).

The search for the origin of the neural crest requires searching for its character in other chordates, providing they are suitable models for craniate ancestors. Amphioxus has been proposed as a model for these ancestors since it has few or single copies of genes which exist in multiple copies in craniates and are expressed at several of their putatively homologous structures (Holland, 1992; Garcia-Fernández and Holland, 1994). Additionally, the fossil record of the earliest chordates such as *Pikaia graciliens* shows an anatomy that closely matches that of amphioxus, indicating general conservation of primitive morphology since their divergence (Conway Morris and Whittington, 1979).

Evidence for neural crest character of craniates has been found in amphioxus where a neural crest was previously assumed not to exist. This evidence is as follows: In amphioxus there are migrating cells at the borders of the neural plate (Holland *et al.*, 1996). (2) These cells are found to express the *distal-less* gene (*AmphiDll*), also present in the neural crest of vertebrates (Holland *et al.*, 1996). (3) Amphioxus possesses a metameric peripheral nervous system which presents various similarities to that of craniates such as the presence of primary sensory neurons known to be derived only from the neural crest in vertebrates (Fritzsch and Northcutt, 1993; see Lacalli (1996) for homology of rostral sensory pathways to those of craniates). (4) In amphioxus, melanocytes can be observed in the cup organs of Hesse, a linear series of photoreceptive groups of cells in the neural tube. This could reflect some kind of neural crest as suggested by Whittaker (1997). However, Holland *et al.* (1996) reported these organs to express *AmphiDll* but related them to neural areas of arthropods known to transmit visual stimuli and express *distal-less*.

The foregoing evidence suggests a neural crestlike system in amphioxus not unlike trunk neural crests of craniates. This proposal must await evidence indicating that primary sensory neurons develop from cells surrounding the neural

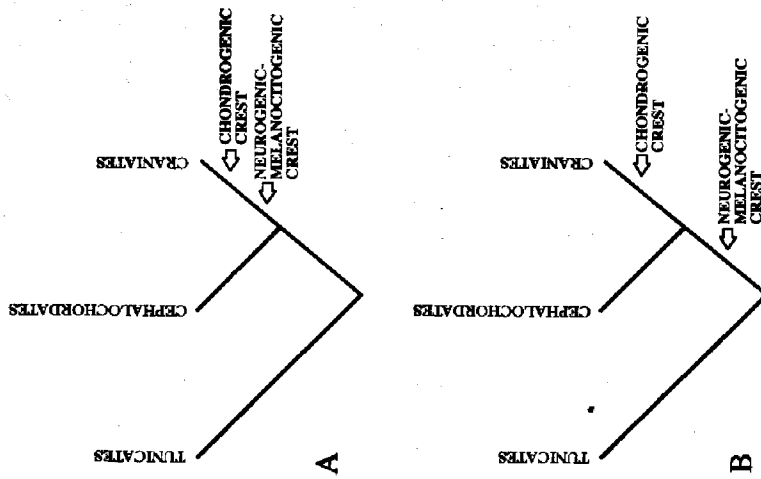


Fig. 7 (A) Gans and Northcutt (1983) denied the presence of any obvious precursor of a neural crest in cephalochordates. (B) The presence of a neurogenic-melanocytogenic crestlike system in Amphioxus proves the secondary establishment of chondrogenesis.

plate as well as analysis of gene expression for more specific neural crest markers such as *Slug* and *Snail* (Essex *et al.*, 1993; Nieto *et al.*, 1994; Mayor *et al.*, 1995). This has been done in the tunicate *Halocynthia roretzi*, which expresses *Snail* at the borders of the neural plate (Corbo *et al.*, 1997). Since amphioxus has no skull, it can be concluded that the cephalic chondrogenic neural crest was established in evolution after the neurogenic-melanocytogenic crest (Fig. 7B). This idea is also supported by transplantation experiments which show that cephalic crest cells can attain trunk crest identity if grafted to trunk migration routes whereas trunk crest cells cannot become chondrogenic by transplantation to those of cephalic regions (Hörstadius, 1950).

