DEVELOPMENTAL BIOLOGY **172,** 591–601 (1995)

Dorsal and Ventral Cell Types Can Arise

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To challenge the developmental potential of dorsal neural tube cells and test whether single neuroepithelial cells can give rise to the full range of neural tube derivatives, we grafted a notochord lateral to the closing neural folds. This results in juxtaposition of dorsal and ventral cell types, by inducing floor plate cells and motor neurons dorsally. Clonal analysis with the vital dye lysinated rhodamine dextran showed that both "dorsal" and "ventral" neural tube derivatives can arise from a single precursor. Cells as diverse as sensory ganglion cells, presumptive pigment cells, roof plate cells, motor neurons, and floor plate cells were observed in the same clone. The presence of such diversity within single clones indicates that the responses to dorsal and ventral signals are not mutually exclusive; even in the early neural tube, neuroepithelial cells are not restricted to form only dorsal or ventral neural tube derivatives.

INTRODUCTION

Just after closure, the neural tube appears to be a somewhat homogeneous neuroepithelium. Shortly thereafter, distinct cell types appear in the dorsal and ventral portions of the neural tube, reflecting the first overt manifestation of dorsoventral polarity. For example, 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, ventrolaterally, some cells differentiate into motor neurons.

A rapidly expanding literature demonstrates that the notochord can influence the dorsoventral polarity of the neural tube (van Straaten *et al.*, 1988, 1989; Yamada *et al.*, 1991), inducing floor plate cells by a contact-mediated signal (Placzek *et al.*, 1993) and motor neurons by a diffusible signal (Yamada *et al.*, 1993). A logical extension of such studies is the proposal that primary interactions specify the most dorsal and ventral fates of the neural tube, followed by secondary interactions within the neural tube that pattern and assign the various cell types. Molecular correlates of this proposal are found in *sonic hedgehog*, which is localized in the notochord and subsequently in the floor plate (Echelard *et al.*, 1993; Roelink *et al.*, 1994), and in *dorsalin-1*, a member of the TGF-β family that is localized in the dorsal neural tube and can induce some neural crest derivatives (Basler *et al.*, 1993).

Although current dogma suggests that both dorsalizing and ventralizing signals function after neural tube closure, increasing evidence suggests that some properties are established earlier. For example, a notochord implanted into the dorsal midline prior to neural tube closure does not suppress formation of either neural crest cells or commissural neurons (Artinger and Bronner-Fraser, 1992), despite its ability to induce an ectopic floor plate and motor neurons (van Straaten *et al.*, 1989). Furthermore, the birth of the first neurons during gastrulation (Sechrist and Bronner-Fraser, 1991) suggests that at least some cell types are specified early.

To test if there is such a stepwise mechanism of neural tube specification, the normal fates of neural tube cells must be challenged. The normal differentiation pattern can be altered by grafting a notochord adjacent to the dorsolateral aspect of the closing neural tube in stage 9 to 11 embryos (Hamburger and Hamilton, 1951). The implanted notochord ventralizes a region of the neural tube that is fated to give rise to neural crest cells, commissural neurons, and roof plate cells, inducing some dorsolateral cells to form ventral motor neurons and floor plate cells (van Straaten *et al.*, 1988, 1989; Yamada *et al.*, 1993). Two disparate scenarios are consistent with the ability of dorsally located cells

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0012-1606/95 $12.00

to form ventral derivatives. First, neural tube cells may have equivalent developmental potentials until relatively late in development. Interactions with either dorsal or ventral signals in their local environments then results in phenotype selection. Alternatively, the neural tube may consist of a mixture of cells with a narrowly restricted range of potential fates (either presumptive dorsal or ventral) scattered throughout the neural tube. Ventral or dorsal phenotypes may arise because of the selective survival or differentiation of the proper cell types in response to ventral or dorsal signals. In the first of these scenarios, the notochord plays an instructive role; in the second, its role is selective.

Single-cell lineage analysis provides a simple and straightforward means to distinguish between these divergent possible scenarios, by determining the fates and assessing the developmental potentials of individual neural tube cells. One good cell lineage marker is lysinated rhodamine dextran (LRD; Gimlich and Braun, 1986), which is a large, membrane impermeant dye that is passed only to progeny by cell division. In our previous experiments, we have analyzed the cell phenotypes that can arise from single cells within the dorsal neural tube injected with LRD and have shown that neural crest progenitors: (1) are multipotent, having the ability to form multiple neural crest phenotypes; and (2) are not segregated from other neural tube precursors, as they also give rise to neuronal types forming in the dorsal neural tube (Bronner-Fraser and Fraser, 1988, 1989).

Here, we test whether a single cell can give rise to the full range of neural tube derivatives. We challenge the developmental potential of dorsal neural tube cells by grafting a notochord lateral to the closing neural folds. This results in juxtaposition of dorsal neuroepithelial cells with ventral cell types, by inducing floor plate cells and motor neurons dorsally. After injecting lineage tracer into individual neuroepithelial cells, the results show that both dorsal and ventral neural tube derivatives can arise from a common precursor. This suggests that neuroepithelial cells are not restricted to form only dorsal or ventral neural tube derivatives in the early neural tube.

MATERIALS AND METHODS

Notochord Grafts

Quail embryos (*Coturnix coturnix japonica*) were incubated at 38°C until they reached stage 10–12 (Hamburger and Hamilton, 1951). The trunk region was dissected out of the embryo using an electrolytically sharpened tungsten needle. The notochords were isolated from surrounding tissues with collagenase (160 units/ml; Worthington Biochemical, Freehold, NJ) for 15 min on ice followed by 7 min at 37°C. The notochords then were allowed to recover in complete medium consisting of modified Eagle's medium plus 15% horse serum and 10% embryo extract for 1 hr on ice.

