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Developmental potential of trunk neural crest cells in the mouse

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SUMMARY

The availability of naturally occurring and engineered mutations in mice which affect the neural crest makes the mouse embryo an important experimental system for studying neural crest cell differentiation. Here, we determine the normal developmental potential of neural crest cells by performing in situ cell lineage analysis in the mouse by microinjecting lysinated rhodamine dextran (LRD) into individual dorsal neural tube cells in the trunk. Labeled progeny derived from single cells were found in the neural tube, dorsal root ganglia, sympathoadrenal derivatives, presumptive Schwann cells and/or pigment cells. Most embryos contained labeled cells both in the neural tube and at least one neural crest derivative, and numerous clones contributed to multiple neural crest derivatives. The time of injection influenced the derivatives populated by the labeled cells. Injections at early stages of migration yielded labeled progeny in both dorsal and ventral neural crest derivatives, whereas those performed at later stages had labeled cells only in more dorsal neural crest derivatives, such as dorsal root ganglion and presumptive pigment cells. The results suggest that in the mouse embryo: (1) there is a common precursor for neural crest and neural tube cells; (2) some neural crest cells are multipotent; and (3) the timing of emigration influences the range of possible neural crest derivatives.

Key words: developmental potential, cell fate, neuron, microinjection

INTRODUCTION

A basic question in developmental neurobiology is: how are a diverse array of descendants generated from an apparently homogeneous neurectoderm? One possibility is that precursor cells are predetermined to form only a selected cell type in a specific location. Alternatively, the descendants of individual precursors might be capable of forming a wide range of cell types in a variety of derivatives, perhaps being instructed in their choice of phenotypes by their final environment. One system in which to study the mechanisms underlying cell type specification in the nervous system is the vertebrate neural crest. This population arises within the dorsal portion of the neural tube during neurulation (Horstadius, 1950; Weston, 1970). From this point of origin, neural crest cells migrate extensively throughout the embryo to give rise to numerous neuronal and non-neuronal cell types (Horstadius, 1950; Weston, 1970; Le Douarin, 1982). In the trunk, these derivatives include pigment cells, neurons and glia of the dorsal root and sympathetic ganglia, adrenomedullary cells, aortic plexuses and Schwann cells (review, Le Douarin, 1982).

In order to understand cell lineage relationships in the developing neural crest, it is necessary to identify individual neural crest cells and their descendants either in situ or in culture. In clonal cultures of neural crest cells, some clones contain only neural cells, whereas others give rise to mixed derivatives (Sieber-Blum and Cohen, 1980; Sieber-Blum, 1991; Stemple and Anderson, 1992), as diverse as neurons and cartilage (Baroffio et al., 1988; 1991). In avian and amphibian embryos, it has been possible to perform in vivo cell lineage experiments by injecting single cells with vital dye or infecting the cells with a replication-incompetent retrovirus; these experiments have shown that at least some premigratory (Bronner-Fraser and Fraser, 1988, 1989; Frank and Sanes, 1991; Collazo et al., 1993) and migrating (Fraser and Bronner-Fraser, 1991) trunk neural crest precursors are multipotent, capable of giving rise to cells in multiple neural crest derivatives. Taken together, these experiments have suggested that many neural crest precursor cells are initially multipotent, becoming progressively restricted in developmental potential with time and environmental influences.

Many open questions remain regarding the factors involved in cell fate segregation of the neural crest. Answers to these questions require manipulation of various aspects of the cellular and extracellular factors influencing the neural crest. The mouse embryo offers a particularly useful model for studies of neural crest development because of the availability of mutant and transgenic embryos that affect this population. A variety of genetic mutations disrupt aspects of neural crest development (review, Morrison-Graham and Weston, 1989). For example, two naturally occurring mutations, *Patch* (Gruneberg and Truslove, 1960) and *Splotch* (Auerbach, 1954), have phenotypes consistent with defects in cranial or trunk neural crest development, respectively. Homozygous

Patch embryos have a cleft face, open neural tube and abnormal heart development (Gruneberg and Truslove, 1960). Homozygous *Splotch* embryos have no sensory ganglia or pigment cells (Auerbach, 1954). In addition to naturally occurring mutants, recent technical advances have made it possible to induce specific mutations that affect both cellular and extracellular molecules (review, Capecchi, 1989). By comparing the differentiation of the neural crest cells in mutant versus wild-type embryos, it will be possible to learn about those molecules involved in cell differentiation. This requires a baseline understanding of the normal developmental potential of individual mouse neural crest cells in situ, about which little is currently known. Unfortunately, the mouse embryo is notoriously difficult to manipulate experimentally, making analysis of individual cell lineage in situ difficult. Although tempting, inference about neural crest cell lineage by analogy to other species may be dangerous. Differences in the timing and pathways of migration are known to exist between species (Serbedzija et al., 1989,1990); these might either result from or be indicative of distinct mechanisms of cell determination.

Here, we successfully have adapted the microinjection techniques previously used to investigate neural crest cell lineage in avian and amphibian development to the mouse embryo. Although the technique of retroviral marking might offer an approach requiring less manipulation (Frank and Sanes, 1991), the axial dispersion of neural crest cells (Bronner-Fraser and Fraser, 1988) makes demonstrating the clonality of marked descendants difficult, although not impossible (cf. Walsh and Cepko, 1992). To circumvent these potential difficulties, individual dorsal neural tube cells were labeled by iontophoretic injection of lysinated rhodamine dextran (LRD) in whole mouse embryos, which were subsequently cultured for 1 to 2 days. The results show that, in the mouse, some neural crest cells are multipotent; furthermore, we find that their timing of emigration appears to restrict the range of available neural crest derivatives and that there is a common precursor for neural crest and neural tube cells.

