

Induction of floor plate differentiation by contact-dependent, homeogenetic signals

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SUMMARY

The floor plate is located at the ventral midline of the neural tube and has been implicated in neural cell patterning and axon guidance. To address the cellular mechanisms involved in floor plate differentiation, we have used an assay that monitors the expression of floor-plate-specific antigens in neural plate explants cultured in the presence of inducing tissues. Contact-mediated signals from both the notochord and the floor plate act directly on neural plate cells to induce floor plate differentiation. Floor plate induction is initiated medially by a signal from the notochord, but appears to be propagated to more lateral cells by homeogenetic signals that

derive from medial floor plate cells. The response of neural plate cells to inductive signals declines with embryonic age, suggesting that the mediolateral extent of the floor plate is limited by a loss of competence of neural cells. The rostral boundary of the floor plate at the midbrain-forebrain junction appears to result from the lack of inducing activity in prechordal mesoderm and the inability of rostral neural plate cells to respond to inductive signals.

Key words: floor plate, notochord, neural plate, induction.

INTRODUCTION

Inductive interactions, in which signals from one cell group control the fate of adjacent cells, constitute one of the major strategies used to generate diverse cell types during vertebrate embryogenesis (Gurdon, 1992; Jessell and Melton, 1992). There is increasing evidence that the differentiation and patterning of cells within the neural plate is controlled by adjacent mesodermal cells. The first neural cells to exhibit overt differentiation are located at the midline of the neural plate (Schoenwolf and Smith, 1990) and later give rise to the floor plate at the ventral midline of the neural tube (Kingsbury, 1930). Evidence that the floor plate is induced by axial mesodermal cells of the notochord has derived from studies in chick embryos. Notochord grafts placed next to the neural tube induce the morphological (van Straaten et al., 1988; Smith and Schoenwolf, 1989), antigenic (Yamada et al., 1991) and functional (Placzek et al., 1990b) properties of the floor plate in adjacent neural cells. Other ventral neural cell types, including motor neurons, also differentiate ectopically in response to notochord grafts (Yamada et al., 1991; Ericson et al., 1992). Inversely, floor plate cells and motor neurons do not develop in the absence of the notochord (Placzek et al., 1990b; Bovolenta and Dodd, 1991; Clarke et al., 1991; Hirano et al., 1991; van Straaten and Hekking, 1991; Yamada et al., 1991;

Ruiz i Altaba, 1992). Thus, the notochord appears to be required for the differentiation of the floor plate and ventral neurons. The ability to induce a floor plate is, however, not restricted to the notochord. Grafts of the floor plate can also induce floor plate cells and motor neurons in vivo (Placzek et al., 1991; Yamada et al., 1991; Ericson et al., 1992), suggesting that the floor plate also contributes to the normal patterning of ventral cell types.

In vivo studies have emphasized the role of the notochord and the floor plate in the control of early neural pattern, but do not permit analysis of the nature or mechanism of action of inducing signals that derive from these midline cells. Floor plate differentiation can be induced in explants of neural plate in vitro as assayed by expression of a floor plate-specific axonal chemoattractant (Placzek et al., 1990b). However, this assay is indirect, requiring the presence of dorsal spinal cord which could contribute cofactors to the induction. Moreover, in this assay, it is not possible to examine whether floor plate cells induce floor plate since they themselves produce the chemoattractant.

To begin to examine the mechanisms underlying floor plate differentiation, we have used surface antigens as cellular markers to develop an in vitro assay that monitors floor plate induction directly. Both the notochord and the floor plate produce a contact-dependent signal that is sufficient to direct competent neural plate cells to a floor plate

fate. Our results show that the earliest floor plate cells to differentiate are induced by the notochord and suggest that recruitment of adjacent neural plate cells to a floor plate fate is mediated by a homeogenetic signal from previously induced floor plate cells. The spatial extent of the floor plate may be limited in part by the loss of competence of neural plate cells to respond to inductive signals propagated from the midline.

MATERIALS AND METHODS

Tissue dissection

Lateral neural plate tissue was isolated from the neural plate region of embryonic day (E) 9 (at level of prospective somites 1-3) or E10 (at level of prospective somites 15-19) rat embryos (see Fig. 1). Rat embryonic age was assessed by somite number: 5-6 somites develop every 12 hours. E9 embryos had 0-2 somites, E10 embryos had 10-12 somites and E11 embryos had 22-24 somites. Tissues were incubated at 22°C in L15 containing 1 mg/ml Dispase (Boehringer-Mannheim) for 10 minutes, then washed in L15 and neural tissue isolated using tungsten needles. Prospective floor plate and roof plate regions were cut off. The remaining tissue constituted the lateral neural plate explant.

Hamburger-Hamilton (1951) stages were used to assess chick development. Notochord and floor plate explants from stage 6-13 chicks were isolated by dissection after Dispase treatment. Notochord explants isolated from stage 6 embryos were occasionally contaminated with endodermal tissue. Stage 14-24 chick notochord and floor plate explants were dissected in the absence of Dispase except as noted in the text. In all the experiments described here, notochord and floor plate were dissected from anterior regions (adjacent to prospective or actual somites 0-5) of chick embryos. This contrasts with our previously published experiments in which inducing tissues were dissected from caudal regions. This difference accounts for the apparent discrepancy in the observed time of loss of inducing activity by the notochord during development *in vitro* (results described here) and *in vivo* (eg. Yamada et al., 1991). Prechordal plate mesoderm and the underlying endoderm were dissected from ectoderm after Dispase treatment. In all cases, Dispase was used at 1 mg/ml for 5 minutes.

Explant culture

Explants were embedded within three-dimensional collagen gels as described previously (Tessier-Lavigne et al., 1988; Placzek et al., 1990a) and cultured in Opti-MEM (Gibco) supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), glutamine (2 mM) and 4% foetal calf serum (Gibco) at 37°C in a 5% CO₂ environment for various lengths of time, usually for approx. 85 hours. Where indicated, cytochalasin D (0.1-0.5 µg/ml) was included in the medium. Neural plate explants were cultured alone, with potential inducing tissue or with conditioned medium (see below). The standard assay used conjugates of E10 rat lateral neural plate and stage 6-7 chick notochord or floor plate (Fig. 1). Unless otherwise indicated, all conjugates were made by intertwining the two tissue pieces to ensure contact. This was most easily achieved by manipulating explants within collagen matrices; however, floor plate induction was also observed when notochord or floor plate explants were plated in contact with neural plate explants on 35 mm tissue culture dishes (Nunc). Within 6 hours of culture in collagen matrices, the explants rounded up and could not be separated unless treated with Dispase. The effect of each experimental paradigm was examined on a minimum of four neural plate explants and in most cases many more (see figures).

In experiments to determine whether floor plate inducing activity is diffusible, two different culture paradigms were used. First neural plate explants were surrounded by a ring of notochord or floor plate explants, placed at a distance of 50-100 µm from the neural plate tissue ($n=8$ each, for notochord and floor plate) in a collagen gel. Second, neural plate explants and notochord or floor plate explants were positioned on opposite sides of Nucleopore (0.2 µm pore-size, $n=4$ each) or dialysis (50×10³ M_r exclusion limit, $n=10$ each) membranes. To examine the time course over which tissues retain inducing activity when cultured *in vitro*, stage 6 notochord or stage 6-24 floor plate were cultured alone for various times before placing them in contact with neural plate explants in fresh collagen gels for a further 80 hours ($n=4-8$ each).

Labelling with BrdU

To assess incorporation of BrdU into cells in neural plate explants cultured alone or with inducing tissue, 30 µg/ml BrdU (Sigma) was added to the culture medium at one of the following time points: 0 hours, 12 hours, 26 hours, 44 hours, 70 hours. After a one hour incubation period, BrdU was removed and explants were immediately processed for immunohistochemical detection of BrdU. To assess the effects of mitomycin C on cell division, mitomycin C-treated and control explants were pulsed for 1 hour with 30 µg/ml BrdU at either 12 hours or 24 hours. Explants were then washed twice in culture medium, and incubated further (up to a total of 100 hours) in culture medium alone.

Immunocytochemistry

Embryos were fixed as previously described (Dodd et al., 1988), transferred to 7% gelatin (300 Bloom, Sigma), 10% sucrose in 0.12 M phosphate buffer at 37°C for 3 hours, then to 4°C overnight. Embryos in gelatin blocks were mounted in Tissue-tek, and 10-15 µm cryostat sections were incubated with antibodies to FP3 and FP4 as described previously (Dodd et al., 1988).