White Leghorn chicken embryos were incubated at 38°C until they reached the 9–11 somite stage (Hamburger and Hamilton, 1951). The eggs were washed with 70% ethanol, 4 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 Ringer's solution was injected under the blastoderm to aid in visualization of the embryo. The vitelline membrane was deflected using an electrolytically sharpened tungsten needle.

Isolated notochords were grafted dorsolateral to the closing neural tube at prospective wing bud levels. A pulled glass needle was used to make an incision 3–5 somites in length in the unsegmented mesoderm region lateral to the neural tube. A donor notochord was transferred in 2 μl of medium using a pipetman and was placed in the vicinity of the incision. The notochord was oriented parallel to the incision and inserted between the dorsal neural tube and the unsegmented mesoderm using a glass needle.

Single-Cell Microinjection

Within 1 hr after implantation of the notochord, the embryos were placed under a Zeiss epifluorescence microscope with oblique lighting from a fiber optic illuminator. Thin-walled aluminosilicate microelectrodes (A-M Systems; resistance of 50–120 MΩ) were filled at the tip with LRD (Molecular Probes; 100 mg/ml) and then back-filled with 1.2 *M* LiCl. The microelectrode was held by a Huxley style 3-axis micromanipulator (Newport Instruments) and a dorsal neural fold cell was impaled by a negative current pulse. Injection of dye was achieved iontophoretically, using 4-nA pulses of current (250-msec pulses, 2 Hz). The membrane resistance and resting potential, which can be used to assess the health of the cell during the injection, were monitored via a Neurodata amplifier and Gould digital storage oscilloscope. After the injection, the microelectrode was withdrawn rapidly from the cell and the labeled cell observed using epifluorescence optics to confirm that only one cell was injected. The embryos were then returned to the incubator until the time of fixation (0–2 days postinjection).

Histology

Embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, embedded in paraffin, and serially sectioned. The slides were deparaffinized and coverslipped in buffered glycerol before observation. For embryos to be processed for neurofilament immunoreactivity, embryos were flash-frozen in isopentane cooled in liquid nitrogen, methanol freeze-substituted at −80°C, brought gradually to 4°C, dehydrated, embedded in paraplast, and serially sectioned at 10 μm (Bronner-Fraser and Fraser, 1989). Slides were deparaffinized in histosol, coverslipped in mineral oil for examining LRD fluorescence, and then stained with neurofilament antibodies as described below. Embryos to be processed for 16.5 HZ, FP-1, anti-

CRABP, and HNK-1 antibodies were dehydrated, embedded in polyester wax (90% polyethylene glycol/10% hexadecanol), and serially sectioned at 10 μm.

Immunocytochemistry

A monoclonal antibody against the nonphosphorylated form of the intermediate molecular weight neurofilament protein (NF-M; provided by Dr. Virginia Lee, Lee *et al.*, 1987) was used to identified neurons and their processes. For verification of the phenotype of motor neurons, floor plate cells, commissural neurons, and dorsal root ganglion cells, respectively, we used monoclonal antibodies 16.5HZ, FP-1, anti-CRABP, and HNK-1. 16.5HZ is a motor neuron marker obtained from the Developmental Studies Hybridoma Bank (maintained by the Department of Pharmacology and Molecular Sciences at the Johns Hopkins University School of Medicine, Baltimore, MD, and the Department of Biology at the University of Iowa, Iowa City, IA, under contract number NO1-HD-2-3144 from the NICHD). CRABP monoclonal antibody (kindly provided by Drs. U. Eriksson and M. Maden) recognizes commissural neurons (Eriksson *et al.*, 1987) and was processed as described previously (Artinger and Bronner-Fraser, 1992). FP-1 antibody (kindly provided by Drs. T. Yamada and T. Jessell) specifically recognizes floor plate cells (Yamada *et al.*, 1991). HNK-1 antibody recognizes neural crest cells and some of their neuronal derivatives including dorsal root ganglion cells.

For neurofilament immunoreactivity, paraffin sections were deparaffinized and rehydrated. Approximately 20 μl of antibody solution, diluted 1:300 in PBS containing 0.1% BSA, 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 in PBS for 5 min and incubated for 1–1.5 hr with FITC-conjugated antibodies against mouse IgGs. Sections were washed in PBS and coverslipped with gelmount (Biomeda). For 16.5 HZ, FP-1, and HNK-1 antibody staining, embryos were processed as follows: Polyester wax sections were rehydrated and undiluted hybridoma supernatant was applied to selected slides, which were incubated in a humidified chamber overnight at 25°C. The sections were then washed in PBS and incubated for 1 hr with secondary antibodies. Highly fluoresceinated antibodies (Antibodies Inc.) against mouse IgGs were used to label 16.5HZ and FP-1 antibodies. HNK-1 antibody was recognized using FITC-conjugated antibodies against mouse IgMs. The slides were then washed in PBS and coverslipped with gelmount for analysis. 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 LRD-labeled cells; a fluorescein filter set was used to visualize immunoreactivity with the various antibodies used in this study. Data were recorded photographically on ektachrome film or electronically using a Hamamatsu SIT video camera and stored on removable Bernouilli discs. Image processing was accomplished using the Vidim software system (Fraser, Stolberg, and Belford, unpublished) for the Imaging Technology LM processor. Most images presented are superimposed fluorescence and transmitted light images, superimposed using the Vidim software program or Adobe Photoshop on a Macintosh computer. Typically, the fluorescence images are presented in the red and/or green channels and the transmitted light is presented primarily in the blue channel.