MATERIALS AND METHODS

Animals

Embryos were obtained by mating CD-1 females with BDF-1 males (Charles Rivers) overnight. The presence of a vaginal plug the following morning was taken to indicate pregnancy and the date that the plug was observed was designated embryonic day 0 (E0). Embryos were removed surgically from anesthetized mothers between E8 and E10.5 (8 to 40 somites) as described previously (Serbedzija et al., 1990, 1991, 1992). Pregnant females were anesthetized with avertin, prepared by mixing 0.5 g of 2,2,2-tribromoethanol (Chemical Dynamics Corporation), 0.31 ml of 2-methyl-2-butanol (Aldrich) and 39.5 ml of distilled water. The dosage was determined by the formula: dosage in ml=(0.1+0.02×weight of the mother in grams). After deep anesthesia was achieved, the abdomen was swabbed with 70% ethanol and the uterus was surgically exposed. An incision was made in the wall of the uterus on the side opposite the uterine arteries. The embryos were removed and placed in dissecting medium consisting of 20% fetal bovine serum (Hyclone), 79% Dulbecco's modified Eagle's medium (DMEM, Whittaker's Bioproducts) and 1% penicillin-streptomyocin L-glutamine (GPS, Whittaker's Bioproducts) at 37°C. Embryos were dissected to detach partially their extraembryonic membranes, but both the embryo and the extraembryonic membranes were left attached to the placenta for the entire culture period.

Intracellular injection

Intracellular injections were performed as previously described (Bronner-Fraser and Fraser, 1988). All injections were made with a 100 mg/ml solution of lysinated rhodamine dextran (LRD; Molecular Probes). The LRD solution was placed in the tips of thin-walled aluminosilicate micropipettes and the micropipettes then were back-filled with 1.2 M LiCl. The micropipettes were mounted on a micromanipulator (Leitz). Single neural tube cells were impaled by the microelectrode and the dye was expelled by iontophoresis using a Getting Microelectrode Amplifier. The membrane potential was monitored before, during and after the impalement to determine the health of the cell and to ensure that the micropipette had not drifted into another cell. To stabilize the embryos during the injection, they were placed in a small depression cut in a 2% agar dish (Bacto-Agar). To minimize the chance of labelling more than one neuroepithelial cell, the embryo was approached with the dye-filled micropipette perpendicular to its long axis. During the injections, embryos were maintained at approximately 30°C.

Embryo culture

Embryos were cultured in medium consisting of 50% rat serum, 49% DMEM, and 1% penicillin-streptomycin L-glutamine mixture (Whittaker Bioproducts) as decribed previously (Serbedzija et al., 1990, 1991, 1992). Embryos, with their extraembryonic membranes and placentas attached, were placed in 15 ml culture tubes containing 4 ml of culture medium. The culture tubes then were placed in a 5% carbon dioxide atmosphere and rotated at 3 revs/minute at 37°C.

Cultured embryos were compared with embryos developed to similar stages in utero to ascertain if the culture period itself affected embryonic maturation. Based on the size of the limb buds and the number of somites, embryos cultured up to 36 hours appeared similar to embryos that developed in utero. In transverse sections, both sets of embryos had comparably sized neural tubes and dorsal aorta.

Rat serum collection and preparation

Adult rats were anesthetized by inhalation of halothane (Fluothane, Ayerst Laboratories Inc.) and decapitated using a guillotine. Blood was collected in serum separation tubes (Vacutainer brand SST tubes, Becton Dickinson), and spun for 30 minutes at 3400 revs/minute. The serum then was decanted from the tube and stored at −70°C.

Histology

Embryos were fixed in 4% paraformaldehyde at 4°C for 4 hours and prepared for cryostat sectioning by washing in 0.1 M phosphate buffer for 1 hour, followed by soaking in a 15% sucrose solution for 8 to 12 hours at 4°C. They were embedded in 15% sucrose and 7.5% gelatin (Oxiod), rapidly frozen in liquid nitrogen and serially sectioned on a cryostat at 25 μm (HM 500 M, Microm). Sections were coverslipped in gel/mount (Biomedia Corp.) and viewed on an epifluorescence microscope equipped with a light-intensifying camera (Hammamatsu-SIT) and an image processing system (Imaging Technologies Series 151), using the VidIm software package (G. Belford, S. E. Fraser and J. Stollberg, unpublished data).

RESULTS

Here, we have adapted the technique of single-cell microinjection of vital dye to analyze neural crest lineage in mouse embryos grown in culture. Single neural tube cells were labeled by iontophoretic injection of lysinated rhodamine dextran (LRD) on embryonic day 8 (E8) to E10.5. Injections were made into the trunk neural tube between the axial levels

Table 1. Distribution of labeled progeny arising from single neural tube cells injected with LRD in embryos labeled between E8 and E10

Class	Number of Embryos	Neural tube	Dorsolateral pathway	Dorsal root ganglia	Sympathetic ganglia	Aortic plexus/ adrenal medulla	Ventral motor root
1	9	X					
2	4	X	X	X			
3	3	X		X	X		
4	4	X			X		
5	5	X	X				
6	1		X	X			
7	4		X				
8	1		X	X	X		
9	2			X	X		
10	1				X		
11	4			X			
12	2				X	X	
13	4	X		X			
14	1	X		X			X

of somites 6 and 18. Embryos in which single cell injection was verified (see below) were placed immediately into culture medium and allowed to develop for 1 to 2 days prior to fixation, sectioning and analysis. Table 1 summarizes the number of clones analyzed and the distribution of the LRD-labeled cells.

Verification of single-cell injection

After injection, living embryos were examined using fluorescence microscopy to visualize the LRD and verify that only one cell had been labeled. In most cases, the labeled cells looked like columnar epithelial cells, spanning the thickness of one wall of the neural tube. Embryos with more than one labeled cell were discarded. To verify that only one cell per embryo was labeled, 10 embryos were fixed, sectioned and analyzed immediately after injection. Of these, 8 contained a single labeled neural tube cell and 2 contained a single labeled ectodermal cell overlying the dorsal neural tube. In transverse section, the injected neural tube cells appeared columnar, extending the width of the neural tube (often the labeled cells spanned two adjacent sections; Fig. 1). Labeled cells in the ectoderm were easily distinguished in section, but could not be unequivocally distinguished from neural tube cells in the living whole mount. At the time of injection, these labeled cells could be taken as either epidermal cells or early migrating neural crest cells. This potential ambiguity is not a problem, because the epidermis and neural crest form different and easily distinguishable derivatives. Cells that gave rise to a patch of labeled epidermal cells were not included in the present analysis.