Explants cultured in collagen gels were processed for immunocytochemistry. Explants were fixed in ice-cold 4% paraformaldehyde in 0.12 M phosphate buffer for 2 hours and then either sucrose-protected and sectioned as previously described (Tessier-Lavigne et al., 1988) or processed as whole mounts. For whole-mount labelling, explants cushioned in surrounding collagen were transferred from the culture dish to a polypropylene test-tube, and incubated at 4°C in 1 ml 10 mM PBS, 1% HIGS, 0.1% Triton (PBS+). After 2 hours, PBS+ was replaced with primary antibody in PBS+, and the explants incubated with agitation overnight on a Nutator at 4°C. Explants were washed in PBS+, at 22°C for 2 hours on a Nutator, then incubated in FITC-conjugated secondary antibody (Boehringer-Mannheim) at 1.200 in PBS+ at 22°C for 2 hours. Explants were washed, placed on a slide and coverslipped in glycerol:carbonate buffer (1:1) containing paraphenylenediamine as described previously (Placzek et al., 1990a).

FP3 was detected using monoclonal antibody (mAb) 6G3 (mouse IgG, S. Morton, J. Dodd and T. Jessell, unpublished) and FP4 was detected using mAb K1/2E7 (mouse IgG1, Dodd and Jessell, 1988). mAbs 4D7 (mouse IgM, Yamamoto et al., 1986), which recognizes the TAG-1 axonal glycoprotein (Dodd et al., 1988), 2H3 (mouse IgG1), which recognizes the M neurofilament subunit (Dodd et al., 1988) and 3A10 (mouse IgG), which recognizes a neuron-specific protein (Furley et al., 1990) were used as neuronal markers. Antibodies directed against FP3, FP4, 4D7 and 2H3 do not cross-react with antigens in chick tissue. mAb Not-1 (mouse IgG, Yamada et al., 1991) was used to detect chick notochord. Hybridoma supernatants were diluted at 1:1 in PBS+. For simultaneous identification of FP3 and FP4, or FP3 and Not-1, in single sections or explants, double labelling was performed using mAb 2E7 or mAb Not-1 with rabbit polyclonal anti-FP3 antibodies (J. Walter, S. Morton and T. Jessell, unpublished) at

1:4000. Rabbit anti-FP3 was detected using rhodamine-conjugated secondary antibody (TAGO) at a 1:200 dilution.

BrdU was detected using anti-BrdU antibody (Becton-Dickinson) diluted at 1:20 in PBS+. Prior to labelling with anti-BrdU, sections were rinsed in PBS, incubated at 22°C in 2 M HCl for 35 minutes and rinsed extensively to neutralise. For double-labelling with Not-1 and anti-BrdU, sections were first processed for Not-1 labelling and then treated with 2 M HCl before performing anti-BrdU labelling. To examine the extent of floor plate induction after mitomycin C treatment, alternate sections were labelled with 6G3 and anti-BrdU. For visualisation of nuclei after cytochalasin D treatment, sections were incubated in Hoechst 33258 at 0.1 µg/ml in PBS for 15 minutes.

Quantitation of BrdU incorporation

The number of BrdU-labelled nuclei per explant was counted in serial 12 µm cryostat sections. Neural plate-notochord conjugates were double-labelled with Not-1 and anti-BrdU antibodies and the BrdU-labelled nuclei in Not-1-labelled tissue excluded from the counts. To determine the percentage of cells that incorporated BrdU within the first 24 hours, the number of cells in a neural plate explant at the time of dissection was calculated. A minimum of 20 explants were incubated in enzyme-free dissociation medium for 5 minutes at 37°C. The tissue was then rinsed, triturated to give a single cell suspension and mixed at 1:1 with 0.4% trypan blue. The numbers of live and dead cells were counted using a haemocytometer.

Mitomycin-C treatment of explants

Neural plate explants were incubated in 10 µg/ml mitomycin C in Opti-MEM at 37°C in 5% CO₂ for 20 minutes, washed for 3 hours at 4°C in three changes of L15 containing 1% heat-inactivated goat serum, then either cultured alone or as conjugates with untreated notochord or floor plate. After 12 hours or 24 hours in culture, explants were pulse-labelled with BrdU for 1 hour, then washed and cultured further up to a total of 100 hours.

Labelling with PKH26 and separation of tissues

PKH26 was made up in Diluent C according to the manufacturers protocol (Zynaxis Cell Science Inc). 5 µl of the dye solution was added to 1 ml 0.01 M PBS, and notochord or floor plate explants incubated in this at 22°C for 30 minutes. The explants were subsequently rinsed extensively in L15, their uniform labelling checked under fluorescent optics and then combined with unlabelled neural plate explants. To separate notochord or floor plate and neural plate explants after periods of contact, conjugates were removed from the collagen and incubated in Dispase (1 mg/ml). Neural plate explants that were free of contaminating labelled notochord tissue (assessed by examination under fluorescent optics) were then cultured further (up to a total of 100 hours) in collagen gels. Neural plate explants were serially sectioned and labelled either with mAb 6G3 alone or with mAb Not-1 and polyclonal anti-FP3.

Preparation of conditioned medium

Floor plate-conditioned medium (FPCM) was prepared as previously described (Placzek et al., 1990a), using 30 E13 rat floor plates to condition 1 ml of serum-free Opti-MEM. Concentrated FPCM was prepared by reducing a 10 ml preparation to a final volume of 1 ml through a 3 × 10⁵ M_r centricon membrane (Amicon). Concentrated FPCM was tested at various concentrations (ranging from 1:1 to 1:20), diluting against Opti-MEM, and

to a final concentration of 4% foetal calf serum. Notochord-conditioned medium was prepared in a similar manner. 100 stage 6 chick notochords were used to condition 1 ml medium.

Factors tested for the ability to induce floor plate differentiation

The following growth factors failed to induce a floor plate within rat neural plate explants, when tested over a concentration range of 1-100 ng/ml: TGFα, TGF 1, TGF 2, IGFI, IGFII, EGF, aFGF, bFGF, IL3a, IL4, IL5, IL6, GM-CSF, Stem Cell Factor, Natural Killer Stimulatory Factor, scatter factor. In addition, retinoic acid was tested over a concentration range of 10⁻⁵ to 10⁻⁸ M.

RESULTS

Cell differentiation in neural plate explants

To establish a direct *in vitro* assay of floor plate induction in rat neural plate, we first determined that floor plate differentiation does not occur in explants of rat lateral neural plate cultured in the absence of the notochord or floor plate. Explants of caudal neural plate dissected from embryonic day (E) 10 (10-somite stage) rat embryos (Fig. 1A,C) were cultured alone for periods up to 100 hours and examined for the expression of the rat floor plate-specific antigens FP3 and FP4 (see Fig. 2 legend). Explants examined after 14-100 hours did not express either antigen (Fig. 3A-D), indicating that floor plate differentiation did not occur. In contrast, there was extensive neuronal differentiation from 14 hours, as assessed by the expression of the neurofilament M subunit, the 3A10 antigen and the TAG-1 axonal glycoprotein (Fig. 3E-G).

In the spinal cord of chick embryos from which the notochord has earlier been removed, neurons characteristic of the dorsal spinal cord differentiate but floor plate cells and motor neurons do not (Yamada et al., 1991; Placzek et al., 1991; Ericson et al., 1992). We therefore examined whether the neurons that differentiated in rat neural plate explants grown alone were dorsal in phenotype. As described above, TAG-1 was expressed in explants, appearing after 30 hours *in vitro*. TAG-1 is a marker for commissural neurons in the dorsal spinal cord of rat embryos but is also expressed by motor neurons in the ventral spinal cord (Dodd et al., 1988). However, another early marker of motor neurons, the Islet-1 homeodomain protein (Thor et al., 1991; Ericson et al., 1992) was not expressed in rat neural plate explants grown alone (T. Yamada and M. Placzek, unpublished observations), suggesting that the TAG-1⁺ cells are commissural neurons and not motor neurons. In support of this, when the neural plate explants were exposed to a chemoattractant selective for dorsal commissural axons (Placzek et al., 1990a), they exhibited extensive axon outgrowth (Fig. 3H). These two results suggest that dorsal neuronal classes differentiate within neural plate explants cultured under conditions in which floor plate differentiation does not occur.