Two of the principal differences distinguishing amphioxus from craniates are the lack of rhombomeres and the extension of somites throughout the whole

length of the neural tube. These cephalic somites are conserved at approximately the same axial level as rhombomeres in primitive craniates such as cyclostomes and elasmobranchs (Fig. 8B). Cephalic somites are absent in amniotes whose cephalic paraxial mesoderm is weakly segmented into somitomeres; rostrocaudally, somites begin where rhombomeres end (Fig. 8C). Fate mapping studies of somitomeres and comparative studies among primitive and derived vertebrates suggest a common developmental mechanism for segmentation of somitomeres/cephalic somites and the neural crest (Gilliland and Baker, 1993; Trainor *et al.*, 1994). The evolution of a morphologically segmented hindbrain in craniates can be said to coincide with the appearance of chondrogenic properties of the cephalic neural crest which develop into the skull, these being the only ectodermal cells capable of differentiating into bone, a tissue mostly derived from mesoderm. A proposal on the origin of neural crest chondrogenesis consistent with the foregoing observations would be the transference of a mesodermal developmental system to the ectoderm in the cephalic region conferring the chondrogenic properties of the cephalic crest and maybe even segmentation of the hindbrain (Fig. 8B). This suggestion would imply paralogy (taken here as homology of structures within the same organism) of segmentation in the hindbrain and, ideally, that of the cephalic somites of primitive vertebrates (somitomeres are clearly derived; Kuratani, 1997). Previous studies comparing somite segmentation to that of rhombomeres have shown few similarities, such as participation of the receptor tyrosine kinase *sek* (Nieto *et al.*, 1992), and have indicated as an important difference that neural tube segmentation develops by the constriction of a structure with a preexisting interior cavity, whereas in somites this is achieved by the condensation of mesenchyme which posteriorly invades to produce a myocel (a process called schizocoely). These studies compare hindbrain to somites present in the same animal. The paralogy specified above is significant since anterior somites of amphioxus and primitive vertebrates develop in a way totally different from their posterior schizocoelic somites. Anterior somites are formed by the pouching of the underlying pharyngeal archenteron roof and since their cavity develops from the endodermal lumen, these are called enterocoelic somites (Pre-sley *et al.*, 1996). This enterocoelic segmentation may prove more similar to hindbrain segmentation than that of schizocoelic somites since they both consist of modification of a structure with a preexisting cavity. If rhombomeres and anterior somites are paralogous, we should search for mesodermal properties of regions other than the neural crest in the hindbrain neural tube. The experiments involving varying degrees of ablation of the neural tube and cell staining done by Scherson *et al.* (1996) have shown that the mid- and hindbrain neural tube is able to regenerate the cephalic neural crest and sustain normal development. This suggests a potential for nondorsal regions of the neural tube to be chondrogenic and eventually form skull.

Chondrogenesis of the cephalic crest as well as its mesenchymal nature makes it more similar to sclerotomes than other somite regions. The fact that scler-