Distribution of labeled progeny from LRD injections into the dorsal neural tube

The progeny of individual LRD-labeled neural tube cells were identified in 45 embryos that were fixed and analyzed 24 to 48 hours after injection. In 20% (9/45) of the embryos, LRD-labeled cells were present only in the neural tube. 47% (21/45) of the embryos contained LRD-labeled cells both in the neural tube and in neural crest derivatives. The remaining 33% (15/45) of the embryos had labeled cells only in neural crest derivatives. The data on the distribution of LRD-labeled progeny are summarized in Table 1. In 11% (5/45) of the embryos, labeled cells were observed on both sides of the embryo, in neural crest derivatives as well as in the neural tube. Fig. 2A shows a section through an embryo fixed 24 hours after injection, which contains labeled neural crest cells on both the left and right sides of the neural tube; Fig. 2B depicts two bilaterally distributed neurons in the neural tube of an embryo fixed 36 hours after injection.

LRD-labeled cells in the neural crest derivatives

36 embryos contained labeled cells in one or more neural crest derivatives. Many LRD-labeled clones were composed of cells

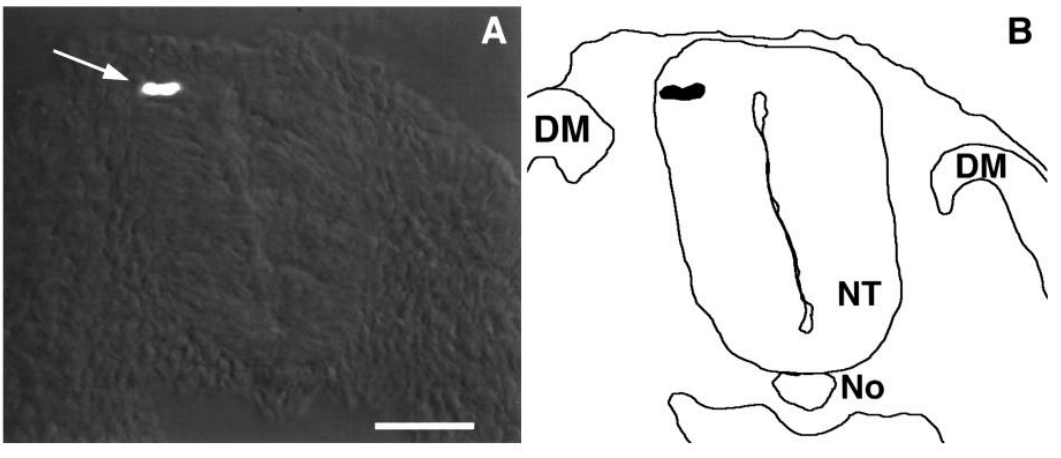

Fig. 1. Microinjection reliably fills single neural tube cells. (A) Phase and fluorescence image of a transverse section through an embryo injected at E8.5 and fixed immediately. A single LRD columnar cell (arrow) is present in the dorsal portion of the neural tube, which gives rise to the neural crest. (B) A line drawing of A. The neural tube (NT), notochord (No) and dermomyotome (DM) are labeled for orientation. Scale bar, 50 μm.

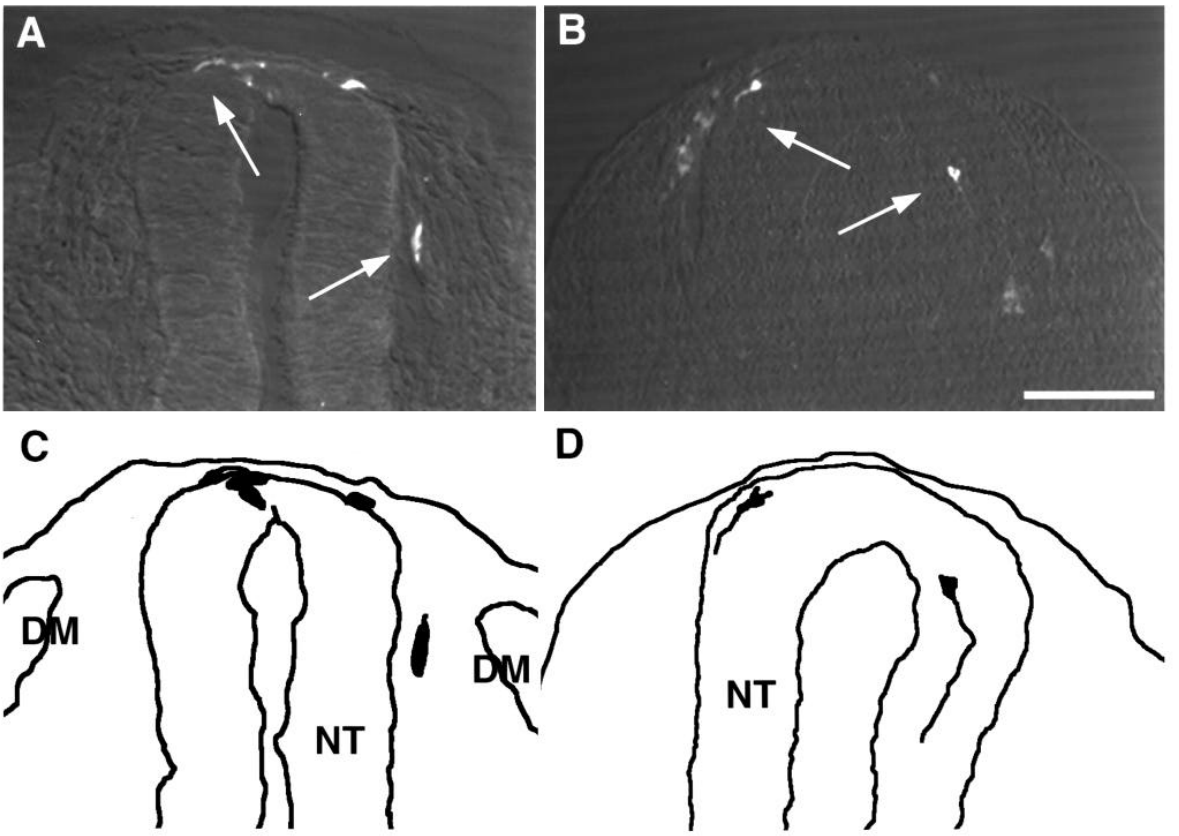

Fig. 2. Bilateral distribution of clones contributing to the neural crest and the neural tube. (A) Phase and fluorescence image of a transverse section through an embryo injected with LRD at E8 and allowed to develop for an additional 24 hours. LRD-labeled cells are found on both sides of the neural tube (arrows). Other LRD-labeled cells outside the neural tube correspond to migratory neural crest cells along the ventromedial pathway, which will contribute to the dorsal root ganglia. (B) A similar image from another embryo injected at E10 and allowed to develop for an additional 36 hours; two bilaterally distributed neurons (arrows) in the neural tube are labeled with LRD. This clone contains another neuron that is three sections (approximately 60 μm) rostral to the section shown here. (C-D) Line drawings of A and B. For orientation, the neural tube (NT) and dermomyotome (DM) are labeled. Scale bar, 100 μm.

