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Ventral Neural Tube Derivatives When Challenged by Transplantation

Seth Ruffins, Kristin Bruk Artinger,[1] and Marianne Bronner-Fraser[2]

Division of Biology, California Institute of Technology 139-74, Pasadena, California 91125

Once neural crest cells undergo an epithelial–mesenchymal transition to leave the neural tube, it has been classically assumed that they are fated to differentiate within the neural crest lineage. To test this idea, we challenged the developmental potential of recently emigrated neural crest cells by transplanting them into the ventral portion of the neural tube at the open neural plate stage. Newly migrating neural crest cells were isolated in tissue culture, labeled with the lipophilic dye DiI, and microinjected into the ventral portion of the neural plate. After 2 days, some neural crest cells became incorporated into the neuroepithelium in positions characteristic of floor plate cells and motor neurons. Some of the labeled cells within the ventral neural tube expressed FP-1, characteristic of floor plate cells. A few labeled cells were found in positions characteristic of motor neurons and expressed islet-1. In contrast, neural crest cells transplanted onto neural crest pathways expressed the HNK-1 epitope, but no ventral neural tube markers. Injection of neural crest cells into the mesenchyme adjacent to the notochord or culturing them in the presence of Sonic hedgehog failed to elicit FP-1 expression. These results suggest that migrating neural crest cells are flexible in their fate and retain the ability to form neural tube derivatives even after emigrating from the neural tube.

INRODUCTION

During neurulation, the midline ectoderm thickens to form the columnar epithelium of the neural plate, in contrast to the cuboidal ectoderm from which these cells are derived. The neural folds then begin to elevate and the neural plate invaginates to form the cylindrical neural tube. Initially, the neural tube appears to be a somewhat homogeneous neuroepithelium. Subsequently, distinct cell types appear in the dorsal and ventral portions of the neural tube, reflecting the first overt manifestation of dorsoventral polarity. Neural crest cells emigrate from the dorsal neural tube and commissural neurons begin to differentiate dorsolaterally. In the ventral neural tube, presumptive floor plate cells in the midline assume a wedge-shaped morphology and begin to express characteristic floor plate markers such as the transcription factor HNF-3β, the cell surface epitope FP-1, and the secreted molecule netrin (rev. Tanabe and Jessell, 1996). Ventrolaterally, neural tube cells differentiate into motor neurons which express islet-1 and various lim transcription factors (Tsuchida *et al.*, 1994).

The notochord and/or its precursors within Hensen's node are responsible for establishing ventral properties within the neural tube. A notochord grafted lateral to the neural tube induces an extra floor plate and motor neuron pools (van Straaten *et al.*, 1988; Yamada *et al.*, 1991), via a Sonic hedgehog (Shh)-mediated signal (Echelard *et al.*, 1993). Shh protein is expressed in the floor plate and notochord (Marti *et al.*, 1995a). Furthermore, addition of Shh-expressing cells or purified peptides adjacent to intermediate neural plate explants leads to induction of floor plate and motor neurons in a dose-dependent fashion (Marti *et al.*, 1995b; Roelink *et al.*, 1995).

Dorsal cell types within the neural tube also arise via inductive interactions. Juxtaposition of the neural plate and surface ectoderm induces the production of neural crest cells (Selleck and Bronner-Fraser, 1995) as well as other dorsally expressed transcripts like Wnt-1, Wnt-3a, and Pax-3 (Dickinson *et al.*, 1995; Liem *et al.*, 1997). The TGF-β family members dorsalin-1 (Basler *et al.*, 1993), BMP-4, and

[1] Present address: Department of Cell Biology, Harvard Medical School, Boston, MA.

[2] To whom correspondence should be addressed. Fax: (626) 395-7717. E-mail: mbronner@caltech.edu.

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BMP-7 (Liem *et al.*, 1995) have been shown to be sufficient to substitute for the nonneural ectoderm in inducing neural crest cells from neural plates. BMPs are expressed in the early ectoderm, downregulated prior to neural tube closure, and then expressed at high levels in the neural folds (Selleck *et al.*, 1998). Thus, this class of molecules represents a good candidate for signal(s) that induce dorsal cell types within the developing spinal cord (Tanabe and Jessell, 1996). Although the factors responsible for inducing certain cell types in the neural tube are beginning to be clarified, many basic questions remain regarding the timing of fate restrictions of neural tube-derived cells.

As neural crest cells emigrate from the neural tube, they undergo an epithelial–mesenchymal transition which has been assumed to correlate with their determination toward a neural crest fate. Here, we challenge the developmental potential of early migrating neural crest cells by microinjecting them into the ventral neural tube—where they are exposed to strong ventralizing signals. Our results suggest that early migrating neural crest cells can become incorporated into the neural tube and can express both morphological features and molecular markers characteristic of floor plate cells and motor neurons. Thus, early migrating neural crest cells may not be restricted to form only neural crest derivatives.

