Developmental Biology **218**, 13–20 (2000)

A Critical Period for Conversion of Ectodermal Cells to a Neural Crest Fate

Seth Ruffins and Marianne Bronner-Fraser[1]
Division of Biology, 139-74, California Institute of Technology, Pasadena, California 91125

Previously, we found that interactions between neural and nonneural ectoderm can generate neural crest cells, with both the ectodermal and the neuroepithelial cells contributing to induced population (M. A. J. Selleck and M. Bronner-Fraser, 1995, *Development* 121, 525–538). To further characterize the ability of ectodermal cells to form neural crest, we have challenged their normal fate by transplanting them into the neural tube. To ensure that the ectoderm was from nonneural regions, we utilized extraembryonic ectoderm (the proamnion) and transplanted it into the presumptive midbrain of 1.5-day-old chick embryos. We observed that the grafted ectoderm has the capacity to adopt a neural crest fate, responding within a few hours of surgery by turning on neural crest markers HNK-1 and *Slug*. However, the competence of the ectoderm to respond to neural crest-inducing signals is time limited, declining rapidly in donors older than the 10-somite stage. Similarly, the inductive capacity of the host midbrain declines in a time-dependent fashion. Our results show that extraembryonic ectoderm has the capacity to form neural crest cells given proper inducing signals, expressing both morphological and molecular markers characteristic of neural crest cells.

Key Words: **neural crest cells; notochord; *Slug;* neural plate.**

INTRODUCTION

The neural crest is a highly migratory cell type that emerges from the dorsal neural tube following neurulation. It was classically assumed that presumptive neural crest cells were a segregated lineage, distinct from presumptive central nervous system cells within the neural tube. Accordingly, expression of neural crest markers like the zinc finger transcription factor, *Slug* (Nieto *et al.*, 1994), in the neural folds and neural tube has been taken as evidence for a fate restriction toward a neural crest lineage. However, single-cell lineage analysis of dorsal neural tube cells has revealed that there is a shared lineage between neural crest and spinal cord cells in the chick (Bronner-Fraser and Fraser, 1988, 1989), frog (Collazo *et al.*, 1993), and mouse (Serbedzija *et al.*, 1992). Even more surprisingly, prior to neural tube closure, single cells within the avian neural folds have the ability to form epidermal derivatives in addition to neural crest and neural tube derivatives (Selleck *et al.*, 1995). These results demonstrate that cells within the neural folds or dorsal neural tube are capable of contributing to the neural crest lineage, but are not uniquely fated to do so.

An interaction between the presumptive epidermis and the neural plate induces the formation of neural crest cells (Moury and Jacobson, 1989; Selleck *et al.*, 1995; Dickinson *et al.*, 1995). This interaction is time dependent, with the competence of the neural plate to respond to inductive signals declining after stage 10 (Dickinson *et al.*, 1995). Interestingly, quail/chick recombinants demonstrated that both neural and nonneural ectoderm are able to contribute neural crest cells as a result of this interaction, suggesting that the neural plate induces ectoderm to form neural crest and vice versa (Selleck *et al.*, 1995). Overexpression of the transcriptional regulator Id2, which is expressed in the cranial neural folds, converts ectodermal cells to neural lineages, demonstrating that proper transcriptional control is necessary for appropriate allocation of cell types to epidermal, neural crest, and neural tube lineages (Martinsen and Bronner-Fraser, 1998). Taken together, these experiments demonstrate an intriguing relationship between ectodermal derivatives (neural crest, epidermis, and neural tube).

Many questions remain about the spatial and temporal ability of the ectoderm to respond to inductive interactions by the neuroepithelium. First, little is known about the time of competence of the ectoderm to respond to induction by the neural plate or the time of competence of the neural plate to induce ectoderm to become neural crest. Second,

[1] To whom correspondence should be addressed. Fax: (626) 395-7717.

0012-1606/00 $35.00

only embryonic ectoderm was recombined with neural plate previously, whereas no attempt was made to examine the ability of the extraembryonic ectoderm to form neural crest cells. The embryonic ectoderm will form epidermis. In contrast, extraembryonic ectoderm is distant from the prospective neural plate and destined to form only extraembryonic membranes.

Here, we challenge the developmental potential of ectodermal cells by transplanting them into the neural tube, from which neural crest cells normally arise. To ensure that the ectoderm was from nonneural regions, we utilized extraembryonic ectoderm (the proamnion) and transplanted it into the presumptive midbrain of 1.5-day-old chick embryos. The results show that the grafted ectoderm has the capacity to express morphological properties and molecular markers characteristic of neural crest cells within a few hours of surgery. The competence of the ectoderm to respond to neural crest-inducing signals decreases with time as does the inductive capacity of the midbrain. These results demonstrate that nonembryonic ectoderm has the capacity to form neural crest cells given proper inducing signals.