- Borca, M. I. (1909). Sur les masses mésodermiques et leurs dérivés chez les Téléostans. *C. R. Acad. Sci. Paris* **149**, 637–644.
- Bracht, A. (1908). Recherches sur l'ontogénèse de la tête chez les amphibiens. *Arch. Biol.* **23**, 165–258.
- Bradley, L. C., Snape, A., Bhatt, S., and Wilkinson, D. G. (1992). The structure and expression of the *Xenopus Krox-20* gene: Conserved and divergent patterns of expression in rhombomeres and neural crest. *Mech. Dev.* **40**, 73–84.
- Bronner-Fraser, M. (1986). Analysis of the early stages of trunk neural crest migration in avian embryos using monoclonal antibody HNK-1. *Dev. Biol.* **115**, 44–55.
- Campuzano, S., and Modolelli, J. (1992). Patterning of the *Drosophila* nervous system: The achaete-scute gene complex. *Trends Genet.* **8**, 202–207.
- Catala, M., Teillet, M.-A., De Robertis, E. M., and Le Douarin, N. M. (1996). A spinal cord fate map in the avian embryo: While regressing, Hensen's node lays down the notochord and floorplate thus joining the spinal chord lateral walls. *Development* **122**, 2599–2610.
- Chitnis, A., and Kintner, C. (1996). Sensitivity of proneural genes to lateral inhibition affects the pattern of primary neurons in *Xenopus* embryos. *Development* **122**, 2295–2301.
- Clarke, J. D. W., Holdier, N., Soffe, S. R., and Storm-Mathisen, J. (1991). Neuroanatomical and functional analysis of neural tube formation in notochordless *Xenopus* embryos; laterality of the ventral spinal chord is lost. *Development* **112**, 499–516.
- Coffman, C. R., Skoglund, P., Harris, W. A., and Kintner, C. R. (1993). Expression of an extracellular deletion of *Xotch* diverts cell fate in *Xenopus* embryos. *Cell* **73**, 659–671.
- Collazo, A., Bronner-Fraser, M., and Fraser, S. E. (1993). Vital dye labelling of *Xenopus laevis* trunk neural crest reveals multipotency and novel pathways of migration. *Development* **118**, 363–376.
- Conway Morris, S., and Whittington, H. B. (1979). The animals of the Burgess Shale. *Sci. Am.* **240**, 122–133.
- Corbo, J. C., Erives, A., Di Gregorio, A., Chang, A., and Levine, M. (1997). Dorsoroventral patterning of the vertebrate neural tube is conserved in a protochordate. *Development* **124**, 2335–2344.
- Cox, W. G., and Hemmati-Brivanlou, A. (1995). Caudalization of neural fate by tissue recombination and bFGF. *Development* **121**, 1007–1015.
- de Beer, G. R. (1947). The differentiation of the neural crest cells into visceral cartilages and odontoblast in *Amphystoma* and re-examination of the germ layer theory. *Proc. R. Soc. London* **134**, 377–398.
- Detwiler, S. R., and Kehoe, K. (1939). Further observations on the origins of the sheath cells of *Schwan*. *J. Exp. Zool.* **81**, 415–436.
- DuShane, G. P. (1935). An experimental study of the origin of pigment cells in Amphibia. *J. Exp. Zool.* **72**, 1–32.
- DuShane, G. P. (1936). The Dopa reaction in Amphibia. *Proc. Soc. Exp. Biol. Med.* **33**, 592–593.
- DuShane, G. P. (1938). Neural fold derivatives in Amphibia: Pigment cells, spinal ganglia and Rohon-Beard cells. *J. Exp. Zool.* **78**, 485–501.
- Epstein, D. J., Vekemans, M., and Gros, P. (1991). *Splotch (Sp2H)*, a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of *PAX-3*. *Cell* **67**, 767–774.
- Essex, L. J., Mayor, R., and Sargent, M. G. (1993). Expression of *Xenopus snail* in mesoderm and prospective neural fold ectoderm. *Dev. Dyn.* **198**, 108–122.
- Ferreiro, B., Kintner, C., Zimmerman, K., Anderson, D., and Harris, W. A. (1994). *XASH* genes promote neurogenesis in *Xenopus* embryos. *Development* **120**, 3649–3655.
- Fritzsch, B., and Northcutt, R. G. (1993). Cranial and spinal nerve organization in amphioxus and lampreys: Evidence for an ancestral cranial pattern. *Acta Anat.* **148**, 96–109.

rotomes and the cephalic crest share the expression of *Twist* (Gitelman, 1997) as well as suffer similar homeotic transformation of their skeletal elements by *Hox* gene disruptions in accordance with "posterior prevalence" (Gendron-Maguire et al., 1993; Rijli et al., 1993) further endorses this view.

