

Control of Cell Pattern in the Developing Nervous System: Polarizing Activity of the Floor Plate and Notochord

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Summary

Individual classes of neural cells differentiate at distinct locations in the developing vertebrate nervous system. We provide evidence that the pattern of cell differentiation along the dorsoventral axis of the chick neural tube is regulated by signals derived from two ventral midline cell groups, the notochord and floor plate. Grafting an additional notochord or floor plate to ectopic positions, or deleting both cell groups, resulted in changes in the fate and position of neural cell types, defined by expression of specific antigens. These results suggest that the differentiation of neural cells is controlled, in part, by their position with respect to the notochord and floor plate.

Introduction

Development of the vertebrate nervous system begins with induction of the neural plate from uncommitted ectoderm in response to signals from adjacent mesoderm (Spemann, 1938). The neural plate then folds along its midline to form the neural tube (Schoenwolf and Smith, 1990). Regional differentiation of the neural epithelium along the anteroposterior (A-P) axis is apparent at the neural plate stage (Roach, 1945; Jacobson, 1964; Hemmati-Brivanlou and Harland, 1989), giving rise anteriorly to the forebrain and midbrain and posteriorly to the hindbrain and spinal cord. The differentiation of cell types along the dorsoventral (D-V) axis of the neural tube occurs later (Hutchinson, 1936; Roach, 1945) and becomes evident as distinct classes of neurons appear at different D-V positions. For example, in the spinal cord, motor neurons are located ventrally, whereas commissural neurons and neural crest cells appear in dorsal positions. Each of these cell types is distributed bilaterally with an organization that is symmetric with reference to the midline of the neural plate.

Cells at the midline of the neural plate give rise to a cell group called the floor plate at the ventral midline of the neural tube (Rosenquist, 1966; Jessell et al., 1988; Fraser et al., 1990; Schoenwolf and Smith, 1990). Differentiation of the floor plate appears to be induced by underlying

mesodermal cells of the notochord (Watterson et al., 1955; van Straaten et al., 1988; Smith and Schoenwolf, 1989; Placzek et al., 1990b; K. Hatta and C. Kimmel, personal communication). Floor plate cells have specialized properties that influence the development of other neural cells. The floor plate releases a diffusible chemoattractant that orients the growth of a subset of developing spinal cord axons (Tessier-Lavigne et al., 1988; Placzek et al., 1990a). Contact between growth cones and the floor plate appears to contribute to the guidance of axons at the ventral midline of the spinal cord (Bovolenta et al., 1988; Bovolenta and Dodd, 1990; Kuwada et al., 1990). In addition, the floor plate and the notochord are sources of a polarizing signal that respecifies cell pattern along the A-P axis of the developing chick limb, mimicking the effect of the zone of polarizing activity and retinoic acid (Hornbruch and Wolpert, 1986; Wagner et al., 1990).

The ability of the notochord and floor plate to polarize embryonic tissues, together with their midline location, raises the possibility that these two cell groups are involved in controlling the pattern of cell differentiation along the D-V axis of the developing nervous system. To test this possibility, we used antibodies directed against cell-specific antigens to determine whether the pattern of cells in the embryonic chick nervous system changes after induction of an additional floor plate at ectopic positions in the neural tube by a notochord graft; grafting of a floor plate next to the neural tube; or removal of the notochord to prevent floor plate differentiation. Addition or deletion of the notochord and floor plate resulted in marked changes in the pattern of neural cell types. Moreover, the fate of neural epithelial precursors appeared to be dependent on the position that they occupy with respect to the notochord and floor plate. These results suggest that cell patterning along the D-V axis of the developing nervous system is controlled, at least in part, by a signal or signals that derive from the notochord and floor plate.

Results

Identification of Cell Type and Position in the Developing Nervous System

Antigens selectively expressed by distinct classes of cells within the developing chick nervous system were used to assess the pattern of cell differentiation. These antigens define the floor plate, motor neurons, commissural neurons, serotonergic neurons, and dorsal spinal cord cells, as well as the notochord (see Experimental Procedures and Table 1). The normal expression pattern of these antigens in the embryonic chick nervous system is shown in Figure 1.

Induction of the Floor Plate by the Notochord

In initial experiments to assess the contribution of the notochord and floor plate to the differentiation of other neural cells, we induced a second floor plate at ectopic lo-

Table 1. Identification of Cell Types in Embryonic Chick Nervous System and Notochord

Cell Type	Antibody	Onset of Expression in Neural Tube (H. H. Stage)	Reference
Floor plate	FP1	10	This study
	SC1	10	Tanaka et al., 1984
Motor neurons	FP2	20	This study
	SC1	15	Tanaka et al., 1984
Serotonergic neurons	α -serotonin	23	Wallace et al., 1985
Commissural neurons	α -CRABP	15	Maden et al., 1989
Differentiated neurons	3A10	15	This study
Dorsal spinal cord cells	AC4	Absent from ventral spinal cord by stage 17	This study
Notochord	Not1	5	This study

cations by grafting a segment of notochord adjacent to the neural tube. Notochord grafts have been shown to induce floor plate differentiation as assessed by changes in cell morphology (van Straaten et al., 1988; Smith and Schoenwolf, 1989) and by the expression of a floor plate chemoattractant (Placzek et al., 1990b). We therefore examined whether the chick floor plate antigens recognized by MAbs FP1, FP2, and SC1 (Figures 1A–1C) could serve as

molecular markers of floor plate induction. The expression of each of these antigens was induced in lateral neural epithelial cells by grafting a piece of notochord next to the neural tube of stage 9–11 chick embryo hosts (Figures 2A–2C and Figure 3A). Expression of these floor plate antigens was used in subsequent experiments to determine the presence of an induced floor plate within the neural tube and its position with respect to other cell types.

Control of Motor Neuron Differentiation

In normal embryos, motor neurons occupy a position close to, but not immediately adjacent to, the floor plate (Figure 1C). The distribution of motor neurons was examined in the spinal cord of embryos in which a segment of notochord had been grafted at different D–V positions. Placing an extra notochord at the dorsal midline of the neural tube (Figures 2D and 2G) resulted in a marked alteration in the pattern of cell differentiation in the spinal cord. The region of spinal cord immediately adjacent to the dorsal notochord expressed the FP1 (Figure 2E) and SC1 antigens (not shown) in 3 of 5 embryos, providing evidence for induction of a floor plate. Expression of the SC1 and 3A10 antigens also revealed a marked change in the location of motor neurons in all 5 embryos examined. Two bilaterally symmetric columns of motor neurons were detected in a normal ventral position; however, the dorsal half of the spinal cord contained two additional motor columns, near but not immediately adjacent to the grafted notochord (Figures 2F and 2H). There was no detectable

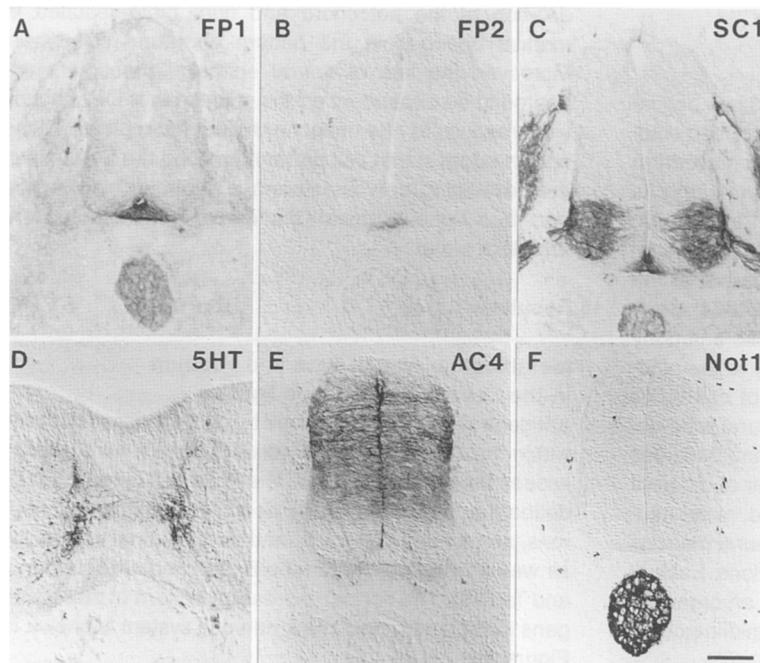


Figure 1. Identification of Cell Classes in Developing Chick Nervous System

(A) Floor plate cells at the ventral midline of the chick spinal cord and the underlying notochord express the FP1 antigen. No other region of the spinal cord expresses this antigen at detectable levels (stage 20).