MATERIALS AND METHODS

Ectoderm Grafts

White Leghorn chick and Japanese quail eggs were obtained through a commercial supplier (Highland International and Karasoulas Farms, respectively). Eggs were incubated at 37°C until the 4- to 21-somite stage and windowed. Ectoderm from the proamnion of quail embryos was dissected using glass needles and transferred to chicken eggs with a finely pulled glass mouth pipette. At midbrain levels, the quail ectoderm was placed: (1) into the lumen of the midbrain neural tube, (2) through the ventral neural tube, or (3) through the host ectoderm into the dorsal neural tube (see Fig. 1). After surgery, Howard Ringers solution was placed on the embryo and the egg was closed with Scotch brand Magic Tape (3M Corp.) and incubated for 8 or 14 h.

Histochemistry and in Situ Hybridization

After the appropriate incubation period, the eggs were reopened and the embryos removed, washed with Howard Ringers solution, and fixed in Carnoy's fixative (10% acetic acid, 60% EtOH, 30% $CHCl_3$) for 5–10 min. The embryos were then dehydrated with 70 and 100% EtOH, washed 3× in Histosol, and embedded in paraffin. Embedded embryos were sectioned at 10 μm, placed on glass slides, deparaffinized, rehydrated, and incubated with monoclonal antibodies QCPN and HNK-1 for at least 4 h at room temperature or overnight at 4°C. The slides were then washed in PBS and incubated with FITC–goat anti-mouse IgM (to detect HNK-1) and RITC–goat anti-mouse IgG (to detect QCPN) at a dilution of 1:500 each for 1–2 h. *In situ* hybridization was performed by the method described in Henrique *et al.* (1995).

Microscopy and Image Processing

Slides of sectioned embryos were viewed with a Zeiss Axiophot epifluorescence microscope. A rhodamine filter set was used to visualize QCPN antibody-stained cells, while a fluorescein filter set was used to visualize HNK-1 antibody staining. Data were recorded photographically using Kodak Elite II 400 film or electronically using a Hamamatsu SIT camera or Bio-Rad MRC 600 LSM and stored on removable Iomega Jaz discs. Image processing was performed using the Vidim software system (Fraser, Stolberg, and Belford, unpublished), NIH Image (available by anonymous FTP at FTP:zippy.nimh.nih.gov/pub/nih-image) and Adobe PhotoShop (Adobe Systems, Inc.). Whole-mount *in situ* hybridization images were recorded photographically using Kodak 160T film. All images were postprocessed and composed using Adobe PhotoShop.