MATERIALS AND METHODS

Neural Crest Culture

Neural crest cultures are prepared using standard techniques (Lallier and Bronner-Fraser, 1991). Quail embryos with 12 to 18 somite pairs were harvested and placed in ice-cold Howard Ringers solution. The embryo was trimmed to include only the neural tube and surrounding tissues, including the segmental plate and the last 6 to 9 somites. The neural tube was isolated by incubating the trimmed tissue in dispase for 20 min at room temperature, followed by gentle trituration and collection in complete medium [DMEM (Gibco) containing 10% horse serum plus 15% embryo extract]. The entire dorsoventral extent of the neural tube was placed on a tissue culture dish coated with 10 μg/ml fibronectin (New York Blood Bank) and allowed to adhere 1 h prior to the addition of F-12 media containing N-2 growth supplement (Gibco). Neural tube explants were incubated at 37°C for 12–18 h, during which time many neural crest cells migrated away from the neural tube. After 12–18 h, the entire neural tube was removed with a tungsten needle and the remaining neural crest cells were visually checked to ascertain that all neural tube cells were removed.

The residual neural crest population was labeled with the lipophilic dye DiI-CM (Molecular Probes, Eugene, OR). Briefly, DiI-CM was dissolved in 100% ethanol at a concentration of 1 mg/ml. The concentrated DiI-CM solution was diluted 1:50 in 0.3 M sucrose which was then directly added to the plated cells. After 1 h incubation at 37°C, the cells were extensively washed with Howard Ringers solution and removed from the culture dish by gentle scraping and pipeting in complete medium containing 10 mM EDTA. The cell suspension was concentrated by centrifugation.

Preparation of Embryos

White Leghorn chicken embryos were incubated at 38°C until they reached the Hamburger–Hamilton stage 9–10 (Hamburger and Hamilton, 1951). The eggs were washed with 70% ethanol, 1.5 ml of albumen was removed, a window was cut in the shell over the embryo, and India ink (Pelikan Fount, Hanover, FGR) diluted 1:10 in Howard Ringers solution was injected under the blastoderm to aid in visualization of the embryo. The vitelline membrane was deflected using an electrolytically sharpened tungsten needle.

Microinjection of Cells into Embryos

DiI-labeled neural crest cells were backfilled into a pulled micropipet connected to a hydraulic micromanipulator (Narashige, Japan). Approximately 3 μl of medium containing cells was placed into the micropipet. The pipet tip was inserted into the open neural plate in stage 9–10 embryos and occasionally by secondarily opening the neural tube in stage 11 embryos. Labeled cells were expelled with a pulse of pressure into the neuroepithelium. Embryos were examined under epifluorescent illumination immediately after injection to verify that cells were implanted into the desired location in or lateral to the ventral midline of the neural tube. Typically, 1 to 10 cells were placed into an injection site.

Immunocytochemistry

Embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, placed into 15% sucrose overnight at 4°C, embedded in 15% sucrose/7.5% gelatin (Sigma), rapidly frozen in liquid nitrogen, and serially sectioned at 10 μm on a cryostat (Zeiss Micron, Heidelberg, Germany). Sections were mounted on gelatin-subbed slides.

The following antibodies were used in this study: (1) FP-1, which recognizes floor plate cells (kindly provided by Dr. T. Jessell; (2) islet-1 antibody, which recognizes a transcription factor expressed by motor neurons and dorsal root ganglion cells (Developmental Studies Hybridoma Bank; University of Iowa); (3) the HNK-1 antibody, which recognizes migrating neural crest cells (American Type Culture); and (4) the QCPN antibody, which recognizes quail cells (Developmental Studies Hybridoma Bank). Antibody solution was applied to each section and incubated overnight in a humidified chamber at either 4 or 25°C. After incubation with primary antibodies, sections were washed twice in PBS for 5 min and incubated for 1–1.5 h with FITC-conjugated antibodies against mouse IgGs or IgM (in the case of HNK-1). Sections were washed in PBS and coverslipped with gelmount (Biomeda). No significant fluorescent signal was detectable with the secondary antibodies alone.

Microscopy

Slides with whole-mounted or sectioned embryos were viewed with an Olympus Vanox or a Zeiss Axiophot epifluorescence microscope. A rhodamine filter set was used to visualize the DiI-labeled cells, while a fluorescein filter set was used to visualize 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 Bernoulli disks. Image processing was performed using Vidim software (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.). All images were postprocessed using Adobe Photoshop.

RESULTS

It has been assumed that the emigration of neural crest cells from the neural tube is an irreversible sign of commitment to a neural crest fate. In the present study, we tested this idea by grafting early migrating neural crest cells into the ventral portion of the neural tube and examining their subsequent differentiation.

Isolation of Early Migrating Neural Crest Cells

When explanted in tissue culture, neural crest cells emigrate from the neural tube and form a monolayer of migratory cells. This makes it possible to isolate neural crest cells in the absence of other cell types. Early migrating neural crest cells were obtained by culturing quail neural tubes on fibronectin substrates for approximately 16 h, during which time neural crest cells migrated away from the neural tube explants. Only the leading edge of migrating cells was used for injections (Fig. 1), whereas those cells remaining close to the neural tube as well as neural tube cells themselves were scraped away. For the purpose of our study, we define ''early migrating neural crest cells'' as those that are in this leading edge, in a monolayer at least 100 μm from the neural tube explant (Figs. 1B and 1C). When left in monolayer culture, neural crest cells fail to express floor plate markers at any time examined (Figs. 1D and 1F), but do express the HNK-1 epitope (Figs. 1E and 1F). In contrast, FP-1 is expressed when the ventral neural tube is included in the explant (Fig. 1G). Similarly dissociated neural tube cells do not express FP-1 (data not shown).