(B) Floor plate cells can also be identified by expression of the FP2 antigen. This antigen is restricted to the basal region of the floor plate and is not expressed by other spinal cord cells or by the notochord (stage 24).

(C) Floor plate cells and motor neurons in chick spinal cord express the SC1 antigen. Within the spinal cord, this antigen is expressed almost exclusively by these two cell types. There is, however, faint staining of roof plate cells in embryos older than stage 22. Dorsal root ganglion neurons and their afferent fibers in the dorsal root entry zone and the notochord are also labeled (stage 22).

(D) Neurons in the medial hindbrain express the neurotransmitter serotonin. Labeled neuronal cell bodies and processes are located immediately adjacent to the floor plate at the midline of the hindbrain (stage 27).

(E) Cells expressing the AC4 antigen are restricted to a band in the intermediate and dor-

sal regions of chick spinal cord. By this stage, the ventral region of the spinal cord containing the floor plate, ventromedial neuroepithelial cells, and motor neurons do not express the AC4 antigen. The region adjacent to the roof plate at the dorsal midline of the spinal cord is also unlabeled. At later embryonic stages, AC4 expression is maintained on cells in the dorsal region of the spinal cord (see Experimental Procedures) (stage 22).

(F) Expression of the Not1 antigen by the notochord. This antigen is expressed selectively by the notochord in stage 5–28 chick embryos and was used to establish that notochord cells are not incorporated into the neural tube in grafting experiments (stage 22).

Scale bar: A, B, C, E, and F = 80 μ m; D = 60 μ m.

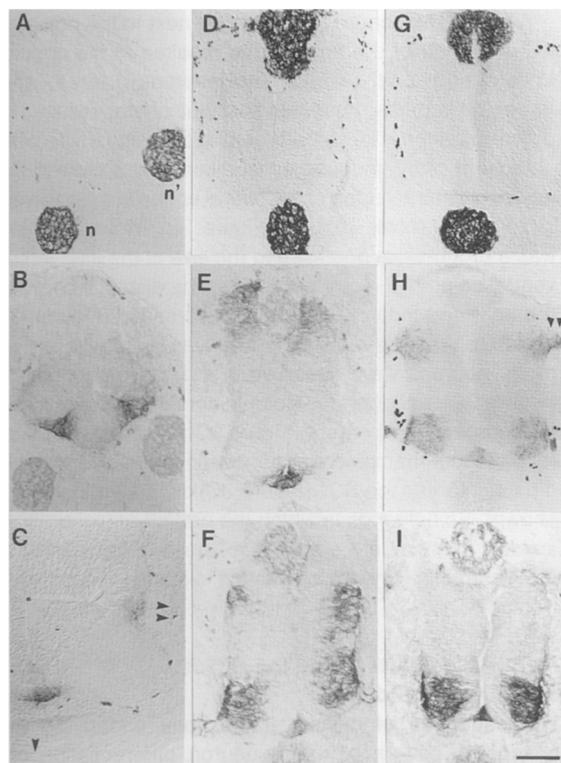


Figure 2. Changes in Cell Pattern in the Developing Spinal Cord in Response to Notochord Grafts

(A) Identification of host (n) and grafted (n') notochord by expression of the Not1 antigen. The spinal cord exhibits a distortion in the neural epithelium adjacent to the grafted notochord, which results from the wedging of neural epithelial cells (stage 22).

(B) Adjacent section through the same embryo, showing expression of the FP1 antigen. The host floor plate is located above the host notochord and expresses the FP1 antigen. Neural epithelial cells near the grafted notochord also express the FP1 antigen. FP1-labeled cells span the distance from the lumen to the external surface of the spinal cord, as does the host floor plate.

(C) The FP2 antigen is also expressed by neural epithelial cells in the region adjacent to a grafted notochord. The FP2 antigen is restricted to the basal region of the host and induced floor plate. The position of the host notochord is marked with a single arrowhead and that of the grafted notochord with two arrowheads. Because the FP2 antigen does not appear until relatively late in spinal cord development, the host embryo was permitted to develop to stage 25. The host and grafted notochords are therefore separated from the spinal cord by a larger distance than in (A) and (B) (stage 25).

Notochord grafts were performed in 17 stage 9–11 host embryos, of which 13 expressed floor plate antigens defined by MAbs FP1, SC1, and FP2.

(D) A grafted notochord located at the dorsal midline of the spinal cord, identified by expression of the Not1 antigen. Grafts were placed in the lumen of the neural groove, so that during tube closure the notochord was displaced dorsally, ending up at the dorsal midline (stage 20). (E) Adjacent section through the same embryo as in (D), showing that dorsal neural epithelial cells near the dorsal notochord express the FP1 antigen, even though there is no morphologically detectable floor plate.

(F) Adjacent section through the same embryo, showing the presence of four discrete columns of SC1-labeled motor neurons. Two of these columns are located in the ventral spinal cord near the host floor plate, which is also labeled. The other two are located in the dorsal spinal cord near dorsal cells expressing the FP1 antigen (see [E]). Both the dorsal and ventral columns of cells were identified as neurons by their expression of the 3A10 antigen (not shown).

(G) A grafted notochord located at the dorsal midline of the spinal cord,

change in the size of the ventral motor neuron columns when compared with unoperated controls (Figures 2F and 2H), indicating that notochord grafts caused an increase in the total number of motor neurons.

In 3 additional embryos with dorsal midline notochord grafts, no expression of the FP1 or SC1 antigens was observed in the dorsal region of the spinal cord, suggesting that an ectopic floor plate and motor neurons were not induced (Figure 2I). In these embryos, the notochord graft did not prevent neural tube closure, and a thin strip of neural cells formed at the dorsal midline in the position normally occupied by the roof plate (Figure 2I). In the 5 embryos described above in which a floor plate and motor neurons were induced dorsally (Figures 2F and 2H), the notochord had prevented fusion of the neural tube and there was no morphologically detectable roof plate (Figures 2G and 2D). Induction of a floor plate and motor neurons in response to a dorsally placed notochord may therefore occur only when fusion of the dorsal midline of the neural tube fails, preventing roof plate differentiation.

Notochord grafts were also placed next to the neural tube, midway between the roof plate and floor plate. An ectopic floor plate was detected adjacent to the grafted notochord, and there was an additional column of motor neurons in the dorsal spinal cord on the side of the graft, separated from the induced floor plate by a region of unlabeled cells (Figure 3A). The axons of these dorsal motor neurons projected out of the spinal cord (Figure 3A). To quantify the change in number of motor neurons in response to lateral notochord grafts, sections labeled with MAb SC1 were counterstained with Hoechst 33258 to label all cell nuclei. There was a significant increase in the number of motor neurons on the side of the spinal cord adjacent to the notochord graft (155 ± 18 labeled cells per section, ipsilateral; 103 ± 9 labeled cells per section, contralateral; mean \pm SEM, five sections from 2 embryos; $p < 0.05$, Student's *t* test). No significant change in the total number of cells on the side of the spinal cord adjacent to the graft (487 ± 26 labeled cells per section) was found, when compared with the contralateral side (453 ± 22 la-

identified by expression of the Not1 antigen. Note that as in (D), the dorsal midline of the spinal cord is separated by the grafted notochord, and note also that there is no morphologically detectable roof plate (stage 20).

(H) Adjacent section through the same embryo as in (G), showing four distinct columns of SC1-labeled motor neurons. In this section, labeled motor neurons in the dorsal spinal cord can be observed to project axons from the spinal cord (arrowheads). The neural epithelium next to the grafted notochord does not express SC1. A total of 5 embryos had dorsal notochord grafts in similar positions. Three expressed dorsal floor plate antigens, and all 5 had ectopic dorsal motor neurons.

(I) A grafted notochord located at the dorsal midline of the spinal cord. In this embryo, the graft is located outside the spinal cord. The dorsal region of the spinal cord contains a thin strip of cells, fused at the midline, which may correspond to the roof plate. Under these conditions, floor plate antigens are not expressed (not shown) and no ectopic SC1-labeled neurons are detected (stage 20). Similar results were obtained in 3 embryos in which the dorsal notochord graft did not prevent neural tube closure.

Scale bar: A and B = $80 \mu\text{m}$; C, G, H, and I = $70 \mu\text{m}$; D, E, and F = $60 \mu\text{m}$.