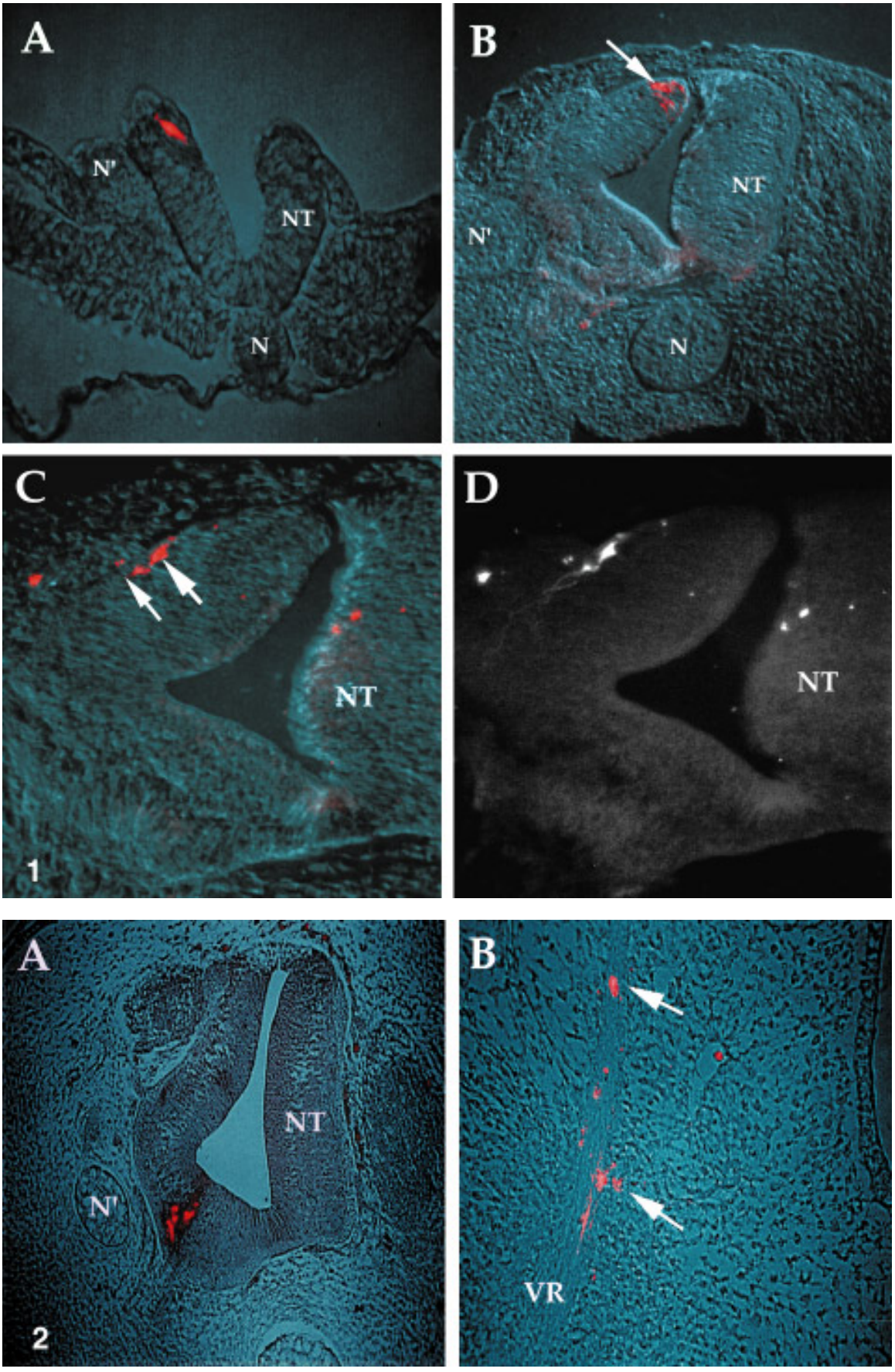
RESULTS

Verification of Single-Cell Injection

We iontophoretically injected LRD into individual cells in the dorsal portion of the closing neural tube after dorsolateral implantation of an exogenous notochord. Following dye injection, we verified the presence of a single dye-labeled cell by direct visualization under the epifluorescence microscope. The labeled cells appeared as elongated, columnar neuroepithelial cells stretching from the luminal to the pial surface of the closing neural tube (Fig. 1A). In a few

FIG. 1. Transverse sections through embryos after grafting a notochord lateral to the closing neural tube, followed by microinjection of lysinated rhodamine dextran (LRD) into a dorsal neural fold cell. (A) In an embryo fixed immediately after injection, a single neuroepithelial cell in the dorsal neural tube (NT) and adjacent to the ectopic notochord (N′) is labeled with dye. (B–D) An embryo fixed 2 days after injection contained labeled cells in neural crest-derived dorsal root ganglia and other dorsal neural tube derivatives. In (B), dye is apparent in roof plate cells (arrow), and in an adjacent section (C, viewed at higher magnification), a labeled cell was observed in the dorsal root ganglion (large arrowhead) and in a commissural neuron (large arrow) and its ventrally directed axon (small arrow) in the dorsolateral neural tube. (D) shows the same view as (C) in fluorescence only to better visualize the axonal process of commissural neurons. N, notochord.

