Control of dorsoventral pattern in vertebrate neural development: induction and polarizing properties of the floor plate

MARYSIA PLACZEK[1], TOSHIYA YAMADA[2], MARC TESSIER-LAVIGNE[2], THOMAS JESSELL[2] and JANE DODD[1]

[1]Department of Physiology and Cellular Biophysics, [2]Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University, New York, New York 10032, USA

Summary

Distinct classes of neural cells differentiate at specific locations within the embryonic vertebrate nervous system. To define the cellular mechanisms that control the identity and pattern of neural cells we have used a combination of functional assays and antigenic markers to examine the differentiation of cells in the developing spinal cord and hindbrain in vivo and in vitro. Our results suggest that a critical step in the dorsoventral patterning of the embryonic CNS is the differentiation of a specialized group of midline neural cells, termed the floor plate, in response to local inductive signals from the underlying notochord. The floor plate and notochord appear to control the pattern of cell types that appear along the dorsoventral axis of the neural tube. The fate of neuroepithelial cells in the ventral neural tube may be defined by cell position with respect to the ventral midline and controlled by polarizing signals that originate from the floor plate and notochord.

Key words: dorsoventral patterning, floor plate, notochord, neural differentiation, spinal cord.

Introduction

A basic problem in vertebrate development is to define the mechanisms that control the identity and patterning of cells in the nervous system. The initial event in neural cell patterning is the induction and regionalization of the neural plate. The establishment of polarity along the anteroposterior axis of the neural plate is evident soon after neural induction; however the patterning of cells along the dorsoventral axis is delayed, occurring as the neural plate folds to form the neural tube (Roach, 1945; Jacobson, 1964) (Fig. 1). The position of neuroepithelial cells along these two primary axes is likely to contribute significantly to their fate. In anterior regions of the neural plate, small differences in cell position along the anteroposterior axis result in markedly different cell fates (Puelles et al. 1987). However, in more posterior regions such as the spinal cord, the overall pattern of cell types is essentially identical at different segmental levels (Hollyday and Hamburger, 1977; Altman and Bayer, 1984; Eisen, 1991). Thus, a major determinant of neural cell identity in the spinal cord is likely to be cell position along the dorsoventral axis of the neural tube.

Each of the neuronal cell groups that differentiates along the dorsoventral axis of the nervous system exhibits bilateral symmetry (Fig. 1). The dorsoventral polarity of the neural tube and the bilateral symmetry of the spinal cord could be established at earlier stages by cells at the midline of the neural plate which provide signals that spread laterally to control the fate of adjacent neuroepithelial cells. The cells located at the midline are one of the first cell groups to undergo differentiation within the neural plate (Bancroft and Bellairs, 1975; Schoenwolf, 1982; Altman and Bayer, 1984). During neurulation these midline cells give rise to the floor plate (Baker, 1927; Kingsbury, 1930), an epithelial cell group that later occupies the ventral midline of the neural tube (Fig. 1) and has been implicated in the patterning of axonal projections in the developing central nervous system (Jessell et al. 1989). The floor plate releases a diffusible chemoattractant that may orient the growth of the axons of commissural neurons towards the ventral midline of the embryonic spinal cord (Tessier-Lavigne et al. 1988; Placzek et al. 1990a, 1990b). The floor plate also appears to act as an intermediate target involved in changes in axonal trajectory at the midline (Bovolenta and Dodd, 1990, 1991; Kuwada et al. 1990a, 1990b; Kuwada and Hatta, 1990; Hatta et al. 1990; Yaginuma et al. 1991). In addition, floor plate cells are the source of a polarizing signal that can respecify the pattern of cell differentiation along the anteroposterior axis of the developing chick limb (Wagner et al. 1990), mimicking the effect of the limb polarizing region and of retinoic acid (Tickle et al. 1975, 1982).

Neural Plate

Neural Folds

Neural Tube

Spinal Cord

roof plate

floor plate

notochord
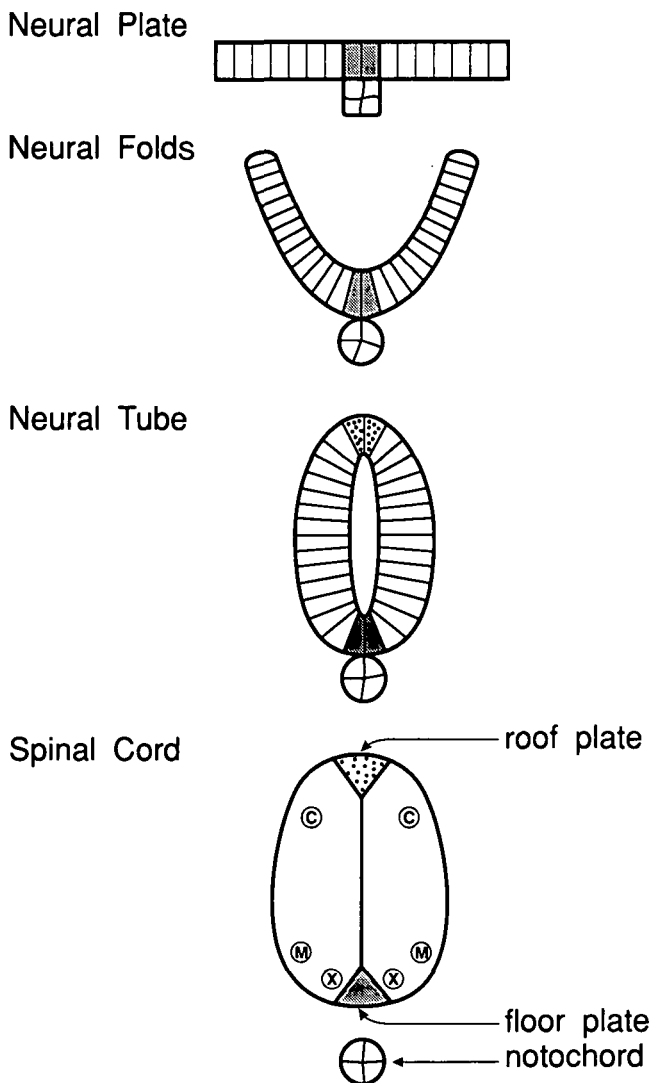
Fig. 1. Establishment of the dorsoventral axis in the developing spinal cord. The four diagrams show successive stages in the development of the neural tube and spinal cord. The neural plate consists initially of a simple columnar epithelium. Cells at the midline of the neural plate are contacted directly by axial mesoderm cells of the notochord. More lateral regions of the neural plate overlie the segmental plate mesoderm (not shown). During neurulation, the neural plate buckles at its midline to form the neural folds. Contact between the midline of the neural plate and the notochord is maintained at this stage. The neural tube is formed when the dorsal tips of the neural folds fuse. Cells in the region of fusion form a specialized group of dorsal midline cells, the roof plate. Cells at the ventral midline of the neural tube retain proximity to the notochord and differentiate into the floor plate. After neural tube closure neuroepithelial cells continue to proliferate and eventually differentiate into defined classes of neurons at different dorsoventral positions within the spinal cord. For example, commissural (C) neurons differentiate dorsally near to the roof plate, and motor (M) neurons differentiate ventrally near to the floor plate, which by this time is no longer in contact with the notochord. Other classes of neurons (X) differentiate in the region of neural epithelium that intervenes between the floor plate and motor neurons. For further details see Schoenwolf and Smith (1990) and Yamada *et al.* (1991).

The characterization of these floor plate properties has led us to investigate the cellular interactions that control floor plate differentiation and to determine whether the floor plate has a role in regulating the identity of other neural cells. Here we discuss experiments that provide evidence firstly, that local inductive signals from the notochord control the differentiated properties of floor plate cells and secondly, that the floor plate and notochord together regulate the differentiation of neural cell types along the dorsoventral axis of the neural tube.

Results

Induction of the floor plate

The first sign of floor plate differentiation is a change in the morphology of cells at the midline of the neural plate (Schoenwolf and Smith, 1990). Floor plate cells exhibit basally located nuclei and apical constrictions, leading to the characteristic wedge-shaped appearance of the floor plate in the neural tube (Schroeder, 1970; Schoenwolf and Franks, 1984). Early studies on the

control of floor plate differentiation examined the morphology of the spinal cord in chick embryos with duplicated notochords (Watterson *et al.* 1955). In these embryos, spinal cord cells with characteristic floor plate morphology were observed adjacent to both notochords, suggesting that the differentiation of the floor plate depends on the notochord. More recent experimental studies have provided additional support for this idea. Grafting of a notochord next to the lateral part of the neural tube results in the appearance of cells with floor plate-like morphology in the adjacent neural epithelium (van Straaten *et al.* 1985a,b, 1988; Smith and Schoenwolf, 1989). In addition, in embryos in which the notochord has been deleted, either experimentally or as a result of mutations, wedge-shaped cells are absent from the midline of the spinal cord (Wolff, 1936; Grabowski, 1956; Youn and Malacinski, 1981; van Straaten and Drukker, 1987; Kuwada and Hatta, 1990; Hatta *et al.* 1990; Bovolenta and Dodd, 1991).

These results are consistent with the idea that the notochord can influence the morphology of the neural tube, generating a region of cells with the appearance of a floor plate. However, comparable changes in neuroepithelial cell shape occur during the development of many regions of the central nervous system (Lewis, 1947; Martins-Green, 1988; Schoenwolf *et al.* 1988; Moury and Jacobson, 1989), making it difficult to identify the floor plate solely by morphology. The characterization of floor plate-specific antigens (Table 1) and functions (Dodd and Jessell, 1988; Tessier-Lavigne *et al.* 1988; Jessell *et al.* 1989) has permitted a more critical test of the dependence of floor plate differentiation on signals that derive from the notochord. To examine the origins of the floor plate, we

Table 1. *Identification of cell types in embryonic chick nervous system and notochord*

Cell type	Marker	Onset of expression in neural tube (H. H. Stage)	Reference
Floor plate	FP1	10	Yamada *et al.* 1991
	SC1	10	Tanaka and Obata, 1984
	FP2	20	Yamada *et al.* 1991
Motor neurons	SC1	15	Tanaka and Obata, 1984
Serotonergic neurons	Serotonin	23	Wallace, 1985
Sensory relay neurons	CRABP	15	Maden *et al.* 1989
			Kitamoto *et al.* 1989
Differentiated neurons	3A10	15	Yamada *et al.* 1991
Dorsal spinal cord cells	AC4	Absent from ventral spinal cord by stage 17	Yamada *et al.* 1991
Notochord	Not1	5	Yamada *et al.* 1991

first determined whether surface antigens expressed by the floor plate are induced in other regions of the neural tube in response to a segment of notochord grafted adjacent to the neural tube of stage 9–11 (Hamburger and Hamilton, 1951) chick embryos. The floor plate antigens, FP1, FP2 and SC1 were induced in neuroepithelial cells adjacent to notochord grafts placed dorsally or laterally to the neural tube (Fig. 2 and Yamada *et al.* 1991). These results show that the expression of specific floor plate antigens can be induced by the notochord.

Previous studies have shown that the embryonic rat floor plate releases a diffusible chemoattractant which promotes the outgrowth and orientation of spinal commissural axons *in vitro* (Tessier-Lavigne *et al.* 1988; Placzek *et al.* 1990a, 1990b). The chick floor plate also releases this chemoattractant (Placzek *et al.* 1990c). In both rat and chick embryos, the chemoattractant is restricted to the floor plate and thus provides a functional marker with which to monitor the differentiation of the floor plate. To assay for induction of the chemoattractant *in vivo*, a segment of notochord was grafted adjacent to the neural tube of stage 9–11 chick embryos. Explants of spinal cord from the region adjacent to the grafted notochord consistently evoked axon outgrowth from rat dorsal spinal cord explants (Placzek *et al.* 1990c). In contrast, neither the neuroepithelium from the contralateral, unoperated side of the spinal cord nor the grafted notochord itself exhibited chemotropic activity (Placzek *et al.* 1990c). Thus, the notochord can induce expression of this chemoattractant in regions of the chick neural tube that normally lack this activity.

There appears to be a marked rostrocaudal gradient in the onset of floor plate differentiation within the neural tube. Midline neural plate cells isolated from the anterior region of stage 6 chick embryos are already capable of expressing differentiated floor plate characteristics as assessed by production of the chemoattractant (Fig. 3C, D and Placzek *et al.* 1990c). In contrast, floor plate differentiation in more caudal regions is delayed, and does not occur until about stage 12 (Placzek *et al.* 1990c). Removal of the notochord before this stage should therefore reveal whether the notochord is required for the differentiation of the floor plate during normal development. To test this, a piece

of notochord from the caudal region of stage 10–12 embryos was removed and the expression of antigenic and functional properties of the floor plate examined at stage 19–23. At segmental levels lacking the notochord, cells at the ventral midline of the spinal cord did not express either the SC1 or FP1 antigens (Yamada *et al.* 1991). Similarly, the chemotropic activity of regions of the ventral midline of the neural tube isolated from the notochordless region and from regions just rostral and caudal was assayed (Placzek *et al.* 1990c). The ventral midline, at levels lacking a notochord, did not evoke axon outgrowth, whereas the ventral midline rostral and caudal to the notochord region evoked extensive outgrowth. These results provide evidence that the notochord is required for the differentiation of the floor plate during normal development.