In light of the foregoing considerations, the question of how such a transference of a mesodermal system to the ectoderm would be reflected in craniate development is raised. In primitive craniates this could have consisted in the movement of cells from the mesoderm to the ectoderm during middle to late gastrulation. The possibility of mesoderm-ectoderm cell exchanges in gastrulation was proven by Catala et al. (1996) for the notochord and floorplate. Alternatively, the transference of a mesodermal system might not require cell movement but the activation of a genetic program for chondrogenesis within the ectoderm, possibly mediated by inductive signals arising from the anterior mesoderm. Additional evidence that supports either of the two alternatives comes from the observation that almost all the genes expressed in the cephalic crest are also expressed in the mesoderm (Section VI). Once this transference occurred, a duplicated system of segmented mesoderm-ectoderm would have appeared. This duplication of functions could have allowed partial loss of anterior somites in vertebrates, lost functions having been assumed by ectodermal cells in the form of a neural crest (Fig. 8C).

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References

- Albers, B. (1987). Competence as the main factor determining the size of the neural plate. *Dev. Growth Differ.* **29**, 535–545.
- Alfandari, D., Wolfsberg, T. G., White, J. M., and DeSimone, D. W. (1997). *ADAM 13*: A novel *ADAM* expressed in somitic mesoderm and neural crest cells during *Xenopus laevis* development. *Dev. Biol.* **182**, 314–330.
- Baker, R. C., and Graves, G. O. (1939). The behavior of the neural crest in the forebrain region of *Amphystoma*. *J. Neurol.* **71**, 389–416.
- Bang, A. G., Papanopolu, N., Kintner, C., and Goulding, M. D. (1997). Expression of *Pax-3* is initiated in the early neural plate by posteriorizing signals produced by the organizer and by posterior non-axial mesoderm. *Development* **124**, 2075–2085.
- Bellefroid, E. J., Kobbe, A., Gruss, P., Pieler, T., Gurdon, J. B., and Papanopolu, N. (1998). *Xiro3* encodes a *Xenopus* homolog of the *Drosophila Irroquois* genes and functions in neural specification. *EMBO J.* **17**, 191–203.