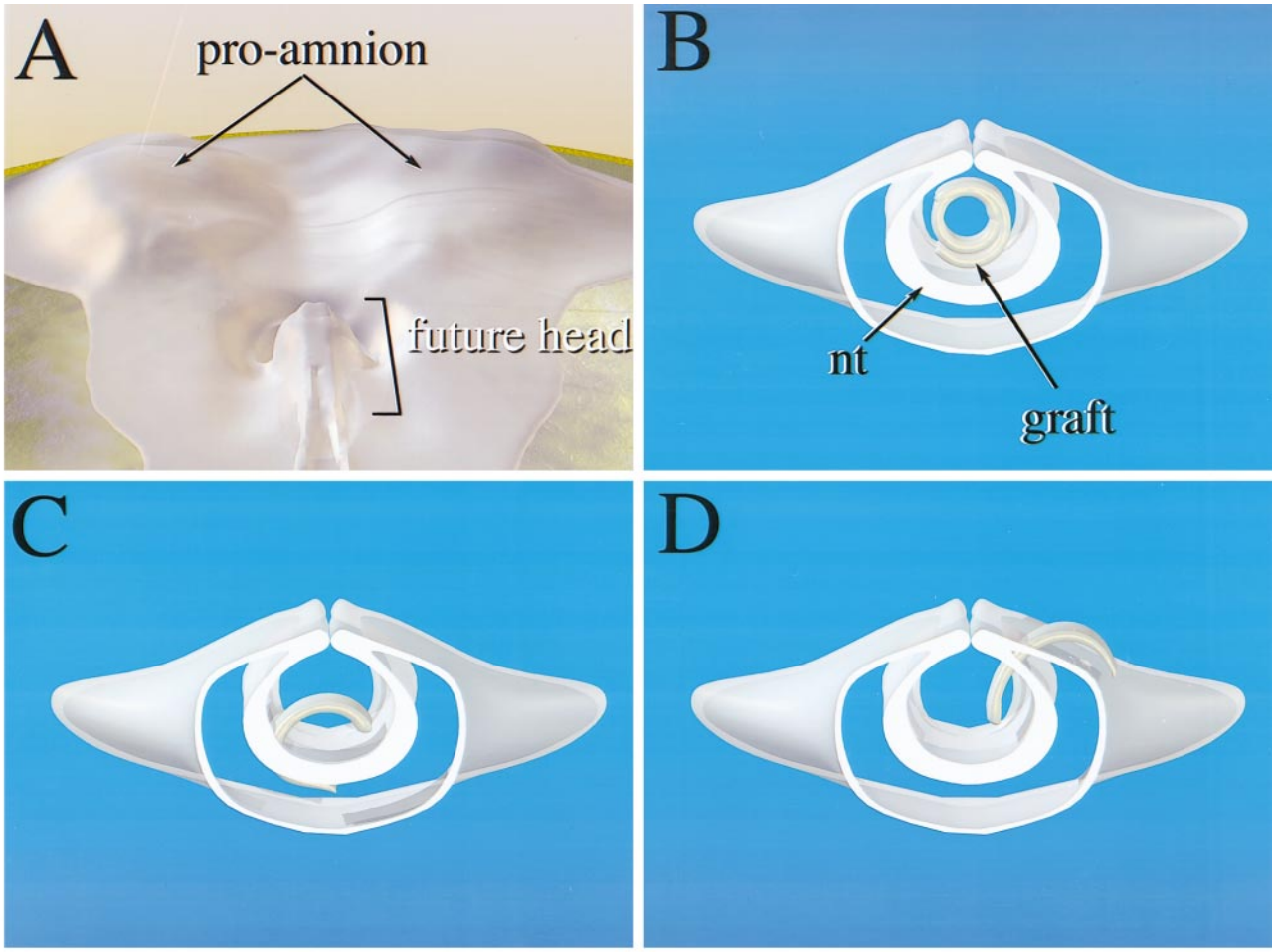
RESULTS

We have examined the ability of nonneural ectoderm to form neural crest cells by grafting extraembryonic ectoderm (the proamnion) into the presumptive midbrain. Donor quail ectoderm was collected from the proamnion region of embryos having from 4 to 21 somites. By the 4-somite stage, proamnion (anterior and lateral to the head) begins to delaminate from the underlying endoderm. This separation allows for the relatively simple collection of pure ectodermal tissue.

Quail ectoderm was grafted at the level of the midbrain into the lumen of the midbrain neural tube, through the ventral neural tube, or through the host ectoderm into the dorsal neural tube (see Fig. 1). All sites of placement within the midbrain gave identical results and, therefore, are presented cumulatively. Embryos were fixed at 8 or 14 h postsurgery. Neural crest formation was assayed using two cell markers: the HNK-1 antibody, which recognizes migrating neural crest cells (Tucker *et al.*, 1984), or the zinc finger transcription factor *Slug*, which recognizes premigratory and early migrating neural crest cells (Nieto *et al.*, 1994). As is the case with most molecular markers, neither of these is unique to neural crest cells. However, both HNK-1 and *Slug* are expressed on early migrating neural crest cells. The combination of two molecular markers together with the migratory morphology of the HNK-1-positive cells is likely to reflect a population with the ability to form neural crest. To verify that the cells were of donor origin, sections were also stained with the QCPN antibody, which specifically recognizes an epitope in the nucleus of quail cells.

Extraembryonic Ectoderm Has the Potential to Form HNK-1-Positive Neural Crest

In embryos examined 8 h after grafting, 15% of the grafts (n = 20 embryos) from donors ranging from the 5- to 10-somite stage contained quail HNK-1-immunoreactive neural crest cells (Fig. 2A). These results suggest that at least a small number of ectoderm cells are able to manifest neural crest characteristics as early as 8 h postgrafting. Given that the HNK-1 epitope is expressed only after neural crest cells emigrate from the neural tube, it is a relatively late marker, indicating that response to induction may be rapid.



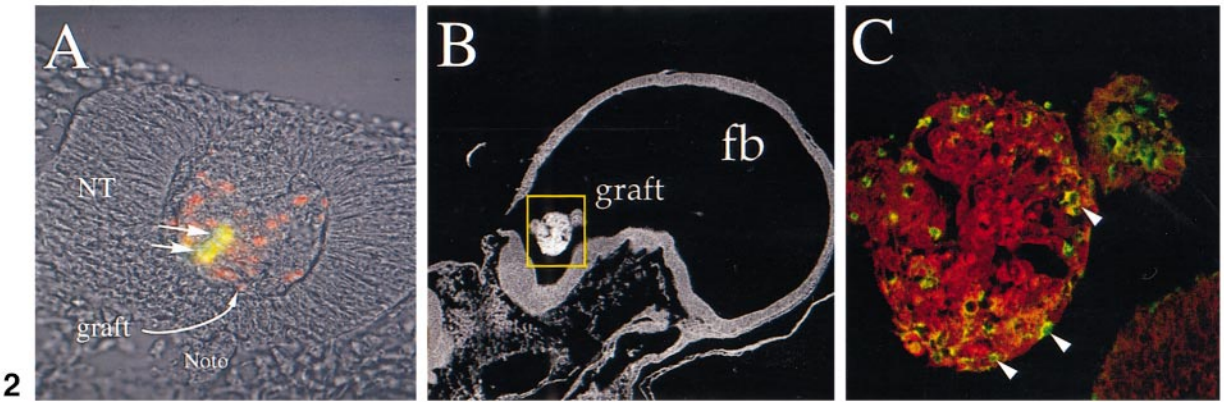


FIG. 1. Schematic diagram illustrating the types of operations performed in the this study. (A) A whole-mount view showing the location of the proamnion, an extraembryonic ectoderm, relative to the embryo. The proamnion begins to delaminate at the 4-somite stage, allowing for relatively easy collection of ectoderm. Quail proamnion was transplanted at the midbrain level of chick embryos into the lumen (B), through the ventral neural tube (C), or through the ectoderm into the neural tube (D). nt, neural tube.