Neural crest cells were directly labeled with the lipophilic dye DiI on the culture dish (Fig. 1C), dissociated, and microinjected into the ventral portion of the open neural plate or recently closed neural tube of stage 9–11 (Hamburger and Hamilton, 1951) host chicken embryos (Fig. 2). As a control, some neural crest cells were injected into the dorsal neural tube. After allowing 2 days for cells to incorporate and differentiate, embryos were fixed and sectioned to identify the location, morphology, and phenotype of the injected cells. By these stages, many neural crest cells had completed their migration and neural tube cells had undergone overt cytodifferentiation. The neural tube forms a wide variety of cell types easily recognized by their characteristic positions and morphology. Motor neurons are distinguished by round cell bodies, axons emanating from the neural tube, and islet-1 immunoreactivity; floor plate cells are identified by their wedge-shaped morphology and FP-1 immunoreactivity.

Some Neural Crest Cells Become Incorporated into the Neural Tube after Injection

Neural crest cells injected into the chick neural tube survived and became incorporated into the neuroepithelium after injection into the ventral neural tube. Typically, between 1 and 10 labeled cells were introduced into each injection site. Because our injections were targeted to the ventral midline, labeled cells were observed at the site of floor plate differentiation in the majority of embryos. Occasionally, labeled cells were observed more laterally, within or adjacent to motor neuron pools. In addition to the neural tube, labeled cells were observed below the neural tube and around the notochord in some embryos. This is not unexpected given the difficulties of injecting cells into a single-layered epithelium. For control injections targeted to the dorsal midline, labeled cells were typically observed in the dorsal or intermediate regions of the neural tube.

To investigate whether other mesenchymal cells could incorporate into the neuroepithelium, we injected DiI-labeled skin fibroblast cells into the ventral neural tube in the same manner as neural crest cells. In 10 embryo, no incorporation of labeled fibroblasts was observed (data not shown). Because fibroblasts are clearly visible within the neuroepithelium immediately after injection, it is likely that they fail to incorporate and subsequently die.

Some Injected Neural Crest Cells Express Floor Plate and Motor Neuron Markers within the Neural Tube

In the majority of injections, DiI-labeled neural crest cells were inserted in or near the embryonic midline. Many of the injected cells assumed an elongated morphology within the floor plate regions, characteristic of the wedge-shaped floor plate cells. To ascertain whether some of the injected neural crest cells assumed appropriate molecular markers of the ventral midline, sections through injected embryos were stained with FP-1 antibody, a marker that unequivocally recognizes floor plate cells (Yamada *et al.*, 1991). Within 43 separate injection sites, we identified one or more DiI-labeled/FP-1-immunoreactive cells (Fig. 3). In two other cases, injected cells bearing the quail nuclear marker QCPN were observed within the floor plate region. These results show that migrating neural crest cells can acquire molecular characteristics unique to floor plate cells after injection into the neural tube. Although it is difficult to know the exact number of cells incorporated per graft as well as the rates of cell division and/or death, all labeled cells that were observed in a floor plate location appeared to express the floor plate marker.

To determine whether some of the injected neural crest cells expressed molecular markers and positions characteristic of motor neurons, sections were stained with anti-islet-1 antibody (Yamada *et al.*, 1991). In two embryos, we found DiI-labeled cells that were islet-1 immunoreactive within the motor neuron pool (Fig. 4). In addition, the cells extended axonal processes. This is consistent with the possibility that neural crest cells have converted to a motor neuron fate. In one other embryo fixed at a slightly earlier developmental time, we found a DiI-labeled cell adjacent to islet-1-positive cells, though the cell itself was not islet-1 reactive. Because most of the injections were targeted to the ventral midline, we noted DiI-labeled cells in the motor pools only in a small number of embryos.