in multiple and varied neural crest locations (39%; Table 1), including cells of the dorsal root and sympathetic ganglia, presumptive pigment cells along the dorsolateral pathway (between the dermomyotome and the ectoderm) and presumptive Schwann cells (aligned along the ventral roots). Because the clones contributed to more than one neural crest derivative, the precursors were at least bipotent. Furthermore, many of these clone contributed to both the neural crest and the neural tube.

21 of the neural-crest-containing clones (58%) contributed to both neural crest derivatives and the neural tube, many of which (n=8) had LRD-labeled cells in the neural tube plus at least two neural crest derivatives. For example, four embryos contained labeled cells in the neural tube, dorsal root ganglia and along the dorsolateral pathway (Fig. 3), and three of the embryos contained labeled cells in the neural tube, dorsal root ganglia and sympathetic ganglia. Another embryo contained LRD-labeled cells in the neural tube, dorsal root ganglia and the ventral motor root. The remaining clones containing neural crest plus neural tube progeny (n=13) contributed to only a single neural crest derivative.

15 of the neural-crest-containing clones (42%) gave rise exclusively to neural crest derivatives. 9 of these clones contributed to only one neural crest derivative (Table 1; Fig. 4). The remaining 6 clones had LRD-labeled cells within two or more neural crest derivatives. 2 of these clones contained labeled cells in the dorsal root and sympathetic ganglia (Fig. 5), and another 2 clones contained cells in the sympathetic ganglion and in the region that will give rise to either presumptive adrenal medulla or aortic plexus (Fig. 6). Another clone contained labeled cells in the dorsal root ganglion and along the dorsolateral pathway taken by presumptive pigment cells (Fig. 7).

LRD-labeled cells confined to the neural tube

A variety of cell phenotypes were found in the 9 embryos that had labeled cells exclusively in the neural tube. 4 of the clones were composed of labeled cells of a neuronal morphology with round cell bodies and long axons that projected ventrally (Fig. 2B), typical of commissural neurons. In 2 other cases, the neural tube clone was composed of labeled cells with a neuroepithelial morphology in the presumptive alar region of the neural tube. The remaining 3 embryos contained rounded cells in the alar portion of the neural tube; if these cells possessed axons, they were too faintly labeled to be clearly discerned.