FIG. 2. An embryo fixed 2 days postinjection of a single neural tube cell which contributed to motor neurons, Schwann cells, and nonneuronal cells. At low magnification (A), a group of labeled cells is apparent close to the ectopic notochord (N′). At higher magnification (B), the ventral root (VR) of the same embryo contains label in Schwann cell bodies (straight arrows) as well as in axonal processes (large arrowhead).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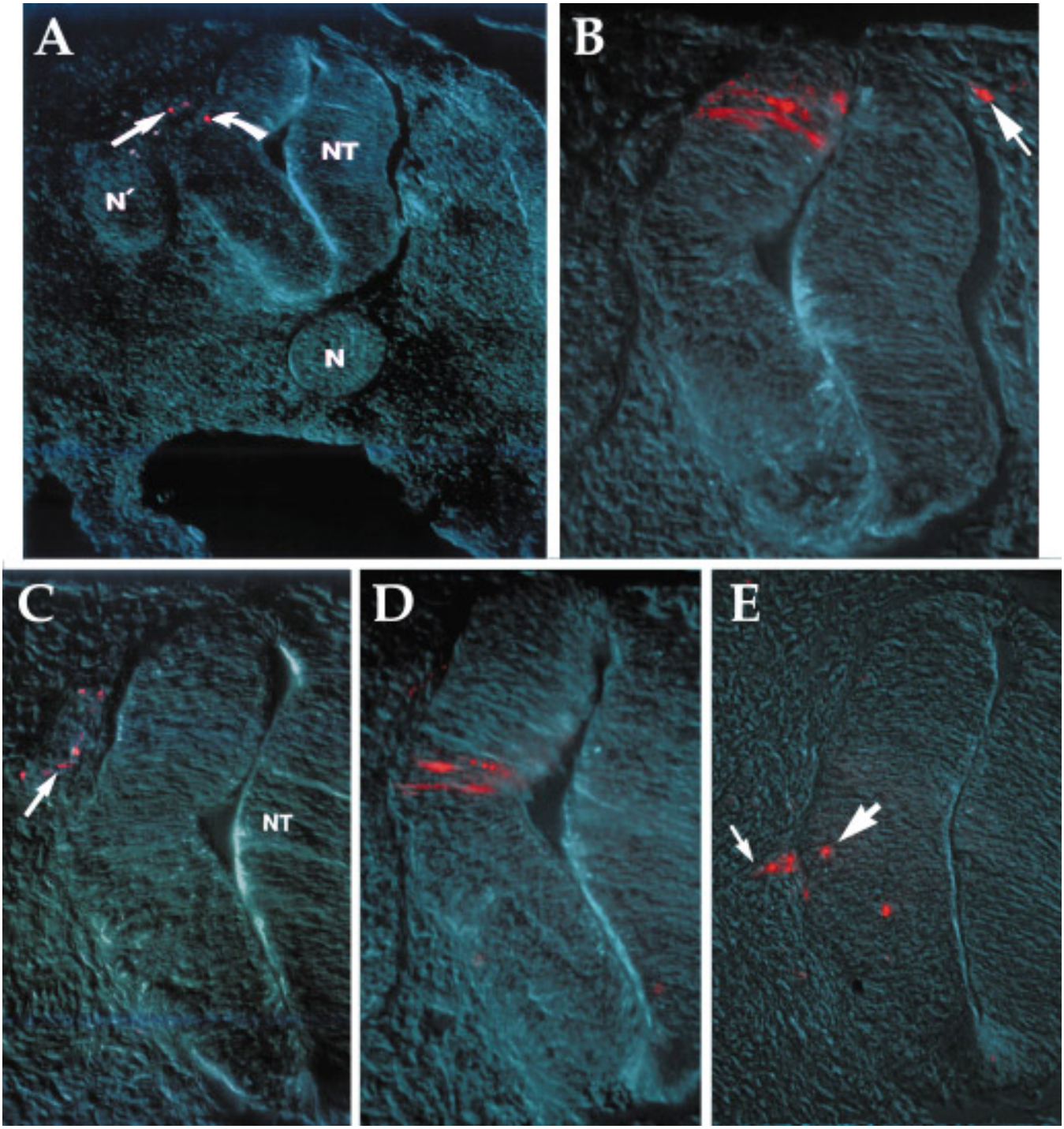


FIG. 3. An embryo fixed 2 days after injection which contained labeled neural crest derivatives, as well as both dorsal and ventral neural tube derivatives. At low magnification (A), labeled cells are seen in the DRG (straight arrow) and a cell that appears to be a commissural neuron (curved arrow). (B) In other sections of this same embryo, other labeled cells form undifferentiated neuroepithelial cells and migrating neural crest cells. In adjacent sections, labeled cells are evident in the DRG (C; arrow), in the induced floor plate (D), and in motor neurons (E; large arrow) and motor axons (small arrow) emanating from the lateral portion of the neural tube ventral to the ectopic notochord.

TABLE 1
Distribution of LRD-Labeled Clones after Notochord Implantation

Class	No. embryos	Type	Dorsal							Ventral		Rostrocaudal extent (μm)
			NT	COM	RP	DRG	SA	PIG	SC	MN	FP	
1	12	D	X									20–150
2	1	D						X				20
3	2	D				X						20
4	10	D	X			X						30–160
5	2	D	X					X				70–140
6	4	D	X	X		X						90–100
7	1	D	X	X	X	X						150
8	1	D	X	X		X	X		X			90
9	1[a]	D	X			X		X				140
10	2	D + V	X							X		90
11	1	D + V	X		X					X		—
12	1	D + V	X						X	X		150
13	2	D + V	X			X		X		X		130–170
14	3	D + V	X			X				X		60–210
15	2	D + V	X			X					X	180
16	1[a]	D + V				X				X	X	120
17	1[a]	D + V	X	X		X				X		160
18	1	D + V	X		X	X				X		130
19	1	D + V	X		X			X		X		110
20	1	D + V	X	X	X	X				X	X	320

Note. TOTAL, 50. NT, Neural tube cell; COM, commissural neuron; RP, roof plate cell; DRG, dorsal root ganglion cell; SA, sympathoadrenal cell; PIG, presumptive pigment cell; SC, Schwann cell; MN, motor neuron; FP, floor plate cell; D, dorsal only clone; D + V, dorsal plus ventral clone.
[a] Derived from apparent mitotic pair.

cases, the labeled cells appeared to be an interconnected mitotic pair (Table 1).

Distribution of LRD-Labeled Clones after Notochord Implantation

After injection, embryos were incubated for 2 additional days (reaching stage 22 to 23 according to the criterion of Hamburger and Hamilton, 1951), by which time many neural crest cells had completed their migration and undergone overt cytodifferentiation. As summarized in Table 1, labeled cells were identified in 50 embryos in which the notochord was properly located adjacent to the neural tube.