These *in vivo* studies do not exclude the possibility that other tissues, for example somites, act in combination with the notochord to induce differentiation of the floor plate. To test whether the notochord alone is sufficient for induction, stage 7 chick notochord was placed *in vitro* together with lateral regions of stages 6 and 7 neural plate (Fig. 3A,B). Explants of the lateral region of the neural plate alone did not exhibit chemotropic activity (Fig. 3C,D). However, significant chemotropic activity was observed when explants of lateral neural plate and notochord were placed in direct contact (Figs 3C,D and 4A) (Placzek *et al.* 1990c). In contrast, chemotropic activity was not detected when the notochord and lateral neural plate were placed 50–100 μm apart (Figs 3C,D and 4B). This argues against the possibility that the uninduced lateral neural plate and notochord release different factors that act in combination to evoke commissural axon outgrowth and suggests that the notochord is sufficient to induce a floor plate.

These results also suggest that proximity or direct contact between notochord cells and the neural plate is necessary for induction of the floor plate. In support of this, during the early development of the neural tube only those cells contacted by the notochord exhibit the shape changes characteristic of early floor plate differentiation (Schoenwolf and Smith, 1990). Because there is little cell death within the chick neural tube before stage 15 (Homma *et al.* 1990) the ectopic
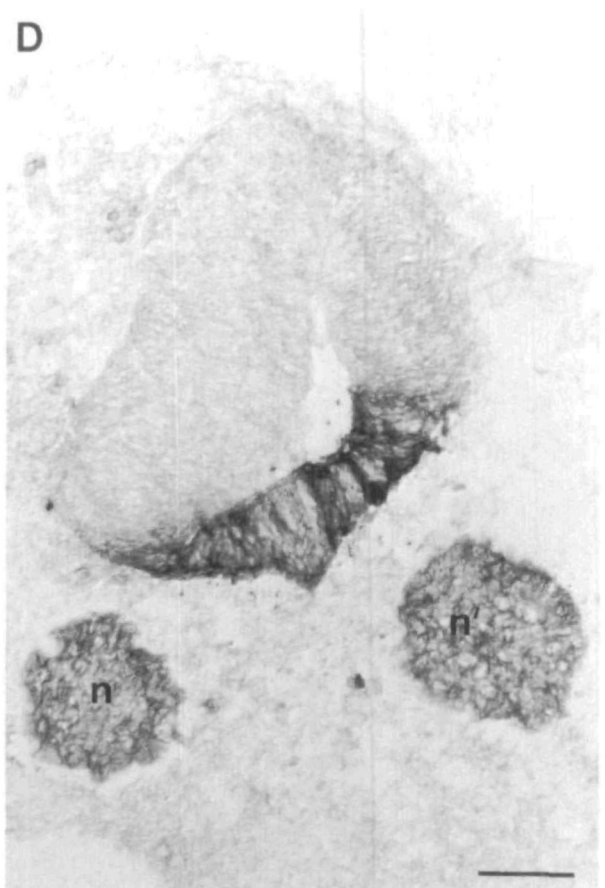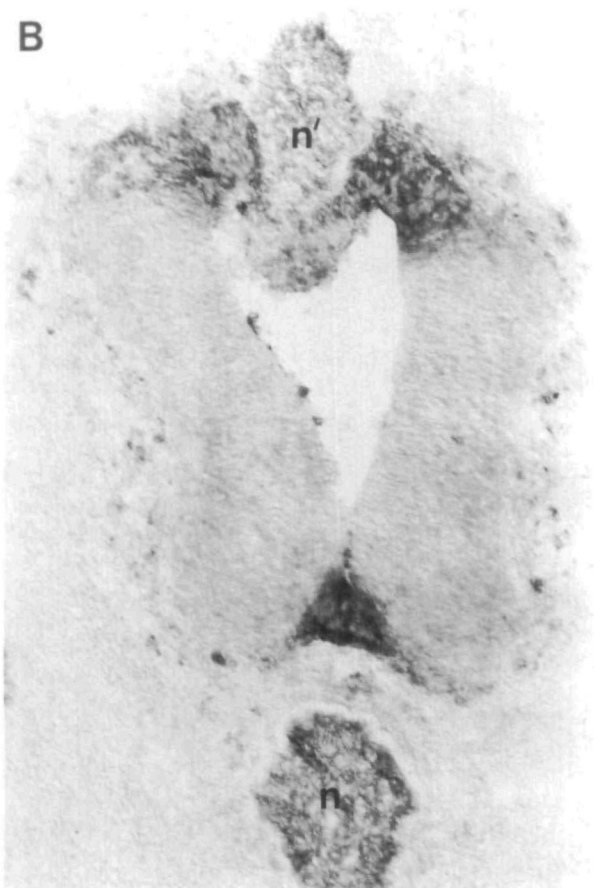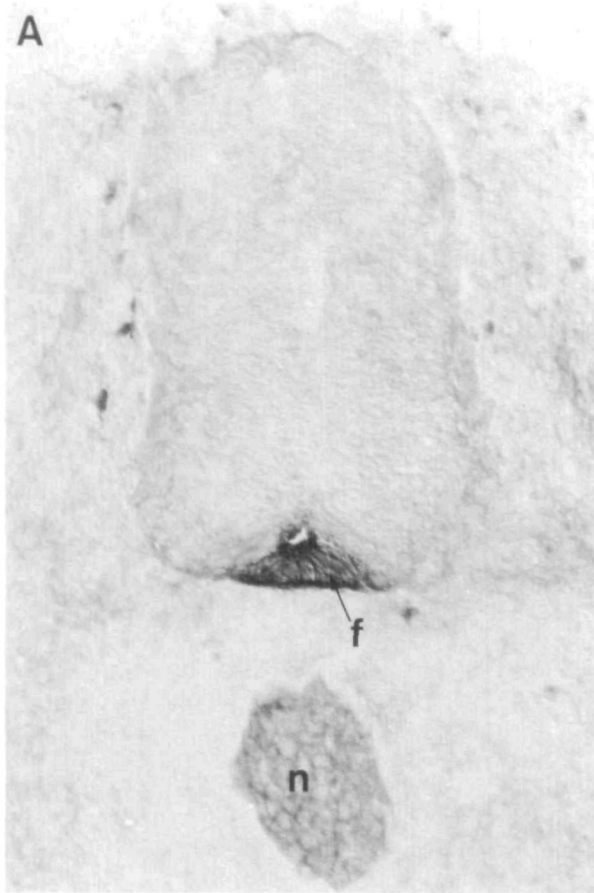
Fig. 2. Induction of a floor plate in the developing spinal cord in response to notochord grafts. (A) In control chick embryos, floor plate (f) cells at the ventral midline of the spinal cord and the underlying notochord (n), express the FP1 antigen. No other region of the spinal cord expresses the FP1 antigen at detectable levels. (B) A grafted notochord (n') placed in the lumen of the neural groove is displaced dorsally and comes to lie at the dorsal midline of the spinal cord. Dorsal neuroepithelial cells near the ectopic notochord express the FP1 antigen. FP1-labelled cells span the distance from the lumen to the external surface of the spinal cord, as does the host floor plate. (C) The FP1 antigen is also expressed by neuroepithelial cells in the region adjacent to a laterally grafted notochord (n'). The spinal cord exhibits a distortion in the neuroepithelium, which presumably results from the wedging of the FP1-positive cells. (D) When the grafted notochord is located in an extreme ventral position a single expanded region of FP1-labelled cells is observed. Abbreviations: f, floor plate; n, host notochord; n', grafted notochord. Bar=60 μm.

expression of floor plate-specific properties in neural tube cells is likely to result from induction rather than from the rescue of committed precursor cells that normally die in the absence of the notochord. The induction of chemotropic activity in isolated explants of lateral neural plate also makes it unlikely that *in vivo* the ectopic notochord simply directs the dorsolateral migration of committed floor plate progenitors originally located at the ventral midline.

More generally, these results suggest that mesodermally derived inductive signals have sequential and progressively more refined roles in regulating neural differentiation. During gastrulation, these signals appear to control the initial induction of the neural plate and its early regionalization along the anteroposterior axis (Mangold, 1933; Spemann, 1938; Dixon and Kintner, 1989; Ruiz i Altaba, 1990). Later, during neural tube development, the mesoderm also determines the identity of a specific subset of cells within the neural plate, inducing them to differentiate into floor plate cells. As discussed below, induction of the floor plate by the notochord appears to be an important step in subsequent aspects of cell patterning in the developing nervous system.

Signals from the floor plate and notochord control cell pattern along the dorsoventral axis of the neural tube

The differentiation of floor plate cells precedes that of other cell types within the neural tube (Fig. 1 and Schoenwolf and Smith, 1990). In the spinal cord, floor plate differentiation is followed by the appearance of motor neurons, commissural neurons and neural crest cells at different dorsoventral positions (Fig. 1). The characterization of the floor plate as a source of polarizing activity in the developing limb (Wagner *et al.* 1990), together with its midline location within the neural tube, raises the possibility that the floor plate controls the pattern of cell differentiation along the dorsoventral axis of the neural tube (Jessell *et al.* 1989).
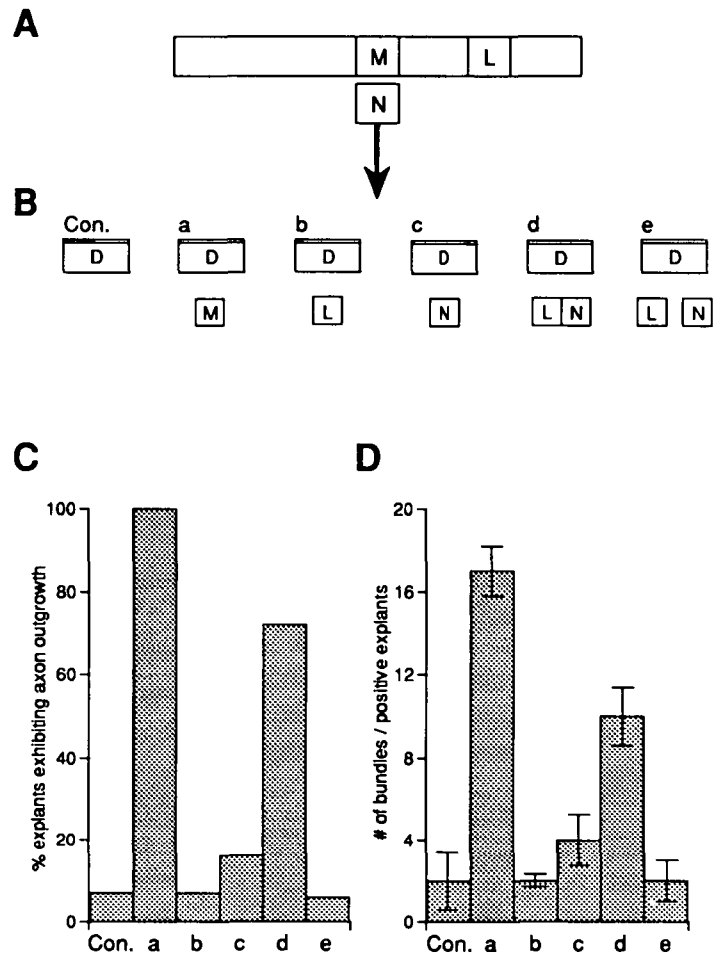
Fig. 3. Induction of chemotropic activity in the lateral neural plate *in vitro*. (A) Schematic cross section through H–H stages 6–7 chick embryo neural plate and underlying notochord. Regions assayed for chemotropic activity are boxed. (B) Dorsal spinal cord explants (D) were cultured for 40–44 h, either alone (Con.), with midline neural plate (a), with lateral neural plate alone (b), with notochord alone (c), and with lateral neural plate and notochord in contact (d) or apart (e). (C,D) The percentage of dorsal explants showing axon outgrowth (C) and the average number of axon bundles from dorsal explants that showed outgrowth (D) is shown. Standard error bars are shown. The number of explants for each column in (C) are as follows: Con, 67; a, 40; b, 59; c, 44; d, 39; e, 31. Abbreviations: D, dorsal spinal cord explant; M, midline neural plate; L, lateral neural plate; N, notochord (From Placzek *et al.* 1990c).