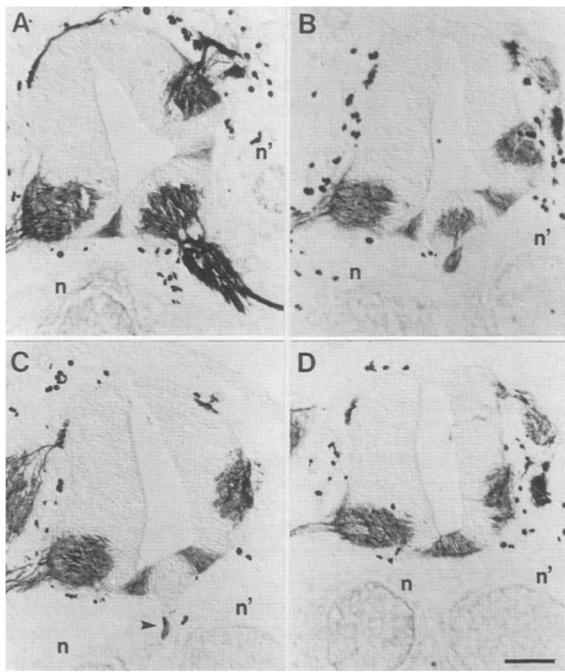


Figure 3. Cell Pattern Varies Depending on the D-V Position of the Grafted Notochord

The notochord graft was positioned at an angle with respect to the host notochord, such that it contacted the neural epithelium at different D-V locations. Serial sections through the spinal cord reveal a change in pattern of cell differentiation.

(A) A grafted notochord (n') positioned lateral to the neural tube, midway between the roof plate and the floor plate, induces a second floor plate and an additional column of motor neurons in the dorsal spinal cord, revealed by expression of the SC1 antigen. With notochord grafts in this position, the sizes of the ipsilateral and contralateral ventral motor neuron columns are about equal. The small labeled cells surrounding the spinal cord in this and subsequent figures are red blood cells which contain endogenous peroxidase activity.

(B) Caudal section from the same embryo in which the segment of grafted notochord (n') is now located more ventrally and nearer to the host notochord (n). Again, a second floor plate and a dorsally located ectopic column of motor neurons are induced. However, SC1 labeling of this section reveals a clear decrease in the size of the ventral column of motor neurons on the side of the graft, compared to the contralateral side.

(C) More caudal section from the same embryo, in which the grafted notochord (n') is in an even more ventral position. A second floor plate and a dorsal motor neuron column are present. However, the column of motor neurons between the host and induced floor plate has now disappeared. A few labeled axons, which derive from neurons in adjacent sections, can be seen outside the neural tube (arrowhead).

(D) Additional caudal section from the same embryo in which the host (n) and grafted (n') notochords are located symmetrically on either side of the ventral midline. A single expanded floor plate is revealed by SC1 labeling together with two columns of motor neurons.

It was not possible to determine the precise spatial relationship between the grafted notochord and induced cells in the neural tube, because analysis of cell position was performed 24–48 hr after placement of the graft, during which time the notochord was displaced from its original position.

Scale Bar: A, B, C, and D = 80 μ m.

beled cells per section; mean \pm SEM, five sections from 2 embryos). Thus, notochord grafts into stage 10 hosts increased the number of motor neurons without changing total cell number.

Although notochord grafts placed next to the dorsal or lateral region of the neural tube resulted in the appearance of additional ectopic motor neurons, grafts located in ventral regions, near the host notochord, resulted in different patterns of cell differentiation. Ventrally located notochord grafts induced an additional floor plate in the adjacent ventral region of the neural epithelium and an ectopic dorsal motor column (Figure 3B). However, there was a progressive decrease in the size of the intervening ventral column of motor neurons as the distance between the host and induced floor plate decreased (Figures 3B and 3C). When this distance was less than \sim 80 μ m, no motor neurons were observed in the intervening region (Figure 3C), although the ectopic dorsal column of motor neurons was still present (Figure 3C). The region of neuroepithelium that intervened between the two floor plates did not express the SC1 (Figure 3C) or FP1 antigens (not shown). Spinal cords in which the grafted notochord was located immediately adjacent to the host notochord at the ventral midline had a single expanded floor plate (Figure 3D).

Floor Plate Grafts Mimic the Effect of the Notochord

The sequence of cellular interactions underlying the change in pattern of motor neuron differentiation observed after notochord grafts is not clear. The notochord could induce both the floor plate and motor neurons, in which case the floor plate might be expected not to have a direct effect on motor neuron differentiation. Alternatively, motor neuron induction may be controlled solely by the floor plate, with the role of the notochord being to induce the floor plate. It is also possible that both the notochord and floor plate can induce motor neurons.

To determine whether the floor plate can influence neural cell differentiation independently of the notochord, segments of chick floor plate were grafted into host embryos. We excluded the possibility that grafted floor plate cells invaded the host neural tube, by performing additional experiments with quail and rat floor plate grafts and species-specific antibodies (see Experimental Procedures). In 6 of 12 embryos, floor plate grafts induced expression of the FP1 and SC1 antigens in regions of the spinal cord adjacent to the graft (Figures 4A and 4B). The morphology of the labeled cells and the lack of 3A10 labeling (not shown) confirm that these are floor plate cells. Recent studies of floor plate differentiation in zebrafish embryos have provided independent support for the idea that floor plate cells can induce other neural tube cells to acquire floor plate properties (K. Hatta and C. Kimmel, personal communication). Floor plate grafts also produced marked changes in the pattern of motor neuron differentiation. In embryos in which an additional floor plate was induced, ectopic motor neurons were also present, separated from the induced floor plate by an intervening region of unlabeled neural epithelium (Figure 4B). Floor plate grafts also induced ectopic motor neurons in the absence of a secondary floor plate (Figures 4C and 4D). Suppression of the host ventral motor neuron column was also observed (Figure 4E). These results show that floor plate

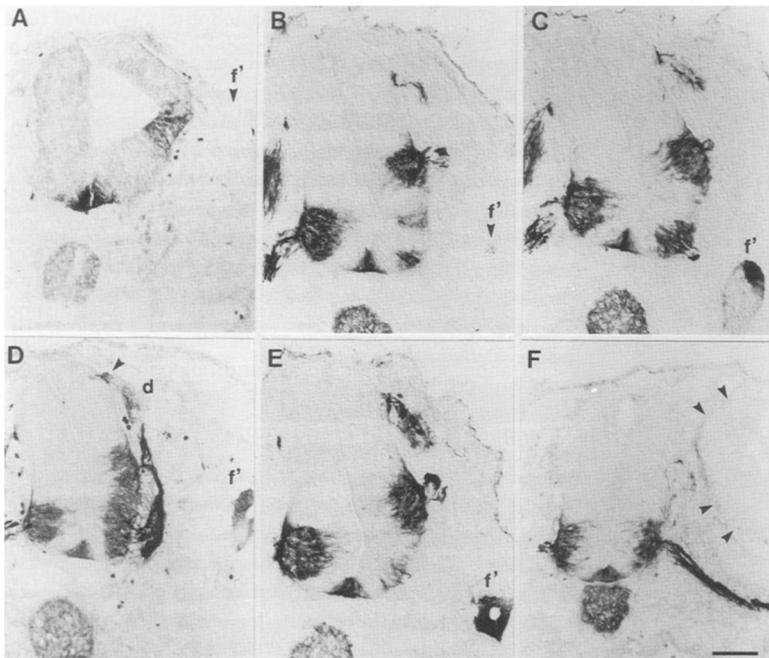


Figure 4. Floor Plate Grafts Mimic the Notochord in Changing Cell Pattern in the Developing Spinal Cord

(A) A graft of quail floor plate (f') induces expression of the FP1 antigen in adjacent neural epithelium. The FP1 antibody does not recognize quail; thus, the floor plate graft is unlabeled (stage 20). A total of 12 stage 9–11 embryos received floor plate grafts (4 chick, 5 quail, and 3 rat), of which 6 embryos expressed floor plate antigens in adjacent neural epithelium. Floor plate tissue from all three species induced a floor plate in the host neural tube. (B) A graft of chick floor plate induces expression of the SC1 antigen on cells with the morphological appearance of a floor plate. These cells do not express the 3A10 antigen (not shown). In addition, there is a dorsally located column of ectopic motor neurons. The size of the ipsilateral ventral column of motor neurons is greatly reduced. The position of the floor plate graft (most of which is present in adjacent sections) is marked " f' ". All 12 stage 9–11 embryos that received floor plate grafts [see (A)] exhibited ectopic motor neurons.

(C) Adjacent section through the same embryo, in which the floor plate graft (f') induces an ectopic column of motor neurons, separated from ventral motor neurons by a region of unlabeled cells, with no evidence of an induced floor plate.

(D) A separate embryo in which a floor plate graft (f') induces a marked increase in the number of motor neurons without evidence for induction of a floor plate. Note that dorsally located motor neurons have SC1-labeled axons, which project out of the spinal cord and join with the axons of ventrally located motor neurons to form an expanded ventral root. SC1-labeled cells in more dorsal regions are dorsal root ganglion neurons (d), which have axons projecting into the dorsal root entry zone (arrowhead).