FIG. 2. Ectoderm transplanted into the neural tube expresses the HNK-1 epitope. (A) A transverse section showing quail ectoderm (identified by QCPN in red) grafted into the neural tube that expresses the HNK-1 epitope (green), a neural crest marker. Double-labeled cells appear yellow. (A and C) Close contact between the graft and the neural tube does not appear to be necessary for induction of HNK-1 expression in ectoderm grafts. (B) A monochrome confocal image of a longitudinal section shows the location of the graft within the midbrain. Note that the graft is within the lumen of the neural tube. (C) Higher magnification of the inset (yellow box in B) shows that grafted cells express both QCPN (red) and the HNK-1 epitope (green). Noto, notochord; NT, neural tube; fb, forebrain. Arrows point to HNK-1-expressing quail cells.

Given that response to induction was low at 8 h postsurgery, embryos were allowed to develop for a total of 14 h. Donor tissue was derived from embryos having 4 to 21 somites. For donor tissue ranging from the 4- to 10-somite stage, there was a robust ability to form HNK-1/QCPN-immunoreactive cells (Fig. 2A). Positive cells were observed in 89% of the grafts (n = 37; Fig. 3). HNK-1/QCPN-positive cells were generally dispersed within the grafted ectoderm and occasionally had migrated away from the graft by the time of fixation. Typically, 1–10 positive cells were observed per embryo. Interestingly, quail neural crest cells appeared to be induced not only when ectoderm and midbrain neural tube were in direct contact, but also when the ectoderm remained segregated within the lumen of the neural tube (Figs. 2B and 2C). This suggests that contact is not requisite for induction of neural crest cells.

Extraembryonic ectoderm collected after the 10-somite stage had a reduced ability to produce neural crest cells (Fig. 3). Only 18% (n = 11) of the grafts from 14-somite-stage donors expressed HNK-1-positive cells and no tissue collected after the 16-somite stage produced any HNK-1-immunoreactive cells. When embryos containing grafts from 11- to 21-somite-stage ectoderm were pooled, only 8% (n = 25) of the donor tissue generated neural crest cells. The precipitous drop in the ability of proamnion to produce HNK-1-positive cells at these later stages indicates that this tissue loses its capacity to respond to the inducing signal(s).

Although these experiments were not designed to quantitate the amount of neural crest cells produced by the neural tube in response to an interaction with the proamnion, there appeared to be an increase in host neural crest generation. In four separate embryos in which ectodermal transplants pierced the neural tube, the neural tube appeared to continue to produce emigrating neural crest cells for longer periods of time on the engrafted side (Fig. 4). In contrast, the ventral extent of neural crest migration was similar on both operated and unoperated sides (Fig. 4).

Analysis of Slug Expression by Grafting Extraembryonic Ectoderm

Although the HNK-1 epitope is a useful neural crest marker, it is later expressed in the neural tube itself and on a variety of cell adhesion molecules (Kruse *et al.*, 1984). Therefore, we employed a second neural crest marker, the transcription factor *Slug* (Nieto *et al.*, 1994), to provide further evidence that the ectoderm grafts to the neural tube were generating neural crest *de novo*. Donor grafts were obtained from embryos having 4 to 9 somites and host embryos were fixed after 14–18 h. After this manipulation, all donor grafts expressed *Slug* (Fig. 5). The signal for *Slug* was comparatively weaker in the grafted quail ectoderm compared with the host neural crest. One possible explanation for the difference in intensity of staining is that the length of interaction resulting in *Slug* expression is much longer in the host tissue than in the donor ectoderm. However, a more likely explanation is that the *Slug* probe is derived from the untranslated region of the chick *Slug* gene, which cross-reacts with quail but is not a perfect match, likely leading to weaker signal.

In contrast to the HNK-1 epitope, which is expressed in a subset of the grafted cells, *Slug* transcripts appear to be expressed uniformly throughout the graft. In normal embryos, *Slug* is an early marker expressed throughout the neural folds in a population that has the potential to form neural crest cells. Our lineage studies, however, show that these neural fold cells give rise not only to neural crest but also to epidermis and the central nervous system (Selleck *et al.*, 1995). Thus, the entire donor ectoderm may express *Slug*, which correlates with the ability to form neural crest cells, whereas a subset of these cells actually goes on to form neural crest. Had our embryos been incubated for longer times, it is conceivable that a larger population would also become HNK-1 immunoreactive.