Induction of floor plate antigens in rat neural plate explants

Experiments *in vivo* show that floor plate differentiation

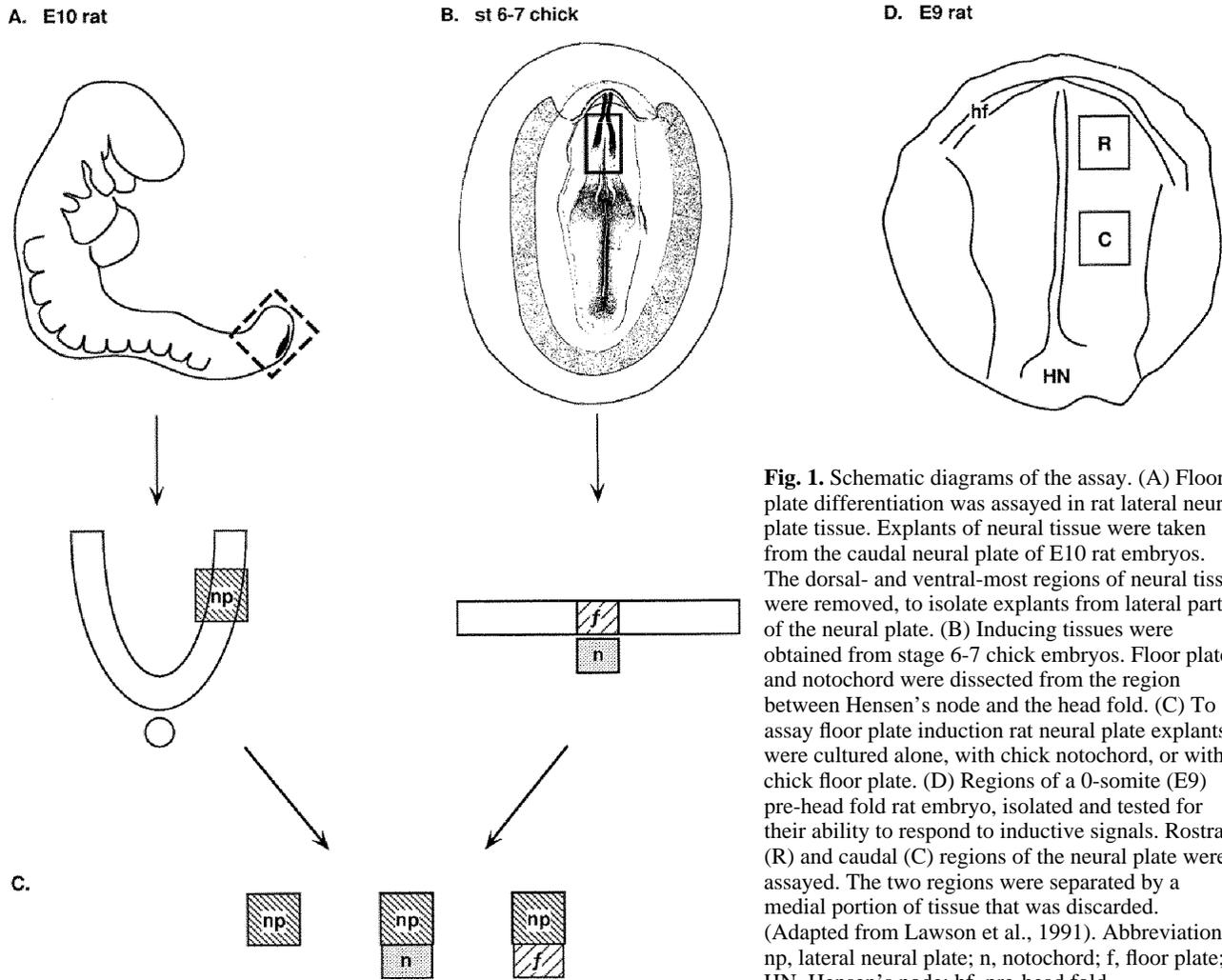


Fig. 1. Schematic diagrams of the assay. (A) Floor plate differentiation was assayed in rat lateral neural plate tissue. Explants of neural tissue were taken from the caudal neural plate of E10 rat embryos. The dorsal- and ventral-most regions of neural tissue were removed, to isolate explants from lateral parts of the neural plate. (B) Inducing tissues were obtained from stage 6-7 chick embryos. Floor plate and notochord were dissected from the region between Hensen's node and the head fold. (C) To assay floor plate induction rat neural plate explants were cultured alone, with chick notochord, or with chick floor plate. (D) Regions of a 0-somite (E9) pre-head fold rat embryo, isolated and tested for their ability to respond to inductive signals. Rostral (R) and caudal (C) regions of the neural plate were assayed. The two regions were separated by a medial portion of tissue that was discarded. (Adapted from Lawson et al., 1991). Abbreviations: np, lateral neural plate; n, notochord; f, floor plate; HN, Hensen's node; hf, pre-head fold.

can be induced by signals from either the notochord or the floor plate (Yamada et al., 1991; Placzek et al., 1991; Hatta et al., 1991). To determine whether signals from each cell group are sufficient to induce floor plate properties, explants of caudal neural plate from E10 rat embryos were grown in contact with explants of notochord or floor plate for up to 100 hours (Fig. 1A-C). Because rat notochord and floor plate both express FP3 and FP4 while chick tissues do not, stage 6 chick notochord and floor plate were used as the inducing tissues. Both notochord and floor plate were able to induce expression of FP3 and FP4 in rat neural plate tissue. FP3 was expressed by neural plate cells adjacent to the inducing tissue within 24 hours (not shown) and its level of expression increased progressively up to 70 hours (Fig. 4). FP4 was also expressed by neural plate cells cultured together with notochord or floor plate but was first expressed at 70 hours (Fig. 4), consistent with its delayed expression *in vivo* relative to that of FP3 (Fig. 2). Moreover, as *in vivo*, FP4 was expressed by only a subset of FP3⁺ cells (Fig. 4). The notochord and floor plate were equally effective as inducers of FP3 and FP4 in neural plate explants (Table 1).

Of many other embryonic tissues tested, only Hensen's

node was able to induce expression of FP3 and FP4 (Table 2). Indeed, the ability of Hensen's node to induce floor plate antigens may result from the differentiation of node tissue into notochord and floor plate that occurs under these *in vitro* conditions (M. P., unpublished observations). These results establish that inductive signals from the notochord and the floor plate are each sufficient to induce floor plate differentiation and show that these signals act directly on neural plate cells.

Prolonged cell contact is required for floor plate induction

The first cells at the ventral midline of the neural tube to express FP3 are in contact with the notochord (Fig. 2B), raising the possibility that contact is required for induction of floor plate properties. To test this possibility, neural plate explants were cultured with notochord or floor plate under conditions in which the tissues were in proximity but not in contact (see methods). FP3 and FP4 were not expressed in neural plate explants under these conditions. In addition, neural plate explants cultured alone for 100 hours in notochord- or floor plate-conditioned medium did not express FP3 or FP4 (not shown). These experiments suggest that

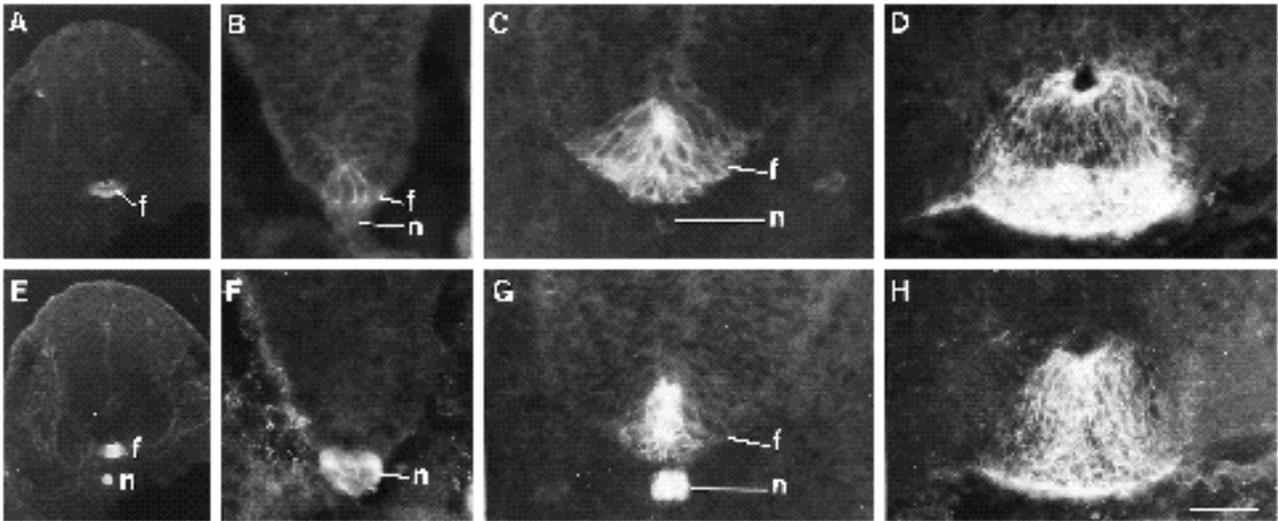


Fig. 2. Expression of the FP3 and FP4 antigens in the embryonic rat spinal cord. (A-D) FP3 expression. (E-H) FP4 expression. A and E show sections of an E12.5 rat embryo spinal cord showing that FP3(A) and FP4(E) are restricted to the floor plate (f). Both antigens are also expressed by the notochord (n), although the expression of FP3 by the notochord is transient and has ceased by E12.5. At the earliest embryonic stages examined (8-somite rat embryos, not shown), FP3 was expressed uniformly, but at extremely low levels, throughout the neural tube and FP4 was expressed selectively by notochord cells. (B,F) A 14-somite-stage embryo (E10.5), double-labelled to detect FP3 and FP4. Expression of FP3 is observed on a midline strip of cells, 2-5 cells wide, that is contacted by the notochord (n). FP4 remains restricted to the notochord at this stage. (C,G) A 24-somite-stage embryo (E11), double-labelled to detect FP3 and FP4. FP3 is now expressed at high levels on a midline strip of 15-20 cells. FP4 expression is first detected in the spinal cord on a 5 cell-wide strip of midline cells that constitute the medial subset of FP3⁺ cells. The notochord (n), has been displaced ventrally and no longer contacts the neural tube. (D,H) An E13 embryo, double labelled to detect FP3 and FP4. The domain of expression of FP4 (H) has expanded, but remains slightly narrower than that of FP3 (D). By late E13 (not shown) the domain of expression of FP4 is almost indistinguishable from that of FP3. The heterogeneity of the floor plate therefore appears only at earlier stages of floor plate development and may reflect the stage of differentiation of individual cells since FP3 is expressed earlier than FP4. Scale bar represents: A,E, 300 μ m; B,F, 36 μ m; C,D,G,H, 62 μ m.

induction of floor plate properties is mediated by a contact-dependent signal.