(E) Section through the same embryo as in (B) and (C), in which a floor plate graft results in a marked reduction in the size of the ipsilateral column of ventral motor neurons with a dorsally displaced group of SC1-labeled motor neurons.

(F) Regions other than the floor plate grafted next to the neural tube do not cause a change in cell pattern. In this embryo, the region of neural tube immediately adjacent to the floor plate in a stage 17 embryo was grafted next to the lateral neural tube. (The position of the graft is shown by arrowheads.) SC1 antigen expression reveals that there is no obvious change in the position of floor plate and motor neurons (stage 20). Similar results were obtained in 4 embryos.

Scale bar: A, B, C, and E = 70 μ m; D and F = 80 μ m.

grafts mimic the effect of the notochord and suggest that the floor plate can regulate motor neuron differentiation independently of the notochord.

To determine whether changes in cell pattern were induced by other neural tissues, the region of neural epithelium immediately adjacent to the floor plate and the ventrolateral and dorsal regions of stage 10–17 neural tube were also tested (see Wagner et al., 1990 for location of grafted regions). No changes in the pattern of cell differentiation, assessed by morphology and antigen expression, were observed with any of these grafts (Figure 4F). Thus, the ability of the floor plate to regulate the pattern of cell differentiation is not shared by other regions of the neural tube.

The Notochord Can Induce Motor Neurons Independently of the Floor Plate

To examine whether the notochord is capable of inducing motor neurons without also inducing a floor plate, we extended previous observations that floor plate properties are not induced when notochord grafts are placed next to the neural tube of older (stage 14–15) embryos (van Straaten et al., 1988; Placzek et al., 1990b). Grafts of stage

10 notochord placed next to the neural tube of stage 15–18 host embryos failed to induce expression of the FP1 antigen (not shown). Despite this, expression of the SC1 and 3A10 antigens revealed a marked increase in the size of the motor column (not shown). These results suggest that the notochord can induce motor neurons by a direct action that does not require induction of a floor plate. In support of this, dorsal notochord grafts into younger embryos were, in some cases, able to induce motor neurons in the absence of expression of floor plate antigens (see Figures 2G and 2H). We cannot rule out the possibility that notochord grafts into older embryos induce floor plate properties that are not detected by our assays, including the ability to induce motor neurons. However, taken together with the results described in the preceding section, it is likely that the floor plate and notochord can independently induce motor neurons.

Induction of Serotonergic Neurons

The consistent appearance of a region of unlabeled neural epithelium intervening between an induced floor plate and motor column suggests that the differentiation of cells in this region is also controlled by the notochord and floor

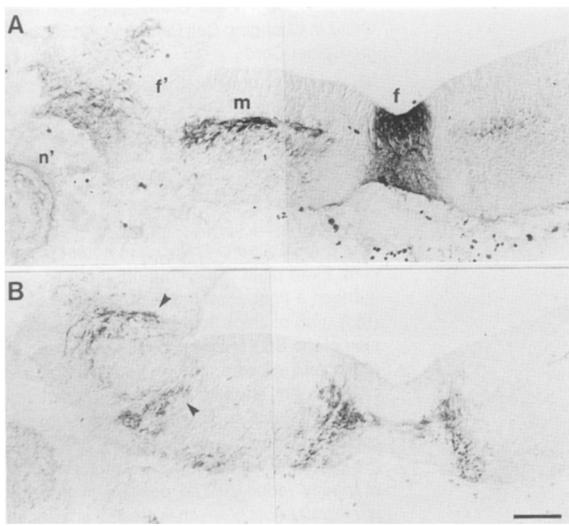


Figure 5. Induction of Serotonergic Neurons by Notochord Grafts Adjacent to the Hindbrain.

A stage 9 notochord was grafted adjacent to the hindbrain of a stage 7–8 chick embryo.

(A) Section through the hindbrain at the level of the second rhombomere labeled for SC1 expression at stage 25. Cells in the medial region of the host floor plate (f) express high levels of SC1; lower levels of antigen are expressed by cells of the induced floor plate (f'). SC1-labeled ventral neurons, presumably cranial motor neurons (m), are also induced between the host and secondary floor plate.

(B) Adjacent section through the same embryo, processed for serotonin immunocytochemistry. The host floor plate is flanked by a group of serotonergic neurons. Ectopic serotonergic neurons are also located immediately adjacent to the induced floor plate. An additional floor plate was induced in 3 embryos, 2 of which had ectopic serotonergic neurons.

Scale bar = 80 μ m.

plate. In the spinal cord, we have not identified markers that identify individual cell types in this region. However, in the hindbrain, cells in the region located between the floor plate and cranial motor neurons differentiate into serotonergic neurons (Wallace, 1985; Figure 1D). We therefore examined whether the notochord can induce the appearance of ectopic serotonergic neurons, by grafting

a piece of caudal 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 MAb SC1 (Figure 5A) and FP1 (not shown). Ectopic serotonergic neurons were present immediately adjacent to the induced floor plate (Figure 5B). In contrast, SC1-labeled neurons, probably cranial motor neurons, were induced at a distance from the ectopic floor plate (Figure 5A). These findings show that the notochord can induce a floor plate at more anterior levels of the neuraxis and that at a given rostrocaudal level, distinct classes of neurons are induced at different D–V positions with respect to the floor plate and notochord.

Changes in Cell Differentiation in Dorsal Neural Tube

The ability of the floor plate and notochord to induce ventral cell types in dorsal regions suggests that the fate of cells that normally differentiate dorsally may be altered. The effect of notochord and floor plate grafts on dorsal neural cells was determined by examining the expression of the AC4 antigen. By stage 17, the AC4 antigen is restricted to the intermediate and dorsal region of the spinal cord and is not expressed by the floor plate, motor neurons, or the region of intervening neural epithelium (Figure 1E and Figure 6A). Notochord or floor plate grafts placed next to the lateral part of the neural tube resulted in a marked decrease in AC4 expression in the dorsal spinal cord adjacent to the graft (Figures 6B and 6C). Changes in the distribution of identified neurons in the intermediate and dorsal region of the spinal cord after grafts were assessed using cellular retinoic acid-binding protein (CRABP) as a marker of spinal relay neurons, in particular commissural neurons (Maden et al., 1989). The number of CRABP-labeled neurons decreased markedly after lateral notochord grafts (not shown). Thus, the appearance of ventral cell types in dorsal regions after notochord or floor plate grafts is accompanied by the loss of markers characteristic of more dorsal cell types.

The effect of dorsal grafts on neural crest cells was not examined in detail. Dorsal root ganglia, which derive from the neural crest, were present near the spinal cord, on the

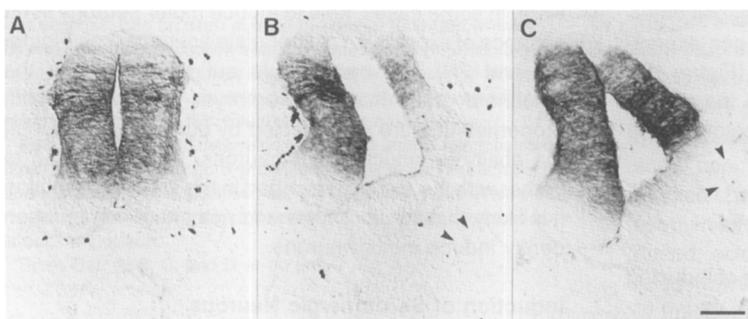


Figure 6. Suppression of AC4 Expression in the Dorsal Spinal Cord

(A) Expression of the AC4 antigen by cells in intermediate and dorsal regions of the chick spinal cord (stage 20–21).

(B) Section through the same embryo shown in (A), at a level adjacent to a segment of grafted notochord (position shown by arrowheads). Expression of the AC4 antigen is greatly reduced in the region of spinal cord adjacent to the graft. Similar results were obtained in 13 embryos.

(C) Section through a different embryo, at a level adjacent to a piece of grafted chick floor

plate (arrowheads). The intermediate region of the spinal cord on the side adjacent to the graft does not express AC4, whereas the equivalent region on the contralateral side expresses high levels. Grafts of other regions of the neural tube do not mimic the effect of the floor plate (not shown) (stage 23). Similar results were obtained in 6 embryos.

Scale bar = 70 μ m.