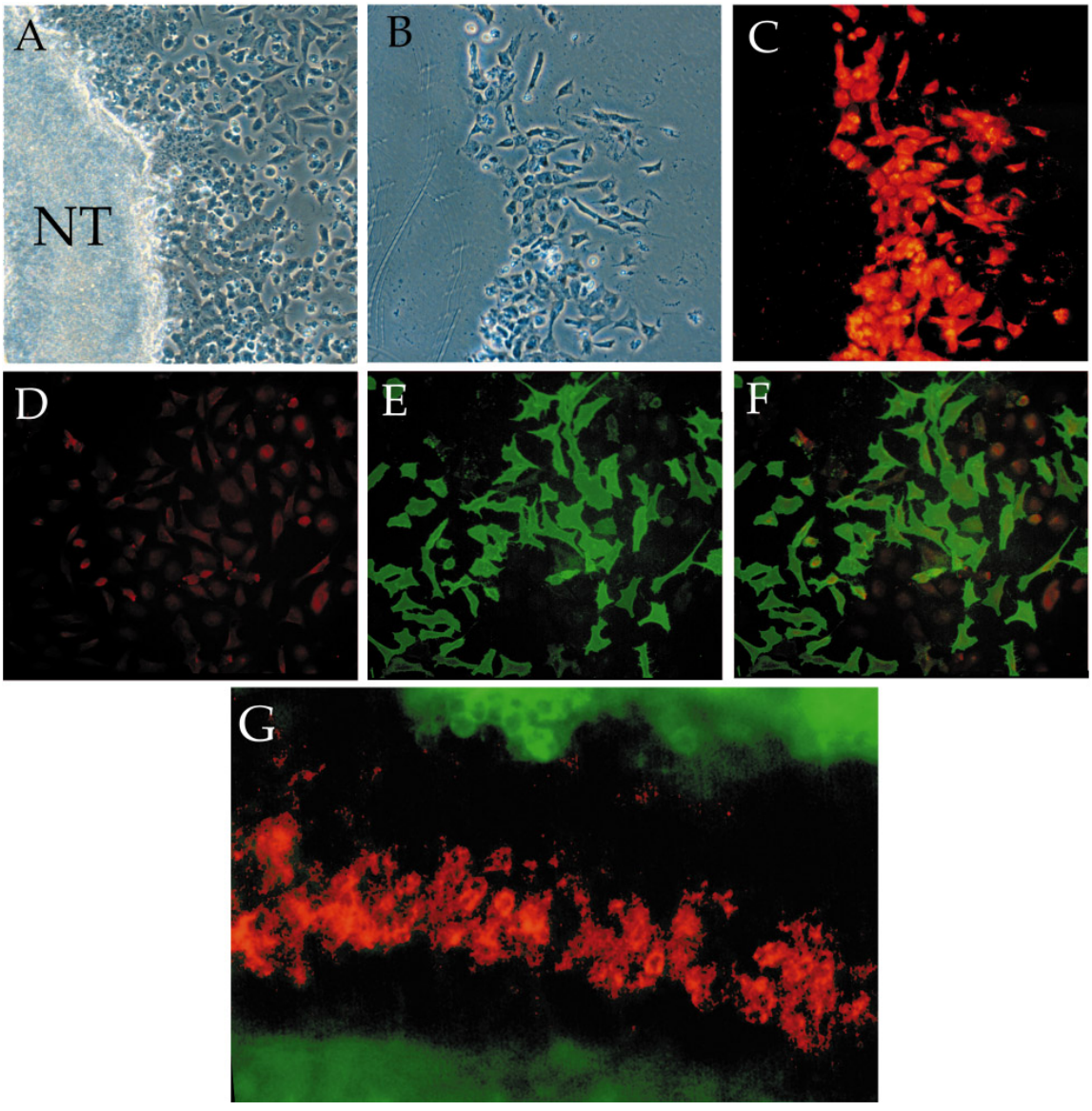

FIG. 1 Migrating neural crest cells in culture. (A) A culture 18 h after plating a neural tube (NT) onto a fibronectin-coated dish. Many neural crest cells have migrated from the neural tube. (B) A culture shortly after the neural tube has been scraped away, leaving a pure population of migrating neural crest cells that are labeled with the lipophilic dye, DiI. (C) The same field as in (B) visualized by epifluorescence through the rhodamine channel illustrates the efficacy of DiI labeling of the neural crest cells. (D) A neural crest culture stained with FP-1 antibody had no FP-1—postive cells. (E) The same field as in (D) stained with the HNK-1 antibody. Numerous HNK-1 immunoreactive cells were observed in the culture in the culture. (F) combine FP-1 (red) and HNK-1 (green) staining. (G) A culture in which the neural tube was left attached to the monolayer of migrating neural crest cells. The culture was subsequently stained with the FP-1 (red) and HNK-1 (green) antibodies. FP-1—postive cells were evident in the ventral midline region, whereas HNK-1—postive neural crest cells emerged from the dorsal aspect of the cultured neural tube.

Neural Crest Cells Injected onto Neural Crest Migratory Pathways Maintain Neural Crest Markers

As a control, neural crest cells were injected either into the dorsal neural tube (Figs. 5A and 5B) or onto the neural crest migratory pathway through the somites (Figs. 5C and 5D) to test whether they maintain neural crest markers after injection. Numerous HNK-1-immunoreactive/DiI-labeled neural crest cells were observed along neural crest migratory pathways (n = 12 embryos). In addition, DiI-labeled neural crest cells that were inadvertently injected