Grafting experiments *in vivo* show that the notochord must be present for a minimum of 18 hours for induction of the chick floor plate antigens, FP1 and FP2 (T. Yamada and M. Placzek, unpublished observations), although the period of contact necessary for induction cannot be determined from these studies. We therefore determined the period of contact that is required for floor plate differentiation in neural plate explants *in vitro* under conditions in which the duration of contact can be controlled. Notochord and floor plate explants were labelled with a lipid-partitioning dye, PHK26 (Horan and Slezak, 1989), and placed in contact with neural plate explants. After 12 hours, 18 hours or 24 hours, the inducing tissue was removed completely, as verified by the absence of dye-labelled cells, and neural plate explants were cultured for a total period of 100 hours. FP3 and FP4 were first detected in neural plate explants that had been contacted by notochord or floor plate for 18 hours (Table 3). This result suggests that a prolonged period of contact between the inducing tissue and the neural plate is required for floor plate induction *in vitro*. However, it remains possible that contact is required for a shorter period with the prolonged action of a second, diffusible, signal required for maintenance or progression of floor plate differentiation. To examine this possibility, we repeated the assay described above in the presence of floor plate-con-

ditioned medium. The minimum time of contact required for induction was not reduced (not shown), supporting the idea that prolonged contact is required for floor plate induction.

The notochord and floor plate provide proliferative signals for neural cells.

Removal of the notochord from early chick embryos results not only in the absence of a floor plate (Placzek et al., 1990; van Straaten and Hekking, 1991; Yamada et al., 1991) but also in a decrease in the number of cells in the neural tube (van Straaten and Hekking, 1991). This finding raises the possibility that the notochord and floor plate provide factors that promote the survival or proliferation of neural plate cells as well as inductive signals. Notochord grafts *in vivo* do not appear to increase the number of cells in the spinal cord (Yamada et al., 1991). However, proliferation of cells in the neural tube *in vivo* may be maximal in the presence of the host notochord and floor plate, masking an effect of ectopic notochord. To test whether notochord and floor plate have a proliferative effect on neural plate, we used bromodeoxyuridine (BrdU) incorporation as an indicator of cell division in neural plate explants that were isolated from host notochord. Explants were incubated with BrdU added for 1 hour at various times after the onset of culture and the number of labelled cells determined by immunocytochemistry. In neural explants grown alone, between 4 and 14% of cells incorporated BrdU over the first 24 hours, after

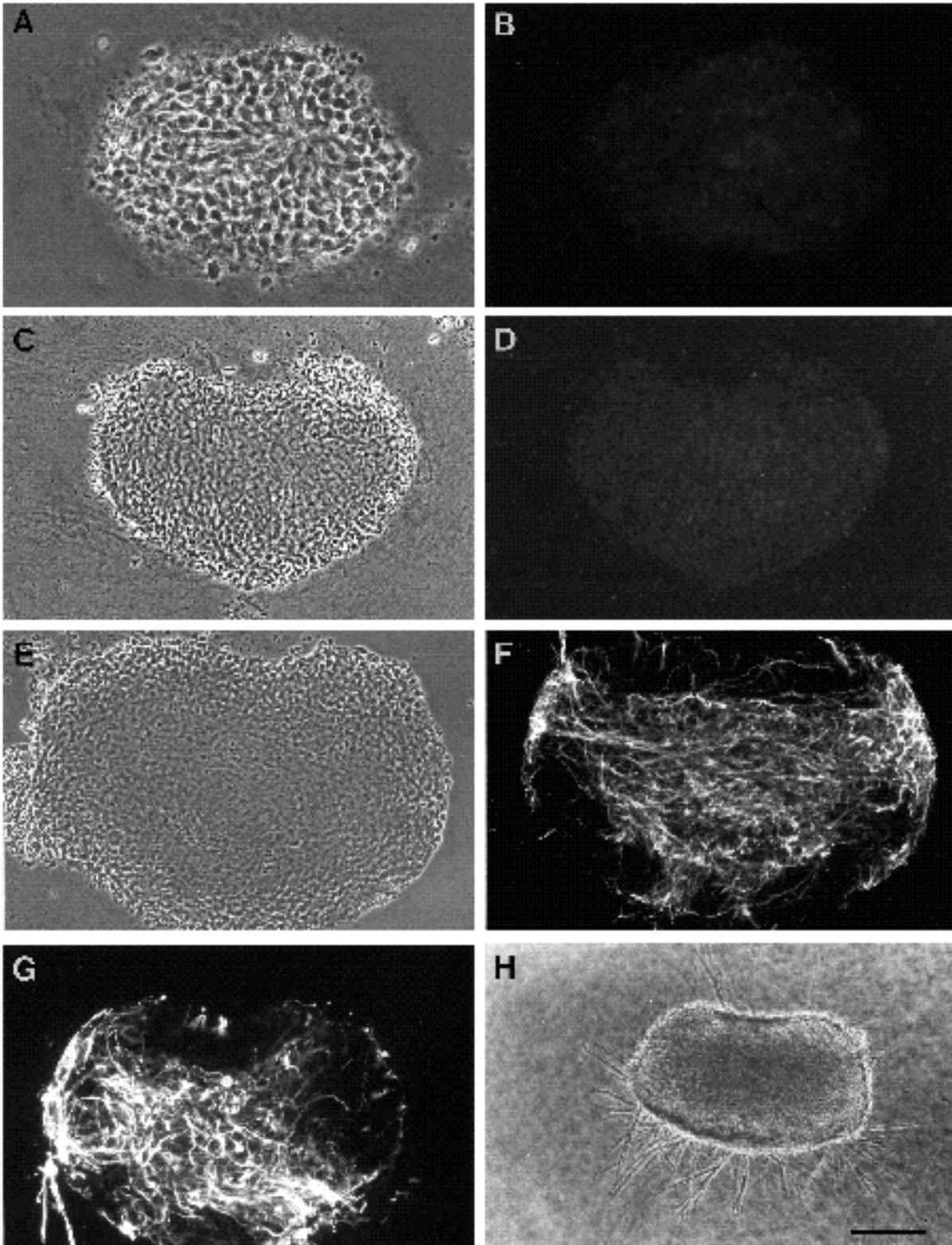


Fig. 3. Cell differentiation in isolated neural plate explants. Phase-contrast micrographs (A,C,E) of sections through neural plate explants cultured in isolation for 85 hours and labelled by immunofluorescence for FP3 (B), FP4 (D) and 2H3 (F) expression. Neither FP3 nor FP4 are detected within neural plate explants. Neuronal cells begin to differentiate within explants after 12 hours in vitro (not shown), and by 85 hours have extended long neurites, that can be detected by expression of neurofilament (F), 3A10 (not shown) and TAG-1 (G) antigens. H shows a phase-contrast micrograph of a neural plate explant cultured for 85 hours in the presence of floor plate-conditioned medium. Extensive neurite outgrowth, characteristic of dorsal commissural axons, is observed. Scale bar represents: A,B, 56 μm ; C-G, 112 μm ; H, 165 μm .