Extraembryonic Ectoderm Grafted into the Head Mesenchyme Does Not Form Neural Crest

To examine whether grafting extraembryonic ectoderm to any location within the embryo would induce production of neural crest cells, we performed grafts similar to those above but inserted the quail ectoderm into the head mesenchyme adjacent to the neural tube rather than in the neural tube itself. Competent quail extraembryonic ectoderm from 4- to 8-somite-stage donors was grafted into the head mesenchyme of similarly staged chick hosts (n = 10). The operated embryos were allowed to develop for 14 h and stained with the HNK-1 antibody. In no cases were HNK-1-positive cells of donor origin observed (Fig. 6). This suggests that the inducing signal is either not present or suppressed within the head mesenchyme.

Stage Dependence of the Ability of Midbrain to Induce Neural Crest Cells from Donor Ectoderm

The above experiments suggest that the midbrain of host chicken embryos ranging from the 4- to 12-somite stage has the ability to induce neural crest formation in donor quail ectoderm. We next examined whether the inducing signal is present in the neural tube at later stages. Competent extraembryonic ectoderm was grafted into somite stage 17–24 chick midbrains which were allowed to develop an additional 14 h. In no cases (n = 12) were HNK-1-positive quail cells found in the grafted tissue (Fig. 3). This suggests that the ability of the midbrain to induce neural crest cells in competent ectoderm has declined by these stages.

DISCUSSION

Neural crest cells arise via an inductive interaction between the neural plate and the nonneural ectoderm when these two tissues are juxtaposed (Moury and Jacobson, 1989; Selleck and Bronner-Fraser, 1995; Dickinson *et al.*, 1995). This interaction sets up a specialized region of cells, the neural folds, that expresses cell-type-selective markers

(e.g., *Slug*) and has the potential to form neural crest cells. However, these cells are not uniquely destined to form neural crest cells. In fact, cell lineage studies have revealed a remarkable plasticity in cells of the neural tube and neural folds. In the neural folds prior to neural tube closure, single cells have the capacity to generate all ectodermal derivatives, including epidermal cells, neural crest cells, and neural tube cells (Selleck and Bronner-Fraser, 1995). Thus, the neural crest does not represent a segregated population in the early neural plate. These experiments raise the intriguing question of when cells become "committed" to a neural crest versus an ectodermal fate. In order to test cell commitment, it is necessary to challenge developmental potential by transplanting either one cell or a group of cells into a region destined to form other embryonic structures. If transplanted cells alter their fates under these circumstances, they cannot have been committed at the time of transplantation.

In the present study, we have challenged the ability of extraembryonic ectoderm to contribute to embryonic tissues and, in particular, to neural crest cells. Our results demonstrate that ectoderm, derived from the extraembryonic proamnion, can be induced to express the neural crest markers HNK-1 and *Slug* when juxtaposed with neural tube cells *in vivo*. Interestingly, the competence of this ectoderm to respond to induction rapidly declines as a function of age. Similarly, the ability of the midbrain neural tube to induce neural crest cells from extraembryonic ectoderm diminishes with time. These results show that the competence of both the inducing and the responding tissue is time limited.

Other data support the idea that inductive interactions may be time limited in the early development of the neural crest and neural tube. Just as juxtaposition of nonneural ectoderm and early neural plate tissue induces the formation of neural crest cells (Selleck and Bronner-Fraser, 1995), interactions using slightly older neural tissues lead to the expression of dorsal tube markers like *Wnt*-1 and *Wnt*-3a (Dickinson *et al.*, 1995), which normally form only from dorsal regions of the neural tube. Thus, induction of particular markers is stage dependent. Recently, BMP-4 and BMP-7 have been shown to be sufficient to substitute for the nonneural ectoderm in inducing neural crest cells (Liem *et al.*, 1995). Although it remains to be demonstrated whether BMPs are necessary for this induction and if their effects are primary, this class of molecules represents good candidates for neural crest inducers. Later, BMPs induce expression of roof plate cells and dorsal sensory neurons within the neural tube (Liem *et al.*, 1998). These data suggest that neural plate/epidermal interactions induce multiple dorsal properties in the developing spinal cord that occur in a temporally distinct sequence. Clearly, the competence of the neural plate to respond to induction changes as a function of time. It remains to be determined whether single or multiple dorsalizing signals are involved in these inductive interactions.

Although molecules like BMPs are good candidates for the ectodermal signal that induces neural crest cells from neural plate tissue, the nature of the reciprocal inducer (i.e., neural plate/tube inducer that generates neural crest cells from ectoderm) is unknown. BMPs are initially expressed in the nonneural ectoderm, but later in the neural folds (Watanabe and LeDouarin, 1996). Therefore, it is possible that BMPs represent the inducing molecules. This possibility is made even more intriguing by the observation that BMPs appear to exert their primary function in neural crest formation when they are expressed in the neural folds rather than in the nonneural ectoderm (Selleck *et al.*, 1998). It is worth noting that the proamnion, itself, does not express BMP-4 (unpublished observation). Other good candidates for inducers are Wnts, since both Wnt-1 and Wnt-3a are expressed at high levels in the dorsal neural tube (Hollyday *et al.*, 1995). In *Xenopus*, Wnts have been shown to be potent neural crest inducers in combination with inhibition of BMP signaling (LaBonne and Bronner-Fraser, 1998).