To determine whether the fate of neuroepithelial precursors within the chick neural tube depends on position with respect to the floor plate, we first identified antigens that are selectively expressed by distinct subsets of neural cells distributed at different positions along the dorsoventral axis of the spinal cord and hindbrain (Table 1, and Yamada *et al.* 1991). The expression of these antigens was monitored to determine how the pattern of cells in the embryonic chick nervous system changes in the presence of an additional floor plate or notochord at ectopic positions in the
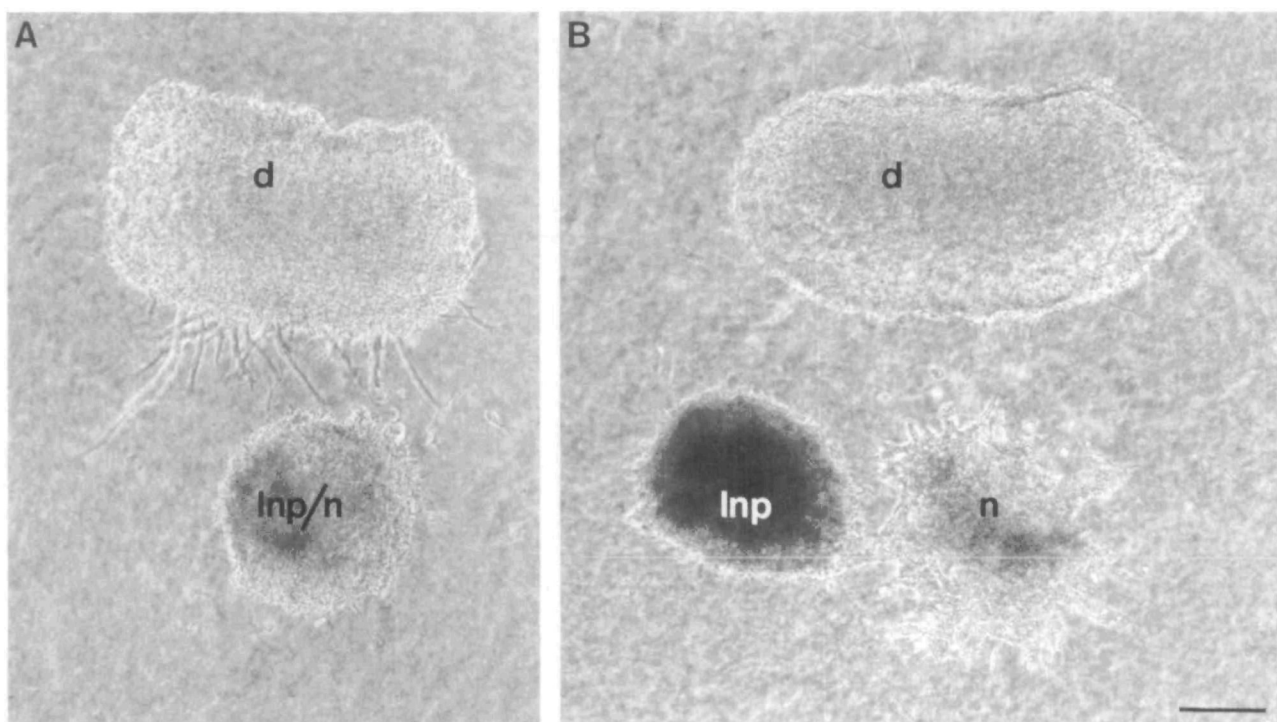
Fig. 4. Proximity is required between notochord and lateral neural plate for induction of chemotropic activity *in vitro*. Phase contrast micrographs showing examples of axon outgrowth from E11 rat dorsal spinal explants (d) cultured for 40 h in a collagen matrix. (A) Axon outgrowth is evoked when stage 6 chick lateral neural plate (lnp) is cultured in direct contact with stage 7 chick notochord (n). (B) Axon outgrowth is not evoked when stage 6 lateral neural plate and notochord are placed 50 μm apart. Abbreviations: d, dorsal spinal cord explant; lnp, lateral neural plate; n, notochord; lnp/n, notochord plus lateral neural plate; Bar=110 μm.

neural tube, and after elimination of the floor plate and notochord.

### Changes in the pattern of neural cell differentiation after notochord grafts

Induction of motor neurons

To determine whether the floor plate controls the differentiation of other neural cells, the location of motor neurons was examined in embryos in which an additional floor plate had been induced at the dorsal midline by a notochord graft. Motor neurons normally differentiate in the ventral spinal cord near, but not immediately adjacent to the floor plate (Fig. 5A). In embryos with dorsal notochord grafts, labelling with MAb SC1 revealed the presence of two columns of motor neurons in a normal ventral position. In addition, the dorsal half of the spinal cord contained two ectopic motor columns that differentiated in a bilaterally symmetric manner around the newly induced floor plate (Fig. 5B) (Yamada *et al.* 1991). When notochord grafts were placed next to the neural tube, midway between the roof plate and the floor plate, an ectopic floor plate was detected adjacent to the graft. In addition, an ectopic column of motor neurons developed in the dorsal spinal cord on the side of the graft, separated from the induced floor plate by a region of unlabelled cells. There was about a 1.5-fold increase in the number of motor neurons on the side of the spinal

cord adjacent to the graft but no significant change in the total number of neural cells (Yamada *et al.* 1991).

Induction of cells adjacent to the floor plate

The expression of the SC1 antigen by floor plate cells and motor neurons revealed a region of unlabelled neuroepithelium that was invariably interposed between the floor plate and motor columns in the spinal cord of normal and operated embryos (Yamada *et al.* 1991). In the chick spinal cord, primitive longitudinal (PL) interneurons differentiate in this region (Yaginuma *et al.* 1990) (termed region X) although antigenic markers that identify, selectively, PL cells or other cells in region X in the spinal cord have not been defined. However, in the hindbrain, a subset of cells in region X differentiate into serotonergic neurons (Wallace, 1985). We therefore examined whether the differentiation of cells in region X is dependent on the floor plate and notochord by monitoring the expression of serotonergic neurons after grafting a piece of notochord adjacent to the neural tube at the level of the hindbrain. Notochord grafts at the level of the hindbrain induced an ectopic floor plate, which could be detected with MAbs SC1 and FP1. Serotonergic neurons were present immediately adjacent to the ectopic floor plate whereas SC1-labelled cranial motor neurons were induced at a distance from the ectopic floor plate (Yamada *et al.* 1991). These findings show that cells characteristic of region X can be induced by midline-derived signals and
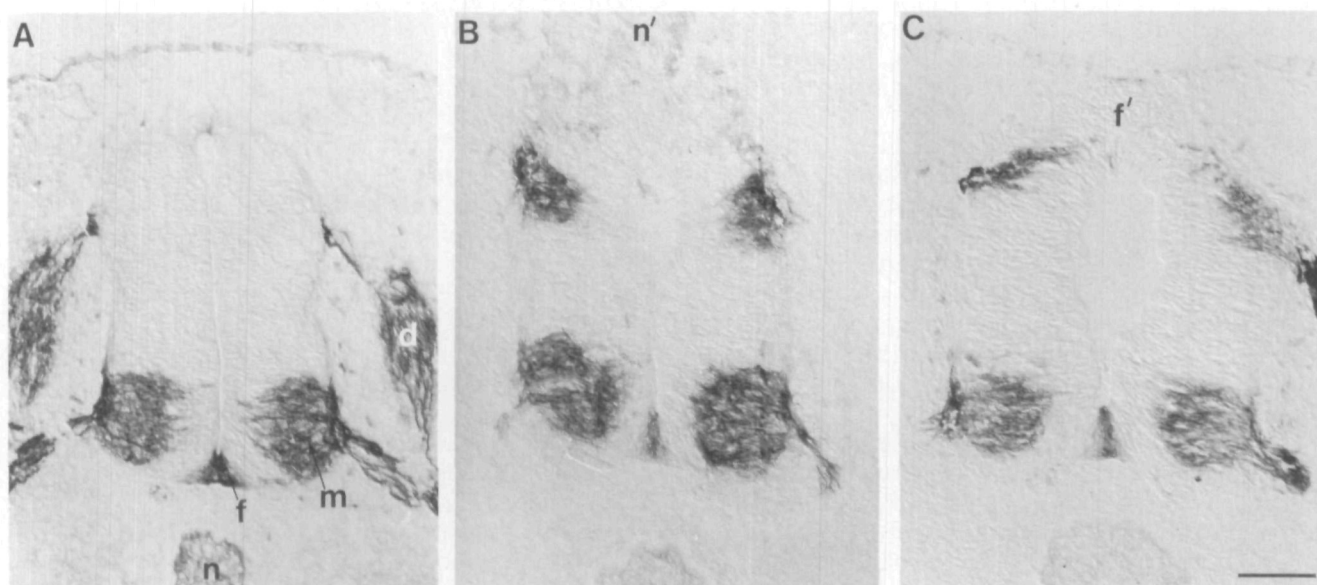
Fig. 5. Changes in the pattern of motor neurons in response to notochord and floor plate grafts. (A) In control stage 22 chick embryos, floor plate (f) cells and motor neurons (m) express the SC1 antigen. In contrast to the floor plate, the motor neuron columns do not span the width of the spinal cord, and labelling does not extend to the lumen. Within the spinal cord of embryos younger than stage 22, the SC1 antigen is expressed exclusively by these two cell types. Faint labelling of roof plate cells and preganglionic autonomic neurons occurs in older embryos. Dorsal root ganglion (d) neurons and their afferent fibres in the dorsal root entry zone, and the notochord (n) are also labelled. (B) A grafted notochord (n') located at the dorsal midline of the spinal cord results in the presence of four discrete columns of SC1-labelled motor neurons. Two of these columns are located in the ventral spinal cord near the host floor plate. The other two are located bilaterally in an ectopic dorsal position around cells that were shown in serial sections to express the FP1 antigen (see also Fig. 2B). (C) A graft of quail floor plate (f') located at the dorsal midline of the spinal cord results in the appearance of four SC1-positive motor neuron columns. The two dorsally-located ectopic motor neuron columns differentiate with bilateral symmetry with respect to the grafted floor plate. Abbreviations: f, floor plate; m, motor neuron column; d, dorsal root ganglion; n, host notochord; n', grafted chick notochord; f', grafted quail floor plate. Bars=(A) 85 μm; (B,C) 60 μm.

occupy a position between ectopic floor plate cells and motor neurons.

Decrease in dorsal cell types

We next examined whether the appearance of ventral cell types in dorsal regions after notochord grafts is accompanied by changes in the pattern of differentiation of dorsal cell types. The AC4 antigen is expressed by essentially all neuroepithelial cells at the neural plate stage. During neurulation, expression of the AC4 antigen disappears from neuroepithelial cells in a ventral to dorsal progression and by stage 17 the antigen is restricted to the intermediate and dorsal regions of the spinal cord with the exception of a small region near to the roof plate (Yamada *et al.* 1991). After this stage expression remains constant and is restricted to the dorsal half of the spinal cord. The progressive dorsal restriction of the AC4 antigen raises the possibility that its expression is repressed by signals that derive from the ventral midline.

To test this possibility, notochord grafts were placed next to the lateral part of the neural tube. A marked decrease in expression of the AC4 antigen was observed in the dorsal spinal cord adjacent to the graft (Yamada *et al.* 1991). Changes in the distribution of identified neurons in the dorsal and intermediate region of the

spinal cord were assessed by monitoring the expression of cellular retinoic acid-binding protein (CRABP), a marker of spinal relay neurons (Maden *et al.* 1989; Kitamoto *et al.* 1989). The number of CRABP-labelled neurons decreased after lateral notochord grafts (not shown). Thus, the appearance of ventral cell types in dorsal regions after notochord grafts is accompanied by a change in the pattern of differentiation of cell types characteristic of the dorsal spinal cord. As discussed later, the signals that control the differentiation of cells in the dorsal neural tube remain unclear.

Changes in neural cell pattern mediated by the floor plate

The change in neural cell pattern observed after notochord grafts raises the possibility that a cascade of local inductive signals may control the pattern of cell differentiation in the developing nervous system. Induction of the floor plate by the notochord would be the first step in this process. Signals from the floor plate may then control the differentiation of other cells along the dorsoventral axis of the neural tube. One prediction of this model is that the floor plate should be able to influence directly the pattern of neural cell differentiation. To test this, segments of floor plate were grafted adjacent to the neural tube of host embryos.

Floor plate grafts were found to produce changes in the pattern of cell differentiation along the dorsoventral axis of the spinal cord that were indistinguishable from the changes evoked by notochord grafts (Yamada et al. 1991). When a floor plate was grafted above the dorsal midline of a host neural tube, labelling with the MAb SC1 revealed the presence of two additional motor columns that differentiated in a bilaterally symmetric position with respect to the grafted floor plate (Fig. 5C). Similarly, lateral floor plate grafts induced an adjacent ectopic column of motor neurons. In addition, floor plate grafts were able to suppress the expression of markers such as the AC4 antigen that are characteristic of dorsal cell types.

To determine whether the change in pattern of cell differentiation was dependent on signals from the floor plate itself and not from flanking neural tissue, the region of neuroepithelium immediately adjacent to the floor plate and, in addition, the ventrolateral and dorsal regions of the neural tube were tested for their ability to induce motor neuron differentiation. No changes in the pattern of motor neuron differentiation were observed with such grafts (Yamada et al. 1991). Thus, the pattern of cell differentiation in the neural tube appears to be modified by signals that originate from the floor plate.
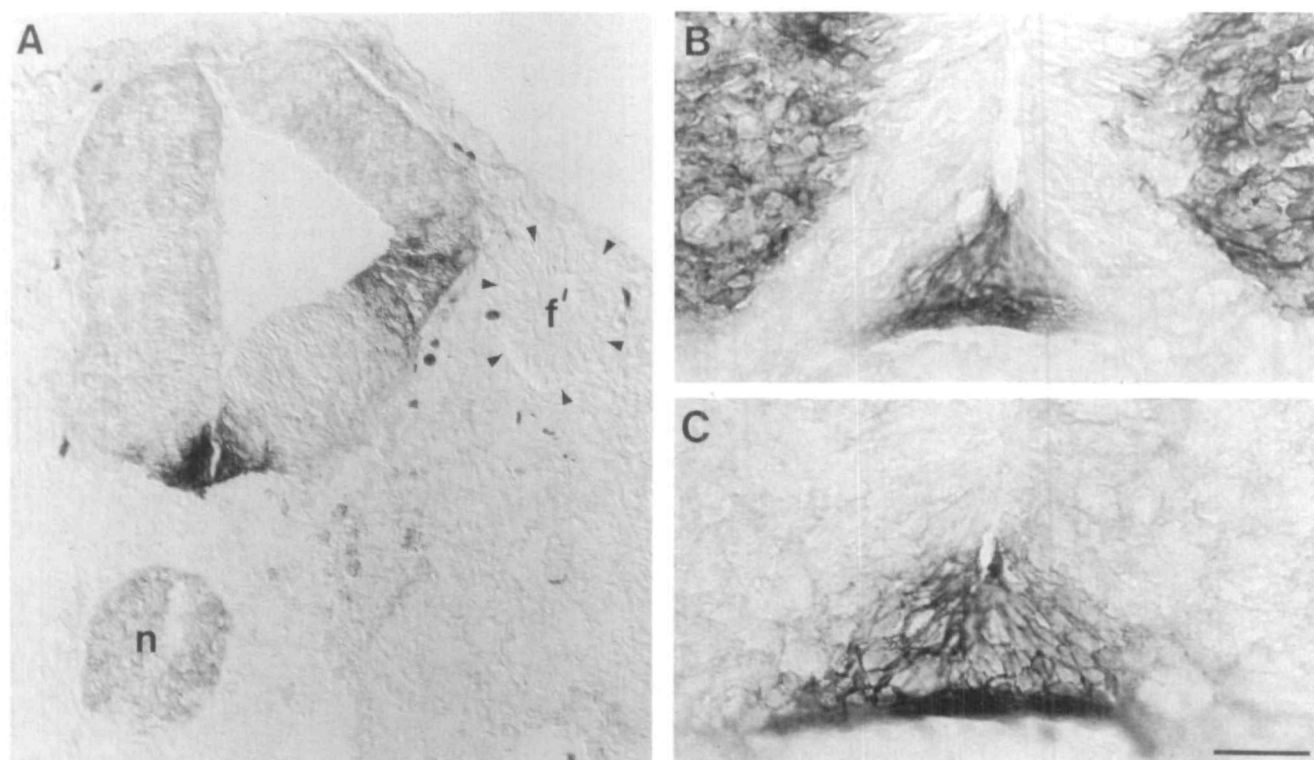
Homeogenetic induction by the floor plate