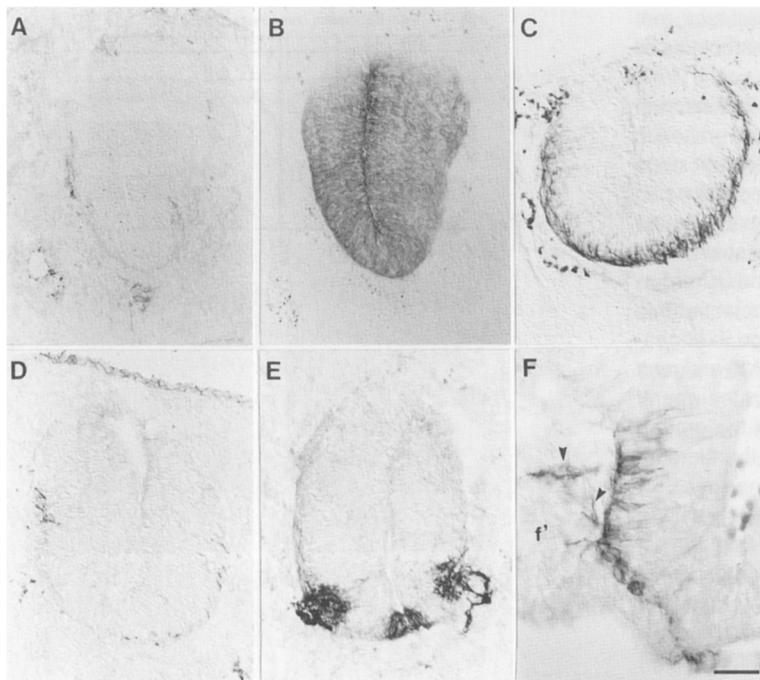


Figure 7. The Absence of a Floor Plate Changes Cell Pattern in the Developing Spinal Cord

(A) Section through the spinal cord of a chick embryo, from which a segment of notochord had been removed at stage 10. No SC1-labeled cells are detected within the spinal cord at levels lacking the notochord, although a few labeled axons are seen adjacent to and under it. Faint labeling of the roof plate is also detectable in this section (stage 20). Similar results were obtained in 4 embryos.

(B) Adjacent section through the embryo shown in (A). Virtually the entire spinal cord, including the ventral midline, expresses the AC4 antigen. Only a small region of neural epithelium adjacent to the roof plate is not labeled.

(C) Differentiated neurons labeled by MAb 3A10 in the spinal cord of a different embryo from which the notochord had been removed. Many of these labeled neurons appear to be commissural neurons, since a similar pattern of labeling was observed with antibodies against CRABP (not shown).

(D) Expression of the SC1 antigen in spinal cord after grafting a 500 μ m segment of stage 9 caudal neural tube into stage 14 chick wing bud region. Grafts were performed before floor plate differentiation had occurred (see Placzek

et al., 1990b). Embryos examined at the equivalent of stage 22 of graft development revealed no SC1-labeled cells in the spinal cord, although few labeled axons were observed. There was no morphological sign of a floor plate at the ventral midline of the spinal cord. Similar results were obtained in 4 embryos.

(E) Stage 12 caudal neural tube grafted into the wing bud prior to motor neuron differentiation. Expression of the SC1 antigen reveals the presence of a floor plate and motor neurons. Similar results were obtained in 3 embryos.

(F) Grafting of caudal stage 9–10 chick neural tube, together with a segment of E13 rat floor plate, results in the appearance of SC1-labeled neurons in the area adjacent to the floor plate graft (f'). SC1-labeled axons (arrowheads) project out of the neural tube. Similar results were obtained in 6 embryos.

Scale bar: A and B = 70 μ m; C, D, and E = 60 μ m; F = 40 μ m.

side to which a notochord or floor plate had been grafted (Figure 3D), although the size of the dorsal root ganglia appeared smaller than that on the contralateral side.

Cell Pattern in the Absence of the Notochord and Floor Plate

The pattern of cell types observed in the developing spinal cord after notochord or floor plate grafts shows that these two cell groups can regulate neural cell differentiation. To examine whether the notochord and floor plate are required to establish the normal pattern of cell differentiation, we eliminated these two cell groups early in neural tube differentiation. Floor plate differentiation does not occur until stage 12 in the caudal region of the chick neural tube, and removal of the notochord before this stage prevents subsequent development of the floor plate (Placzek et al., 1990b). We therefore removed a piece of notochord from the caudal region of stage 10–11 embryos and examined the resulting pattern of cell differentiation within the spinal cord. At segmental levels lacking the notochord, cells at the ventral midline of the spinal cord did not express either the SC1 (Figure 7A) or FP1 (not shown) antigens, providing further evidence that removal of the notochord prevents the differentiation of the floor plate. The absence of the SC1 antigen also showed that motor neurons were absent. Conversely, the AC4 antigen, which

is normally absent ventrally (Figure 6A), was expressed along the entire D–V extent of the spinal cord, except for the small area near the roof plate (Figure 7B). Although motor neurons were absent, other neurons were present in the spinal cord, many of which coexpressed the 3A10 antigen (Figure 7C) and CRABP (not shown), suggesting that they are commissural neurons. These neurons were present at abnormally ventral positions, including the region normally occupied by motor neurons and the floor plate (Figure 7C). Thus, removal of the notochord and floor plate appears to prevent the appearance of motor neurons but not other classes of spinal neurons.

To further examine the contribution of different cell types to normal cell patterning, the neural tube was isolated from both the notochord and somitic mesoderm, which has been suggested to influence neural tube development (Watterson, 1965). To achieve this, segments of caudal neural tube from stage 9 embryos were grafted into the wing bud region of stage 14 host embryos. The SC1 antigen was not detected in the grafted neural tube (Figure 7D), indicating that both the floor plate and motor neurons were absent. In contrast, when the caudal neural tube was grafted from stage 13 embryos, after the onset of floor plate differentiation but before the appearance of motor neurons, both a floor plate and motor neurons were present in the spinal cord (Figure 7E). Thus, after the on-

set of floor plate differentiation within the neural tube, motor neurons can develop in the absence of the notochord and somites.

In all of the experiments described above, induction of neural cells at ectopic positions occurred in the presence of a host notochord and floor plate. The finding that stage 9 caudal neural tube fails to develop a floor plate when grafted into the wing bud was used to test whether the floor plate can induce motor neurons in the absence of any contribution from the host floor plate or notochord. A small piece of quail or rat floor plate was implanted into the wing bud together with a segment of stage 9–10 caudal neural tube. Expression of the SC1 and 3A10 antigens revealed that motor neurons were present in the neural tube in the region adjacent to the location of the grafted floor plate (Figure 7F). Floor plate grafts can therefore induce motor neuron differentiation in the absence of a host floor plate. We did not observe an induced floor plate in these grafts; however, this may have resulted from the difficulty in maintaining the two grafted pieces in proximity in the wing bud. Previous studies suggest that a host floor plate is not necessary for induction of a floor plate at ectopic sites (Placzek et al., 1990b).

Conservation of Positional Relationship between Motor Neurons and the Floor Plate

The grafting experiments described above suggest that motor neurons appear at a constant distance from the notochord and floor plate. If this is the case, then removal of a segment of notochord and the subsequent failure of floor plate differentiation might be expected to result in the appearance of additional motor neurons at the ventral midline of the neural tube, at a fixed position relative to the cut end of the notochord and floor plate (see Figure 8A). To examine the spatial relationship between the notochord, floor plate, and motor neurons in greater detail, we therefore removed a segment of notochord before floor plate differentiation. Serial sections through the spinal cord were analyzed at different positions within the notochord-free region (Figure 8A). The floor plate extended for ~60 μm beyond the severed notochord (Figure 8B). Further into the notochord-free region, expression of floor plate antigens disappeared, although distinct lateral motor columns were still present, separated by a wide expanse of unlabeled cells at the ventral midline of the neural tube (Figure 8C). At ~120 μm into the notochord-free region, the bilaterally distinct motor columns disappeared and a single expanded region of motor neurons was present at the ventral midline of the neural tube (Figure 8D). At distances greater than ~160 μm from the cut end of the notochord, motor neurons were absent (Figure 8E). Thus, ectopic motor neurons can be induced by removal of the notochord and floor plate as well as by ectopic grafts. In all cases, the ectopic motor neurons appeared at a constant distance from the floor plate and notochord.