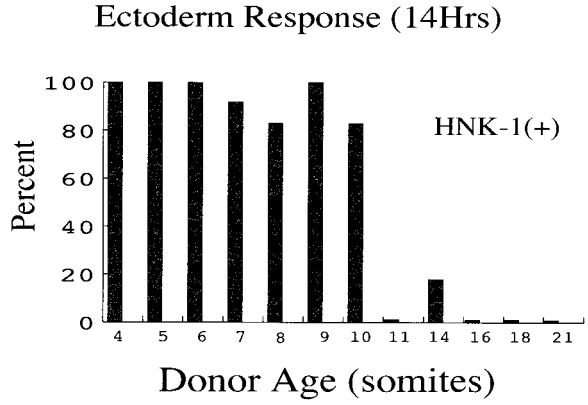
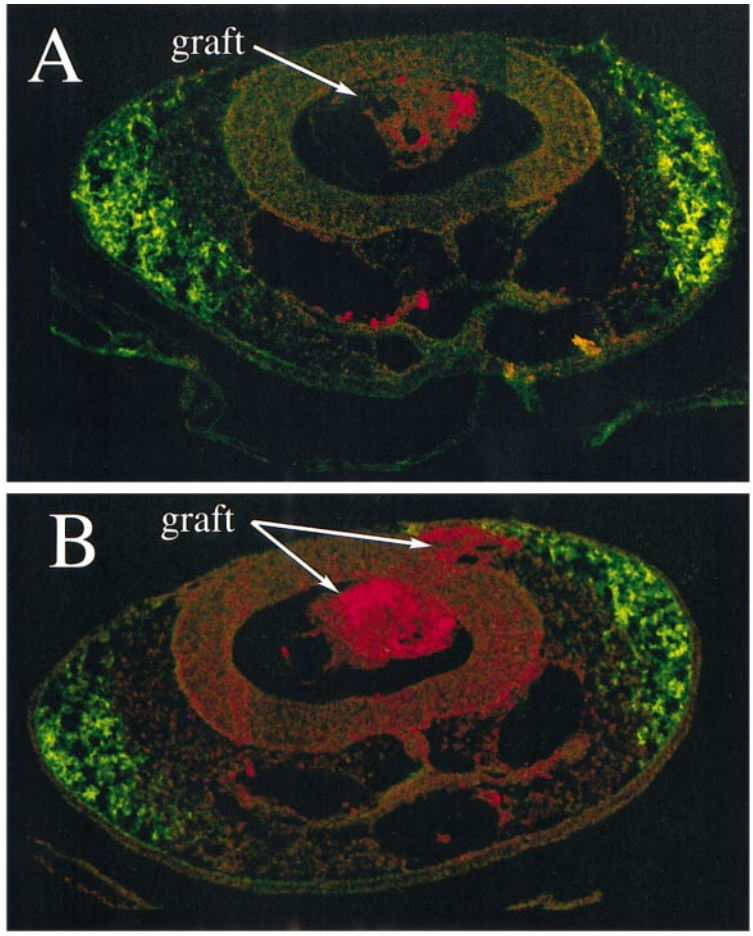


FIG. 3. The capacity for the proamnion to express the HNK-1 epitope diminishes rapidly after the 10-somite stage.

In numerous grafts, we observed expression of neural crest markers even when the graft was within the neural tube lumen and not directly contacting the neural tube. This suggests that the inducing signal is diffusible. In the donor tissue, *Slug* was expressed throughout the graft, whereas HNK-1 immunoreactivity arose in a few cells within the graft. The uniform *Slug* expression is likely to reflect two things. First, *Slug* is an earlier marker, coming in premigratory regions, whereas HNK-1 is first expressed after cells emigrate from the neural tube in normal embryos. Second, *Slug* is expressed in cells with the "potential" to form neural crest. However, only a subset is likely to actually differentiate into neural crest cells. This is similar to the situation in the neural folds, where *Slug*-expressing precursors can form neural crest, ectodermal, and neural tube derivatives (Selleck *et al.*, 1995).

By challenging the fate of ectodermal and neural plate cells of various developmental stages, these experiments tell us: (1) the stages at which the neural tube produces inductive signals leading to neural crest formation and (2) the stages at which ectodermally derived tissue can respond