- Gans, C., and Northcutt, R. G. (1983). Neural crest and the origin of vertebrates: A new head. *Science* **220**, 268–273.
- García-Fernández, J., and Holland, P. W. H. (1994). Archetypical organization of the amphioxus *Hox* gene cluster. *Nature* **370**, 563–566.
- Gendron-Maguire, M., Mallo, M., Zhang, M., and Gridley, T. (1993). Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. *Cell* **75**, 1317–1331.
- Gilland, E., and Baker, R. (1993). Conservation of neuroepithelial and mesodermal segments in the embryonic vertebrate head. *Acta Anat.* **148**, 110–123.
- Gitelman, I. (1997). Twist proteins in mouse embryogenesis. *Dev. Biol.* **189**, 205–214.
- Gómez-Skarmeta, J. L., Rodríguez, I., Martínez, C., Cufí, J., Ferrés-Marcó, M. D., Beamonte, D., and Modolell, J. (1995). Cis-regulation of *achaete* and *scute*: Shared enhancer-like elements drive their coexpression in proneural clusters of the imaginal discs. *Genes Dev.* **9**, 1869–1882.
- Gómez-Skarmeta, J. L., Díez del Corral, R., de la Calle-Mustienes, E., Ferrés-Marcó, M. D., and Modolell, J. (1996). *araucan* and *caupolican*, two members of the novel *Iroquois* complex, encode homeoproteins that control proneural and vein forming genes. *Cell* **85**, 95–105.
- Gómez-Skarmeta, J. L., Glavie, A., de la Calle-Mustienes, E., Modolell, J., and Mayor, R. (1998). *Xiro*, a *Xenopus* homolog of the *Drosophila Iroquois* complex genes, controls development at the neural plate. *EMBO J.* **17**, 181–190.
- Green, J. B. A., Naw, H. B., and Smith, J. C. (1992). Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* **71**, 731–739.
- Harrison, R. G. (1910). The outgrowth of the nerve fiber as a mode of protoplasmic movement. *J. Exp. Zool.* **9**, 787–841.
- Harrison, R. G. (1924). Neuroblast versus sheath cell in the development of peripheral nerves. *J. Comp. Neurol.* **37**, 123–206.
- Harrison, R. G. (1935). On the origin and development of the nervous system studied by the methods of experimental embryology. *Proc. R. Soc. London* **118**, 155–196.
- Hemmati-Brivanlou, A., and Melton, D. A. (1997). Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell* **88**, 13–17.
- Hirano, S., Fuse, S., and Sohal, G. S. (1991). The effect of the floor plate on pattern and polarity in the developing central nervous system. *Science* **251**, 310–313.
- His, N. (1868). "Untersuchungen über die erste Anlage des Wirbelthierleibes." Die erste Entwicklung des Hühnchens im Ei. F. C. W. Vogel, Leipzig.
- Hofreiter, J., and Hamburger, V. (1955). Embryogenesis: Progressive differentiation. In "Analysis of Development" (B. H. Willier, P. A. Weiss, and V. Hamburger, Eds.), Chapter 1, pp. 230–296. Saunders, Philadelphia.
- Holland, N. D., Panganiban, G., Henyey, E. L., and Holland, L. Z. (1996). Sequence and developmental expression of *Amphidil*, an Amphioxus distal-less gene transcribed in the ectoderm, epidermis and nervous system: Insights into the evolution of craniate forebrain and neural crest. *Development* **122**, 2911–2920.
- Holland, P. W. H. (1992). Homeobox genes in vertebrate evolution. *BioEssays* **14**, 267–273.
- Holland, P. W. H. (1996). Molecular biology of lancelets: Insights into development and evolution. *Isr. J. Zool.* **42**, 247–272.
- Hopwood, N. D., Pluck, A., and Gurdon, J. B. (1989). A *Xenopus* mRNA related to *Drosophila twist* is expressed in response to induction in the mesoderm and the neural crest. *Cell* **59**, 893–903.
- Hörstadius, S. (1950). "The Neural Crest." Oxford University Press, Oxford.
- Keller, R. E., and Spith, J. (1984). Neural crest cell behavior in white and dark larvae of *Ambystoma mexicanum*: Time-lapse cinemicrographic analysis of pigment cell movement *in vivo* and in culture. *J. Exp. Zool.* **229**, 109–126.
3. Neural Crest in *Xenopus*
- Knecht, A. K., and Harland, R. M. (1997). Mechanisms of dorsal-ventral patterning in noggin-induced neural tissue. *Development* **124**, 2477–2488.