Criteria for classification of phenotype. The labeled neural tube cells formed a wide variety of cell types easily recognized by their characteristic positions and morphology; these include dorsal root ganglion cells (DRG) adjacent to the neural tube, sympathoadrenal cells (SA) around the dorsal aorta, Schwann cells (SC) along the ventral roots, and pigment cells (PIG) under the epidermis. Within the neural tube, commissural neurons (COM) were recognizable by their rounded cell bodies and ventrally directed axons (Serbedzija, Bronner-Fraser, and Fraser, 1992; Yaginuma and Oppenheim, 1989; Yaginuma *et al.*, 1990), whereas roof plate cells (RP) were identified as arc-shaped cells in the dorsal midline. Motor neurons (MN) were distinguished by round cell bodies and axons emanating from the neural tube; floor plate (FP) cells were identified by their wedge-shaped morphology.

Although morphological criteria coupled with a cell's position can be quite reliable for scoring cell phenotype, we used antibody markers to verify differentiated cell types in several embryos. The following antibodies were used as molecular markers: (1) anti-neurofilament antibody was used to distinguish neuronal from nonneuronal cells; (2) HNK-1 antibody, together with position, was used to identify dorsal root ganglion cells; (3) CRABP antibody was used to identify commissural neurons; (4) FP-1 was used to identify floor plate cells; and (5) the 16.5 HZ antibody was used to identify motor neurons.

Clones contributing to single-cell types. In 12 embryos (Class 1), only undifferentiated labeled columnar neuroepithelial cells were found in the neural tube. Three other embryos only had derivatives in the neural crest, with 1 (Class 2) contributing to pigment cells and 2 others (Class 3) only to DRG cells. The remaining 35 clones produced cells in multiple derivatives, including cells in the neural tube and neural crest. Clones tended to be relatively compact in their rostrocaudal extent, ranging from 20 to 210

μm for the majority of clones, although a single clone (Class 20) extended over 320 μm (Table 1).

Clones contributing to dorsal neural crest/tube derivatives only. Nineteen clones (Classes 4–9) comprised both neural crest derivatives and various neural tube cell types (commissural neurons, roof plate cells, and undifferentiated dorsal neuroepithelial cells). These dorsal neural tube/crest clones comprised neural tube cells plus cells in a single (Classes 4–7) or in multiple (Classes 8 and 9) neural crest derivatives. A typical dorsal clone (Class 7), which contained neuroepithelial cells, roof plate cells, commissural neurons, and DRG cells, is illustrated in Figs. 1B–1D.

Clones contributing to both dorsal and ventral neural tube derivatives. In 16/50 embryos, clones contributed progeny to both dorsal and ventral derivatives in various combinations. Three clones contained neuroepithelial cells and/or roof plate cells and motor neurons either with or without roof plate cells (Classes 10 and 11). One clone (Class 12) contained motor neurons and Schwann cells along the ventral root (Fig. 2), as well as undifferentiated neuroepithelial cells. Twelve other clones (Classes 13–20) were comprised of neural crest derivatives as well as dorsal and ventral neural tube derivatives in multiple combinations (Table 1). Some clones had motor neurons or floor plate cells in combination with DRG cells (Classes 14–16) and pigment cells (Class 13). Other clones (Classes 17–20) contained more complex arrays of derivatives. For example, one clone (Class 20) was composed of COM, DRG, RP, MN and FP cells in addition to undifferentiated neuroepithelial cells and migrating neural crest cells (Fig. 3). The presence of such diverse cell types in single clones suggests that precursor cells in the closing neural tube are not restricted to form dorsal or ventral derivatives.

Determination of Cell Phenotype Using Antibody Markers

Selected sections from ten of the embryos described above were treated with molecular markers to verify cell phenotype. The typical appearance of HNK-1-immunoreactive, LRD-labeled DRG cells is illustrated in Fig. 4A (n = 3). LRD-labeled commissural neurons were identified by CRABP immunoreactivity (Fig. 4B; n = 3). Motor neurons in portions of the neural tube near the grafted notochord were identified by neurofilament immunoreactivity (Fig. 4C) or by using the 16.5HZ antibody (n = 3). Floor plate cells in the neural tube immediately adjacent to the grafted notochord were recognized by FP-1 immunoreactivity (n = 1; Fig. 4D). In no cases did the antibody staining contradict the classification made by morphological criteria alone.

LRD-Labeled Clones from Ventral Neural Tube Injections in Normal Embryos

Interestingly, two of the embryos described above contained both floor plate cells and motor neurons arising from a common precursor. To test if there is such a common precursor in the presence of the endogenous notochord alone, we injected single neural tube cells near the ventral midline of normal stage 11–12 embryos in the midtrunk region. Of the seven clones identified, three gave rise exclusively to motor neurons and one gave rise to motor neurons plus small cells with the appearance of interneurons. Two clones contained both motor neurons (Figs. 5A and 5B; neurofilament-positive) and wedge-shaped floor plate cells (Fig. 5C; neurofilament-negative); one contained motor neurons and commissural neurons. Thus, floor plate cells and neurons can share a common lineage in the early neural tube, consistent with previous fate mapping experiments in the hindbrain (Fraser *et al.*, 1990). The distribution of labeled cells indicates a large degree of lateromedial dispersal of cells, consistent with previous analyses at hindbrain (Fraser *et al.*, 1990) and trunk (Leber *et al.*, 1990; Stern *et al.*, 1991a) levels.