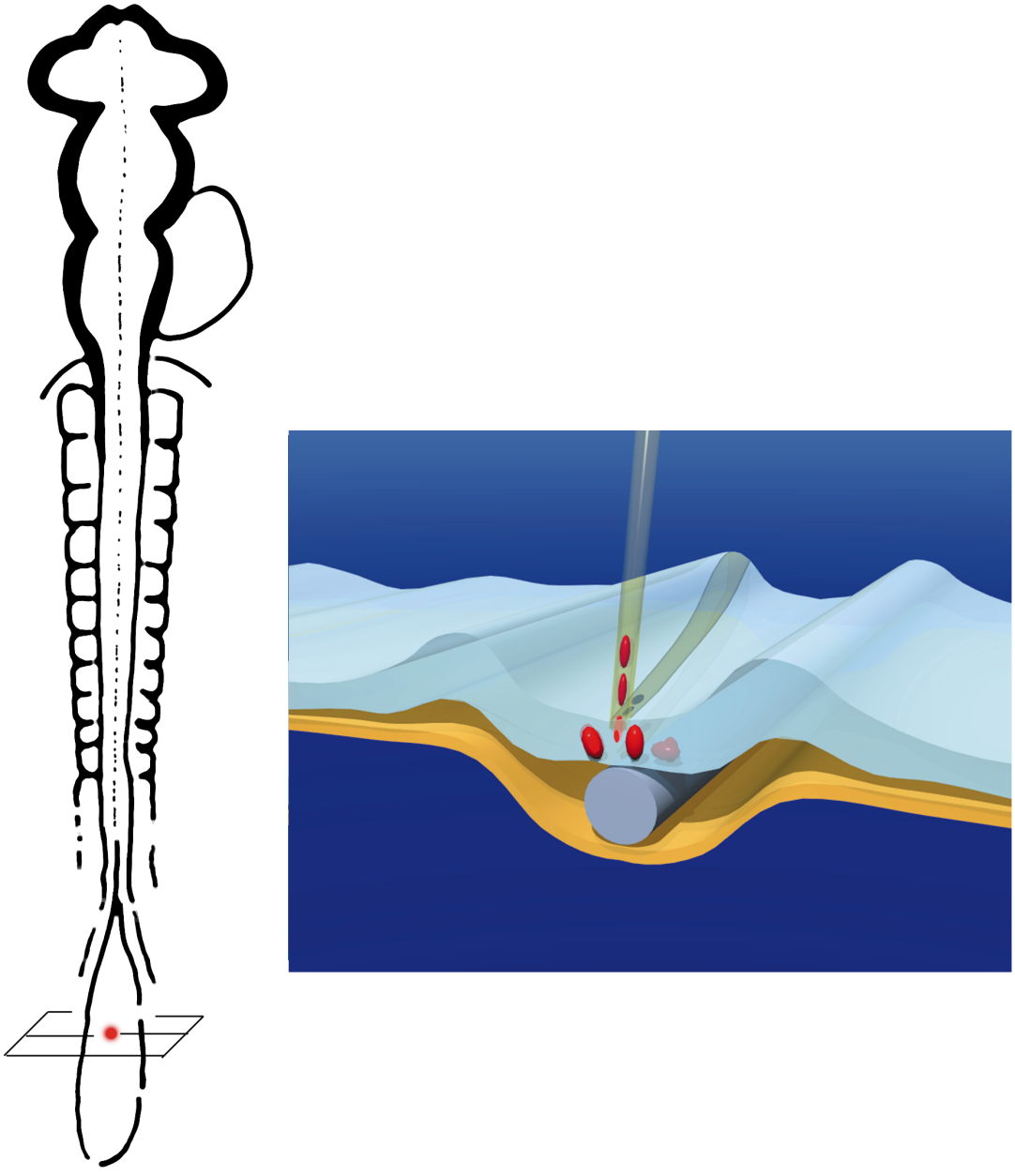

FIG. 2. Schematic diagram illustrating the procedure of injecting DiI-labeled neural crest cells into the ventral neural tube. In a stage 10 (10 somite) embryo (left), injections were made into the open neural plate at the level indicated in red. Some injections were performed into the newly closed neural tube (not shown). A three-dimensional rendering (right) illustrates the location of injection of the DiI-labeled cells (red) into the midline region immediately above the notochord.

into deeper regions of the embryo remained HNK-1 positive (data not shown). In contrast, neural crest cells transplanted into the dorsal neural tube often assumed the morphology of neuroepithelial cells, but generally did not express the HNK-1 epitope. Similarly, DiI-labeled neural crest cells within the ventral neural tube failed to express the HNK-1 epitope (data not shown).

Neural Crest Cells Injected around the Notochord or Grown in the Presence of Shh Fail to Express Floor Plate Markers

Since the notochord is the source of the ventralizing signal Sonic hedgehog (Marti *et al.*, 1995a,b), it is possible that injecting neural crest cells in its vicinity would be