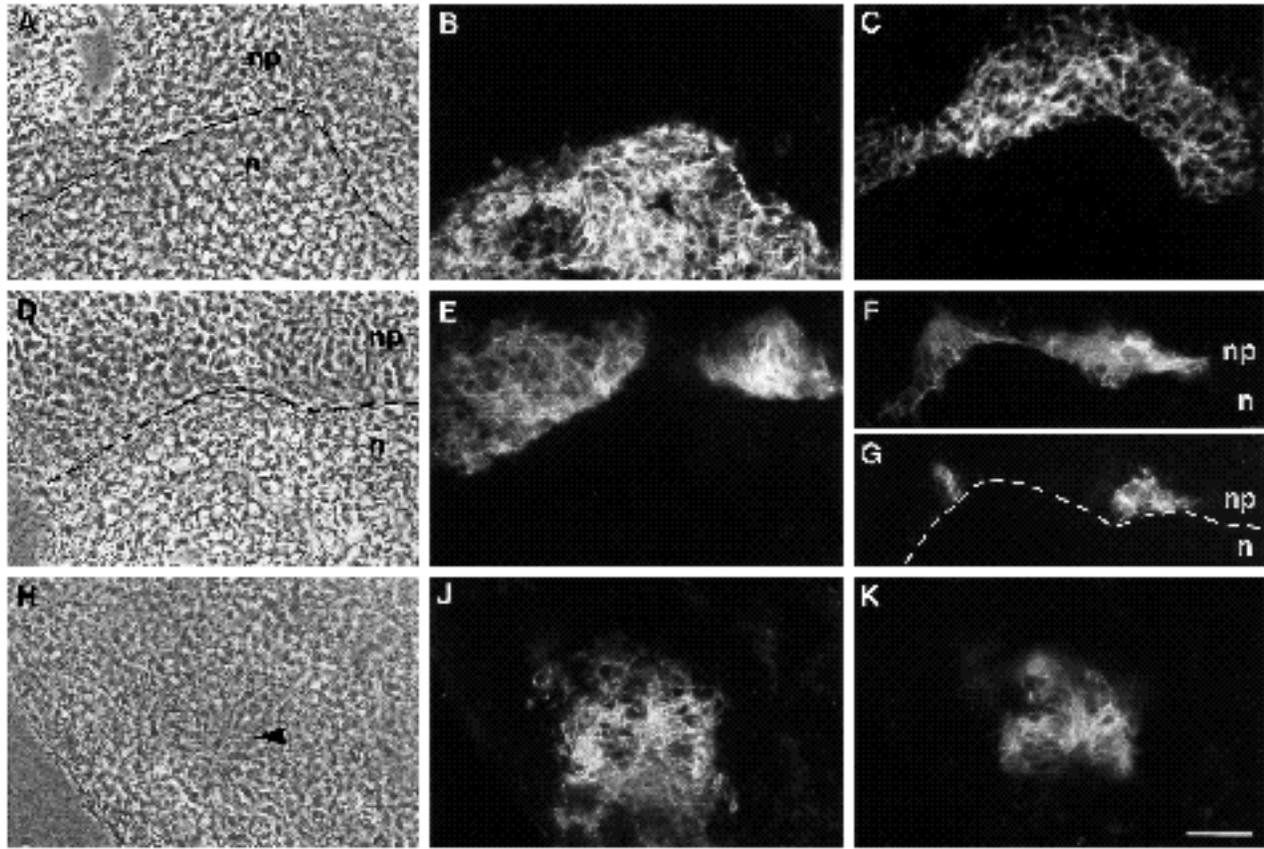


Fig. 4. Induction of floor plate differentiation in rat neural plate explants by chick notochord and floor plate. (A-G) Induction of floor plate by notochord. (H-K) Induction of floor plate by floor plate. (A-C) Phase-contrast micrograph (A) of a section through a rat neural plate (np)-chick notochord (n) conjugate cultured for 70 hours and double-labelled to detect expression of the chick Not-1 (B) and rat FP3 (C) antigens. Neural plate cells adjacent to the notochord express FP3. The domain of FP3 expression extends approximately 10 cells from the junction of the two explants (indicated by a dotted line in A). (D) Phase-contrast micrograph of a section through a rat neural plate (np)-chick notochord (n) conjugate cultured for 100 hours. The dotted line indicates the junction of two explants. (E) FP4 expression in the same section shown in D. Cells that express FP4 are located some distance from the border of the two tissues. (F,G) Section through a rat neural plate (np)-chick notochord (n) conjugate cultured for 90 hours and double-labelled to detect expression of the FP3 (F) and FP4 (G) antigens. The dotted line in G indicates the junction of the notochord and neural plate explants. FP4 is expressed by only a subset of cells that express FP3. (H) Phase-contrast micrograph of a section through a rat neural plate-chick floor plate conjugate cultured for 85 hours, and double-labelled to detect expression of FP3 (J) and FP4 (K). FP4 expression (K) is confined to a subset of FP3⁺ cells (J). Cells that express FP3 and FP4 exhibit an epithelial morphology (indicated by an arrowhead in H). Scale bar, 56 μ m.

which time the number of labelled cells decreased (Fig. 5). When neural plate explants were grown together with notochord, there was a marked increase in the number of BrdU-labelled cells which was most obvious at 44 hours (Fig. 5). Incorporation of BrdU into neural plate cells grown in the presence of notochord had virtually ceased by 70 hours.

Table 1. Induction of floor plate antigen expression in neural plate explants

	FP3	FP4
np alone	0% ($n=65$)	0% ($n=40$)
np+n	85% ($n=65$)	63% ($n=30$)
np+f	86% ($n=50$)	60% ($n=30$)

Rat neural plate explants (np) were cultured alone in contact with stage 6 chick notochord (n) or floor plate (f) for 80-100 hours and assayed for expression of FP3 and FP4 by immunocytochemistry.

Similar results were obtained using floor plate as the inducing tissue (not shown). These results suggest that, in addition to inductive signals, the notochord and the floor plate provide trophic signals, promoting either cell division or cell survival in neural plate explants.

Two lines of evidence suggest that the trophic and inductive effects of the notochord and the floor plate can be separated. As shown above, contact is required for floor plate induction. In contrast, neural plate explants grown at a distance from notochord or floor plate, or in the presence of floor plate-conditioned medium, showed enhanced proliferation (not shown). In addition, the peptide growth factors, acidic and basic fibroblast growth factor and transforming growth factor-2, enhanced proliferation within neural plate explants (not shown) but did not induce floor plate differentiation (see methods).

It remains possible that cell division is a prerequisite for

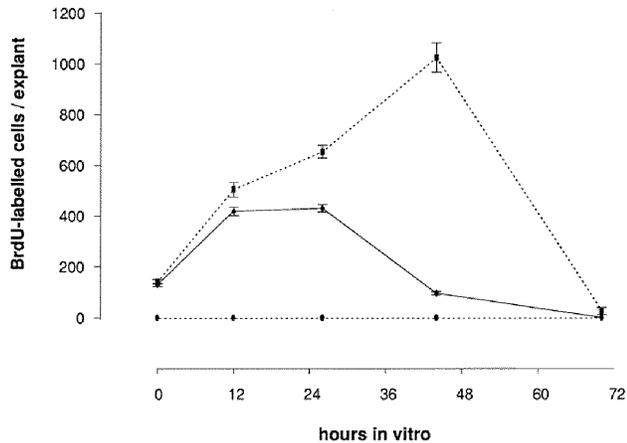


Fig. 5. BrdU incorporation by cells within neural plate explants is enhanced by the notochord. Graph shows the number of cells that incorporate BrdU in neural plate explants grown alone (diamonds), in the presence of notochord with (circles) or without (squares) mitomycin C. The mean number of cells that incorporate BrdU is plotted as a function of time in vitro. The number of cells in explants at the time of dissection is 2900 ± 82 (mean \pm s.e.m., $n=4$ experiments). Bars show s.e.m.; $n=6$ explants.

floor plate differentiation. To address this, floor plate induction by notochord and floor plate was examined in neural plate explants in which cell division had been blocked by mitomycin C (Figs 5, 6). In the absence of cell division, FP3 expression was still induced by notochord (Fig. 6) and floor plate (not shown) to an extent similar to that observed in the absence of mitomycin C. Therefore cell division is not required for floor plate induction.

Floor plate cells as a source of inducing activity

Although both the notochord and the floor plate can induce floor plate differentiation, this could reflect the synthesis of

inducing factor(s) by both cell types or the sequestration and subsequent release by the floor plate of a factor that is synthesized by only the notochord. An examination of the time period over which the notochord and floor plate are able to induce floor plate antigens in vitro suggests that the floor plate does synthesize the inducing factor. Notochord and floor plate explants from the region adjacent to prospective or actual somites 0-5 of stage 6 to 24 chick embryos were dissected and co-cultured in contact with neural plate tissue for up to 100 hours. Both notochord and floor plate from stage 6 embryos induced floor plate. However, whereas floor plate dissected from later stages retained a high level of inducing activity, notochord from the same stages did not (Fig. 7). The abrupt loss of inducing activity by the notochord was dependent on the use of the enzyme Dispase during isolation of notochord tissue. In the absence of enzyme, the activity was detectable, albeit at greatly reduced levels, until stage 24 (data not shown). In contrast, the floor plate maintained a high level of inducing activity until stage 24, even when enzymatically isolated (Fig. 7). Enzyme treatment may remove surface-associated inducing activity such that only those cells capable of its de novo synthesis can induce floor plate differentiation. Thus notochord explants appear to lose the ability to induce the floor plate earlier in development than do floor plate cells. Further support for this comes from the observation that notochord explants matured in vitro lost the ability to induce the expression of FP3 and FP4 within 12 hours whereas floor plate explants retained strong inducing activity for as long as 72 hours in culture.