Discussion

The distinct cell types found in different regions of the vertebrate nervous system are thought to be generated as a

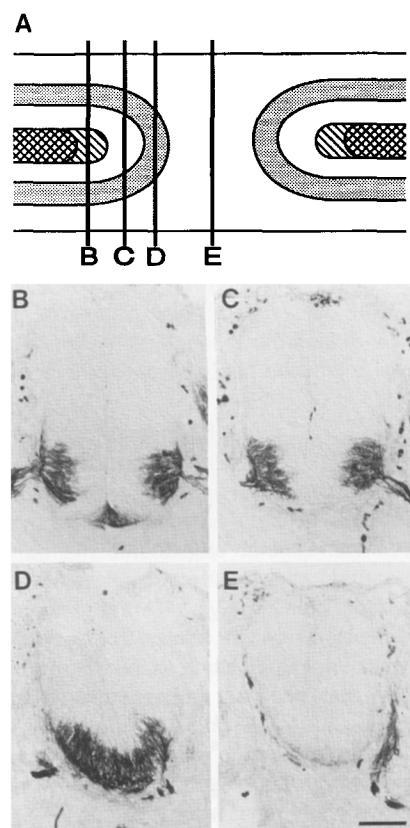


Figure 8. Motor Neuron Differentiation Occurs at a Constant Distance from the Floor Plate after Notochord Removal

(A) Schematic diagram showing a top-down view of the spinal cord of a stage 20 chick embryo from which a 200–300 μm segment of notochord had been removed at stage 10. The approximate positions of the notochord (cross hatching), floor plate (hatching) and motor neurons (stipple) in and around the operated region are shown and assessed by reconstruction from several embryos. Vertical lines indicate the levels from which the cross sections shown in (B) through (E) were obtained.

(B) Cross section of spinal cord at level B. Even though the notochord is absent, there is a normal pattern of SC1 labeling on the floor plate and the bilaterally symmetric columns of motor neurons.

(C) Section at level C. SC1 labeling has disappeared from the ventral midline. Two bilateral columns of motor neurons are still present.

(D) Section at level D, in which there is a single ventral midline group of SC1-labeled motor neurons.

(E) Section at level E, in which no SC1-labeled cells are detected in the spinal cord. Note the smaller cross sectioned area of the spinal cord in this region. Similar results were obtained in 4 embryos.

Scale bar: B, C, D, and E = 70 μm.

consequence of local interactions early in neural development. The results presented here provide evidence that signals from the notochord and floor plate have an instructive role in establishing the identity and position of distinct classes of cells along the D-V axis of the nervous system. The normal program of cell differentiation is changed when an additional floor plate or notochord is present at ectopic locations. Neuronal differentiation is also perturbed by removal of the notochord and floor plate. In both conditions, defined subsets of neurons appear at constant

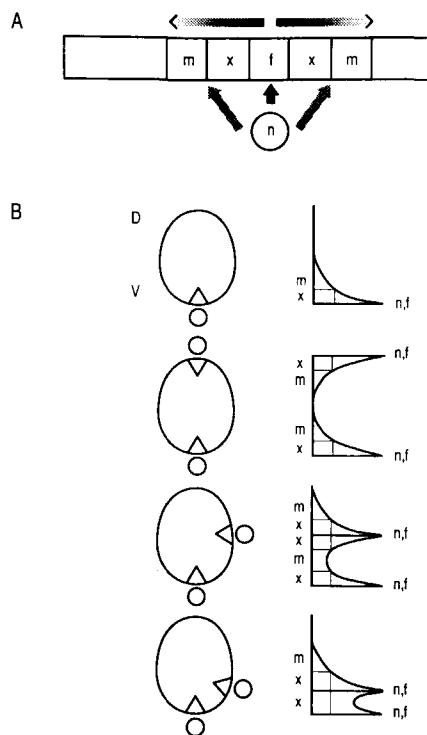


Figure 9. A Model for the Control of Cell Pattern along the Dorsoventral Axis of the Developing Nervous System by the Notochord and Floor Plate

(A) Diagram showing the possible inductive and signaling properties of the notochord and floor plate. The floor plate is induced by local signals from the underlying notochord. Once the floor plate is induced, it mimics the notochord in its signaling properties as assayed by changes in the pattern of cell differentiation in the developing spinal cord. Both structures may contribute to dorsoventral patterning during normal development. For simplicity, the diagram shows these interactions at the neural plate stage, although it is likely that these interactions continue throughout neurulation.

(B) A tentative model consistent with the results of grafting experiments assumes that the notochord and floor plate are sources of a diffusible signal(s), which is distributed in a graded manner through the adjacent neural tube. Cell identity and the overall pattern of cell types is controlled by the concentration of diffusible signal to which precursor neuroepithelial cells are exposed. Additional details of this scheme are discussed in the text. This scheme is based loosely on a model for the role of the ZPA and retinoic acid in controlling A-P polarity in the developing chick limb (see Tickle et al., 1975; Eichele, 1989). n, notochord; f, floorplate; m, motor neurons; x, intervening area.

distances from the host and secondary floor plate and notochord.

Control of Cell Differentiation by the Notochord and Floor Plate

Induction of the floor plate by the notochord appears to be the first step in establishing the pattern of cell differentiation along the D-V axis of the neural tube (Figure 9A). Notochord grafts can induce cell shape changes characteristic of the floor plate (van Straaten et al., 1988; Smith and Schoenwolf, 1989) and the expression of a floor plate-specific chemoattractant (Placzek et al., 1990b) at ectopic locations in the neural tube. The expression of

floor plate antigens in response to notochord grafts provides additional evidence that differentiation of the floor plate is induced by signals from the notochord. The experiments in which caudal neural tube was grafted into the wing bud, however, suggest that later stages of floor plate differentiation can occur independently of the notochord. Removal of the notochord at different stages of development in amphibian and chick embryos has also provided evidence that floor plate differentiation does not require the continued presence of the notochord (Horstadius, 1944; Kitchin, 1949; Watterson et al., 1954).

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 from stage 15 (Langman and Hayden, 1970; Hollyday and Hamburger, 1977). Previous studies have examined the role of mesodermal tissues in the control of ventral neuroblasts in the chick neural tube. Grafts of notochord and muscle next to the spinal cord of older embryos have been shown to increase the proliferation of ventral neuroblasts (van Straaten et al., 1985; van Straaten and Drukker, 1987; van Straaten et al., 1989; Fontaine-Pérus et al., 1989). It remains to be determined whether the mitogenic effects of grafts into older embryos are related to the patterning events reported here in young embryos. Rotation of stage 11–12 neural tube 180° along the D–V axis 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). Our results support and extend the conclusions of that study.

The mechanisms by which the notochord and floor plate control motor neuron differentiation are not known. The increase in the total number of motor neurons eliminates the possibility that notochord and floor plate grafts simply cause the redistribution of a fixed number of motor neurons. There is very little cell death in the lateral neural tube before stage 15 (Homma et al., 1990), but we cannot exclude the possibility that the appearance of ectopic motor neurons results from the rescue of a population of motor neuron progenitors. It is more likely that the differentiation of motor neurons in ectopic regions of the spinal cord results from a change in the fate of motor neuron progenitors. 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). Our results suggest that these progenitors are distributed throughout the neural tube and that signals from the floor plate and notochord are required to promote motor neuron differentiation. The differentiation of other classes of neurons in the ventral region of the spinal cord and hindbrain (for example, serotonergic neurons) may be controlled in a manner similar to that of motor neurons.

The rostral-most group of primary motor neurons is located in the oculomotor nucleus in the midbrain, approximately at the level of the rostral end of the floor plate (Kingsbury, 1930). The experiments presented in this article provide evidence that motor neurons are induced by the floor plate, supporting the suggestions of Ahlborn (1883) and Kingsbury (1930) that the rostrocaudal domain

of the neuraxis over which motor neurons can differentiate is dependent on the notochord and 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, other classes of spinal neurons, including commissural neurons, were present. The differentiation of commissural neurons and neural crest cells in the dorsal neural tube is therefore not dependent on the notochord and floor plate. The differentiation of dorsal cell types may require instructive signals from other cell groups, perhaps the roof plate. Alternatively, neural ectodermal cells may differentiate into dorsal cell types in the absence of any additional signals, in which case the notochord and floor plate may promote the appearance of ventral cell types by modifying a constitutive program of dorsal differentiation.

Control of Cell Pattern within the Neural Tube

Grafting and deletion experiments revealed a constancy in the resulting pattern of ventral cell types, which was most evident when examining motor neurons and the floor plate. Regardless of the position within the spinal cord at which these two cell groups were located, motor neurons never appeared immediately adjacent to the floor plate and were always separated by a narrow region of neural epithelium (see Figures 3A and 3B). In the hindbrain, this region contains a distinct class of neurons that expresses serotonin.

The pattern of cell differentiation within the neural tube could be established by a cascade of locally acting, possibly contact-dependent signals originating at the floor plate, with each cell type responsible for inducing its dorsolateral neighbors. The inability of grafts of the region of neural epithelium immediately adjacent to the floor plate to induce motor neurons provides some evidence against such a model. Alternatively, cell differentiation in the ventral neural tube could be controlled solely by signals from the notochord and floor plate.

