DEVELOPMENTAL BIOLOGY 177, 30–42 (1996)
ARTICLE NO. 0142

Determination of Neuroepithelial Cell Fate: Induction of the Oligodendrocyte Lineage by Ventral Midline Cells and Sonic Hedgehog

Nigel P. Pringle,[1] Wei-Ping Yu,[1,2] Sarah Guthrie,* Henk Roelink,† Andrew Lumsden,* Alan C. Peterson,‡ and William D. Richardson[3]

*MRC Laboratory for Molecular Cell Biology and Department of Biology, University College London, Gower Street, London WC1E 6BT, United Kingdom; *Division of Anatomy and Cell Biology, United Medical and Dental Schools, Guy's Hospital Campus, St. Thomas Street, London Bridge, London SE1 9RT, United Kingdom; ‡Molecular Oncology Group, Royal Victoria Hospital, McGill University, Hershey Pavilion (H5), 687 Pine Avenue West, Montreal, Quebec H3A 1A1, Canada; and †Department of Biological Structures, University of Washington School of Medicine, P.O. Box 537420 Seattle, Washington 98195*

Near the floor plate of the embryonic neural tube there is a group of neuroepithelial precursor cells that are specialized for production of the oligodendrocyte lineage. We performed experiments to test whether specification of these neuroepithelial oligodendrocyte precursors, like other ventral neural cell types, depends on signals from the notochord and/or floor plate. We analyzed heterozygous Danforth's short tail (*Sd*/+) mutant mice, which lack a notochord and floor plate in caudal regions of the neural tube, and found that oligodendrocyte precursors did not appear at the ventricular surface where there was no floor plate. Moreover, oligodendrocytes did not develop in explant cultures of *Sd*/+ spinal cord in the absence of a floor plate. When a second notochord was grafted into an ectopic position dorsolateral to the endogenous notochord of a chicken embryo, an additional floor plate was induced along with an ectopic focus of oligodendrocyte precursors at the

immunohistochemistry with an antiserum against myelin basic

fied from *Escherichia coli* (Roelink *et al.*,

erozygous *Sd*/ / mice are often viable but the notochord is discontinuous in caudal regions of the embryo, leading to

against myelin basic protein (MBP) to visualize differentiated oligodendrocytes (Fig. 4). MBP-positive oligodendro-