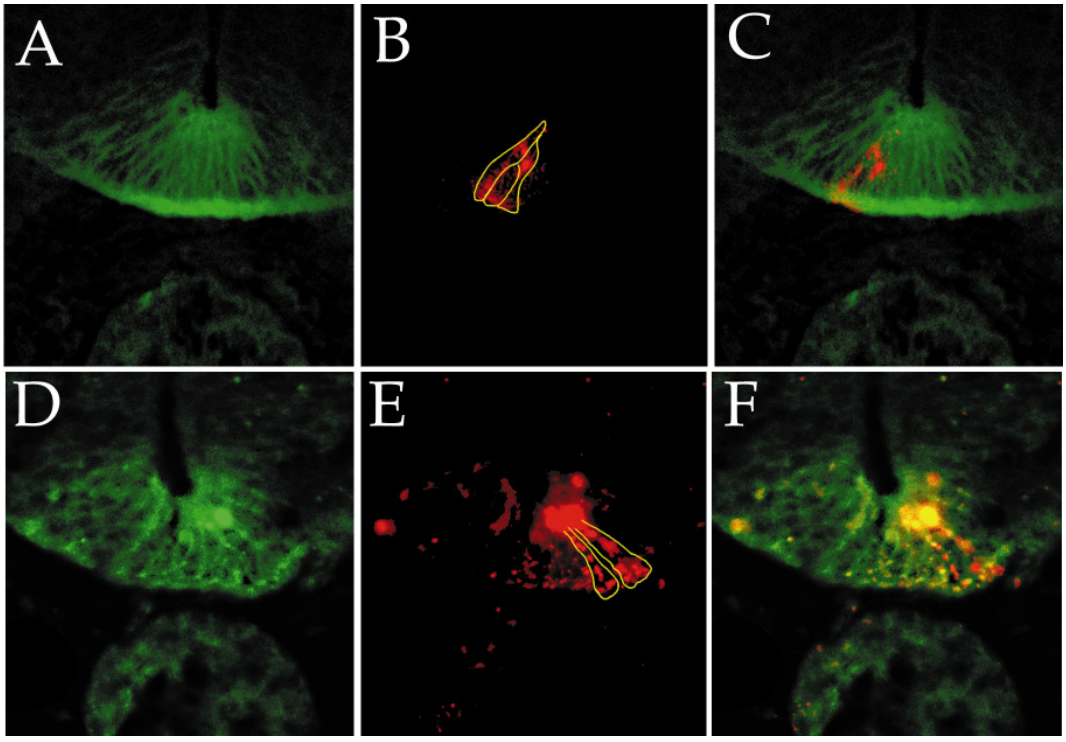

FIG. 3. Neural crest-derived cells become incorporated into the floor plate and express FP-1.(A–C) A section through an embryo in which FP-1 immunoreactivity (A) is observed in wedge-shaped cells within the floor plate; two DiI-labeled cells (B) assume a wedge-shaped morphology and are FP-1 immunoreactive (C) by 2 days after injection. The yellow outline illustrates the shape of the incorporated cells. (D–F) Another embryo with the FP-1—positive floor plate (D) containing several DiI-labeled cells (E) as clearly seen in the double-exposure (F).

sufficient to elicit expression of ventral markers. In eight embryos, labeled neural crest cells were found around the notochord, but not within the neural tube. These cells failed to express FP-1, suggesting that the notochordal environment is not sufficient to elicit neural crest cell differentiation into a floor plate fate. In parallel experiments *in vitro,* we added purified Sonic hedgehog protein (10 μg/ml; kindly provided by Dr. Andy McMahon) to cultured neural crest cells grown in defined medium. These concentrations were based on those reported to elicit floor plate and motor neuron differentiation (Marti *et al.,* 1995b). We were unable to detect FP-1 immunoreactive cells in these cultures (data not shown). These results suggest that a Shh signal in not sufficient to elicit floor plate differentiation from neural crest cells; rather, their incorporation into the neuroepithelium may render them competent to respond to a Shh signal.

DISCUSSION

It has classically been assumed that presumptive neural crest cells, which lie in the neural folds between the neural plate and ectoderm, are segregated from the remaining neural tube from early stages of development. However, single cell lineage analysis of neuroepithelial cells in the dorsal neural tube reveals that a common precursor can give rise to both neural crest- and neural tube-derived cells (Bronner-Fraser and Fraser, 1988, 1989). Furthermore, a single cell in the neural folds prior to neural tube closure can form ectodermal derivatives in addition to neural crest and neural tube derivatives (Selleck *et al.,* 1995). Following tube closure, single-cell lineage analyses have shown that neural crest cells, dorsal neural tube cells (Bronner-Fraser and Fraser, 1988, 1989), and ventral neural tube cells (Artinger *et al.,* 1995) can share common ancestors. Thus, cells in the prospective neural folds and later the dorsal neural tube are not fated solely to give rise to neural crest cells. Rather, these cells have a broad developmental potential with the ability to form multiple peripheral nervous system, central nervous system, and other derivatives.

When do restrictions in developmental potential occur? Neural crest cells undergo an epithelial to mesenchymal transition and migrate out of the neural tube shortly after its closure. One possibility is that neural crest cells lose the ability to form neural tube derivatives as they leave the neural tube. Indeed, single-cell lineage analysis of migrating neural crest cells reveals that they