DISCUSSION

The neural tube is polarized along the dorsoventral axis such that its dorsal portion forms neural crest cells, roof plate cells, and commissural neurons, whereas its ventral portion forms motor neurons and floor plate cells. Previous single-cell lineage analyses have shown that neural crest cells and dorsal neural tube cells share common ancestors (Bronner-Fraser and Fraser, 1988, 1989); however, no clones contained descendants in both the dorsal and ventral portion of the neural tube. In the present study, we have challenged the developmental potential of dorsal neural tube cells and tested whether single neuroepithelial cells can give rise to the full range of neural tube derivatives. Clonal analysis after notochord grafting definitively shows that both dorsal and ventral neural tube derivatives can arise from a single precursor. Cells as diverse as sensory ganglion cells, presumptive pigment cells, roof plate cells, motor neurons, and floor plate cells were observed in the same clone. The wide variety of phenotypes coexisting within single clones (Table 1) argues strongly for the presence of multipotent precursors in the early neural tube. After notochord implantation, 30% of the clones gave rise to both dorsal neural tube/neural crest and ventral neural tube derivatives, extending previous analyses of cell fate using retroviral infection (Leber *et al.*, 1990) or dye injection (Stern *et al.*, 1991a). Even clones that remained in ventral regions were often multipotent, giving rise to divergent cell types ranging from motor neurons to floor plate cells and other unidentified cell types. Because the position of the implanted notochord varies from embryo to embryo, there may be considerable variability regarding the relative proportions of dorsalizing and ventralizing signals; for example, some clones in which the notochord is more distant from the neural tube may have received a dominant dorsalizing signal. However, the results clearly show that a single neu-

roepithelial cell can generate both dorsal and ventral cell types.

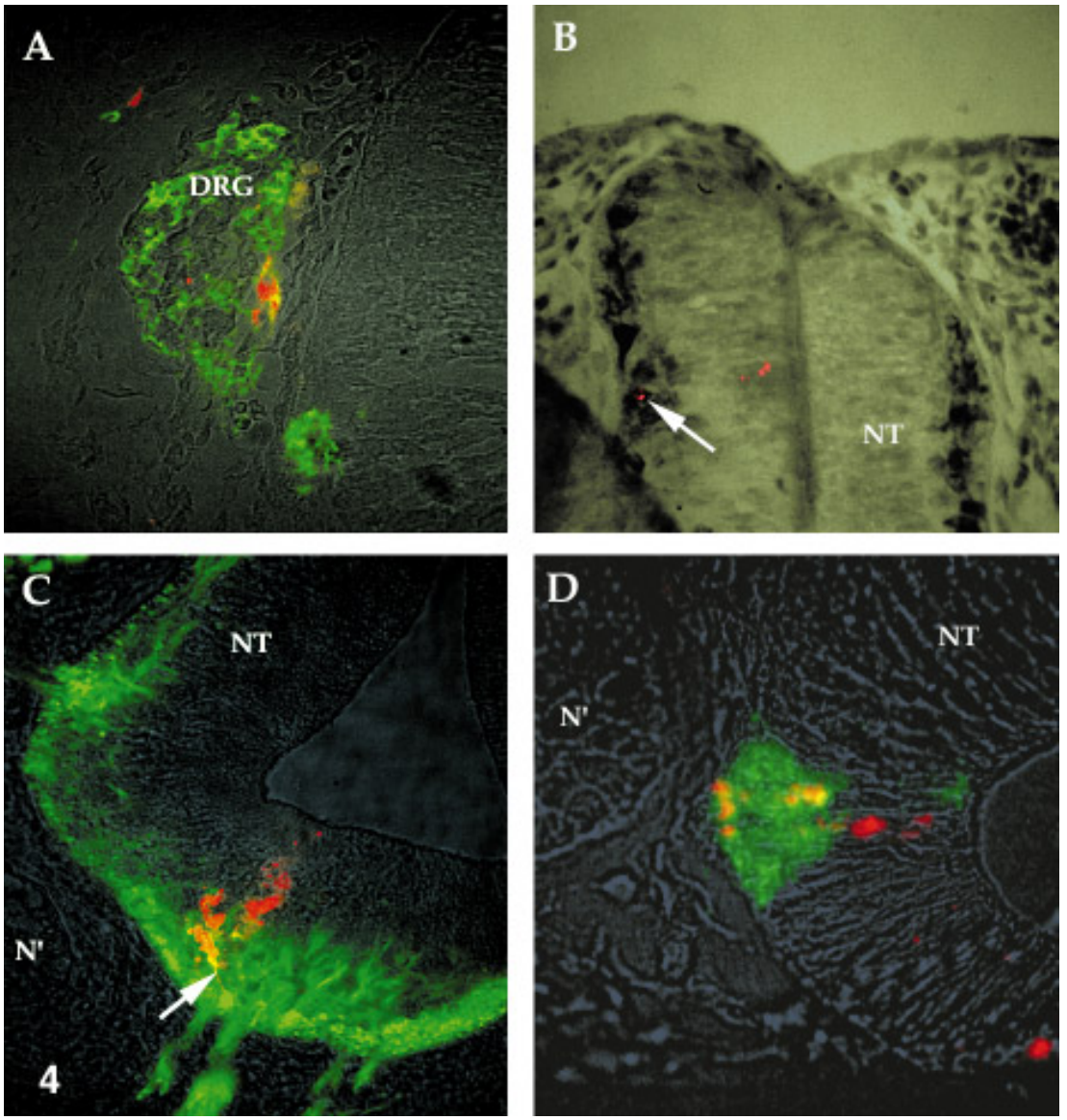
The range of phenotypes within single clones argues against any model in which position directly dictates phenotype or in which a stepwise commitment of neural tube cells progresses from a primary ventral or dorsal state to final phenotype. Consistent with this lack of dorsoventral restriction, recent experiments in which the cranial neural crest is ablated show that ventrally positioned neural tube cells can regulate to reform the neural crest (Scherson *et al.*, 1993; Sechrist *et al.*, 1995). Despite the clear asymmetries observed with molecular and morphological markers in the early neural tube, we find no evidence for commitment at a single-cell level. Instead, such gene expression domains must be viewed as rather dynamic, persisting in the face of the considerable cell mixing seen here and in previous studies (Leber *et al.*, 1990).