Evidence for propagation of a floor plate-inducing signal

There is a progressive expansion in the mediolateral extent of the floor plate during development between E10 and E13 such that the lateral dimension of the floor plate comes greatly to exceed that of the notochord (Fig. 2). The expan-

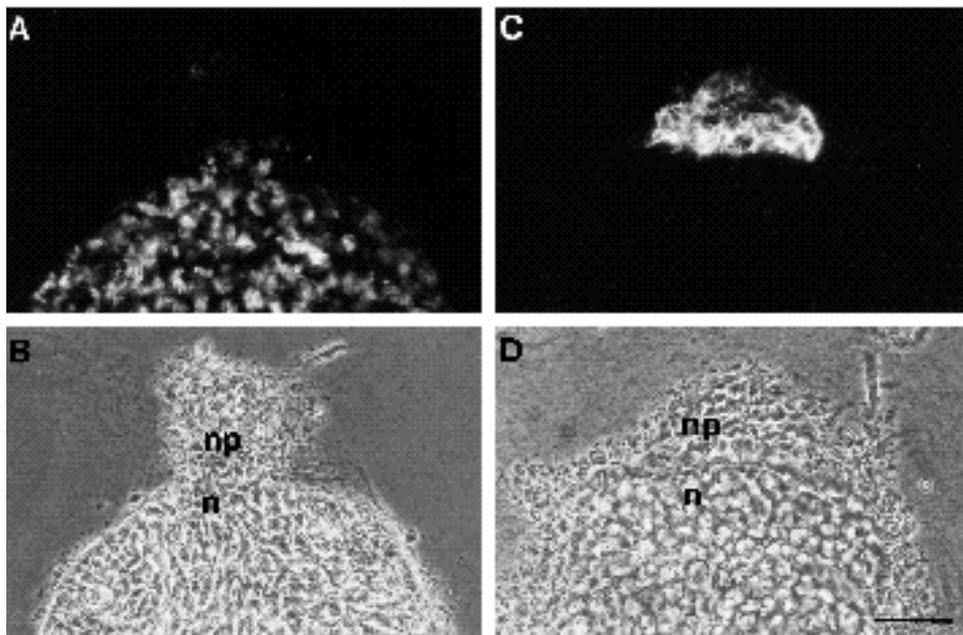


Fig. 6. Floor plate induction occurs in the absence of cell division. (A) A section through a neural plate-notochord conjugate cultured for 80 hours and immunolabelled with anti-BrdU antibody. The neural plate explant had been treated for 20 minutes with mitomycin C, and the conjugate pulsed with BrdU after 24 hours in culture. Notochord, but not neural plate cells, appear to have incorporated BrdU. (B) Phase-contrast micrograph of the section in A. (C) FP3 expression in a section adjacent to that shown in A. The majority of cells within the neural plate explant express FP3. (D) Phase-contrast micrograph of the section shown in C. Similar results were obtained in neural plate-floor plate conjugates (not shown). Scale bar, 56 μ m. Abbreviations: np, neural plate; n, notochord.

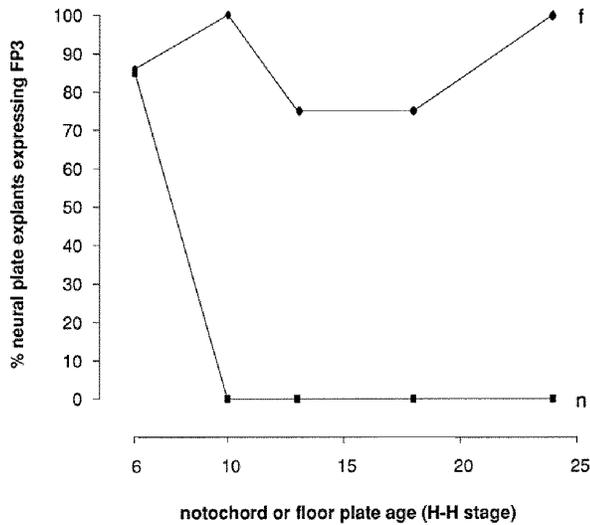


Fig. 7. The notochord loses inductive activity before the floor plate. Neural plate explants were placed in contact with notochord (n) or floor plate (f) explants dissected from chick embryos of different ages and examined for FP3 expression after 100 hours. To standardize the assay against stage 6 tissue (when only rostral tissue is present) tissues from all other ages were dissected from rostral regions. All tissues were dissected in the presence of Dispase. The percentage of neural plate explants expressing FP3 is plotted (ordinate) against the stage of chick embryo from which inducing tissue was obtained (abscissa). Each point represents the mean value from 6-10 different conjugates.

sion occurs after the notochord has descended and contact between the two tissues has been lost, suggesting that the notochord may be responsible for the induction of medial floor plate cells but not for the lateral expansion of the floor plate. This possibility is supported by the observation that the lateral expansion of the floor plate occurs after the loss of floor plate-inducing activity by the notochord. The lateral expansion of the floor plate may instead be mediated by the propagation through the neural plate of a signal from the midline floor plate cells.

To determine whether propagation of floor plate-inducing signals occurs, we first examined the extent of FP3 and FP4 expression within neural plate explants that had been co-cultured in contact with a notochord or a floor plate. FP3- and FP4-expressing cells were found up to ten cell diameters from the junction of the explants (Fig. 4). The division or migration of floor plate cells could contribute to this expanded domain of antigen expression. However, as described above, inhibition of proliferation of neural plate cells by treatment with mitomycin C did not decrease the domain over which FP3-expressing cells were observed when compared with that in control co-cultures (Fig. 6). Similarly, in neural plate explants cultured in the presence of cytochalasin D, a drug that blocks cell migration (Gurdon, 1988), FP3 and FP4 were still expressed at a distance from the inducing tissue (Fig. 8). Together, these results suggest that the spread of floor plate induction is mediated, at least in part, by the propagation of a contact-dependent, homeogenetic inductive signal.

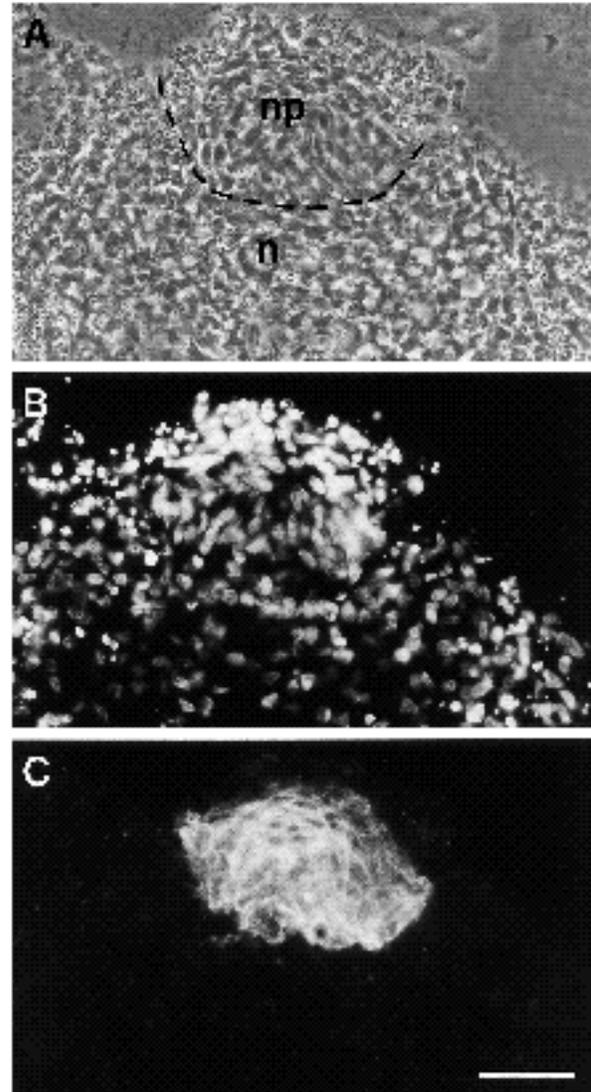


Fig. 8. Floor plate induction in the absence of cell migration. (A) Phase-contrast micrograph of a section through a neural plate (np)-notochord (n) conjugate cultured for 85 hours in the continued presence of 0.2 $\mu\text{g/ml}$ cytochalasin D. The dotted line indicates the junction between the two explants. The size of the neural plate explant is small when compared to that of explants in the absence of cytochalasin D. (B) Fluorescence micrograph of the section shown in A, labelled with the nuclear dye Hoeschst 33258 indicates the number of cells in the neural plate. (C) Immunolabelling of the section shown in A reveals that most cells within the neural explant express FP3. FP3⁺ cells are observed up to six cell diameters from the junction of the explants. Scale bar, 58 μm .