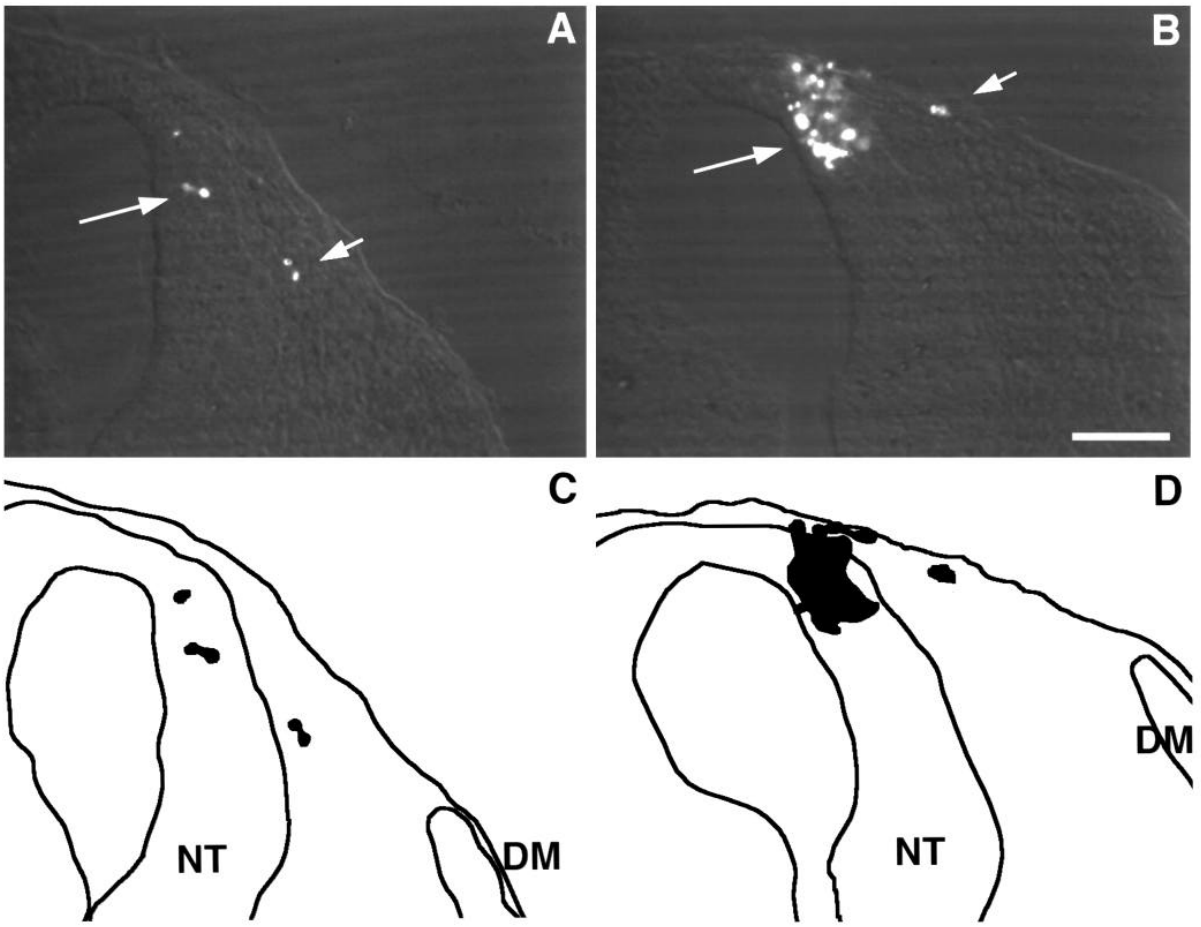

Fig. 3. Single clones contribute to multiple neural crest derivatives and the neural tube. (A-B) Combined phase and fluorescence images of two adjacent transverse sections from an embryo injected at E9 and allowed to develop for an additional 24 hours. (A) LRD-labeled cells in the dorsal root ganglia (short arrow) and in the neural tube (long arrow). (B) The adjacent section, just caudal to A, contains LRD-labeled cells in the neural tube (long arrow) and along the dorsolateral pathway (short arrow) adjacent to the ectoderm. Based on their position, the cells adjacent to the ectoderm are pigment cells. (C-D) Line drawings of A and B with the neural tube (NT) and dermomyotome (DM) labeled for orientation. Scale bar, 50 μm.

Table 2. Compilation of the distribution of labeled progeny arising from LRD injections at different stages of neural crest cell migration

Stage at injection	Number of embryos	Neural tube	Dorsolateral pathway	Dorsal root ganglia	Sympathetic ganglia	Aortic plexus/ adrenal medulla	Ventral motor root
E8-E9	27	X	X	X	X	X	
E9-E10	14	X	X	X			X
>E10	4	X					

LRD injections performed at different stages of neural crest cell migration

To examine the possibility that there is progressive restriction in the developmental potential of neural crest cells generated at later stages of development, we compared the distribution of the labeled progeny in embryos labeled at progressively older stages. Table 2 summarizes the number of clones analyzed and the overall distribution of the LRD-labeled cells at each stage. Table 3 presents the details of the cell phenotypes found together within single clones; the number in each cell of the table presents the number of clones observed with that combination of phenotypes.

Injections of LRD into neural tubes at the level of the forelimb between E8 and E9 produced clones within the neural tube and/or neural crest derivatives (Table 2,3). These early staged injections contributed labeled cells to almost every possible combination of neural crest derivatives, with no obvious pattern (Tables 2,3). Embryos injected between late E9 and E10 contained labeled cells in the more dorsally located derivatives, including the neural tube, dorsal root ganglia, ventral roots and along the dorsolateral pigment pathway (Table 2,3). However, no labeled cells were found in the sympathetic ganglia or around the dorsal aorta. Thus, injections performed at later times yield labeled cells in the dorsal, but

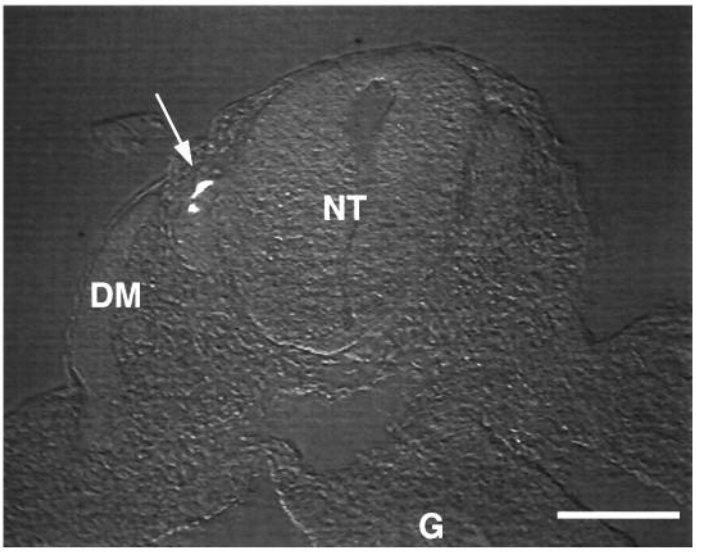

Fig. 4. Some clones contribute to single derivatives. Combined phase and fluorescence image of a transverse section through an embryo injected at E9 and allowed to develop for an additional 36 hours. The descendants of the LRD-injected neural tube cell are contained within a single neural crest derivative, the dorsal root ganglion (arrow). For orientation, the neural tube (NT), gut (G) and dermomyotome (DM) are labeled. Scale bar = 100 μm.

not ventral, derivatives. LRD-labeled progeny arising from single cell injections after E10 were found only in the neural tube (Table 2), suggesting that neural crest cell emigration had ended by this time.

Rostrocaudal extent of clonally related cells.

To determine the rostrocaudal extent of representative clones, the number of sections containing LRD-labeled cells was determined for 28 labeled embryos. Clones that contributed to neural crest derivatives extended from 20 to 150 μm (up to approximately one somite length). There was no obvious correlation between the number of derivatives containing clonally related cells and either the clones' rostrocaudal extent or its number of cells. For example, Figs 5 and 6 both show clones which span 100 to 125 μm and contain labeled cells in two neural crest derivatives. However, the clone in Fig. 6 has many more labeled cells than the clone in Fig. 5. In contrast, Fig. 3 illustrates a clone that contributes to both the dorsal root ganglia and the presumptive pigment cells, but contains only 4 labeled cells, spanning 40 μm.

Labeled cells confined to the neural tube extended from 20 to 80 μm along the rostrocaudal axis, being considerably less dispersed than those observed within neural crest derivatives. There was no obvious correlation between the number of labeled cells in the neural tube and their rostrocaudal extent. Fig. 3 is an example of a clone with a large number of labeled cells in the neural tube which spanned only 40 μm. In contrast, Fig. 2 illustrates a clone containing only three labeled neurons, spanning 80 μm (the third cell, not shown, is located three serial sections rostral to the section in Fig. 2B).