- Krotoski, D. M., Fraser, S. E., and Bronner-Fraser, M. (1988). Mapping of neural crest pathways in *Xenopus laevis* using inter- and intra-specific cell markers. *Dev. Biol.* **127**, 119–132.
- Krull, C. E., Lansford, R., Gale, N. W., Collazo, A., Marcelle, C., Yancopoulos, G. D., Fraser, S. E., and Bronner-Fraser, M. (1997). Interactions of Eph-related receptors and ligands confer rostrocaudal pattern to trunk neural crest migration. *Curr. Biol.* **7**, 571–580.
- Kuratani, S. (1997). Spatial distribution of postotic crest cells defines the head/trunk interface of the vertebrate body; embryological interpretation of peripheral nerve morphology and evolution of the vertebrate head. *Anat. Embryol.* **195**, 1–13.
- Lacalli, T. C. (1996). Frontal eye circuitry, rostral sensory pathways and brain organization in Amphioxus larvae: Evidence from 3D reconstructions. *Philos. Trans. R. Soc. London* **351**, 243–263.
- Landacre, F. L. (1921). The fate of the neural crest in the head of the Urodeles. *J. Comp. Neurol.* **33**, 1–44.
- Le Douarin, N. M. (1969). Particularités du noyau interfasciique chez la caillie japonaise (*Coturnix coturnix japonica*). Utilisation de ces particularités comme "marquage biologique" dans les recherches sur les interactions tissulaires et les migrations cellulaires au cours le l'ontogénèse. *Bull. Biol. Fr. Belg.* **103**, 435–452.
- Le Douarin, N. M. (1982). "The Neural Crest." Cambridge University Press, Cambridge, U.K.
- Liem, K. F., Jr., Tremml, G., Roelink, H., and Jessell, T. M. (1995). Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* **82**, 969–979.
- Löfberg, J., Ahlfors, K., and Fällström, C. (1980). Neural crest cell migration in relation to extracellular matrix organization in the embryonic axolotl trunk. *Dev. Biol.* **75**, 148–167.
- Ma, Q., Kintner, C., and Anderson, D. J. (1996). Identification of *neurogenin*, a vertebrate neuronal determination gene. *Cell* **87**, 43–52.
- Mancilla, A., and Mayor, R. (1996). Neural crest formation in *Xenopus laevis*: Mechanisms of *Xistag* induction. *Dev. Biol.* **177**, 580–589.
- Marchant, L., Linker, C., Ruiz, P., Guerrero, I., and Mayor, R. (1998). The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. *Dev. Biol.* **198**, 319–329.
- Mayor, R., Essex, L. J., Bennett, M. F., and Sargent, M. G. (1993). Distinct elements of the *Xenopus* promoter are required for mesodermal and ectodermal expression. *Development* **119**, 661–671.
- Mayor, R., Morgan, R., and Sargent, M. G. (1995). Induction of the prospective neural crest in *Xenopus*. *Development* **121**, 767–777.
- Mayor, R., Guerrero, I., and Martínez, C. (1997). Role of FGF and *noggin* in neural crest induction. *Dev. Biol.* **189**, 1–12.
- Meyer, D., Wolff, C. M., Stuegler, P., Senan, F., Befort, J. J., and Remy, P. (1993). *Xlfi*, the *Xenopus* homologue of the *flr-1* gene, is expressed during embryogenesis in a restricted pattern evocative of neural crest cell distribution. *Mech. Dev.* **44**, 109–121.
- Meyer, D., Durrhat, M., Senan, F., Wolff, M., Andre, M., Hourdry, J., and Remy, P. (1997). *Eis-1* and *Eis-2* proto-oncogenes exhibit differential and restricted expression patterns during *Xenopus laevis* oogenesis and embryogenesis. *Int. J. Dev. Biol.* **41**, 607–620.
- Morgan, R., and Sargent, M. G. (1997). The role in neural patterning of translation initiation factor eIF4AIII: induction of neural fold genes. *Development* **124**, 2751–2760.
- Murphy, M., and Bartlett, P. E. (1993). Molecular regulation of neural crest development. *Mol. Neurobiol.* **7**, 111–135.
- Nakata, K., Nagai, T., Aruga, J., and Mikoshiba, K. (1997). *Xenopus Zic3*, a primary regulator both in neural and neural crest development. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11980–11985.
- Nieto, M. A., Gilardi-Hebenstreit, P., Charnay, P., and Wilkinson, D. G. (1992). A receptor pro-