Fate mapping studies in avian embryos have shown that the floor plate and notochord derive from overlapping regions of the epiblast (Rosenquist, 1966, 1983; Nicolet, 1970; Hara, 1978; Schoenwolf et al. 1989; Selleck and Stern, 1991). Moreover, these two cell groups exhibit many common biochemical properties (Jessell et al. 1989) and signalling functions (Wagner et al. 1990). These observations raise the possibility that the floor plate mimics the notochord in its ability to induce floor plate differentiation. To test this, a segment of chick floor plate was grafted adjacent to the neural tube, and floor plate differentiation within the neural tube assayed by expression of antigenic markers. Grafts of quail and rat floor plate in combination with antibodies specific for chick floor plate antigens excluded the possibility that grafted floor plate cells invaded the host neural tube. Both the FP1 and SC1 antigens were induced in adjacent regions of the spinal cord after floor plate grafts (Fig. 6A) (Yamada et al. 1991). These results show that the floor plate can mimic the notochord in inducing floor plate differentiation in adjacent regions of the neuroepithelium, providing evidence that the floor plate has homeogenetic inductive properties.

The notochord may induce motor neurons independently of the floor plate

The results described in the preceding sections suggest that a sequential cascade of inductive interactions initiated by the notochord and perpetuated by the floor plate can operate to control the dorsoventral pattern of

Fig. 6. Homeogenetic induction by the floor plate. (A) A graft of quail floor plate (f′) positioned lateral to the neural tube induces expression of the FP1 antigen on cells in the adjacent neuroepithelium. Arrowheads indicate the extent of the floor plate graft. (B) Labelling of a control chick embryo (stage 20) with MAb SC1. (C) Labelling of an adjacent section from the same chick embryo as in (B) with MAb FP1. The expression of SC1 is restricted to the median population of floor plate cells. Abbreviations: n, notochord; f′, grafted quail floor plate. Bar=(A) 65 μm; (B,C) 40 μm.
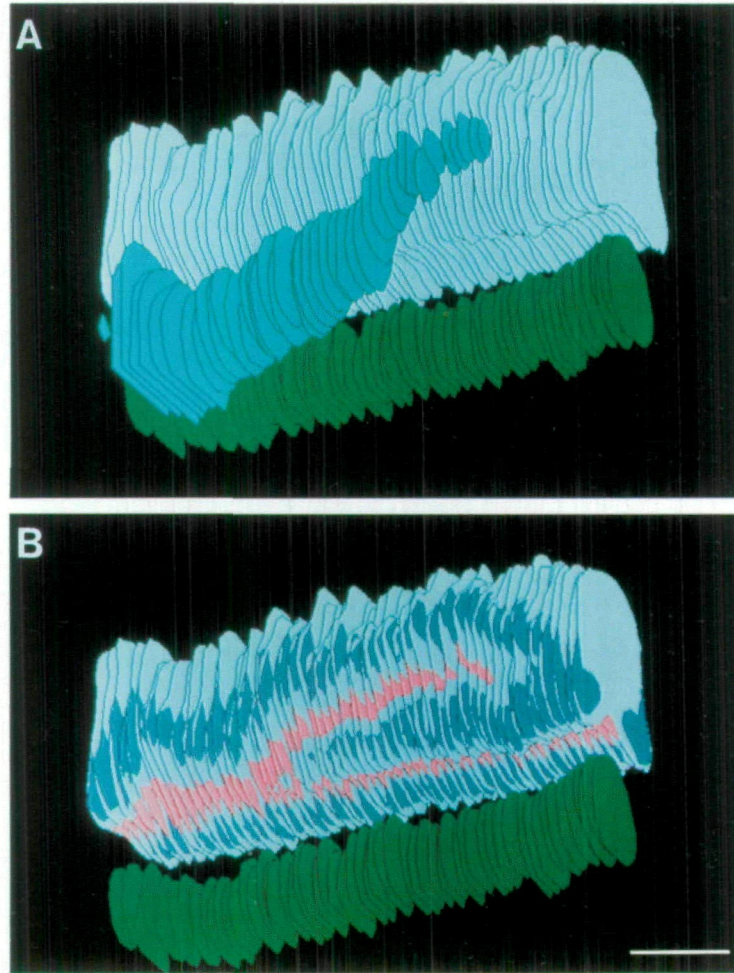
Fig. 7. Cell pattern varies according to the dorsoventral position of a grafted notochord. Three-dimensional reconstruction of an embryo in which the grafted notochord lies skewed with respect to the longitudinal axis of the host spinal cord. Reconstructions were performed by analyzing serial sections labelled, alternately, with MAbs Not-1 and SC1. (A) The relative positions with respect to the spinal cord and the sizes of the host (green) and donor (blue) notochords are shown. (B) The pattern of differentiation of both host and induced floor plates (pink) and host and induced motor neuron columns (dark blue). Regardless of the location of the floor plate along the dorsoventral axis of the spinal cord, the spacing between the floor plate and motor neuron columns is approximately constant. At the anterior end of the induced floor plate, an arc of motor neurons is induced. In the example shown, the end of the induced floor plate lies mid-way between the roof-plate and the floor plate. With the induced floor plate at this position, the sizes of the ipsilateral and contralateral ventral motor neuron columns are about equal. However, as the induced floor plate comes to lie more ventrally, there is a gradual decrease in the size of the ventral motor neuron column ipsilateral to the graft compared to that on the contralateral side. Finally, the column of motor neurons between the host and induced floor plates disappears. However, a region of unlabelled cells remains interposed between the floor plate and motor neurons. Bar=200 μm.

cell differentiation in the neural tube. However, it also possible that signals from the notochord c; regulate motor neuron differentiation directly witho inducing an intermediary floor plate.

To test this possibility a stage 10 notochord w grafted next to a stage 15–18 neural tube, which is 1 longer competent to respond by forming a recognizat floor plate (van Straaten *et al.* 1985*b*; Placzek *et* ι 1990*c*). In contrast to the results of notochord grafts younger hosts, expression of the FP1 antigen was n detected. Despite this, expression of the SC1 and 3A antigens revealed an increase in the size of the mot column adjacent to the notochord graft (Yamada *et* ι 1991). Further evidence for a direct effect of t notochord on motor neuron differentiation is provid-- by dorsal notochord grafts into younger embryos which, in some cases, were able to induce motor neurons in the absence of expression of floor plate antigens (Yamada *et al.* 1991). These results provide some evidence that the notochord can induce motor neurons without prior induction of a floor plate. It remains possible, however, that in both experiments, notochord grafts induced partial floor plate properties which include the ability to induce motor neurons.

Neural cell identity is defined by position with respect to the floor plate

One feature to emerge from these results is the consistency of the position at which motor neurons appear in relation to the floor plate. This is apparent in reconstructions of embryos in which notochord grafts were placed diagonally with respect to the dorsoventral axis and thus occupied a series of different dorsoventral positions adjacent to the spinal cord (Fig. 7A). In dorsal regions, the notochord graft induced an additional floor plate in the adjacent neuroectoderm and an ectopic dorsal motor neuron column at a distance from the induced floor plate (Fig. 7B). As the graft approached more ventral positions the distance between the host and induced floor plates decreased and there was a progressive decrease in the size of the intervening ventral column of motor neurons (Fig. 7B). The decrease in the area occupied by the ventral motor neuron column was greater, proportionally, than the decrease in area of the region of neuroepithelium between the two floor plates (Fig. 8). Thus, at distances between the two floor plates of less than 80 μm, motor neurons were absent from the intervening region. When the grafted notochord was located immediately adjacent to the host notochord the ventral midline had a single expanded floor plate (Fig. 2D). The simplest interpretation of these findings is that motor neuron differentiation is determined primarily by position with respect to the floor plate.

Changes in neural cell pattern in the absence of the floor plate and notochord

To examine whether the notochord and floor plate are required for regulating the pattern of cell differentiation in the neural tube during normal development, a segment of notochord underlying the caudal ventral
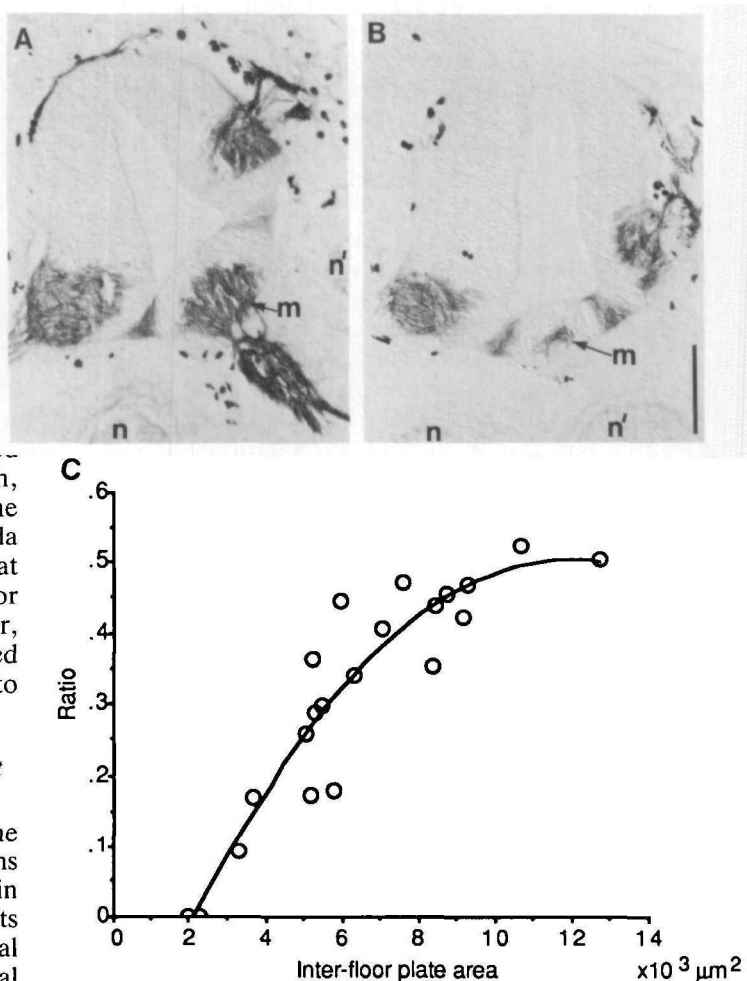
Fig. 8. Suppression of motoneuron differentiation by two closely apposed floor plates. (A) A grafted notochord (n') that lies lateral to the host neural tube induces an ectopic floor plate and a dorsally-located ectopic motor neuron column, revealed by labelling with MAb SC1. The size of the motor neuron column (m) between the host and induced floor plates, is comparable to that of the contralateral motor column. (B) A section taken from a more caudal region of the same embryo. Labelling with MAb SC1 reveals that the grafted notochord (n'), occupies a more ventral position and has thus induced a floor plate ventrally. The size of the motor neuron column (m) between the host and induced floor plates is significantly smaller than that on the control side. (C) The area of the motor neuron pool (m) between host and induced floor plates, measured as a function of the inter-floor plate area. The decrease in total area of the neuroepithelium between the two floor plates is less than the decrease in area of the motor column. The ratio of the motor neuron column area to the inter-floor plate neuroepithelial area is plotted (abscissa) against the inter-floor plate area (ordinate). Abbreviations: n, host notochord; n' grafted notochord; m, motor neuron column between host and induced floor plates. Bar=(A,B) 120 μm.

midline of stage 10–12 embryos was removed, thus preventing the development of the floor plate at these segmental levels (Placzek *et al.* 1990*c*; Yamada *et al.* 1991). The floor plate extended for about 60 μm into the

region of neural tube from which the underlying notochord had been removed. Further into the notochord-free region, the expression of floor plate antigens disappeared although distinct lateral motor columns were still present for a further 60 μm. Beyond this point, the bilateral motor columns merged to form a single expanded column at the ventral midline of the neural tube. Thus an arc of motor neurons differentiates around the end of the floor plate. At distances greater than 100 μm from the end of the floor plate, motor neurons were absent. These results support the idea that motor neurons differentiate at a constant position with respect to the floor plate.