Loss of competence of neural plate cells to floor plate induction

Although floor plate cells induce neighbouring cells to differentiate as floor plate, not all neural plate cells acquire floor plate properties *in vivo*. However, floor plate induction is not observed *in vivo* when notochord is grafted adjacent to the spinal cord of older chick embryos (stage 15: van Straaten et al., 1988; Yamada et al., 1991). Thus the

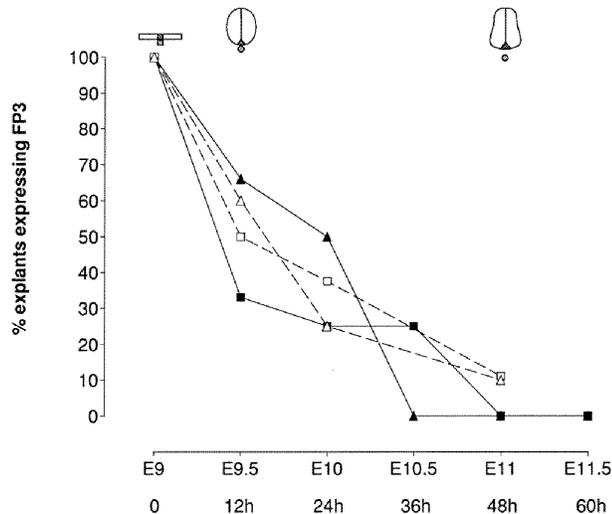


Fig. 9. The neural plate loses competence to respond to floor plate-inducing signals. Rat neural plate explants were recombined with stage 6 chick notochord or floor plate and examined for expression of FP3. The neural plate explants were obtained by dissecting tissue from rat embryos of different ages and by dissecting neural plate from 0-somite (E9) rat embryos, ageing it in vitro and then culturing it with notochord or floor plate. Neural plate explants aged in vitro or in vivo lose competence to respond to inducing signals with similar time courses. Symbols: Neural plate aged in vivo and combined with notochord (closed squares) or floor plate (closed triangles). Neural plate aged in vitro and combined with notochord (open squares) or floor plate (open triangles). Between 6 and 16 explants were examined at each time point for each value. For neural plate explants dissected from embryos of different ages, the percentage of explants expressing FP3 is plotted (ordinate) against the stage of embryo from which it was obtained (E9-E11.5 abscissa). For neural plate explants aged in vitro, the percentage of explants expressing FP3 is plotted (ordinate) against the time of ageing in vitro (hours; abscissa). Diagrams show schematic cross sections through embryonic rat neural tissue, depicting the relevant stage of development of the neural plate and neural tube.

extent of floor plate induction could be limited by the loss of competence of neural plate cells to respond to inductive signals. To test this possibility, rostral neural plate tissue adjacent to somites 1-2 was dissected from rat embryos ranging from the 2-somite to the 28-somite stage. The tissue was co-cultured in contact with notochord or floor plate, and induction assessed by expression of FP3. Over a 48 hour period in vivo, neural plate tissue progressively lost competence to respond to both notochord and floor plate signals (Fig. 9).

This loss in competence could reflect the commitment in vivo of neural plate cells to other ventral cell fates under the influence of notochord- and floor plate-derived signals. To examine whether the loss of competence depends on ventral midline signals, neural plate cells were explanted early (at the 2-somite stage), aged in vitro in isolation from notochord and floor plate, and examined for their ability to respond to inducing signals. The time course over which neural plate explants lost competence to respond to notochord and floor plate signals paralleled that of neural tissue

aged in vivo (Fig. 9). These results suggest that the loss of competence of neural plate cells can occur independently of midline-derived signals that promote ventral cell type differentiation. In addition, these results show that the loss of competence is intrinsic to the neural plate.

Rostral limits to floor plate induction

The floor plate and notochord do not extend into the forebrain (Kingsbury, 1930; Puelles et al., 1987). Expression of FP3, FP4 and chemotropic activity terminate approximately at the midbrain-forebrain boundary (Dodd and Placzek, unpublished observations). The lack of a floor plate in the forebrain could result from the absence of inducing activity in the midline mesoderm underlying the rostral neural plate or from the inability of rostral neural plate cells to respond to floor plate-inducing signals.

To examine these possibilities, the prechordal plate mesoderm was tested for its ability to induce a floor plate in competent neural ectoderm. Midline mesoderm obtained from the region rostral to the notochord in stage 6 chick embryos was co-cultured for 100 hours with neural plate explants taken from the caudal region of 10 somite-stage rat embryos. Prechordal plate mesoderm did not induce FP3 or FP4 in neural plate explants (Tables 2 and 4A). Thus prechordal plate mesoderm is not capable of inducing floor plate properties in competent neural ectoderm.

We next examined whether rostral neural plate can respond to floor plate-inducing signals. Rostral neural plate tissue from E9 (neural plate stage) rat embryos (Fig. 1D) was cultured in contact with stage 6 chick notochord or floor plate. FP3 and FP4 were not induced in explants from the rostral regions of the neural plate (Table 4B). These results suggest that, as early as the pre-headfold stage of development, the prospective forebrain region of the neural

Table 2. Tissue tested for ability to induce floor plate antigens

	% FP3 ⁺ explants	n
st5 chick Hensen's node	100	5
st5 primitive streak	0	4
st6 prechordal mesoderm	0	10
st15-24 chick midbrain	0	6
E11-14 rat midbrain	0	8
E13 rat ventral spinal cord	0	4
E13 rat dorsal spinal cord	0	3
E13 rat roof plate	0	4
E11-14 rat heart	0	4
E14 rat kidney	0	4
E14 rat adrenal	0	4
E14 rat lung	0	4
E14 rat liver	0	4
E14 rat limb bud	0	4
E14 rat gut	0	4
E14 rat malpighian tubules	0	4
E14 rat Wolffian ducts	0	4
E14 rat stomach	0	4
E14 rat testis	0	6
E14 rat Rathke's pouch	0	4

Rat neural plate explants were cultured in contact with other embryonic tissues for 100 hours and assayed for floor plate differentiation by expression of FP3.

Table 3.

	Tissues separated at 12 hours		Tissues separated at 18 hours	
	% FP3 ⁺	%FP4 ⁺	%FP3 ⁺	%FP4 ⁺
np-n	0	0	75	25
np-f	0	0	50	25
np-n + FPCM	0	0	50	50
np-f + FPCM	0	0	75	50

Rat neural plate explants (np) were cultured in contact with stage 6 chick notochord (n) or floor plate (f) for 12 hours or 18 hours, at which time the conjugates were separated and the neural plate explants were cultured alone for a further period to bring the total time to 100 hours.

Neural plate explants were assayed for expression of FP3 and FP4. The experiment was performed both in the basence and in the presence of floor plate-conditioned medium (FPCM). Four explants were used to generate each data point.

Table 4. Floor plate induction in neural plate conjugates derived from different rostrocaudal levels

	% FP3 ⁺ explants	<i>n</i>
A. E10 rat caudal neural plate and:		
st6 chick notochord	80%	10
st6 chick floor plate	70%	10
st6 chick prechordal mesoderm	0%	10
B. E9 rat rostral neural plate and:		
st6 chick notochord	0%	8
st6 chick floor plate	0%	8
E9 rat caudal neural plate and:		
st6 chick notochord	100%	8
st6 chick floor plate	100%	8

Rat neural explants obtained from different rostrocaudal levels of the neural plate (see Fig. 1) were cultured in contact with stage 6 chick notochord, floor plate or prechordal plate mesoderm and assayed for floor plate differentiation by expression of FP3.

plate is unable to respond to floor plate-inducing signals. Thus, the absence of a floor plate in the forebrain appears to be a consequence of the lack of inducing activity in rostral mesoderm and the lack of competence of the rostral neural plate.

DISCUSSION

We have used surface markers expressed by embryonic rat floor plate cells to study the induction of floor plate differentiation in the neural plate. Of many embryonic cell groups tested, only Hensen's node, the notochord and the floor plate itself can induce floor plate differentiation. Floor plate induction requires contact between the inducing tissue and neural plate cells. The inducing activity of the notochord is transient whereas that of the floor plate persists, providing evidence that the floor plate synthesises inducing activity. Floor plate induction is initiated by the notochord but appears to be continued by the propagation through the neural plate of a floor plate-derived homeogenetic signal. In caudal regions of the neuraxis, the mediolateral extent of the floor plate may be limited by a loss of competence

of the neural plate to respond to inductive signals. The absence of the floor plate from the forebrain can be explained by the lack of inducing activity in prechordal mesoderm and by the inability of rostral neural plate cells to respond to inductive signals.

Signals from the notochord and floor plate change the fate of neural plate cells

Cells in neural plate explants maintained in isolation do not acquire antigenic or functional properties of the floor plate but can differentiate into neurons. The expression of TAG-1 by neurons in explants and the extension of axons in response to a floor plate-derived chemoattractant provides evidence that many of the neurons that differentiate are dorsal commissural neurons. Thus, the fate of cells in neural plate explants in vitro is similar to that of cells in the neural tube of chick embryos from which the notochord has been removed. In the spinal cord of such embryos, the floor plate and motor neurons are absent but dorsal cell types differentiate (Yamada et al., 1991, Placzek et al., 1991, Ericson et al., 1992).