For example, the notochord and floor plate could act as local sources of a factor that diffuses through the adjacent neural epithelium, establishing a concentration gradient with its high point at the ventral midline (Figure 9B). In this scheme, the differentiation of neural epithelial cells into distinct cell classes during normal development would be controlled by the concentration of this factor, to which they are exposed. Neural plate cells overlying the notochord are exposed to a high concentration of this factor and differentiate into the floor plate (Figure 9A). The floor plate then acquires signaling properties that mimic those of the notochord. Cells adjacent to the floor plate and notochord are exposed to intermediate concentrations of this factor and differentiate into the intervening area (x in Figure 9B) in the spinal cord and into serotonergic neurons in the hindbrain. Cells further from the floor plate and notochord are exposed to lower concentrations and differentiate into motor neurons. The differentiation of cells in the dorsal region of the neural tube occurs independently but can be subverted by this ventral midline-derived factor.

The grafting and deletion experiments described here are consistent with this general scheme. For example, in experimental embryos in which the distance between the host and ectopic floor plate is sufficiently great, the minimum concentration of the graded signal falls within a range that directs neural epithelial cell differentiation into motor neurons (Figure 9B). Thus, the original motor neuron pool is maintained, together with a new ectopic dorsal group of motor neurons (Figure 3A). However, when the distance between the host and ectopic floor plate decreases, the minimum concentration is maintained within a range that leads to the differentiation of area x (Figure 9B), with the result that motor neurons fail to appear (Figure 3C). Removal of a segment of notochord also results in a change in the pattern of cell differentiation in the surrounding neural tube (Figure 8) that is consistent with the possibility that the fate of a neural epithelial precursor cell is dependent on its position with respect to the notochord and floor plate. The observation that floor plate grafts can induce an additional floor plate raises the question of why the signals responsible for induction of the floor plate do not propagate laterally and convert all neural tube cells into the floor plate. One potential reason is that notochord cells begin to induce the differentiation of cells adjacent to the floor plate (Figure 9A), making them refractory to subsequent signals from the floor plate.

A similar gradient model has been proposed to explain the pattern of cell differentiation along the A–P axis of the developing chick wing bud (Tickle et al., 1975). In the wing bud, A–P pattern appears to be under the control of a specialized region of posterior mesenchyme known as the zone of polarizing activity (ZPA), which can respecify A–P polarity when grafted to ectopic sites (Saunders and Gas-seling, 1968; Tickle et al., 1975). Retinoic acid mimics the effects of the ZPA (Tickle et al., 1982) and appears to be distributed in a graded manner along the A–P axis of the limb bud within 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 (Hornbruch and Wolpert, 1986; Wagner et al., 1990) and floor plate (Wagner et al., 1990), 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. Moreover, biochemical studies show that the floor plate can synthesize morphogenetically active retinoids *in vitro* (Wagner et al., 1990). These observations raise the possibility that 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 have a common molecular basis, possibly involving retinoids.

Studies of amphibian neural development have provided evidence that regional differentiation along the A–P axis is established early in the development of the neural plate (Roach, 1945; Jacobson, 1964; Hemmati-Brivanlou and Harland, 1989), under the control of signals from dorsal mesoderm (Mangold, 1933; Ruiz i Altaba and Melton, 1989). The fate of a cell within the nervous system may

therefore be restricted initially by its A-P position within the neural plate. The position of the cell along the D-V axis of the neural tube with reference to the notochord and floor plate may later determine which of the limited number of possible fates, defined by its A-P position, is selected. For example, regional differences along the A-P axis may determine that cells in the hindbrain located immediately adjacent to the floor plate differentiate into serotonergic neurons, whereas in the spinal cord cells located in an equivalent position differentiate into a distinct (Yaginuma et al., 1990) class of neurons.

Taken together, our results provide evidence that a series of inductive interactions occurring early in the development of the vertebrate nervous system has a central role in the patterning of neural cells. Signals from the notochord induce the floor plate at the midline of the neural plate and neural tube. The floor plate and notochord then appear to control the differentiation of other neural cells and to contribute to the D-V pattern of the nervous system. Thus, as in many other developing tissues (Spemann, 1938; Wolpert, 1969; Tickle et al., 1975; Melton et al., 1988), the pattern of cell differentiation in the neural tube may depend critically on the organizing properties of specialized cell groups.

Experimental Procedures

Animals

Chick and quail eggs (Spafas; Truslow Farms) were incubated at 38°C in a humidified forced draft incubator, and embryos were staged according to the criteria of Hamburger and Hamilton (1951).

The results described in this paper were derived from 86 operated embryos.

Surgical Procedures

Notochord and Floor Plate Grafts

A small region of egg shell and the underlying vitelline membrane were removed. The embryo was submerged in a few drops of warm (37°C–38°C) L-15 medium to prevent dehydration, and India ink was injected under the embryo to enhance visual contrast. An incision was made into the ectoderm and underlying mesoderm to expose one side of the neural tube at the level of the 10th–15th somite. Tissue to be grafted was inserted in the cut. Notochord grafts (500–700 μm long) were obtained from stage 10–12 chick embryos. Floor plate grafts were taken from stage 17–20 chick embryos, E2–E3 quail embryos, and E13 rat embryos. Notochord grafts into the hindbrain of stage 8–9 host embryos were performed using similar procedures, using the position of the otic vesicle as a landmark.

After insertion of grafts, excess medium was removed and the window in the shell was sealed with parafilm. Embryos were incubated at 37°C–38°C for another 40–48 hr after floor plate and notochord grafts in the spinal cord and for another 60–72 hr after the notochord grafts to the hindbrain.

Notochord Removal

Eggs were prepared as described above, and embryos were exposed to 0.15% Trypsin in L-15 medium for 20 min. The ectoderm overlying the caudal region of the embryo was reflected, and the neural tube was separated from the surrounding mesoderm and the underlying notochord. A small segment (200–400 μm) of notochord was then removed from the most caudal region of stage 10 embryos, and the neural tube was returned to its original position. Excess medium was removed, the window was sealed with a coverslip, and embryos were incubated at 37°C–38°C for another 48 hr. In control experiments, the neural tube was separated from the notochord in an identical manner, but the notochord was left intact before replacement of the neural tube.

Neural Tube Grafts into the Wing Bud

Segments (200–400 μm long) of neural tube from the caudal region of stage 9–15 embryos were isolated and inserted into the wing bud re-

gion of stage 12–14 host embryos through a small slit in the ectoderm. The ectoderm was resealed, and embryos were allowed to develop for an additional 40–48 hr. In some experiments, a small piece of E13 rat floor plate or E2–3 quail floor plate was grafted together with stage 9–10 caudal neural tube.

Immunohistochemistry

Control and operated embryos were fixed by immersion, 4% paraformaldehyde in 0.12 M phosphate buffer for 2 hr at 4°C. Frozen 10 μm cryostat sections were mounted on gelatin-coated slides and washed with 10 mM phosphate buffered saline (PBS). Sections were incubated in primary antibodies diluted 1:1 for MAbs and 1:1000 for rabbit antisera in 10 mM PBS, 0.2% Triton X-100. Then sections were rinsed with 10 mM PBS containing 1% heat-inactivated goat serum and incubated in secondary antibodies (HRP-conjugated goat anti-mouse IgG or IgM; HRP-conjugated goat anti-rabbit, both 1:100) in 10 mM PBS with 1% heat-inactivated goat serum overnight at 4°C. The HRP reaction product was visualized with diaminobenzidine (0.5 mg/ml in 10 mM PBS) and H₂O₂ (0.003% final concentration). Sections were then washed, dehydrated, and mounted in Permount.

Cell-Specific Antigens Recognized by Monoclonal and Polyclonal Antibodies

Floor plate cells express antigens recognized by MAb FP1 (Figure 1A), FP2 (Figure 1B), and SC1 (Figure 1C) (Tanaka et al., 1984). The FP1 and SC1 antigens appear on the floor plate soon after neural tube closure. Initially, the FP1 and SC1 antigens are expressed by the same population of ventral midline cells, but by stage 15, FP1 is expressed in a few more lateral cells than SC1. The difference in the lateral extent of expression of the FP1 and SC1 antigens raises the possibility that there are distinct subsets of cells within the floor plate, although we have not examined this in detail. In the study we report on here, we have defined the floor plate by cell morphology combined with the expression of the FP1 or SC1 antigens. The FP2 antigen appears at later stages and is restricted to the basal region of the floor plate (Figure 1B).

MAb SC1 also labels motor neurons, but it does not label other classes of cells within the spinal cord and hindbrain. Although both motor neurons and floor plate express the SC1 antigen, these two cell types could be distinguished by the expression of other floor plate-specific or neuron-specific markers such as MAb 3A10. In addition, in some experiments, motor neuron cell bodies in normal and ectopic positions were identified by retrograde transport and accumulation of Dil after injection of the dye into the limb musculature (not shown).