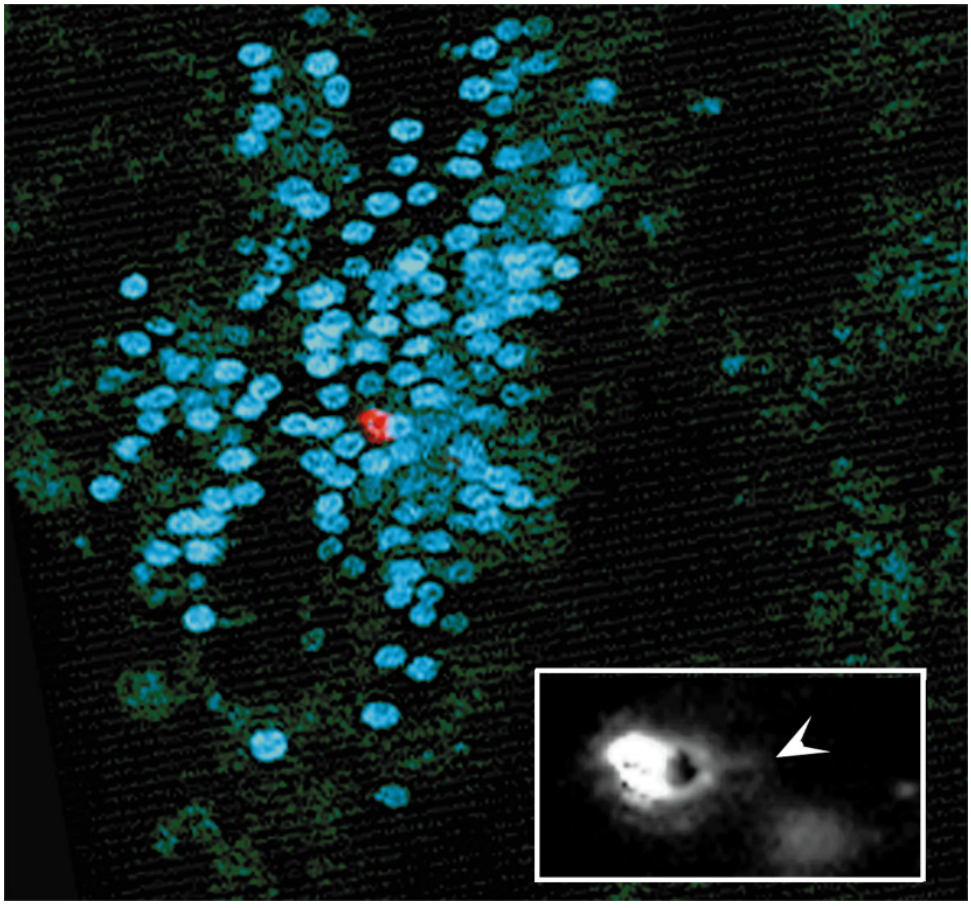

FIG. 4. Some migrating neural crests cells incorporate into the motor neuron pools and express islet-1. A section through an embryo illustrating islet-1 immunoreactivity (blue) in the motor neuron pools into which a DiI-labeled neural crest cell (red) became incorporated. The cell body, but not the nucleus, is DiI-positive. In the inset, the DiI-labeled cell has a small axon (arrowhead).

are fated to form multiple neural crest derivatives but no neural tube derivatives (Fraser and Bronner-Fraser, 1991). However, given that premigratory neural crest cells share a lineage with both neural tube and ectodermal cells, emigration from the neural tube may not necessarily represent a cell lineage restriction. In the present study, we have tested this idea by challenging the fate of early migrating neural crest cells by injecting them into the ventral neural tube. Neural crest-derived cells express ventral neural tube markers after injection, suggesting that they were not irreversibly committed to a neural crest cell fate prior to injection. Thus, these cells retain a more extensive developmental potential than reflected by their normal fate even after departure from the neural tube. Late migrating cranial neural crest cells have been shown to have a broader developmental repertoire than they normally express (Baker *et al.*, 1997). Heterochronic grafting of late migrating neural crest cells in place of the early migrating population revealed that both had equivalent developmental potential, although the later migrating population normally expressed a more restricted range of cell fates (*ibid.*).

Although our data reflect a surprising plasticity in the early migrating neural crest population, there are definite examples of changes in the competence of neural tissue to form particular derivatives as a function of time. For example, juxtaposition of neural plate and ectoderm results in the production of neural crest cells (Selleck *et al.*, 1995). Similar neural plate/ectoderm interactions result in the upregulation of dorsal neural tube markers such as Wnt-1 and Wnt-3a (Dickinson *et al.*, 1995). However, the competence of the neural plate changes as a function of time such that in combination with nonneural ectoderm, early neural plate (stage 4) can generate neural crest cells but not express Wnt-1/3a, whereas later neural plate can generate and express both neural crest cells and Wnt-1/3a (Dickinson *et al.*, 1995). At still later stages, similar interactions mediated by BMPs and activin-like molecules lead to the differentiation of dorsal sensory neurons in the neural tube, but no longer yield neural crest cells (Liem *et al.*, 1997).