Analysis of the cell types remaining in the spinal cord after elimination of the notochord and floor plate revealed a marked difference in the dependency of distinct neuronal classes on ventral midline-derived signals. Although there was a complete absence of motor neurons (Fig. 9A,B), other cell types normally found in the dorsal spinal cord were present over an expanded dorsoventral domain. First, after removal of the notochord, the AC4 antigen which is normally absent from ventral regions, was expressed along the entire dorsoventral extent of the spinal cord, except for the small region near the roof plate. Second, sensory relay neurons that normally differentiate in the dorsal and intermediate regions of the spinal cord and express a cellular retinoic acid binding protein (CRABP) (Maden *et al.* 1989; Kitamoto *et al.* 1989) (Fig. 9C) were located at all dorsoventral positions including the ventral midline after notochord removal (Fig. 9D).

Moreover, in normal embryos, sensory relay axons remain within the spinal cord with commissural axons projecting to and across the floor plate (Fig. 9C). However, after notochord removal, the axons of CRABP-labelled sensory relay neurons projected out of the spinal cord (Fig. 9D). These results show that in the absence of a floor plate and motor neurons, sensory relay neurons in the ventral spinal cord exhibit an abnormal axonal projection pattern reminiscent of that of motor neurons in normal embryos. The outgrowth of commissural axons into the mesenchyme surrounding the spinal cord has also been observed after ectopic floor plate grafts (Placzek *et al.* 1990*b*) and in mouse embryos homozygous for mutations at the *Danforth's short tail* locus in which the floor plate is absent (Bovolenta and Dodd, 1991). These results emphasize the importance of using cell-type specific markers rather than cell position and axonal trajectory to analyze the control of cell identity in the developing nervous system.

More generally, the changes in neural cell pattern observed in the absence of the notochord and floor plate support the idea that the differentiation and patterning of many distinct cell types in the ventral region of the nervous system is controlled by midline-derived signals.

Discussion

The experiments described here provide evidence that

cell patterning within the neural tube is controlled by signals that derive from two axial midline cell groups, the notochord and the floor plate. An early step in the establishment of cell pattern within the neural tube is the induction of the floor plate by the notochord. The floor plate may then regulate the pattern of cell differentiation along the dorsoventral axis of the neural tube. Although the mechanisms underlying these patterning events have not been resolved, the identity of neuroepithelial cells in the ventral region of the neural tube appears to be defined by position with respect to the ventral midline (Fig. 10). These observations support the idea that cell fate within the developing nervous system, as in other embryonic tissues (Chasan and Anderson, 1991; Tickle *et al.* 1975), depends largely on the interpretation of positional signals that derive from local sources (Wolpert, 1969). In vertebrates such polarizing signals appear to derive from discrete cell groups with specialized organizing properties (Spemann, 1938; Gerhart and Keller, 1986).

Induction of the floor plate

Studies of amphibian and avian embryos have provided evidence that the cells that will eventually populate the midline of the neural plate undergo extensive cell rearrangements and possess specialized migratory properties prior to overt signs of neural differentiation (Jacobson, 1981; Keller *et al.* 1985; Alvarez and Schoenwolf, 1991). However, the acquisition of the specialized signalling properties of the floor plate that control neural cell pattern and axonal trajectory require local inductive signals from the notochord. The demonstration of floor plate induction *in vitro* (Figs 3 and 4) indicates that notochord-derived signals are sufficient to induce floor plate properties in the absence of factors supplied by other embryonic cell types. In addition, these *in vitro* studies suggest that contact between the notochord and neural plate is required for induction of the floor plate. The contact-dependence of floor plate induction could reflect the membrane anchoring of an inductive ligand, the requirement for a high concentration of a diffusible factor, or the sequestration of a diffusible signal in the extracellular matrix that surrounds the notochord.

Studies in other vertebrate species have provided evidence consistent with the induction of the floor plate by the notochord. UV-treatment of fertilized *Xenopus* eggs can lead to the development of tadpoles lacking a notochord. The neural tube of these embryos lacks ventral midline cells with characteristics of the floor plate (Youn and Malacinski, 1981; Clarke *et al.* 1991). In addition, analysis of neural differentiation in the embryonic mouse mutants *Danforth's short tail* and *truncate* in which the notochord fails to differentiate or undergoes early degeneration has shown that the caudal region of the spinal cord lacks a morphogenetically- and antigenically-defined floor plate (Theiler, 1959; Bovolenta and Dodd, 1991). However, at more rostral regions of the spinal cord of *Danforth's short tail* embryos the floor plate is present even though the notochord is absent (Bovolenta and Dodd, 1991). This
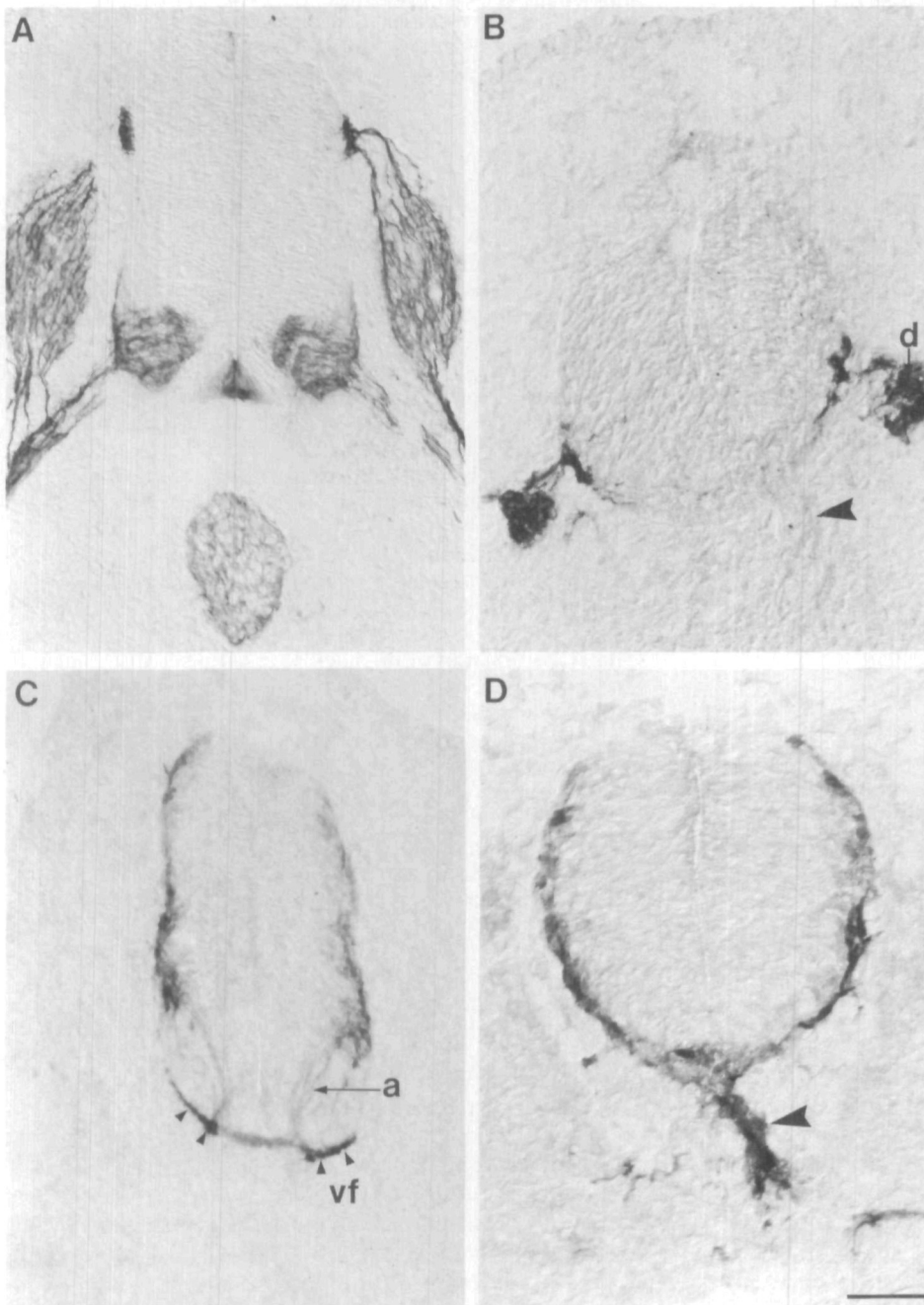
Fig. 9. Dorsal cell types differentiate in ventral positions in the absence of a floor plate. (A) Section through the spinal cord of a control stage 20 chick embryo, labelled with MAb SC1. In the spinal cord, floor plate cells and motor neurons are labelled. Dorsal root ganglion neurons and their fibres are also labelled, as is the notochord. (B) Section through the spinal cord of a chick embryo from which a segment of notochord had been removed at stage 10, labelled with MAb SC1. Notochord, floor plate cells and motor neurons are absent. Dorsal root ganglia (d) form in the absence of the notochord and floor plate. However, the ganglia often occupy an unusually ventral position. A ventral fibre bundle can be seen leaving the spinal cord (arrowhead). (C) Section through a control chick embryo labelled with an antibody against cellular retinoic acid binding protein (CRABP). Sensory relay neurons differentiate in the dorsal and intermediate regions of the spinal cord, and project axons (a) to and across the ventral midline. At the floor plate axons turn to join the ventral funiculi (vf and small arrowheads). Axons do not project out of the spinal cord. (D) Adjacent section through the embryo shown in (B). Differentiated sensory interneurons, labelled with an antibody against CRABP are found along the entire dorso-ventral axis of the neural tube. Their axons project out of the spinal cord. These axons are not labelled by MAb SC1 (see arrow in B). Bar=(A) 80 μm; (C) 90 μm; (B,D) 50 μm.
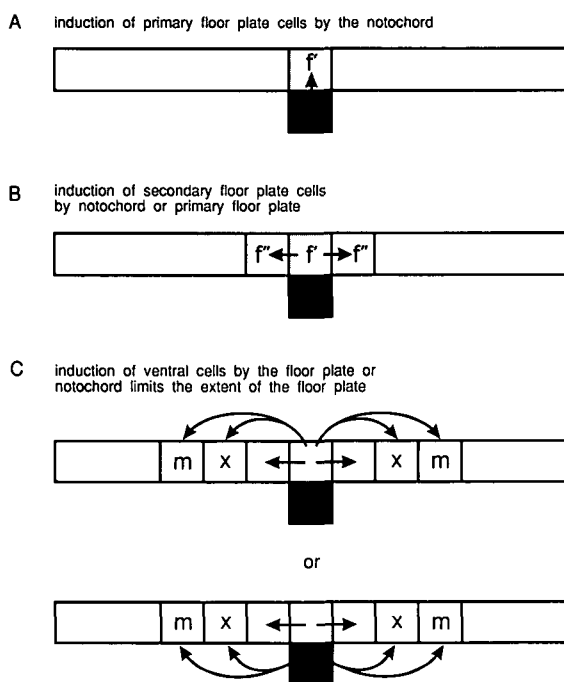
may result from the persistence of primitive notochord cells for a time sufficient to induce a floor plate in anterior regions.

Relative contributions of the notochord and floor plate to the control of neural cell pattern

The dependence of floor plate differentiation on inductive signals from the notochord makes it difficult to distinguish whether the dorsoventral patterning of the neural tube is controlled by signals from the notochord, the floor plate or from both cell groups.

Evidence discussed below suggests that signals from either the floor plate or the notochord may be sufficient to control the differentiation of distinct cell types in the ventral neural tube. Nevertheless, it is difficult at present to rule out the possibility that polarizing signals are synthesized only by the notochord and merely sequestered by the floor plate. The slow release of such signals could then account for the apparent polarizing activity of the floor plate in grafting experiments.

The advantage of two midline cell groups with similar or identical signalling properties is not clear. This

A   Induction of primary floor plate cells by the notochord

B   Induction of secondary floor plate cells by notochord or primary floor plate

C   Induction of ventral cells by the floor plate or notochord limits the extent of the floor plate

or

Fig. 10. Sequential induction of ventral neural tube cells by midline-derived signals. Schematic diagrams which provide a model for the sequence of inductive interactions that establish the identity of neural cells in the ventral neural tube and CNS. (A) The initial acquisition of floor plate (f') properties by cells at the midline of the neural plate (and neural tube) depends on inductive signals from the notochord (n). The induction of the floor plate is likely to require proximity or contact with the notochord since only neural plate cells in direct contact with the notochord acquire early floor plate properties. In vitro studies on floor plate induction also indicate that contact between the notochord and neural plate is required for the expression of floor plate properties (Placzek et al. 1990c). (B) The floor plate mimics the notochord in its ability to induce floor plate (f'') properties in undifferentiated neuroepithelial cells. Grafting experiments in chick and zebrafish embryos have revealed that floor plate cells have homeogenetic inductive properties. In the context of normal neural tube development, the initially induced (primary) floor plate cells (f') may induce floor plate properties in adjacent neuroepithelial cells. These later differentiating floor plate cells may not acquire all the antigenic and functional properties of the floor plate and may, therefore, correspond to secondary (f'') floor plate cells. One function of the lateral recruitment of secondary floor plate cells may be to maintain the lateral dimensions of floor plate despite the intercalation of primary floor plate cells along the midline that occurs during elongation of the neural tube (Jacobson, 1981; Keller and Danilchick, 1988; Wilson et al. 1989; Fraser et al. 1990). The notochord could also be the source of signals that induce secondary floor plate differentiation (C). The ability of the floor plate and notochord to induce the differentiation of other ventral cell types, for example, P–L neurons in the X region (x) and motor neurons (m) may prevent the lateral propagation of the floor plate-derived homeogenetic inductive cascade. The onset of differentiation of x and m cells may deplete the neuroepithelium of undifferentiated precursors that can respond to floor plate-derived signals. This model suggests that midline-derived signals that specify the fate of x and m cells act over a longer range than the signal responsible for floor plate induction.

apparent redundancy may increase the fidelity with which the pattern of cell differentiation within the neural tube is established. At the neural plate stage, the notochord is in direct contact with the midline of the neural plate and may initiate the mediolateral patterning of the nervous system by inducing the floor plate. However, during subsequent stages of neurulation, the notochord is displaced ventrally, becomes surrounded by an extracellular matrix and is separated from the neural tube by mesenchymal cells (Jurand, 1962). Thus, if the notochord were the sole source of signals that control the pattern of cell differentiation along the dorsoventral axis of the neural tube, the entire pattern would have to be established at a time when the notochord is still in proximity to the neural ectoderm. The acquisition of polarizing properties by the floor plate provides a mechanism by which midline-derived signals could continue to impose pattern on the adjacent neuroepithelium at stages after the displacement of the notochord.