Using MAb SC1 to identify both floor plate cells and motor neurons, it is evident that motor neuron cell bodies are located near to the floor plate but are separated by a region of unlabeled neuroepithelial cells (Figure 1C). The identity of these intervening cells has not been defined, although cells in this region appear to give rise to a longitudinally-oriented set of neurons located immediately adjacent to the floor plate (Yaginuma et al., 1990). Although no molecular markers are available for cells that occupy the region between the floor plate and motor neurons at the spinal cord level, in the hindbrain serotonergic neurons are located immediately adjacent to floor plate and can be identified using rabbit anti-serotonin antiserum (Figure 1D).

Cells in the dorsal region of the spinal cord, with the exception of those near the roof plate, express an antigen recognized by MAb AC4 (Dodd and Jessell, 1986). The AC4 antigen is expressed on all chick neural ectodermal cells from the neural plate stage until neural tube closure. The antigen then begins to disappear from the ventral neural tube and spinal cord in a ventral-to-dorsal progression. After the onset of neuronal differentiation, the AC4 antigen is expressed on the majority of cells, including neurons, in the intermediate and dorsal region of the spinal cord (with the exception of the roof plate) but is not expressed by floor plate cells, motor neurons, or other ventral cells (Figure 1E). Expression of AC4 persists in the dorsal region of the spinal cord at later stages of embryonic development, until at least stage 40. Expression of the AC4 antigen therefore provides an antigenic marker of cells located in intermediate and dorsal regions of the spinal cord. The cell bodies and axons of commissural neurons, and possibly other relay neurons in the dorsal region of the spinal cord, can be identified by antibodies directed against CRABP (not shown; see Maden et al., 1989).

The notochord is located immediately underneath the midline of the

neural plate and neural tube and can be identified by expression of a surface antigen recognized by MAB Not1 (Figure 1F).

In some experiments, MAbs directed against a chick-specific SC1 epitope (Tanaka, unpublished data) and a rat-specific floor plate antigen, K1 (Dodd and Jessell, 1988) were used in combination with the antibodies described above to distinguish graft from host floor plate and motor neurons.

Nuclear Labeling for Cell Counting

To count the number of motor neurons and the total number of cells in the spinal cord after notochord grafts, sections were labeled with SC1 antibody and FITC-conjugated secondary antibody for identification of motor neurons; Hoechst 33258 (1:1000 dilution in 10 mM PBS with 0.1% Triton X-100) was then applied to sections for 10–15 min at 22°C for identification of all cell nuclei.

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References

- Ahlborn, F. (1883). Untersuchungen über das Gehirn der Petromyzonten. *Z. Wiss. Zool.* 39, 191–294.
- Bovolenta, P., and Dodd, J. (1990). Guidance of commissural growth cones at the floor plate in embryonic rat spinal cord. *Development* 109, 435–447.
- Bovolenta, P., Jessell, T. M., and Dodd, J. (1988). Disruption of commissural axon guidance in the absence of the midline floor plate. *Soc. Neurosci. Abstract* 14, 271.
- Brockes, J. P. (1989). Retinoids, homeobox genes, and limb morphogenesis. *Neuron* 2, 1285–1294.
- Dodd, J., and Jessell, T. M. (1986). Cell surface glycoconjugates and carbohydrate-binding proteins: possible recognition signals in sensory neurone development. *J. Exp. Biol.* 124, 225–238.
- Dodd, J., and Jessell, T. M. (1988). Axon guidance and the patterning of axonal projections in vertebrates. *Science* 242, 692–699.
- Eichele, G. (1989). Retinoids and vertebrate limb pattern formation. *Trends Genet.* 5, 246–251.
- 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.
- Hamburger, V., and Hamilton, H. (1951). A series of normal stages in the development of chick embryo. *J. Morph.* 88, 49–92.
- Hemmati-Brivanlou, A., and Harland, R. M. (1989). Expression of an engrailed related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* 106, 611–617.
- 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.
- Hornbruch, A., and Wolpert, L. (1986). Positional signalling by Hensen's node when grafted to the chick limb bud. *J. Embryol. Exp. Morphol.* 94, 257–265.
- Horstadius, S. (1944). Über die Folgen von Chordaeextirpation an spaten Gastrulae und Neurulae von *Ambystoma punctatum*. *Acta Zool.* 25, 257–265.
- Hutchinson, D. (1936). Reconstitution in the nervous system following unilateral reversal of the dorsoventral axis in part of the spinal cord of *ambystoma punctatum*. *J. Comp. Neurol.* 63, 465–487.
- 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. I. Bilateral operations. *Zool. Bidrag. Uppsala* 36, 73–160.
- Jessell, T. M., Bovolenta, P., Placzek, M., Tessier-Lavigne, M., and Dodd, J. (1988). Polarity and patterning in the neural tube: the origin and role of the floor plate. *Ciba Found. Symp.* 144, pp. 255–280.
- Kingsbury, B. F. (1930). The developmental significance of the floor-plate of the brain and spinal cord. *J. Comp. Neurol.* 50, 177–207.
- Kitchin, I. C. (1949). The effects of notochordectomy in *ambystoma mexicanum*. *J. Exp. Zool.* 112, 393–415.
- Kuwada, J. Y., Bernhardt, R. R., and Chitnis, A. B. (1990). Pathfinding by identified growth cones in the spinal cord of zebrafish embryos. *J. Neurosci.* 10, 1299–1308.
- Langman, J., and Haden, C. C. (1970). Formation and migration of neuroblasts in the spinal cord of the chick embryo. *J. Comp. Neurol.* 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.
- 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. *Dev. Biol.* 135, 124–132.
- Mangold, O. (1933). Über die Induktionsfähigkeit der verschiedenen Bezirk der Neurula von Urodelen. *Naturwissenschaften* 21, 761–766.
- Melton, D. A., Ruiz i Altaba, A., Yisraeli, J., and Sokol, S. (1988). Localization of mRNA and axis formation during *Xenopus* embryogenesis. *Ciba Foundation Symposium* 144, pp. 16–36.
- Placzek, M., Tessier-Lavigne, M., Jessell, T., 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., Jessell, T., and Dodd, J. (1990b). Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science* 250, 985–988.
- Roach, F. C. (1945). Differentiation of the central nervous system after axial reversals of the medullary plate of *ambystoma*. *J. Exp. Zool.* 99, 53–77.
- Rosenquist, G. C. (1966). A radioautographic study of labelled grafts in the chick blastoderm. *Contrib. Embryol. Carnegie Inst.* 38, 71–110.
- 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.
- Saunders, J. W., Jr., and Gasseling, M. T. (1968). Ectodermal-mesenchymal interactions in the origin of limb symmetry. In *Epithelial-Mesenchymal Interactions*, R. Fleischmajer and R. E. Billingham, eds. (Baltimore: The Williams and Wilkins Co.), pp. 78–97.
- Schoenwolf, G. C., and Smith, J. L. (1990). Mechanisms of neurulation: traditional viewpoint and recent advances. *Development* 109, 243–270.
- 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.
- Spemann, H. (1938). *Embryonic Development and Induction* (New Haven: Yale University Press).
- Steding, G. V. (1962). Experimente zur morphogenese des Rückenmarkes. *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. *Dev. 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.
- Tickle, C., Summerbell, D., and Wolpert, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature* **254**, 199–202.
- 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.
- van Straaten, H. W. M., and Drukker, J. (1987). Influence of the notochord on the morphogenesis of the neural tube. In *Mesenchymal-Epithelial 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., Thors, F., Wiertz-Hoessels, E. L., Hekking, J. W. M., and Drukker, J. (1985). Effect of a notochordal implant on the early morphogenesis of the neural tube and neuroblasts: histometrical and histological results. *Dev. Biol.* **110**, 247–254.
- 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 differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol.* **177**, 317–324.
- 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 differentiation in the neural tube of the chick embryo. *Development* **107**, 793–803.
- 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. (1965). Structure and mitotic behavior of the early neural tube. In *Organogenesis*, R. L. DeHaan and H. Ursprung, eds. (New York: Rinehart and Winston), pp. 129–159.
- Watterson, R. L., Fowler, I., and Fowler, B. J. (1954). The role of the neural tube and notochord in development of the axial skeleton of the chick. *Am. J. Anat.* **95**, 337–400.
- 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.
- Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1–47.
- 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.

Note Added In Proof

The work referred to as K. Hatta and C. Kimmel, personal communication is now in press: Hatta, K., Kimmel, C. B., Ho, R. K., and Walker, C. (1991). "Cyclops," a mutation that blocks specification of the floor-plate of the zebrafish CNS. *Nature*, in press.