The induction of floor plate antigens in neural plate explants grown in contact with notochord or floor plate shows that signals from either midline cell group is sufficient to initiate floor plate differentiation. In previous studies, the notochord has been shown to induce chemotropic activity in chick neural plate tissue (Placzek et al., 1990b), but this assay requires the presence of spinal cord explants which could contribute factors that cooperate with notochord signals to induce floor plate differentiation. The use of antigenic markers of floor plate differentiation eliminates the requirement for other tissues and provides evidence that signals from both the notochord and the floor plate are sufficient for floor plate induction. The induction of floor plate-specific antigens in vitro is frequently accompanied by the acquisition of an epithelial morphology similar to that exhibited by floor plate cells in situ (Holtfreter, 1939; Placzek, unpublished observations). Thus, functional and morphological properties of the floor plate are induced in parallel with surface antigens, suggesting that a complete range of floor plate properties can be induced in vitro.

Floor plate induction requires contact with inducing cells

Contact between the inducing tissues and the neural plate is required for floor plate induction in vitro, supporting suggestions made on the basis of in vivo grafting studies (van Straaten et al., 1985). Although we cannot exclude the possibility that floor plate induction can be achieved by a high concentration of a diffusible factor, floor plate-conditioned medium at concentrations up to seventy five times that which induces motor neuron differentiation (T. Yamada, T. Edlund and T. Jessell, in preparation) did not induce floor plate antigen expression. During embryogenesis, the notochord contacts cells at the midline of the neural plate (Jurand, 1974) and the two cell groups are coupled via gap junctions (Sheridan, 1968). In addition, the change in cell shape and the initial expression of FP3 and FP4 are limited to midline neural plate cells located immediately above the notochord. Taken together, these observations suggest that, in vivo, contact between the notochord and neural plate is

required to initiate floor plate differentiation. The nature of the inducing signal is not known and could involve the action of a membrane-anchored ligand or of a secreted factor that is trapped in the extracellular matrix surrounding the notochord.

The period of contact required for induction of floor plate properties in neural plate cells is uncertain. In rostral regions of the neuraxis, chemotropic activity (Placzek et al., 1990) and floor plate-inducing activity are expressed by explants removed from the midline of the neural plate and grown in isolation. At the time of removal, the midline of the neural plate has been in contact with the notochord for less than 1 hour, suggesting that a brief period of contact is sufficient for the induction of floor plate properties. However, fate-mapping experiments indicate that cells that give rise to the notochord and midline of the neural plate are intermingled in Hensen's node at stage 3-4 (Rosenquist, 1966; Schoenwolf et al., 1989; Selleck and Stern, 1991). Thus, prospective notochord cells may begin to induce floor plate properties in neural plate cells prior to the deposition of the notochord. In contrast, in caudal regions of E10 rat embryos, explants taken from the ventral midline of the neural tube do not express FP3 and FP4 when grown in vitro even though these cells have been contacted by the notochord for several hours. Thus, the generation of a floor plate at caudal levels of the neuraxis appears to require a longer period of contact than at rostral levels. In vitro, a period of contact of 18 hours is required for induction of floor plate antigens, which is greater than the time required for induction in vivo, even at caudal levels of the neuraxis (Placzek et al., 1990; Yamada et al., 1991). The extended period of contact required for floor plate induction in vitro could reflect a delay in the onset of inductive signalling caused by the necessity to resynthesize or concentrate inducing activity after enzymatic treatments required for tissue dissection.

Homeogenetic induction by the floor plate

The ability of the floor plate to induce floor plate differentiation has been observed in grafting studies in chick embryos (Yamada et al., 1991; Placzek et al., 1991), and has been inferred from the ability of wild-type cells introduced into the neural plate of *cyclops* mutant zebrafish embryos to restore the floor plate (Hatta et al., 1991). The in vitro studies presented here provide direct evidence for homeogenetic induction by the floor plate. The floor plate does not appear to serve as a passive carrier of notochord-derived inductive signals. Indeed floor plate cells appear to synthesize inducing signals for a much longer period than the notochord.

Inducing signals from floor plate cells may regulate the final dimensions of the floor plate. As assessed by FP3 and FP4 expression, the floor plate originates as a narrow strip of midline cells, and later expands significantly in the mediolateral dimension. The notochord does not undergo a similar lateral expansion and, indeed, later becomes displaced ventrally, losing contact with the neural tube. The requirement for contact in floor plate induction argues that the lateral expansion of the floor plate is not mediated by signals from the notochord. Three different mechanisms could

account for the spread of floor plate differentiation observed in vivo and in neural plate explants. First, a contact-mediated signal could be propagated through the neural plate as floor plate cells differentiate and acquire inducing activity. Second, committed floor plate precursors may divide, thus expanding the number of floor plate cells. Third, induced floor plate cells may undergo cell rearrangements or migrate, thus contributing to a lateral expansion of the floor plate. The studies of floor plate induction in vitro in the presence of mitomycin C and cytochalasin D suggest that neither cell division nor cell migration are required for the expansion in the domain of floor plate differentiation. Cell division and migration could, however, contribute together with a propagated signal to establish the final dimensions of the floor plate in vivo. In rat embryos, cells at the midline of the spinal cord continue to divide late into embryogenesis (Altman and Bayer, 1984). In addition, injection of lineage tracer into single midline cells in the hindbrain region of chick embryos shows that floor plate cells or their precursors divide and migrate extensively along the rostrocaudal axis (Fraser et al., 1990). Thus, the division and subsequent rostrocaudal extension of floor plate cells is likely to operate together with homeogenetic induction to generate the final dimensions of the floor plate.

The propagation of floor plate-derived homeogenetic signals in vivo is clearly attenuated since floor plate differentiation does not normally occur throughout the entire neural plate. One possibility is that the most lateral floor plate cells do not acquire the ability to induce floor plate. A second mechanism that could limit the propagation of this inductive signal is the loss of competence of neural cells (Nieuwkoop and Albers, 1990; Servetnick and Grainger, 1991). Our studies show that neural plate cells lose competence to respond to inductive signals over a 48 hour period. This time period concurs well with the progression of floor plate induction and expansion in vivo that occurs between E10 and E13 (see Fig. 2). The loss of competence in neural plate cells may result from the induction of other ventral cell types in vivo under the influence of midline signals. However, the loss of competence does not depend on the differentiation of ventral cell types since a similar loss occurs in isolated neural plate explants. In this in vitro situation, the loss of competence could reflect the differentiation of neural plate cells into dorsal cell types, although this is unlikely to be the mechanism by which competence is lost in vivo. Studies in amphibian embryos have provided evidence that the lateral dimension of the neural plate is defined by the loss of competence of ectodermal cells to mesodermal inductive signals (Nieuwkoop and Albers, 1990). Thus, several aspects of early neural patterning initiated by mesodermal signals may be regulated by changes in the competence of ectodermal cells.

The ability of both notochord and floor plate to induce floor plate differentiation raises the question of whether the same inducing signal is produced by both cell types. Although the functional properties of the notochord- and floor plate-derived signals appear to be the same, our experiments do not establish whether or not the floor-plate-inducing signals are identical. In the zebrafish *cyc-1* mutant, introduced wild-type cells that contribute to the floor plate, but not those that populate notochord, can restore floor plate morphology and antigen expression in adjacent mutant

neural tube cells (Hatta et al., 1991). This raises the possibility that the inducing factors derived from the notochord and floor plate are distinct. However, if the *cyc-1* mutation attenuates the response of neural cells to this signal the same factor provided at greater concentration by the floor plate could account for the observed results.

Limiting the floor plate along the rostrocaudal axis

The notochord and floor plate are coextensive along the rostrocaudal axis, terminating at the boundary between the midbrain and forebrain (Kingsbury, 1930; Puelles et al., 1987; J. Dodd and M. Placzek, unpublished observations). The *in vitro* experiments described here show that prechordal mesoderm is unable to induce floor plate antigens when placed in contact with competent neural plate explants. In addition, notochord or floor plate tissue is unable to induce floor plate antigens in the rostral-most region of the neural plate in neural plate-stage embryos. This does not reflect a complete restriction in the fate of rostral neural plate cells since their properties can be changed by signals from mesencephalic tissue (Martinez et al., 1991; Gardner and Barald, 1991; Itasaki et al., 1991). It remains possible that signals from the notochord or floor plate induce the differentiation of other cell classes, perhaps those characteristic of the ventral diencephalon, in rostral neural plate explants.

Taken together, these results suggest that the rostral boundary of the floor plate is defined by the restriction of inducing activity and neural competence to caudal regions of the embryonic axis. The differential competence of the neural plate along its rostrocaudal axis may arise at earlier stages of neural development by the planar spread of patterning signals through the ectoderm at the time of neural induction (Ruiz i Altaba, 1990, 1992; Doniach et al., 1992).

We wish to thank Drs S. Cairns Smith, J. Heemskerk, C. Hume and A. Ruiz i Altaba for helpful discussions and comments on the manuscript and T. Yamada for communicating experimental results before publication. We are also very grateful to Suquin Fung for excellent technical assistance, to Vicki Leon for typing, to Eric Hubel for photographic assistance and to Dan Felsenfeld, Andy Furley and Ira Schieren for computer graphics. This work was funded by grants from the NIH and The Esther A and Joseph Klingenstein Fund. T. M. J. is an Investigator of the Howard Hughes Medical Institute.

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(Accepted 29 September 1992)