Homeogenetic induction and heterogeneity of the floor plate

One of the apparent signalling properties of the floor plate is the ability to induce additional floor plate cells (Fig. 10). Evidence for this derives from grafting experiments in chick embryos, in which rat, quail or chick floor plate can induce floor plate-specific antigens in the adjacent neuroepithelium (Fig. 6A and Yamada et al. 1991). Similar conclusions have been reached from an analysis of zebrafish *cyclops* mutant embryos which lack a recognizable floor plate even though the notochord is present (Hatta et al. 1991). Wild-type ectodermal cells transplanted into a *cyclops* host embryo can populate the midline of the neural tube and acquire antigenic and morphological characteristics of the floor plate (Hatta et al. 1991). This suggests that the

cyclops mutation affects the response properties of the neural ectoderm to notochord-derived signals rather than the signalling properties of the notochord. In addition, mutant host midline cells that are adjacent to wild-type floor plate cells also acquire floor plate morphology. These observations suggest that wild-type cells can be induced by the notochord to differentiate into floor plate cells, and then are able to induce some floor plate properties in adjacent *cyclops* neuroepithelial cells. Both the notochord and floor plate, therefore, appear to have homeogenetic inductive properties. The notochord induces notochord-like signalling properties in overlying neuroepithelial cells and newly differentiated floor plate cells induce floor plate properties in adjacent neuroepithelial cells.

The ability of wild-type floor plate cells to induce floor plate properties in *cyclops* neuroepithelial cells that are refractory to notochord-derived signals (Hatta et al. 1991) also raises the possibility that the notochord and floor plate are sources of distinct signalling

molecules, each with the capacity to induce the floor plate. However, the *cyclops* result could also be obtained if the mutation attenuates the response of neuroepithelial cells to a common signal that is provided at greater concentration by the floor plate than by the notochord.

The ability of the floor plate to induce additional floor plate cells together with the apparent contact-dependence of floor plate induction provides a potential mechanism for maintaining the lateral dimensions of the floor plate during early neural tube development. In chick and amphibian embryos, cells that eventually occupy the midline of the neural plate undergo convergent extension movements in which cell intercalation results in the longitudinal expansion of the set of cells that initially occupy the midline (Jacobson *et al.* 1986; Schoenwolf and Alvarez, 1989; Schoenwolf and Smith, 1990; Fraser *et al.* 1990). If the number of floor plate cells remained constant, these movements would result in a progressive decrease in the lateral dimensions of the floor plate. However, analysis of floor plate antigen expression in chick embryos indicates that the width of the floor plate does not decrease during elongation of the neural tube and, if anything, increases (Yamada *et al.* unpublished observations). Maintenance of the lateral dimensions of the floor plate could be achieved if the initial population of midline floor plate cells were to recruit lateral neuroepithelial cells into the floor plate by homeogenetic induction. The notochord may therefore induce primary floor plate properties in those cells at the midline of the neural plate that are in direct contact with it. A second phase of floor plate development appears to involve the induction of secondary floor plate properties in cells of the adjacent neuroepithelium (Fig. 10).

The homeogenetic inducing activity of the floor plate requires a mechanism for preventing the lateral propagation of this signal throughout the neuroepithelium, converting the entire neural tube into a floor plate. One possibility is that secondary floor plate cells are not able to recruit additional floor plate cells. A second possibility is that the notochord and/or floor plate provide long range signals that induce more lateral neural plate cells to differentiate into ventral neural cell types such as cells in region X and motor neurons (Fig. 10). The specification of these ventral cell types may occur over the same time period, or shortly after the time that the midline cells acquire primary floor plate properties. Thus, by the time that secondary floor plate cells have acquired homeogenetic inducing properties only a small number of cells remain uncommitted and thus available for additional recruitment into the floor plate.

A distinction between primary and secondary floor plate cells is further suggested by the heterogeneity observed in the antigenic properties of floor plate cells in vertebrate embryos. In the chick, the SC1 antigen is restricted to the medial subset of floor plate cells that express the FP1 antigen (Fig. 6B, C and Yamada *et al.* 1991). Similarly in rat embryos the K1 antigen (Dodd and Jessell, 1988) defines a medial subset of floor plate cells that express the P84/6G3 antigen (Chang and Lagenauer, 1990; Jessell and Morton, unpublished observations) and the P35 protein (McKanna and Cohen, 1989). There may also be cellular heterogeneity in the floor plate of zebrafish embryos. The morphological and antigenic features of the single cell that occupies the ventral midline of the spinal cord differ from those of the adjacent lateral cells (Hatta *et al.* 1991). These two lateral cells in the zebrafish spinal cord exhibit properties characteristic of the rat floor plate (Bovolenta and Dodd, 1990) in that commissural axons appear to alter their trajectory from transverse to longitudinal growth in contact with these lateral cells rather than with the midline cell (Kuwada *et al.* 1990*a,b*).

Control of motor neuron differentiation

The cellular mechanisms involved in the differentiation of discrete neuronal cell types, for example motor neurons, in response to notochord and floor plate-derived signals remain unclear. Motor neuroblasts derive from the ventricular zone in the ventral region of the neural tube and migrate laterally to form a discrete cluster of motor neurons beginning at stage 15 (Langman and Haden, 1970; Hollyday and Hamburger, 1977). The increase in the total number of motor neurons after notochord and floor plate grafts (Yamada *et al.* 1991) excludes the possibility that the appearance of ectopic motor neurons results from the redistribution of a fixed number of motor neuron precursors. Moreover, there is very little cell death in the lateral neural tube before stage 15 (Homma *et al.* 1990) which makes it unlikely that the appearance of ectopic motor neurons results from the rescue of a population of committed motor neuron progenitors. It seems more likely that the differentiation of motor neurons in ectopic regions of the spinal cord, in response to notochord or floor plate grafts, results from a change in the fate of pluripotent neuroepithelial progenitors. In support of this, lineage analysis of retrovirally marked cells in the chick neural tube indicates that motor neurons derive from progenitors that give rise to other classes of neurons and also to glial cells (Leber *et al.* 1990). Such multipotential progenitors may be distributed throughout the neural tube with signals from the floor plate and notochord required for their differentiation into motor neurons (Fig. 10). Progenitors that are not exposed to floor plate signals appear to acquire dorsal cell fates (see following section).

This view of the interactions controlling motor neuron differentiation differs from that of Hirano *et al.* (1991), who surgically deprived one side of the neural tube of stage 12 chick embryos of a floor plate. Histological examination of such embryos led to the proposal that neurons located in dorsal regions of the neural tube can give rise to motor neurons but normally are inhibited from doing so by signals from the floor plate. Our experiments suggest that by stage 12 the floor plate has already differentiated, and that the specification of motor neurons by the floor plate and/or notochord has already occurred (Yamada *et al.* 1991).

Thus, elimination of the notochord or separation of the floor plate from one side of the neural tube at stage 12 or later may not result in the conversion of dorsal neural tube cells into motor neurons as proposed by Hirano *et al.* (1991). Instead, the presence of motor neurons in dorsal regions may reflect the dorsal displacement of the normal column of motor neurons as a result of distortion of the neuroepithelium caused by slitting the ventral midline of the neural tube.

The influence of the notochord and other mesodermal tissues on the differentiation of ventral neuroblasts in the chick neural tube has been examined previously. Inversion of the neural tube along its dorsoventral axis at stage 11–12 has been reported to result in the appearance of ventral characteristics in the formerly dorsal spinal cord, although specific cell types were not identified (Steding, 1962). Similarly, notochord grafts placed next to the neural tube have been shown to lead to the proliferation of ventral neuroblasts (van Straaten *et al.* 1985*b*, 1988). In addition, grafts of notochord and muscle next to the spinal cord of older embryos have been shown to increase the proliferation of ventral neurons (van Straaten *et al.* 1985*b*; van Straaten and Drukker, 1987; van Straaten *et al.* 1989; Fontaine-Perus *et al.* 1989). However, it is unclear if the proliferative effects of grafts into older embryos are related to the changes in cell pattern observed in young embryos.

Control of cell differentiation in the dorsal neural tube
Distinct neuronal cells within the spinal cord differ markedly in their dependency on ventral midline-derived signals (Yamada *et al.* 1991). Elimination of the notochord and floor plate results in the absence of motor neurons but other classes of cells including neural crest cells and commissural neurons, are present. The differentiation of the dorsal neural tube therefore does not appear to depend on the notochord and floor plate. Thus, the acquisition of dorsal cell properties may represent the constitutive fate of neuroepithelial cells. An alternative possibility is that neuroepithelial cells in the ventral region of the neural tube require floor plate and notochord-derived signals for their proliferation. In the absence of these signals, these ventral cells fail to divide, resulting primarily in a deletion rather than a change in fate of cells in the ventral neural tube.

The differentiation of neuroepithelial cells into dorsal cell types may require signals from other cell groups, perhaps the roof plate. Indirect support for the existence of roof plate-derived signals that affect neural tube patterning comes from analysis of chick embryos which have received dorsal grafts of notochord or floor plate (Yamada *et al.* 1991). The induction of a floor plate in response to a dorsally placed notochord or floor plate occurs only when fusion of the dorsal midline of the neural tube fails, presumably preventing roof plate differentiation. Thus, signals from the roof plate may render the adjacent dorsal neural tube refractory to notochord and floor plate-derived signals. The nature of such roof plate-derived signals is not known but several members of the *wnt* family of molecules are

localized in and around the roof plate (Wilkinson *et al.* 1987; Roelink and Nusse, 1991).

Control of neural cell pattern by contact-dependent or diffusible signals
The floor plate and notochord could influence the pattern of cell differentiation within the neural tube by at least three different strategies.

First, a cascade of locally-acting, possibly contact-dependent signals might originate from the floor plate, with each cell type responsible for inducing its dorsolateral neighbors (Fig. 11A). The inability of grafts of the region of neuroepithelium immediately adjacent to the floor plate to induce motor neurons provides evidence against such a model. However, this does not exclude a second scheme (Fig. 11B), in which the specification of different cell types depends both on contact-dependent signals and a long range diffusible signal that derives solely from the notochord and/or floor plate. Thirdly, cell differentiation in the ventral neural tube could be controlled solely by signals from the floor plate and notochord (Fig. 11C). For example, the notochord and floor plate could act as local sources of a factor that diffuses through the adjacent neuroectoderm, establishing a concentration gradient with its high point at the ventral midline (Fig. 11C). In this scheme, cell identity and the overall pattern of cell types would be defined by the concentration of diffusible signal to which precursor neuroepithelial cells are exposed (Yamada *et al.* 1991). Neural plate cells exposed to highest concentrations of this factor would differentiate into floor plate; cells exposed to a lower concentration would differentiate into cells of region X and cells exposed to a still lower concentration would differentiate into motor neurons. A variation on this model is that multiple signals from the floor plate or notochord have different ranges of action, thus specifying distinct cell types at different distances from the ventral midline.

Graded signals have been invoked in the generation of cell pattern along the anteroposterior axis of the developing chick wing bud (Tickle *et al.* 1975). There, anteroposterior pattern appears to be under the control of a specialized region of posterior mesoderm known as the zone of polarizing activity (ZPA), which can evoke mirror-image digit duplications when grafted to ectopic sites (Tickle *et al.* 1975). Retinoic acid mimics the effects of the ZPA (Tickle *et al.* 1982) and appears to be distributed unevenly along the anteroposterior axis of the limb bud with its highest concentration in the posterior mesenchyme (Thaller and Eichele, 1987). On this basis, it has been suggested that retinoic acid functions as an endogenous morphogen involved in establishing axial polarity in the developing chick limb (see Brockes, 1989; Eichele, 1989).