DISCUSSION

In this study, the developmental potential of individual trunk neural crest precursors was assessed by labelling individual cells in the dorsal portion of the mouse neural tube. Iontophoretic injection of lysinated rhodamine dextran (LRD) into single cells permitted the progeny of one precursor to be recognized as they contributed to various sites of neural crest cell localization, including the dorsolateral pigment pathway, the dorsal root ganglia, the sympathetic ganglia, the presumptive adrenal medulla and cells along the ventral roots (Table 1). 39% of the clones that gave rise to the neural crest contributed cells to multiple neural crest derivatives. The presence of labeled descendants in both the neural tube and at least one neural crest derivative in 47% of the cases suggests that the neural crest is not a presegregated popula-

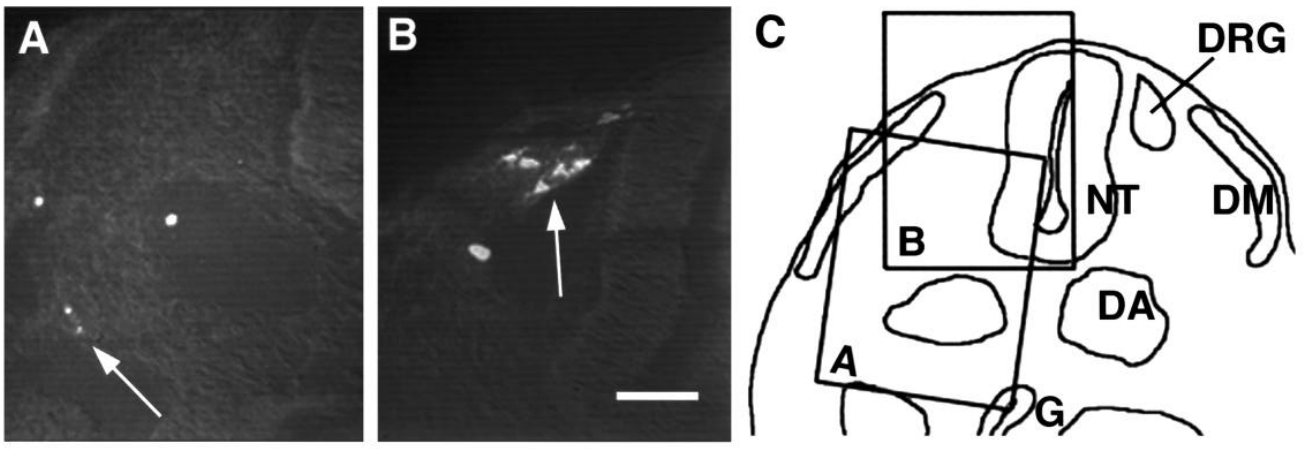

Fig. 5. Clone contributing to both sympathetic and dorsal root ganglia. (A) Combined phase and fluorescence image of an embryo injected at E8.5 and allowed to develop for an additional 24 hours. This section contains two LRD-labeled cells in the sympathetic ganglion (arrow). (B) Two sections, caudal to that shown in A, have LRD-labeled cells within the dorsal root ganglion (arrow). Other LRD-labeled cells (not shown) in the same embryo are found in the dorsal root ganglion two sections caudal to B. (C) Line drawing of a lower magnification view of A with the neural tube (NT), dorsal root ganglion (DRG), gut (G), dorsal aorta (DA) and dermomyotome (DM) labeled. Box A and box B correspond to images (A) and (B), respectively. Scale bar, 50 μm.