This lack of strict lineage restriction cannot be taken as evidence for an absence of any dorsoventral predisposition within the closing neural tube. For example, there is evidence that ventral midline cells in the neural plate may be biased toward a floor plate fate at very early stages. Removal of the notochord from stage 10 embryos results in the absence of a floor plate 2 days later (Yamada *et al.*, 1993) but delayed differentiation of the floor plate occurs after 4 days (Artinger and Bronner-Fraser, 1993). Furthermore, floor plate cells can develop in isolated neural plates (Artinger and Bronner-Fraser, 1993), suggesting that the cells in the neural plate are not completely equivalent, perhaps because of earlier interactions with the chordamesoderm in Hensen's node. Consistent with this scenario, dorsoventral rotation of the neural tube (without the notochord) shortly after its closure results in an upside-down neural tube, with neural crest cells emerging ventrally and motor axons emerging dorsally (Weston and Butler, 1966; Stern *et al.*, 1991b). Similarly, early interactions may predispose the dorsal aspect of the neural tube to give rise to neural crest, as a notochord grafted dorsally during neural tube closure cannot prevent neural crest emigration (Artinger and Bronner-Fraser, 1992). Interactions between the nonneural ectoderm and neural plate can result in the induction of neural crest cells as well as dorsal neural tube markers (Selleck and Bronner-Fraser, 1995; Dickinson *et al.*, 1995). Because these interactions start during gastrulation, this lends further support to the idea that dorsoventral polarity may begin at early stages. The results presented here show that these tissue-level asymmetries do not reflect single cell restrictions; instead, cells moving through distinct territories must adopt and lose the appropriate dispositions as they enter and leave a territory.

Our results demonstrate that individual early neural tube cells are multipotent in their ability to form a variety of neural tube derivatives (including both dorsal and ventral cell types) as well as various neural crest derivatives. In combination with other studies on dorsoventral patterning of the neural tube (reviewed in Jessell and Dodd, 1993), the present results shed light on the signaling mechanisms within the neural tube and suggest the model presented in Fig. 6. Prior to neural tube closure, there is likely to be planar signaling between the nonneural ectoderm and the presumptive neural plate that promotes dorsal properties; such interactions are suggested by tissue culture experiments in which juxtaposition of the ectoderm and the early ventral neural plate results in the formation of neural crest cells (Selleck and Bronner-Fraser, 1995; Dickinson *et al.*, 1995). Similarly, juxtaposition of epidermis and neural plate in amphibians results in generation of neural crest cells apparently from both tissues (Moury and Jacobson, 1989, 1990). Concurrently, signals such as *sonic hedgehog* from the notochord (Echelard *et al.*, 1993), and perhaps its precursor in Hensen's node, promote ventral properties. Although these signals can generate striking dorsoventral differences in the neural tube, they cannot be producing a stepwise commitment of neural tube cells to strict dorsal or ventral fates. Implantation of a notochord during tube closure results in cells that respond to both dorsal and ventral signals. The diverse cell types observed in our single-cell data indicate that some neuroepithelial cells are uncommitted at the time of injection and can respond to both signals. The results of grafting experiments and analyses of normal development suggest that dorsal and ventral signals are provided continuously, beginning during gastrulation and continuing past the time of neural tube closure, and may be composed of multiple and/or overlapping signals. Thus, the specification of dorsal and ventral fates cannot be a unitary event in response to one or two signals. Instead, the results presented

FIG. 4. Embryos labeled with monoclonal antibodies to identify selective cell types. (A) An embryo with an HNK-1 immunoreactive (green), LRD-labeled cell (red) in the dorsal root ganglion (DRG). (B) Another embryo after immunoperoxidase staining with anti-CRABP (brown) in the lateral portion of the neural tube (NT) which also contains LRD (arrow). (C) A section through the embryo shown in Fig. 2, after staining with neurofilament antibodies (green), shows that the labeled cells (red) include motor neurons (large arrowhead) with axons coursing toward the ventral root (straight arrow) as well as other LRD-labeled neurofilament-negative cells. (D) An embryo in which an induced floor plate, recognized by FP-1 staining (green), is present adjacent to the implanted notochord (N′). Some of the LRD-labeled cells (red) within the floor plate are FP-1 immunoreactive (appearing yellow).