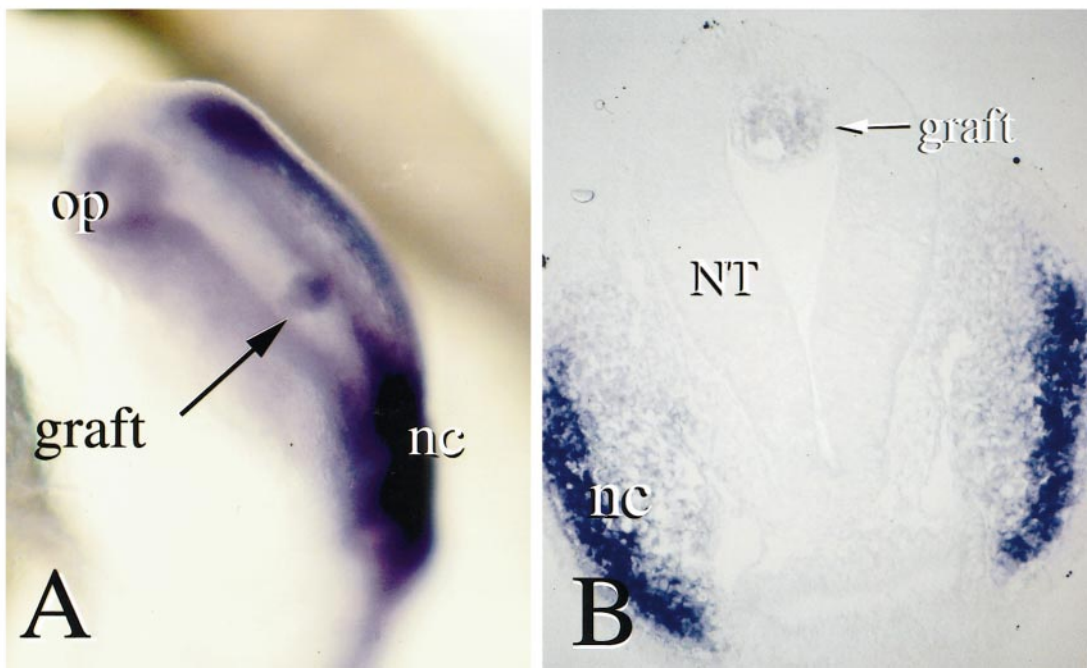


FIG. 4. Transplanted ectoderm appears to increase the length of time that neural crest emigrates from the neural tube. (A and B) Sections from a single embryo at different axial levels. (A) At the level at which the ectoderm (red) is contained within the neural tube, no alterations