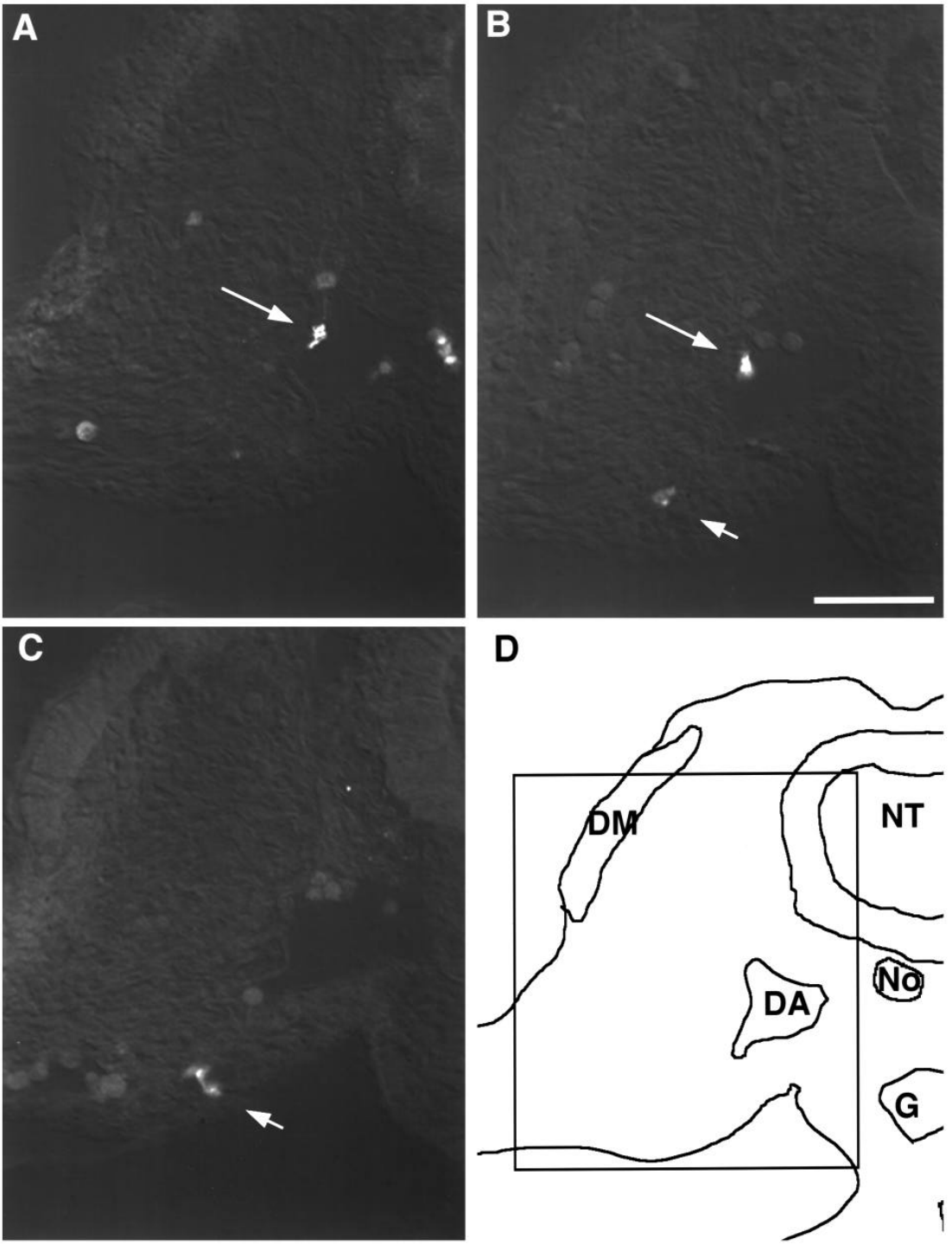

Fig. 6. Sympathoadrenal descendants in labeled clones. Phase and fluorescence images of three adjacent serial transverse sections from an embryo injected at E8 and allowed to develop for an additional 24 hours. (A) LRD-labeled cells (long arrow) in the sympathetic ganglion. (B) The adjacent section, immediately caudal to A, contains cells in both the sympathetic ganglion (long arrow) and in the region that will give rise to the adrenomedulla (short arrow). (C) The next section contains LRD-labeled cells only in the adrenomedullary region (short arrow). (D) Line drawing of a lower magnification view of A with the neural tube (NT), notochord (No), gut (G), dorsal aorta (DA) and dermomyotome (DM) labeled. The box outlines the area corresponding to the images in A-C. Scale bar, 50 μm.

tion in the neural tube. The time of injection influenced the derivatives populated by the labeled cells; those injections performed at early stages contributed to both dorsal and ventral neural crest derivatives, whereas those done later contributed only to dorsal derivatives. Taken together, the results demonstrate that: (1) many murine neural crest precursors within the neural tube are multipotent; (2) neural crest cells can share a common precursor with dorsal neural tube cells; and (3) the timing of injection influences the range of derivatives. In addition, neural tube and neural crest clones arising from a verified single cell injection are often bilaterally distributed.

In our previous work on mouse neural crest migration pathways, we demonstrated two temporally and spatially distinct pathways of migration (Serbedzija et al., 1990). An early wave of cells moves through the ventrolateral portion of the sclerotome to populate the more ventral sites (sympathetic ganglia, the adrenal medulla and the aortic plexuses); a later wave migrates through the ventromedial portion of the sclerotome to populate the dorsal root ganglia. This non-random

Table 3. Details of phenotypes found together within individual clones resulting from injections performed during early and late phases of neural crest migration

	Neural tube	Dorsolateral pathway	Dorsal root ganglia	Sympathetic ganglia	Aortic plexus/ adrenal medulla	Ventral motor root
Early stages of neural crest cell migration						
Neural tube	17	5	7	7		
Dorsolateral pathway	5	8	3	1		
Dorsal root ganglia	7	3	12	6		
Sympathetic ganglia	7	1	6	13	2	
Aortic plexus/adrenal medulla				2	2	
Ventral motor root						
Late stages of neural crest cell migration						
Neural tube	9	4	5			1
Dorsolateral pathway	4	7	3			
Dorsal root ganglia	5	3	8			1
Sympathetic ganglia						
Aortic plexus/adrenal medulla						
Ventral motor root	1		1		1	

order might either result from or cause differences in cell phenotype decisions. For example, there may be two populations of neural crest precursor cells with distinct developmental potentials: an early emigrating population, which is fated to give rise to the more ventral sympathoadrenal derivatives, and a later emigrating population, which gives rise to the dorsal root ganglia. In contrast, these distinct migratory waves might not reflect any intrinsic differences in the neural crest cells themselves, but instead result from extrinsic factors such as differences in the environment's ability to support cell migration. Our injections performed at different stages of embryonic development provide a test between these possibilities. Embryos in which neural crest cell precusors within the neural tube were injected during the early stages of neural crest cell emigration contained labeled cells in both the ventral (sympathoadrenal) and dorsal neural crest (dorsal root ganglia, Schwann cells and presumptive pigment cells) derivatives (Tables 2,3). Thus, our results refute the first scenario in the strict sense and suggest that there cannot be a complete segregation between precursors for 'dorsal' and 'ventral' derivatives. This shows the danger of inferring mechanisms of fate restriction from the migration pathways or the molecular cytology of neural crest cells; thus, scenarios in which features of the cell migration pathway such as a waiting period (Weston, 1991) are proposed to play a major role in cell fate decisions must be viewed as merely suggestive.

The pluripotency observed here cannot rule out the possible existence of some subpopulations fated to give rise to specific derivatives or stage-dependent changes in the potency of the neural crest cells. Partially or totally committed subpopulations of neural crest cell have been shown to exist at later stages in both rat and avian neural crest cells. Using cell sorting in com-