FIG. 5. Transverse sections through a normal embryo 2 days after LRD was injected into a single cell at the ventral midline. (A and B) A labeled cell (arrow) in the ventral neural tube (NT) in the position of a motor neuron, containing neurofilament immunoreactivity. (C) An adjacent section illustrates a floor plate cell, characterized by its wedge-shaped morphology, in the same clone as the motor neuron. The oval-shaped cell is a blood cell which autofluoresces.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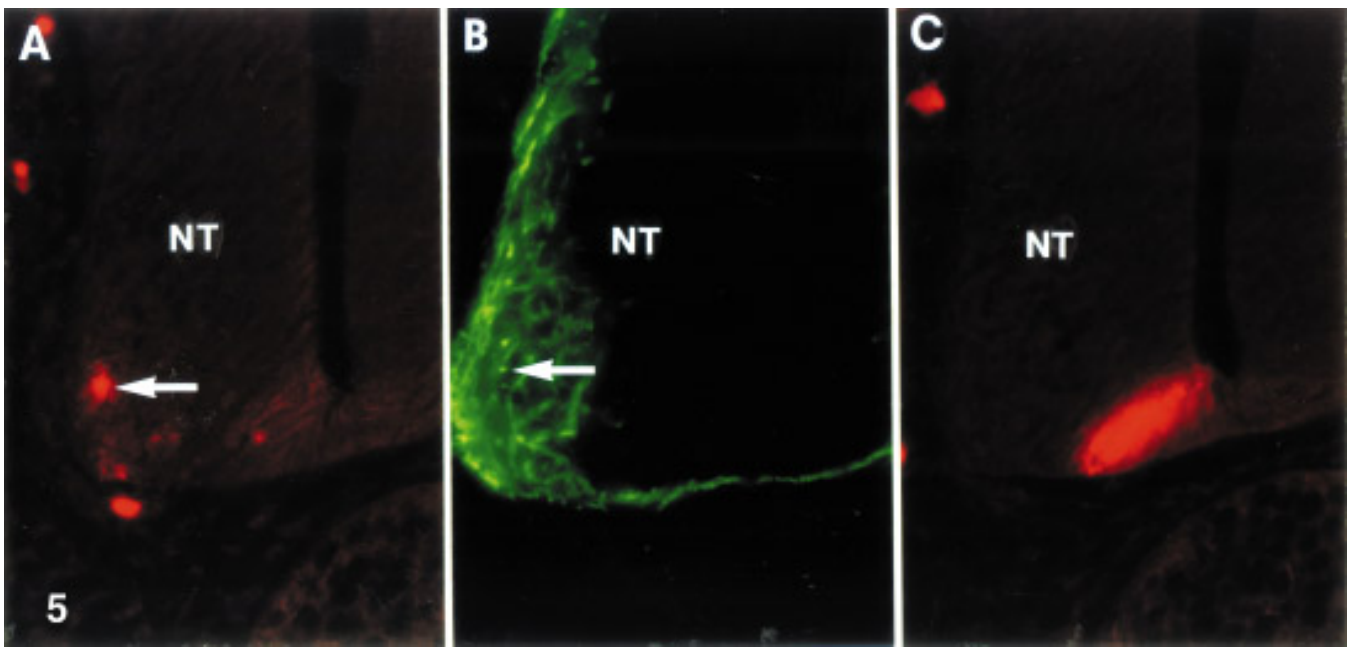
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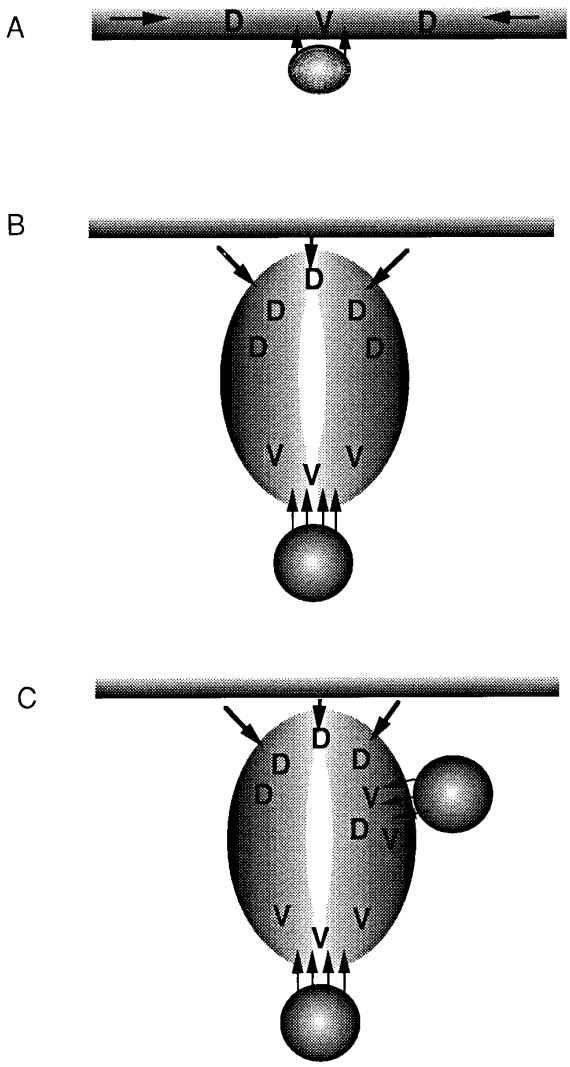

FIG. 6. Schematic diagram illustrating a model for the signaling mechanisms that might be involved in patterning of the neural tube. (A) Prior to its closure, planar signals travel within the nonneural ectoderm and induce dorsal (D) properties. Grafting operations suggest that these probably continue throughout neural tube closure. Concurrently, signals from the notochord, and perhaps its precursor within Hensen's node, induce ventral (V) properties. (B) These primary signals (together with both positive and negative secondary interactions that are likely to occur within the neural tube) lead to the establishment of dorsal cell types (neural crest cells, roof plate cells, commissural neurons) in the dorsal neural tube and ventral cell types (motor neurons and floor plate cells) in the ventral neural tube. Extensive movements of individual cells do not disrupt these patterns, as the progeny of single cells remain responsive to both extrinsic and intrinsic signals. (C) Implantation of a notochord during tube closure results in superimposition of dorsal and ventral signals. The progeny of a single cell can respond to both of these cues, giving rise to both dorsal and ventral derivatives.

here and elsewhere are most consistent with a mechanism in which the specification of cell fate is a continuous process, based on signaling beginning during gastrulation and continuing past the time of neural tube closure.

ACKNOWLEDGMENTS

We thank Drs. A. Collazo, M. Dickinson, C. Krull, J. Sechrist, M. Selleck, and J. Shih for helpful comments on the manuscript, Drs. S. Ruffins and M. Selleck for help with image analysis, and B. Metscher and S. Bryant for help with the polyester wax protocol. We gratefully acknowledge the generous gifts of antibodies from a number of colleagues: Drs. H. Tanaka and T. Yamada for the FP-1 antibody, Dr. Virginia Lee for the neurofilament antibody, and Drs. Ulf Eriksson and Malcom Maden for the anti-CRABP antibodies. This work was supported by USPHS HD-25138 and MH-49176.

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Received for publication June 30, 1995
Accepted August 22, 1995