The notochord and floor plate, but not other regions of the neural tube, mimic the action of the ZPA and retinoic acid in respecifying digit pattern in the chick limb (Wagner *et al.* 1990). Biochemical studies show that the floor plate can synthesize and release active retinoids *in vitro* (Wagner *et al.* 1990). Moreover, the γ
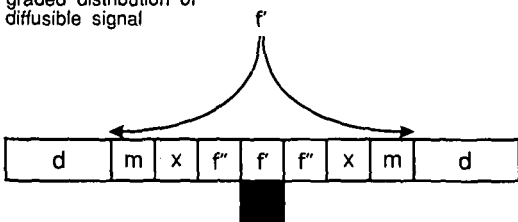
A cascade of contact-dependent signals
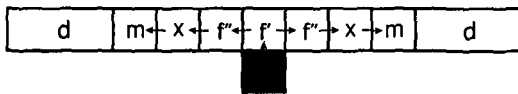
B cooperation of diffusible and contact signals
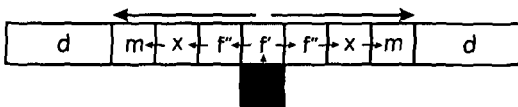
C graded distribution of diffusible signal

Fig. 11. Models for the patterning of cell types in the developing neural plate and neural tube. (A) This model invokes the lateral propagation of a cascade of inductive signals that are dependent on contact between neuroepithelial cells. A single signal, or multiple distinct signals are transmitted through the neuroepithelium. The identity of individual cells may depend on the qualitative or quantitative nature of the signal or on temporal changes in the response of neuroepithelial cells to this signal. This contact-dependent signal does not affect more lateral regions of the neural plate. One major feature of this model is that the notochord and floor plate provide only an initial trigger for the patterning of neural cells with subsequent steps occurring independently of midline cells. (B) In this model a contact-dependent cascade similar to that in (A) operates but in conjunction with a midline-derived signal from the floor plate or notochord. This midline-derived signal may provide a single threshold with information about cell pattern requiring contact-dependent interactions between neural cells. For further details see text. (C) In this model, the floor plate and/or notochord provide the source of a single diffusible signal which establishes a concentration gradient in the adjacent neuroepithelium. Cells located close to the midline are exposed to a high concentration of this signal and differentiate into primary (f') or secondary (f'') floor plate cells. Cells located more laterally differentiate into x or m cells depending on the concentration of the signal to which they are exposed. Cells in more lateral or dorsal regions differentiate into dorsal cells types independently of this ventral midline derived signal. The differentiation of dorsal cells may require a distinct instructive signal. For further details see text.

and β retinoic acid receptors are expressed at high levels in the neural plate and neural tube (Smith and Eichele, 1991; Ruberte *et al.* 1991). The ability of the notochord and floor plate to control the pattern of cell differentiation in both the developing limb bud and neural tube may therefore have a common molecular basis, possibly involving retinoids. However, grafts of the ZPA or of retinoic acid-impregnated beads do not mimic the floor plate or notochord in changing the pattern of cell differentiation in the neural tube (Yamada, unpublished observations). Thus, retinoids released by the floor plate may have functions independent of dorsoventral patterning or act only in combination with additional factors.

Candidate genes that may be involved in the response of undifferentiated neuroepithelial cells to floor plate and notochord-derived signals have been identified. Several of the Hox genes exhibit dorsoventral restrictions in their expression pattern in the early spinal cord (Graham *et al.* 1991). Similarly, Pax genes are expressed in restricted regions along the dorsoventral axis of the neural tube and spinal cord (Goulding *et al.* 1991*a*). In particular, Pax-3 exhibits a striking dorsal restriction within the neural tube (Goulding *et al.* 1991*b*). Finally, Mash-1, a mammalian helix-loop-helix protein is expressed in cells in region X, immediately adjacent to the floor plate (Lo *et al.* 1991).

Control of neural cell pattern in different vertebrates

The cellular signals involved in the control of neural cell pattern are likely to be conserved in different vertebrate species. However, recent studies on *Xenopus* embryos suggest that the time of action and cellular origin of these signals may differ. For example, in UV-treated *Xenopus* embryos which lack a notochord and floor plate the spinal cord contains primary motor neurons, but appears to lack secondary motor neurons (Clarke *et al.* 1991). Other neural cell classes, in particular Rohon-Beard neurons (Lamborghini, 1980) and Kolmer-Agdhur cells (Dale *et al.* 1987) also appear in UV-treated embryos (Clarke *et al.* 1991). A likely reason for this difference is that in *Xenopus* the specification of neuronal cell types such as primary motor neurons (Hughes, 1959; van Mier *et al.* 1985; Nordlander, 1986) and Rohon-Beard neurons (Lamborghini, 1980) occurs during early gastrulation, before overt signs of neural plate formation. These results indicate that in *Xenopus* the determination of primary neurons must be controlled by signals that derive from cells other than the floor plate.

The absence of secondary motor neurons in the spinal cord of notochordless *Xenopus* embryos (Clarke *et al.* 1991) suggests that the differentiation of the majority of motor neurons and perhaps other neurons that are specified at later stages is dependent on the notochord and floor plate. Recent studies of the development of neural cell types in the ectoderm of *Xenopus* exogastrulae have provided support for this idea (Ruiz i Altaba, personal communication). Neural cells characteristic of secondary motor neurons are virtually absent from the exogastrula ectoderm even

though many other classes of neural cells are present. Secondary motor neurons can, however, be induced in exogastrula ectoderm by implanting a notochord, consistent with the idea that signals from the notochord and floor plate are required. In birds and mammals, the determination of most classes of neurons including motor neurons does not appear to occur until after the onset of neurulation (Hollyday and Hamburger, 1977). Thus, most neurons in the avian and mammalian spinal cord may correspond to the secondary neurons in *Xenopus* with respect to the source of the signals involved in their differentiation.

In apparent contrast to observations in *Xenopus* (Clarke *et al.* 1991) the analysis of motor neuron differentiation in the zebrafish *cyclops* embryos indicates that both primary and secondary motor neurons are present despite the apparent absence of a floor plate (Kimmel *et al.* 1991; Eisen, 1991). The presence of primary motor neurons in *cyclops* embryos is likely to reflect the existence of inductive signals that operate during gastrulation, as described above in *Xenopus*. However, the presence of secondary motor neurons in *cyclops* differs from expectations based on analysis of UV-treated *Xenopus* embryos and notochordless chick embryos. There are two possible explanations for this difference. First, the absence of morphological and antigenic features of the midline floor plate cell in *cyclops* embryos does not rule out that partial floor plate properties remain and that these are sufficient to induce other ventral cell types including secondary motor neurons. Alternatively, as discussed above, the notochord may be able to control motor neuron differentiation directly, in the absence of a floor plate. If this is the case the *cyclops* mutation would appear to block the signalling pathway involved in floor plate differentiation without affecting the pathway involved in motor neuron specification. This raises the possibility that the notochord activates distinct signalling pathways that control floor plate and motor neuron differentiation.

Control of neural cell identity along the anteroposterior axis

The notochord and floor plate may also contribute to the pattern of neural cell differentiation along the anteroposterior axis of the neural tube. Many classes of neurons are absent from the forebrain and restricted to more posterior regions of the CNS. For example, the most anterior group of motor neurons, the oculomotor nucleus, is located in the midbrain near the anterior end of the floor plate. Thus, motor neurons represent one of several classes of neurons found at more posterior regions of the CNS which appear to be dependent on the floor plate for their differentiation. These observations reinforce the suggestions of Ahlborn (1883) and Kingsbury (1930) that the anteroposterior domain of the neuraxis over which motor neurons differentiate is defined by the notochord and floor plate.

Studies of amphibian neural development have provided evidence that regional differentiation along the anteroposterior axis is established during the early development of the neural plate, before that along the dorsoventral axis (Mangold, 1933; Roach,1945; Jacobson, 1964; Ruiz i Altaba and Melton, 1989). These findings, together with the present results suggest that the fate of a cell within the nervous system is restricted initially by its anteroposterior position within the neural plate. The location of cells along the dorsoventral axis of the neural tube with respect to the floor plate appears later to determine which of a limited number of possible fates, appropriate for a given anteroposterior position, is selected.

References

AHLBORN, F. (1883). Untersuchungen uber das Gehirn der Petromyzonten. *Z. wiss. Zool.* **39**, 191–294.

ALTMAN, J. AND BAYER, S. (1984). The development of the rat spinal cord. *Adv. Anat. Embryol. Cell Biol.* **85**, 1–164.

ALVAREZ, I. AND SCHOENWOLF, G. C. (1991). Patterns of neuroepithelial cell rearrangement during avian neurulation are determined prior to notochordal inductive interactions. *Devl Biol.* **143**, 78–92.

BAKER, R. C. (1927). The early development of the ventral part of the neural plate of amblystoma. *J. comp. Neurol.* **44**, 1–27.

BANCROFT, M. AND BELLAIRS, R. (1975). Differentiation of the neural plate and neural tube in the young chick embryo. A study by scanning and transmission electron microscopy. *Anat. Embryol.* **147**, 309–335.

BOVOLENTA, P. AND DODD, J. (1990). Guidance of commissural growth cones at the floor plate in the embryonic rat spinal cord. *Development* **109**, 435–447.

BOVOLENTA, P. AND DODD, J. (1991). Perturbation of neuronal differentiation and axon guidance in the spinal cord of mouse embryos lacking a floor plate: analysis of Danforth's short-tail mutation. *Development* **113**, 625–640.

BROCKES, J. P. (1989). Retinoids, homeobox genes, and limb morphogenesis. *Neuron* **2**, 1285–1294.

CHANG, W. AND LAGENAUER, C. F. (1990). Central nervous system antigen P84 can serve as a substrate for neurite outgrowth. *Devl Biol.* **137**, 219–232.

CHASAN, R. AND ANDERSON, K. V. (1991). Maternal control of dorsal–ventral polarity and pattern in the embryo. *In Press*

CLARKE, J. D. W., HOLDER, N., SOFF, S. R. AND STORM-MATHISSEN, J. (1991). Neuroanatomical and functional analysis of neural tube formation in notochordless *Xenopus* embryos; laterality of the ventral spinal cord is lost. *Development* **112**, 499–516.

DALE, N., ROBERTS, A., OTTERSEN, O. P. AND STORM-MATHISSEN, J. (1987). The morphology and distribution of Kolmer-Agduhr cells, a class of cerebrospinal fluid contacting neurons revealed in the frog embryo spinal cord by GABA immunocytochemistry. *Proc. R. Soc. Lond. B.* **232**, 193–203.

DIXON, J. E. AND KINTNER, C. R. (1989). Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals. *Development* **106**, 749–757.

DODD, J. AND JESSELL, T. M. (1988). Axon guidance and the patterning of neuronal projections in vertebrates. *Science* **242**, 692–699.

EICHELE, G. (1989). Retinoids and vertebrate limb pattern formation. *Trends Genet.* **5**, 246–251.

EISEN, J. S. (1991). Determination of primary motoneuron identity in developing zebrafish embryos. *Science* **252**, 569–572.

FONTAINE-PERUS, J., CHANCONIE, M., LEDOUARIN, N. M., GERSHON, M. D. AND ROTHMAN, T. P. (1989). Mitogenic effect of muscle on the neuroepithelium of the developing spinal cord. *Development* **107**, 413–422.

FRASER, S., KEYNES, R. AND LUMSDEN, A. (1990). Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Nature* **344**, 431–435.

GERHART, J. AND KELLER, R. E. (1986). Region-specific cell activities in amphibian gastrulation. *A. Rev. Cell Biol.* **2**, 201–229.

GOULDING, M. D., CHALEPAKIS, G., DEUTSCH, U., ERSELIUS, J. R. AND GRUSS, P. (1991b). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO. J.* **10**, 1135–1147.

GOULDING, M. D., WALTHER, C., JOSTES, B., PLACHOV, D. AND GRUSS, P. (1991a). Expression of developmentally regulated genes in the embryonic mouse nervous system. *Discussions in Neuroscience* **1**, 17–24.

GRABOWSKI, C. T. (1956). The effects of the excision of Hensen's Node on the early development of the chick embryo. *J. exp. Zool.* **133**, 301–343.

GRAHAM, A., MADEN, M. AND KRUMLAUF, R. (1991). The murine Hox-2 genes display dynamic dorsoventral patterns of expression during central nervous system development. *Development* **112**, 255–264.

HAMBURGER, V. AND HAMILTON, H. (1951). A series of normal stages in the development of chick embryo. *J. Morph.* **88**, 49–92.

HARA, K. (1978). Spemann's organizer, in birds. In *Organizer – A Milestone of a Half-century from Spemann*. O. Nakomura and S. Toivonen (Eds) Elsevier/North-Holland Biomedical Press. pp. 221–265.

HATTA, K., HO, R. K., WALKER, C. AND KIMMEL, C. B. (1990). A mutation that deletes the floor plate and disturbs axonal pathfinding in Zebrafish. *Soc. Neurosci. Abstr.* **16**, 139.3.

HATTA, K., KIMMEL, C. B., HO, R. K. AND WALKER, C. (1991). The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**, 339–341.

HIRANO, S., FUSE, S. AND SOHAL, G. S. (1991). The effect of the floor plate on pattern and polarity in the developing central nervous system. *Science* **251**, 310–313.

HOLLYDAY, M. AND HAMBURGER, V. (1977). An autoradiographic study of the formation of the lateral motor column in the chick embryo. *Brain Res.* **132**, 197–208.

HOMMA, S., YAGINUMA, H. AND OPPENHEIM, R. W. (1990). Cell death during the earliest stages of spinal cord development in the chick embryo. *Soc. Neurosci. Abstract* **16**, 836.

HUGHES, A. (1959). Studies in embryonic and larval development in amphibia. 11. The spinal motor root. *J. Embryol. exp. Morph.* **7**, 128–145.