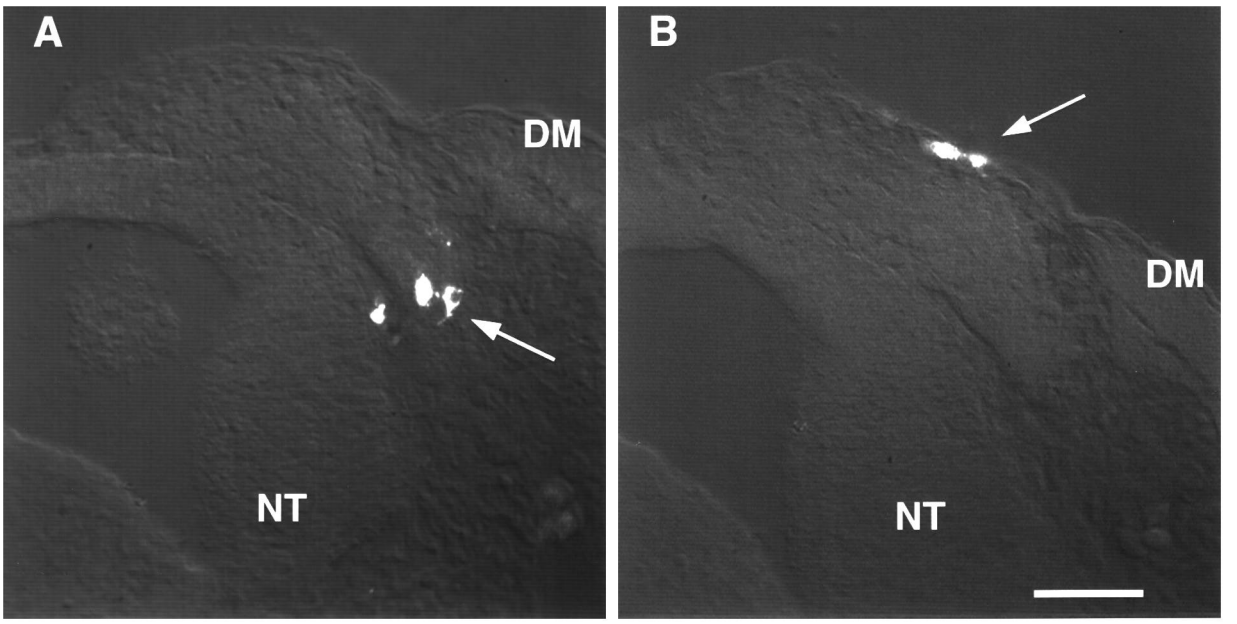

Fig. 7. Dorsal root ganglion cells and pigment cells in a clone without descendants in the neural tube. (A) Phase and fluorescence image of an embryo injected at E9 and allowed to develop for an additional 24 hours. This section contains LRD-labeled cells in the dorsal root ganglion (arrow). (B) Two sections caudal to A, two presumptive pigment cells (arrow) are present in the ectoderm overlying the dorsal root ganglion. For orientation, the neural tube (NT) and dermomyotome (D) are labeled. Scale bar, 50 μm).

bination with clonal analysis to isolate individual trunk neural crest cells, Stemple and Anderson (1992) have demonstrated the existence of a multipotent rat neural crest 'stem' cell with a limited ability to self-renew. Under certain environmental conditions, these multipotent stem cells produce 'blast' cells which give rise to only limited cell types, the nature of which appear to be dictated by the local environment. Examples of partially restricted neural crest-derived blast cells include the 'sympathoadrenal' sublineage, which are precursors to sympathetic neurons, small intensely fluorescent cells and adrenomedullary cells (Doupe et al., 1985; Anderson and Axel, 1986). This lineage appears to be segregated by the time neural crest-derived cells reach the region around the dorsal aorta, consistent with the idea that initially multipotent cells become more limited in developmental potential at late migratory stages or at their final destinations. Similarly, Artinger and Bronner-Fraser (1992) found that trunk neural crest cells that migrate away from young neural tubes in culture, differentiated into melanocytes, sensory and catecholamine-positive cells in culture; in contrast, neural crest cells derived from older neural tubes never became catecholamine positive, even after injecting them into ventral regions of a young host embryo.

The existence of a common neural crest/tube ancestor in the mouse is analogous to that observed in aves and amphibians (Bronner-Fraser and Fraser, 1988, 1989; Frank and Sanes, 1991; Collazo et al., 1993). These results suggest that divergence between the neural crest and dorsal neural tube lineages occurs only at later stages. In fact, under some conditions, even ventral neural tube cells can form neural crest cells if the endogenous neural crest is removed (Scherson et al., 1993). For example, in the cranial region of avian embryos, ventral neural tube cells normally destined to form central nervous system derivatives can adjust their prospective fates to form peripheral nervous system and other neural crest derivatives if challenged by ablating the dorsal neural tube before completion of neural crest cell emigration (Scherson et al., 1993).

Our results on lineage analysis of single neural crest cells in the mouse are in general agreement with those obtained in the chick (Bronner-Fraser and Fraser, 1988; 1989) and *Xenopus* embryos (Collazo et al., 1993) using dextran injection or infection with a recombinant retrovirus (Frank and Sanes, 1991). All three species have multipotent neural crest precursors, whose progeny cells are distributed bilaterally. The existence of such multipotent precursors is substantiated by clonal analysis in vitro in avian (Sieber-Blum and Cohen, 1980; Baroffio et al., 1991) and rat (Stemple and Anderson, 1992) embryos. As in any fate mapping experiment, our results can provide only a lower estimate of the developmental potential of the individual labeled cells. It is possible that the cells could have differentiated into a wider range of phenotypes had they been exposed to all possible environments. Therefore, our results cannot be taken as evidence for the presence of unipotent or restricted subpopulations of neural crest cells. They do, however, offer direct evidence for the multipotentiality of many mouse neural crest precursors.

The present study demonstrates the utility of performing cell marking experiments in mouse neural crest. By using non-invasive approaches, the results show that at least some murine neural crest cells are multipotent. The present single cell labelling results confirm our previous results (Serbedzija et al, 1990), showing that later emigrating neural crest cells gave rise to dorsal but not ventral derivatives (Table 2), similar to the findings in chick embryos (Weston and Butler, 1966; Serbedzija et al., 1989). Interestingly, the exact pathways of migration appear to differ. Unlike avian neural crest cells which follow a single pathway to populate both dorsal root ganglia and sympathoadrenal derivatives (Rickmann et al., 1985; Bronner-Fraser, 1986, Teillet et al., 1987), two distinct paths are seen in mouse. In addition, mouse neural crest cells do not appear to disperse rostrocaudally to as great an extent as neural crest cells in the chick (mouse: 1-somite length; chick: 3-somite lengths; Bronner-Fraser and Fraser, 1989). Without knowledge of the final fates of the clones, these apparent differences in pathways and dispersion might be taken to suggest that those factors regulating neural crest cell differentiation are dissimilar in these two species. The strengths of comparative analyses are that common properties, such as the multipotentiality of many premigratory neural crest cells, become apparent; differences, such as in the timing and pathways of migration emerge as possible species-specific environmental effects. Analysis of neural crest cell lineage in the mouse has the additional advantage of exploiting a system which is poised for genetic analysis. Future experiments, applying the techniques used here for wild-type embryos, to developmental mutants and transgenic animals with defects in neural crest cell migration and differentiation, should offer insights into the molecular basis of cell fate decisions in the neural crest.

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