- tein kinase implicated in the segmental patterning of the hindbrain and mesoderm. *Development* **116**, 1137–1150.
- Nieto, M. A., Sargent, M. G., Wilkinson, D. G., and Cooke, J. (1994). Control of cell behavior during vertebrate development by *Slug*, a zinc finger gene. *Science* **264**, 835–839.
- Nieuwkoop, P. D., and Albers, B. (1990). The role of competence in the cranio-caudal segregation of the central nervous system. *Dev. Growth Differ.* **32**, 23–31.
- Niu, M. C. (1947). The axial organization of the neural crest, studies with particular reference to its pigmentary component. *J. Exp. Zool.* **105**, 79–114.
- Northcutt, R. G., and Gans, C. (1983). The genesis of neural crest and epidermal placodes: A reinterpretation of vertebrate origins. *Q. Rev. Biol.* **58**, 1–28.
- Placzek, M., Tessier-Lavigne, M., Jessell, T., and Dodd, J. (1990). Orientation of commissural axons *in vitro* in response to a floor plate-derived chemoattractant. *Development* **110**, 19–30.
- Presley, R., Horder, T. J., and Slipka, J. (1996). Lancelet development as evidence of ancestral chordate structure. *Isr. J. Zool.* **42**, 97–116.
- Raven, C. P. (1935). Zur Entwicklung der Ganglienleiste. IV. *Roux's Arch.* **132**, 33–575.
- Raven, C. P. (1937). Experiment on the origin of the sheath cells and sympathetic neuroblast in amphibia. *J. Comp. Neurol.* **67**, 221–240.
- Raven, C. P., and Kloos, J. (1945). Induction by medial and lateral pieces of the archenteron roof, with special reference to the determination of the neural crest. *Acta Neerland. Morphol. Norm. Pathol.* **55**, 348–362.
- Rickmann, M., Fawcett, J. W., and Keynes, R. J. (1985). The migration of neural crest cells and the growth of motor axons through the rostral half of the chick somite. *J. Embryol. Exp. Morphol.* **90**, 437–455.
- Rijli, F. M., Mark, M., Lakkaraju, S., Dierich, A., Dolle, P., and Chambon, P. (1993). A homeotic transformation is generated in the rostral branchial region of the head by disruption of *Hoxa-2*, which acts as a selector gene. *Cell* **75**, 1333–1349.
- Sadaghiani, B., and Thiébaud, C. H. (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, H. E., and Dawid, I. B. (1997). Regulation of dorsal fate in the neuraxis by *Wnt-1* and *Wnt-3a*. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13713–13718.
- Sasai, Y., Lu, B., Steinbeisser, H., Geisert, D., Gont, L. K., and De Robertis, E. M. (1994). *Xenopus* chordin: A novel dorsaling factor activated by organizer-specific homeobox genes. *Cell* **79**, 779–790.
- Scherson, T., Serbedžija, G., Fraser, S., and Bronner-Fraser, M. (1996). Regulative capacity of the cranial neural tube to form neural crest. *Development* **118**, 1049–1061.
- Scheucher, M., Dege, P., Lef, J., Hille, S., and Knöchel, 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**, 203–211.
- Schmidt, J. E., Suzuki, A., Ueno, N., and Kimelman, D. (1995). Localized BMP-4 mediates dorsal/ventral patterning in the early *Xenopus* embryo. *Dev. Biol.* **169**, 37–50.
- Selleck, M. A. J., and Bronner-Fraser, M. (1995). Origins of the avian neural crest: The role of the neural plate-epidermal interactions. *Development* **121**, 525–538.
- Serbedžija, G., N., Bronner-Fraser, M., and Fraser, S. E. (1989). A vital dye analysis of the timing and pathways of avian trunk neural crest cell migration. *Development* **106**, 809–816.
- Servetnick, M., and Grainger, R. M. (1991). Changes in neural and lens competence in *Xenopus* ectoderm: Evidence for an autonomous developmental timer. *Development* **112**, 177–188.
- Sharpe, C. R. (1992). Two isoforms of retinoic acid receptor alpha expressed during *Xenopus* development respond to retinoic acid. *Mech. Dev.* **39**, 81–93.
- Smith, A., Robinson, V., Patel, K., and Wilkinson, D. G. (1997). The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. *Curr. Biol.* **7**, 561–570.