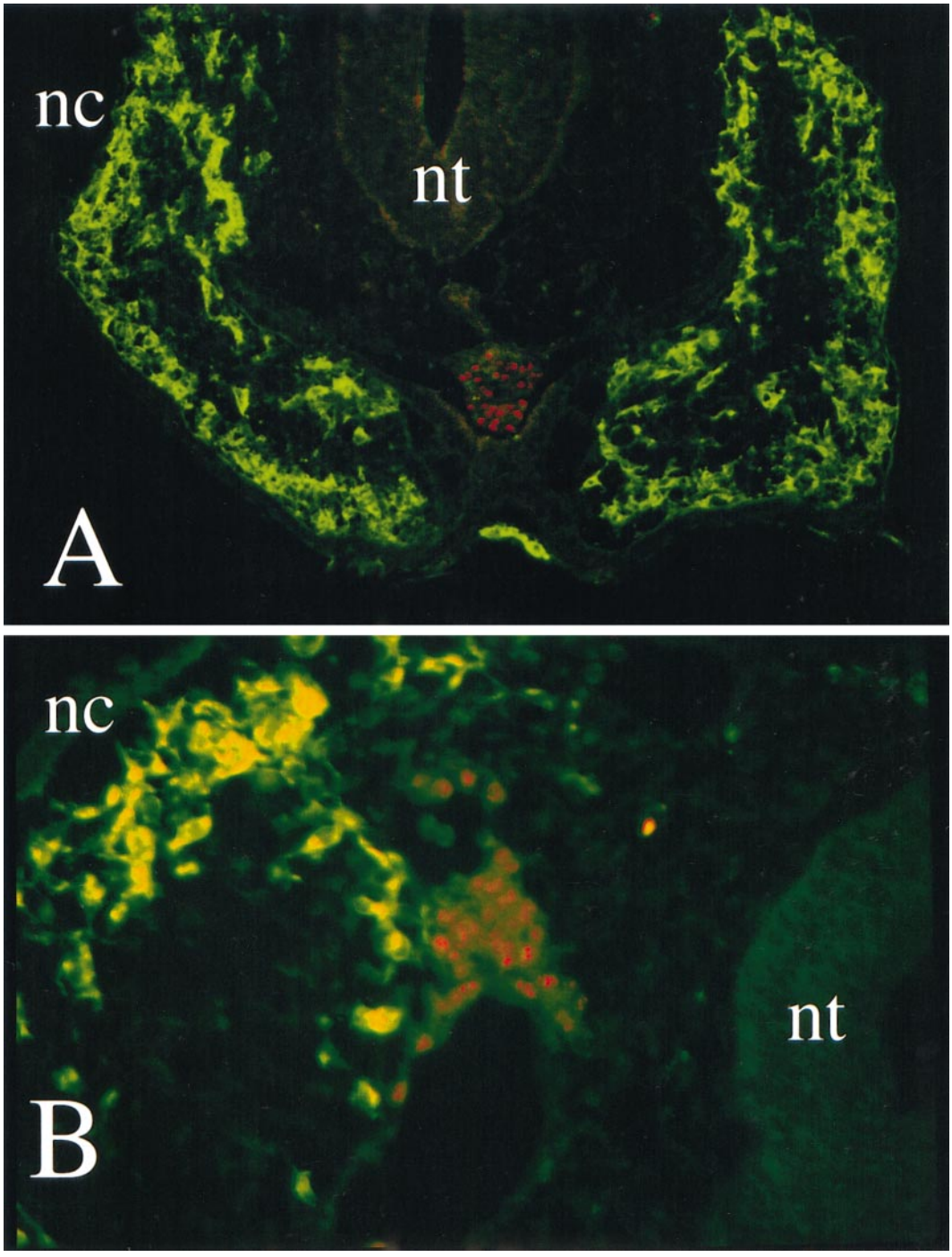


FIG. 6. Grafts of donor ectoderm into the head mesenchyme just below the notochord (A) midline or adjacent to the neural tube (B) do not express the HNK-1 epitope.

in neural crest production were seen. (B) In contrast, donor ectoderm traversing the neural tube appeared to increase production of neural crest (green) by the host embryo on the engrafted side. Note that neural crest emigration is complete on the unoperated side. This embryo was fixed 8 h postgraft, at which time little or no expression of neural crest markers was observed in donor tissue.

FIG. 5. *Slug* is expressed in the grafted tissue. (A) Whole-mount *in situ* hybridization reveals *Slug* expression within the grafted ectoderm. (B) A section at the level of the graft shows *Slug* expression in the graft, but less intense than that observed in the host neural crest. op, optic vesicle; nc, neural crest; NT, neural tube.

to inductive signals to form neural crest. The present study is the first to directly demonstrate that extraembryonic ectoderm can be converted into neural crest cells. The results highlight the incredible plasticity in early vertebrate development and the important role of inductive interactions in this plasticity.

ACKNOWLEDGMENTS

This work was supported by NIH Grants USPHS NS34671 and NS36585 to M.B.F. and NRSA NS11008-1 to S.R.

REFERENCES

Bronner-Fraser, M., and Fraser, S. (1988). Cell lineage analysis shows multipotentiality of some avian neural crest cells. *Nature* **335,** 161–164.

Bronner-Fraser, M., and Fraser, S. (1989). Developmental potential of avian trunk neural crest cells in situ. *Neuron* **3,** 755–766.

Collazo, A., Bronner-Fraser, M., and Fraser, S. E. (1993). Vital dye labelling of *Xenopus laevis* neural crest cells reveals multipotentiality and novel pathways of migration. *Development* **118,** 363–376.

Dickinson, M., Selleck, M., McMahon, A., and Bronner-Fraser, M. (1995). Dorsalization of the neural tube by the non-neural ectoderm. *Development* **121,** 2099–2106.

Henrique, D., Adam, J., Myat, A., Chitnis, A., Lewis, J., and Ish-Horowicz, D. (1995). Expression of a Delta homologue in prospective neurons in the chick. *Nature* **375,** 787–790.

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

Kruse, J., Mailhammer, R., Wenecke, H., Faissner, A., Sommer, I., Goridis, C., and Schachner, M. (1984). Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. *Nature* **311,** 153–155.

LaBonne, C., and Bronner-Fraser, M. (1998). Neural crest cell induction in Xenopus: Evidence for a two-signal model. *Development* **125,** 2403–2414.

Liem, K. F., 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.

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

Martinsen, B., and Bronner-Fraser, M. (1998). Neural crest cell fate is regulated by the helix-loop-helix transcriptional regulator, Id2. *Science* **281,** 988–991.

Moury, J. D., and Jacobson, A. G. (1989). Neural fold formation at newly created boundaries between neural plate and epidermis in the axolotl. *Dev. Biol.* **133,** 44–57.

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.

Selleck, M. A. J., and Bronner-Fraser, M. (1995). Origins of the avian neural crest: The role of neural plate/epidermal interactions. *Development* **121,** 525–538.

Selleck, M. A. J., Garcia-Castro, M., Artinger, K. B., and Bronner-Fraser, M. (1998). Effects of Shh and noggin on neural crest formation demonstrate that BMP is required in the neural tube but not ectoderm. *Development* **125,** 4919–4930.

Serbedzija, G., Bronner-Fraser, M., and Fraser, S. E. (1992). Vital dye analysis of cranial neural crest cell migration in the mouse embryo. *Development* **116,** 297–307.

Tucker, G. C., Aoyama, H., Lipinski, M., Tursz, T., and Thiery, J. P. (1984). Identical reactivity of monoclonal antibodies HNK-1 and NC-1: Conservation in vertebrates on cells derived from the neural primordium and on some leukocytes. *Cell Differ.* **14,** 223–230.

Watanabe, Y., and Le Douarin, N. M. (1996). A role for BMP-4 in the development of subcutaneous cartilage. *Mech. Dev.* **57,** 69–78.

Received for publication September 17, 1999
Revised October 28, 1999
Accepted October 28, 1999