JACOBSON, A. G. (1981). Morphogenesis of the neural plate and tube. In *Morphogenesis and Pattern Formation*. Connelly, T. G. et al. (eds). Raven Press, New York, 233–263.

JACOBSON, A. G., OSTER, G. F., ODEL, G. M. AND CHANG, L. Y. (1986). Neurulation and the cortical tractor model for epithelial folding. *J. Embryol. exp. Morphol.* **96**, 19–49.

JACOBSON, C. O. (1964). Motor nuclei, cranial nerve roots and fiber pattern in the medulla oblongata after reversal experiments on the neural plate of axolotl larvae. 1. Bilateral operations. *Zool. Bidrag. Uppsala* **36**, 73–160.

JESSELL, T. M., BOVOLENTA, P., PLACZEK, M., TESSIER-LAVIGNE, M. AND DODD, J. (1989). Polarity and patterning in the neural tube: the origin and role of the floor plate. *Ciba Found. Symp.* **144**, 255–280.

JURAND, A. (1962). The development of the notochord in chick embryos. *J. Embryol. exp. Morph.* **10**, 602–621.

KELLER, R. E. AND DANILCHIK, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development* **103**, 193–209.

KELLER, R. E., DANILCHIK, M. AND GIMLICH, R. (1985). The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. exp. Morph.* **89** Supplement, 185–209.

KIMMEL, C. B., HATTA, K. AND EISEN, J. S. (1991). Control of primary neuronal development in zebrafish. *This volume*.

KINGSBURY, B. F. (1930). The developmental significance of the floor plate of the brain and spinal cord. *J. comp. Neurol.* **50**, 177–207.

KITAMOTO, T., MOMOI, M. AND MOMOI, T. (1989). Expression of cellular retinoic acid binding protein II (chick-CRABP II) in the chick embryo. *Biochem. biophys. Res. Commun.* **164**, 531–536.

KUWADA, J. Y., BERNHARDT, R. R. AND CHITNIS, A. B. (1990a). Pathfinding by identified growth cones in the spinal cord of zebrafish embryos. *J. Neurosci.* **10**, 1299–1308.

KUWADA, J. Y., BERNHARDT, R. R. AND NGUYEN, N. (1990b). Development of spinal neurons and tracts in the zebrafish embryo. *J. comp. Neurol.* **302**, 617–628.

KUWADA, J. Y. AND HATTA, K. (1990). Axonal pathfinding in the spinal cord of zebrafish mutants missing the floor plate. *Soc. Neurosci. Abst.* **16**, 139.1.

LAMBORGHINI, J. E. (1980). Rohon-beard cells and other large neurons in *Xenopus* embyros originate during gastrulation. *J. comp. Neurol.* **189**, 323–333.

LANGMAN, J. AND HADEN, C. C. (1970). Formation and migration of neuroblasts in the spinal cord of the chick embryo. *J. comp. Neur.* **138**, 419–432.

LEBER, S. M., BREEDLOVE, S. M. AND SANES, J. R. (1990). Lineage, arrangement, and death of clonally related motoneurons in chick spinal cord. *J. Neurosci.* **10**, 2451–2462.

LEWIS, W. H. (1947). Mechanics of invagination. *Anat. Rec.* **97**, 139–156.

LO, L. C., JOHNSON, J. E., WUENSCHELL, C. W., SAITO, T. AND ANDERSON, D. J. (1991). Mammalian achaete-scute homolog 1 is transiently expressed by spatially restricted subsets of early neuroepithelial and neural crest cells. *Genes Dev.* **5**, 1524–1537.

MADEN, M., ONG, D. E., SUMMERBELL, D., CHYTIL, F. AND HIRST, E. A. (1989). Cellular retinoic acid-binding protein and the role of retinoic acid in the development of the chick embryo. *Devl Biol.* **135**, 124–132.

MANGOLD, O. (1933). Uber die Induktionsfahigkeit der verschiedenen Bezirk der Neurula von Urodelen. *Naturwissenschaften* **21**, 761–766.

MARTINS-GREEN, M. (1988). Origins of the dorsal surface of the neural tube by progressive delamination of epidermal ectoderm and neuroepithelium: implications for neurulation and neural tube defects. *Development* **103**, 687–706.

MCKANNA, J. A. AND COHEN, S. (1989). The EGF receptor kinase substrate p35 in the floor plate of embryonic rat CNS. *Science* **243**, 1477–1479.

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

NICOLET, P. G. (1970). Analyze autoradiographique de la localisation des differentes ebauches presomptives dans la ligne primitive de l'embryon de poulet. *Embryol. ex. Morph.* **23**, 79–108.

NORDLANDER, R. H. (1986). Motoneurons of the tail of young *Xenopus* tadpoles. *J. comp. Neurol.* **253**, 403–413.

PLACZEK, M., TESSIER-LAVIGNE, M., JESSELL, T. M. AND DODD, J. (1990a). Orientation of commissural axons *in vitro* in response to a floor plate derived chemoattractant. *Development* **110**, 19–30.

PLACZEK, M., TESSIER-LAVIGNE, M., YAMADA, T., DODD, J. AND JESSELL, T. M. (1990b). The guidance of developing axons by diffusible chemoattractants. *Cold Spring Harbor Symp. quant. Biol.* **55**, 279–289.

PLACZEK, M., TESSIER-LAVIGNE, M., YAMADA, T., JESSELL, T. M. AND DODD, J. (1990c). Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science* **250**, 985–988.

PUELLES, L., ARIAT, J. A. AND MARTINEZ-DE-LA-TORRE, M. (1987). Segment related mosaic neurogenetic pattern in the forebrain and mesencephalon of early chick embryos: 1. Topography of AChE-positive neuroblasts up to stage HH18. *J. comp. Neurol.* **266**, 247–268.

ROACH, F. C. (1945). Differentiation of the central nervous system after axial reversals of the medullary plate of amblystoma. *J. exp. Zool.* **99**, 53–77.

ROELINK, H. AND NUSSE, R. (1991). Expression of two members of the wnt family during mouse development – restricted temporal and spatial patterns in the developing neural tube. *Genes Dev.* **5**, 381–388.

ROSENQUIST, G. C. (1966). A radioautographic study of labelled grafts in the chick blastoderm. *Contrib. Embryol. Carnegie Inst.* **38**, 71–110.

ROSENQUIST, G. C. (1983). The chorda center in Hensen's node of the chick embryo. *Anat. Rec.* **207**, 349–355.

RUBERTE, E., DOLLE, P., CHAMBON, P. AND MORRISS-KAY, G. (1991). Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. *Development* **111**, 45–60.

RUIZ I ALTABA, A. (1990). Neural expression of the *Xenopus* homeobox gene Xhox3: evidence for a patterning neural signal that spreads through the ectoderm. *Development* **108**, 595–604.

RUIZ I ALTABA, A. AND MELTON, D. A. (1989). Interaction between peptide growth factors and homeobox genes in the establishment of antero-posterior polarity in frog embryos. *Nature* **341**, 33–38.

SCHOENWOLF, G. C. (1982). On the morphogenesis of the early rudiments of the developing central nervous system. *Scan. Electron. Microsc.* (Pt 1) P. 289–308.

SCHOENWOLF, G. C. AND ALVAREZ, I. S. (1989). Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* **106**, 427–439.

SCHOENWOLF, G. C., EVERAERT, S., BORTIER, H. AND VAKAET, L. (1989). Neural plate- and neural tube-forming potential of isolated epiblast areas in avian embryos. *Anat. Embryol.* **179**, 541–549.

SCHOENWOLF, G. C., FOLSOM, D. AND MOE, A. (1988). A reexamination of the role of microfilaments in neurulation in the chick embryo. *Anat. Rec.* **220**, 87–102.

SCHOENWOLF, G. C. AND FRANKS, M. V. (1984). Quantitative analysis of changes in cell shapes during bending of the avian neural plate. *Devl Biol.* **105**, 257–272.

SCHOENWOLF, G. C. AND SMITH, J. L. (1990). Mechanisms of neurulation: traditional viewpoint and recent advances. *Development* **109**, 243–270.

SCHROEDER, T. E. (1970). Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy. *J. Embryol. exp. Morphol.* **23**, 427–462.

SELLECK, M. A. J. AND STERN, C. D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* **112**, 615–626.

SMITH, J. L. AND SCHOENWOLF, G. C. (1989). Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation. *J. exp. Zool.* **250**, 49–62.

SMITH, S. M. AND EICHELE, G. (1991). Temporal and regional differences in the expression pattern of distinct retinoic acid receptor-β transcripts in the chick embryo. *Development* **111**, 245–252.

SPEMANN, H. (1938). Embryonic Development and Induction. (New Haven: Yale University Press).

STEDING, G. V. (1962). Experiments zur morphogenese des Ruckenmarkes. *Acta Anat.* **49**, 199–231.

TANAKA, H. AND OBATA, K. (1984). Developmental changes in unique cell surface antigens of chick embryo spinal motor neurons and ganglion cells. *Devl Biol.* **106**, 26–37.

TESSIER-LAVIGNE, M., PLACZEK, M., LUMSDEN, A. G. S., DODD, J. AND JESSELL, T. M. (1988). Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature* **336**, 775–778.

THALLER, C. AND EICHELE, G. (1987). Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature* **327**, 625–628.

THEILER, K. (1959). Anatomy and development of the 'truncate' (boneless) mutation in the mouse. *Am. J. Anat.* **104**, 319–343.

TICKLE, C., ALBERTS, B., WOLPERT, L. AND LEE, J. (1982). Local application of retinoic acid to the limb bud mimics the action of the polarizing region. *Nature* **296**, 564–566.

TICKLE, C., SUMMERBELL, D. AND WOLPERT, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature* **254**, 199–202.

VAN MIER, R., VAN RHEDEN, R. AND EEN DONKELAAR, H. J. (1985). The development of the dendritic organization of primary and secondary motoneurons in the spinal cord of *Xenopus*. *Anat. Embryol.* **172**, 311–324.

VAN STRAATEN, H. W. M. AND DRUKKER, J. (1987). Influence of the notochord on the morphogenesis of the neural tube. In *Mesenchymal-Epithelian Interactions in Neural Development* (J. R. Wolff *et al.* eds). NATO ASI Series Vol. H5 (Berlin: Springer-Verlag), pp. 153–162.

VAN STRAATEN, H. W. M., HEKKING, J. W. M., BEURSGENS, J. P. W. M., TERWINDT-ROUWENHORST, E. AND DRUKKER, J. (1989). Effect of the notochord on proliferation and differentation in the neural tube of the chick embryo. *Development* **107**, 793–803.

VAN STRAATEN, H. W. M., HEKKING, J. W. M., THORN, F., WIERTZ-HOESSELS, E. L. M. AND DRUKKER, J. (1985a). Induction of an additional floor plate in the neural tube. *Acta Morphol. Neerl. Scand.* **23**, 91–97.

VAN STRAATEN, H. W. M., HEKKING, J. W. M., WIERTZ-HOESSELS, E. L., THORS, F. AND DRUKKER, J. (1988). Effect of the notochord on the differentation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol.* **177**, 317–324.

VAN STRAATEN, H. W. M., THORS, F., WIERTZ-HOESSELS, E. L., HEKKING, J. W. M. AND DRUKKER, J. (1985b). Effect of a notochordal implant on the early morphogenesis of the neural tube and neuroblasts: histometrical and histological results. *Devl Biol.* **110**, 247–254.

WAGNER, M., THALLER, C., JESSELL, T. AND EICHELE, G. (1990). Polarizing activity and retinoid synthesis in the floor plate of the neural tube. *Nature* **345**, 819–822.

WALLACE, J. A. (1985). An immunocytochemical study of the development of central serotonergic neurons in the chick embryo. *J. comp. Neurol.* **236**, 444–453.

WATTERSON, R. L., GOODHEART, C. R. AND LINDBERG, G. (1955). The influence of adjacent structures upon the shape of the neural tube and neural plate of chick embryos. *Anat. Rec.* **122**, 539–559.

WILKINSON, D. G., BAILES, J. A. AND McMAHON, A. P. (1987). Expression of the proto-oncogene int-1 is restricted to specific neural cells in the developing mouse embryo. *Cell* **50**, 79–88.

WILSON, P. A., OSTER, G. AND KELLER, R. (1989). Cell rearrangement and segmentation in *Xenopus*: direct observation of cultured explants. *Development* **105**, 155–166.

WOLFF, E. (1936). Les bases de la teratogenese experimentale des vertebres amniotes d'apres les resultats de method, directes. *Arch. Anat. Histol. Embryol.* **22**, 1–375.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.

YAGINUMA, H., HOMMA, S., KUNZI, R. AND OPPENHEIM, R. W. (1991). Pathfinding by growth cones of commissural interneurons in the chick embryo spinal cord: a light and electron microscopic study. *J. comp. Neurol.* **304**, 78–102.

YAGINUMA, H., SHIGA, T., HOMMA, S., ISHIHARA, R. AND OPPENHEIM, R. W. (1990). Identification of early developing axon projections from spinal interneurons in the chick embryo with a neuron specific β-tubulin antibody: evidence for a new 'pioneer' pathway in the spinal cord. *Development* **108**, 705–716.

YAMADA, T., PLACZEK, M., TANAKA, H., DODD, J. AND JESSELL, T. M. (1991). Control of cell pattern in the developing nervous system: Polarizing activity of the floor plate and notochord. *Cell* **64**, 635–647.

YOUN, B. W. AND MALACINSKI (1981). Axial structure development in ultraviolet-irradiated (notochord-defective) amphibian embryos. *Devl Biol.* **83**, 339–352.