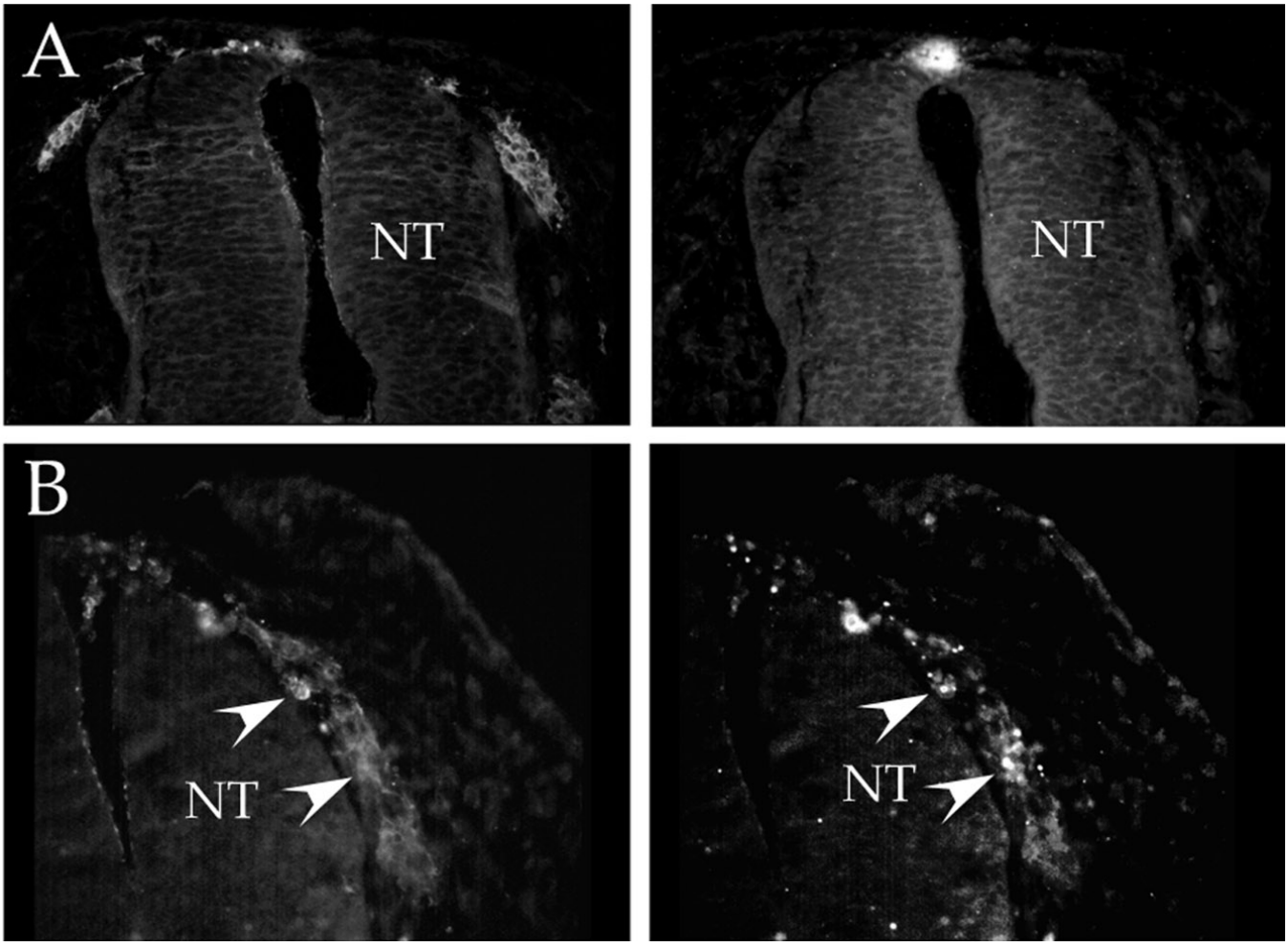

FIG. 5. DiI-labeled neural crest cells placed into the dorsal neural tube or onto neural crest migratory pathways express the HNK-1 epitope. (A, B) Sections stained with HNK-1 antibody (A) through an embryo in which DiI-labeled neural crest cells (B) were injected into the dorsal neural tube (NT). The injected cells were HNK-1 immunoreactive (green). (C, D) Neural crest cells injected onto the neural crest ventral pathway remained HNK-1 immunoreactive (C) and were DiI labeled (D).

Sonic hedgehog is well-known to be the signaling molecule within the notochord responsible for induction of floor plate and motor neuron cell fate (Marti *et al.*, 1995). Since neural crest cells normally arise from the portion of the neural tube most distant from the notochord, it is possible that the absence of Shh maintains their identity as neural crest cells. This scenario seems unlikely given that addition of Shh to neural crest cells *in vitro* is not sufficient to elicit FP-1 expression. Furthermore, we failed to observe FP-1 immunoreactivity in injected neural crest cells that were not incorporated directly into the neuroepithelium. Therefore, it seems likely that acquisition of ventral properties in neural crest-derived cells is a two-step process. The first step, incorporation into the ventral neural tube, may bestow the competence to respond to Shh. The second step may be the response of incorporated cells to ventralizing signals. Thus, neighbor relationships within the neural tube may be important for conferring the ability to respond to Shh. Neural crest cells injected into the dorsal neural tube also assumed a neuroepithelial morphology, supporting the notion that incorporation into the neural tube may be a separable event from acquisition of a particular cell fate.

Although our data suggest that there remains some interchangeability between different neural tube-derived cell types, this does not preclude the possibility that neural tube cells are predisposed to some extent. For example, mounting evidence suggests that ventral midline cells may be determined at early stages. For example, stage 9–10 floor plate cells can develop in isolated neural plates, in the absence of the notochord (Artinger and Bronner-Fraser, 1993). Similarly, ventralization of the dorsal neural tube by a notochord graft cannot prevent formation of neural crest

cells (Artinger and Bronner-Fraser, 1992) after the time of neural tube closure. Thus, there may be early and time-dependent biases in the developing neural plate/tube. However, these do not necessarily represent lineage restrictions.

After emigration from the neural tube, neural crest cells migrate along specific pathways and differentiate into peripheral ganglia as well as nonneuronal derivatives. Various factors encountered during migration may influence their fate decisions. For example, BMP-2 induces neurogenesis and smooth muscle cell differentiation from neural crest stem cells (Shah *et al.*, 1996). BMP-2, -4, and -7 elicit differentiation into adrenergic neurons, perhaps in combination with factors from other embryonic tissues (Varley *et al.*, 1995; Reissman *et al.*, 1996). Interestingly, the spinal cord contains a stem cell population with the ability to produce neural crest-like cells even at stages past the time during which neural crest migration conventionally has been thought to occur (Sharma *et al.*, 1995). These cells retain the ability to form all neural crest derivatives well past the stage of normal neural crest formation (Korade and Frank, 1996).

Our results demonstrate that individual neural crest cells retain a larger developmental potential than they express after leaving the neural tube. When placed in the neural tube milieu, they are able to respond to cues similarly to the neural tube cells from which they are derived. Because our embryos have been analyzed shortly after differentiation of floor plate cells and motor neurons, we cannot preclude the possibility that these do not fully differentiate and/or are eliminated at later times. However, our results suggest that neural crest cells retain the ability to respond to ventralizing signals and to upregulate molecular markers appropriate for ventral midline cells and motor neurons, even after emigration from the neural tube.

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