- Smith, W. C., Knecht, A. K., Wu, M., and Harland, R. M. (1993). Secreted noggin protein mimics the Spemann organizer in dorsalizing mesoderm. *Nature* **361**, 547–549.
- Stern, C. (1954). Two or three bristles. *Am. Sci.* **42**, 213–247.
- Stone, L. S. (1922). Experiments on the development of the cranial ganglia and the lateral line sense organs in *Amphibia punctatum*. *J. Exp. Zool.* **35**, 421–496.
- Suzuki, A., Ueno, N., and Henmati-Brivanlou, A. (1997). *Xenopus msx1* mediates epidermal induction and neural inhibition by BMP4. *Development* **124**, 3037–3044.
- Takebayashi, K., Takahashi, S., Yokota, C., Tsuda, H., Nakamishi, S., Asashima, M., and Kageyama, R. (1997). Conversion of ectoderm into a neural fate by *ATF-3*, a vertebrate basic helix-loop-helix gene homologous to *Drosophila* proneural gene *atonal*. *EMBO J.* **16**, 384–395.
- Tanabe, Y., and Jessell, T. M. (1996). Diversity and pattern in the developing spinal cord. *Science* **274**, 1115–1123.
- Tanabe, Y., Roelink, H., and Jessell, T. (1995). Induction of motor neurons by sonic hedgehog is independent of floor plate differentiation. *Curr. Biol.* **5**, 651–658.
- Thomsen, G. H. (1996). *Xenopus* mothers against decapentaplegic is an embryonic ventralizing agent that acts downstream of the BMP-2/4 receptor. *Development* **122**, 2359–2366.
- Traitor, P. A., Tan, S., and Tam, P. L. (1994). Cranial paraxial mesoderm: Regionalisation of cell fate and impact on craniofacial development in mouse embryos. *Development* **120**, 2397–2408.
- Turner, D. L., and Weintraub, H. (1994). Expression of *achaete-scute* homolog 3 in *Xenopus* embryos converts ectodermal cells to a neural fate. *Genes Dev.* **12**, 1434–1447.
- van Straaten, H. W., Hekking, J. W., Wientz-Hoessels, E. J., Thors, F., and Drukker, J. (1988). Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol.* **177**, 317–324.
- Wang, Y., and Sassoon, D. (1995). Ectoderm-mesenchyme and mesenchyme-mesenchyme interactions regulate *Msx-1* expression and cellular differentiation in the murine limb bud. *Dev. Biol.* **168**, 374–382.
- Whittaker, J. R. (1997). Cephalochordates, the lancelets. In "Embryology: Constructing the Organism" (S. F. Gilbert and M. Raunio, Eds.), pp. 375–376. Sinauer, Sunderland, MA.
- Wilson, P. A., Lagna, G., Suzuki, A., and Hemmati-Brivanlou, A. (1997). Concentration-dependent patterning of the *Xenopus* ectoderm by BMP4 and its signal transducer Smad1. *Development* **124**, 3177–3184.
- Winning, R. S., and Sargent, T. D. (1994). Pagliaccito, a member of the Eph family of receptor tyrosine kinase genes, has localized expression in a subset of neural crest and neural tissues in *Xenopus laevis* embryos. *Mech. Dev.* **46**, 219–229.
- Wolda, S. L., Moody, C. J., and Moon, R. T. (1993). Overlapping expression of *Xwnt-3a* and *Xwnt-1* in neural tissue of *Xenopus laevis* embryos. *Dev. Biol.* **155**, 46–57.
- Wolff, C. M., Stiegler, B., Baltzinger, M., Meyer, D., Ghysdael, J., Stehelin, D., Befort, N., and Remy, P. (1990). Isolation of two different c-ets-2 proto-oncogenes in *Xenopus laevis*. *Nucleic Acids Res.* **18**, 4603–4604.
- Xu, Q., Aldus, G., Holder, N., and Wilkinson, D. G. (1995). Expression of truncated Sek-1 receptor tyrosine kinase disrupts the segmental restriction of gene expression in the *Xenopus* and zebrafish hindbrain. *Development* **121**, 4005–4016.
- Yamada, T. (1990). Regulations in the induction of the organized neural system in amphibian embryos. *Development* **110**, 653–659.
- Yntema, C. L. (1943). An experimental study on the origin of the sensory neurons and sheath cells of the IXth and Xth cranial nerves in *Ambystoma punctatum*. *J. Exp. Zool.* **92**, 93–120.
- Zimmerman, K., Shih, J., Bars, J., Collazo, A., and Anderson, D. J. (1993). *XASH-3*, a novel *Xenopus* *achaete-scute* homolog, provides an early marker of planar neural induction and position along the mediolateral axis of the neural plate. *Development* **119**, 221–232.