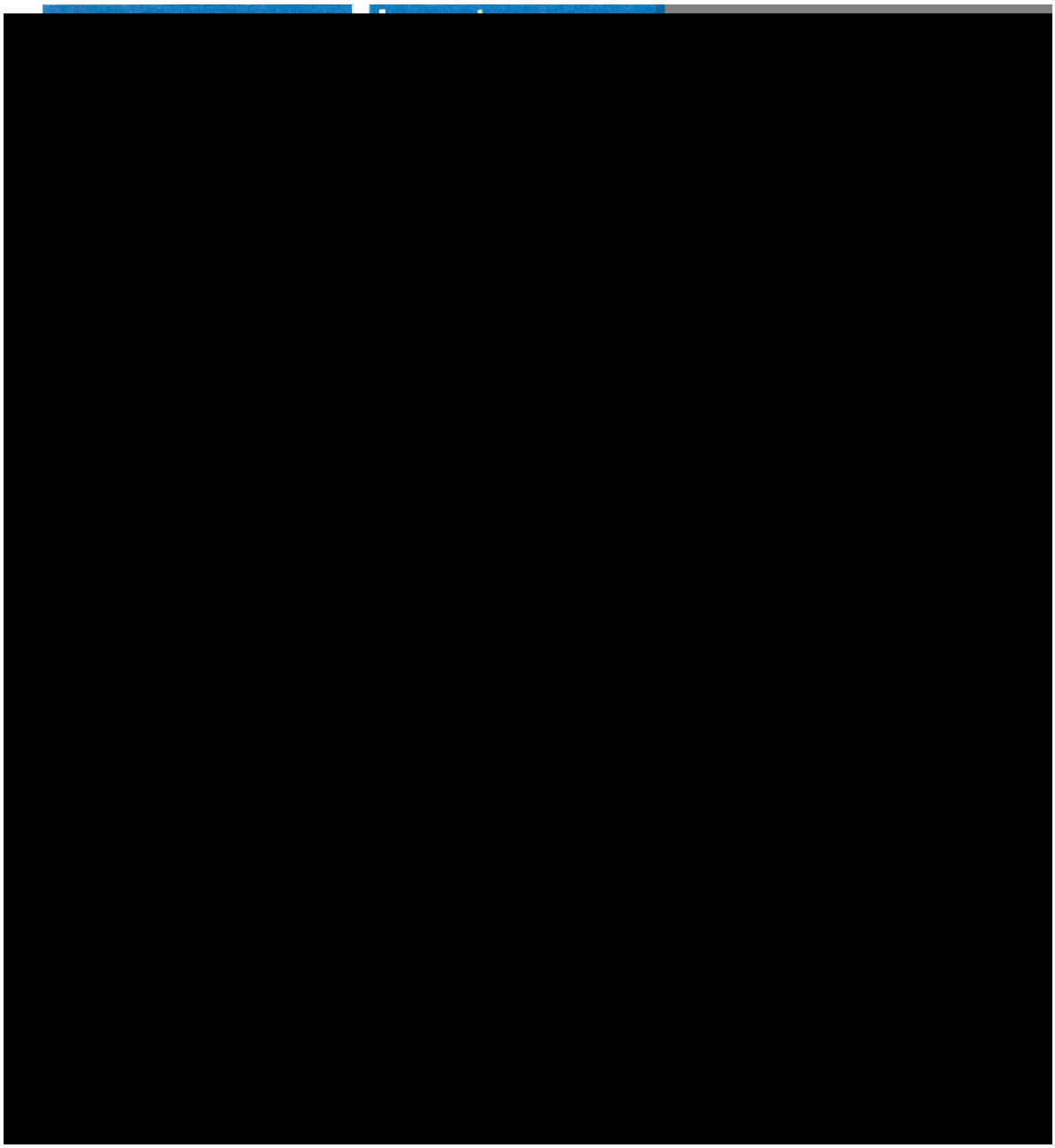

FIG. 1. The appearance of oligodendrocyte precursors at the ventricular surface of Danforth's short-tail mouse mutant depends on the presence of a notochord/floor plate. Transverse cryosections (10 mm) were cut through the spinal cords of heterozygous E13 *Sd*/ / embryos, from the tip of the vestigial tail to the upper thoracic region. In three embryos that we examined, the most caudal sections did possess a floor plate (fp; a), but anterior to this was a region approximately 600 mm in length that lacked a floor plate (b). More anterior still, the floor plate reappeared (c). The sections depicted in the figure are evenly spaced by approximately 1 mm; section (a) came from the caudal spinal cord, 2 mm from the tip of the tail. After hybridization to the PDGFRa probe, the sections were autoradiographed, photographed under dark-field illumination in a dissecting microscope, stained with hematoxylin, and rephotographed under bright-field illumination. The bright- and dark-field images were converted to digital format using a video camera and frame grabber attached to a Macintosh

FIG. 3. Oligodendrocyte progenitor cells invade floor plate-less regions of *Sd*/ / mouse spinal cord by longitudinal migration from neighboring regions of normal spinal cord. Sections were taken from the caudal region of an E15 *Sd*/ / embryo and hybridized *in situ* with the PDGFRa probe (see the legend to Fig. 1). The sections of (a) and (b) lack a morphologically recognizable floor plate, whereas the more anterior section of (c) possesses a floor plate (fp). Note that the abnormal morphology of the spinal cord in (a) and (b) results from the loss of motor neurons and other ventral cell types due to the lack of a notochord/floor plate. The distance between (a) and (b) is approximately 150 mm; the distance between (b) and (c) is 300 mm. PDGFRa$^{/}$ cells appear in (b) despite the absence of a floor plate, presumably because of longitudinal migration of these PDGFRa$^{/}$ cells from the more anterior, normal region of spinal cord. Since the region lacking a floor plate (and PDGFRa$^{/}$ cells) in E13 *Sd*/ / mice is around 600 mm (see Fig. 1), and PDGFRa$^{/}$ cells are found at all levels of the neuraxis at E19 (see text), this suggests that

(data not shown). In the remaining manipulated embryos (5 animals), the grafted notochord was displaced from, and dorsolateral to, the endogenous notochord. Judging by morphology and immunolabeling with monoclonal antibody FP1 (Yamada *et al.*, 1991), an additional floor plate was induced adjacent to the ectopic notochord in each of these embryos (Fig. 6). One of these embryos was developmentally too immature and no PDGFRa$^+$ cells were found anywhere in the ventral half of the cord. Another was developmentally too advanced and many PDGFRa$^+$ cells were distributed throughout the cross section of the cord. In the remaining three manipulated embryos there was a new focus of PDGFRa$^+$ cells at the ventricular surface, the same distance away from the induced floor plate as the original foci were from the endogenous floor plate (Fig. 6). However, a new focus of PDGFRa$^+$ cells was induced only to one side of the ectopic floor plate, and simultaneously one of the original pair of foci was repressed, so that no PDGFRa$^+$ cells appeared in the neuroepithelium between the two floor plates (Fig. 6). This phenomenon was observed consistently in all the sections that we analyzed from these grafts (approximately 10 sections total). The reason for this observation was *not* because there was insufficient space between the two floor plates: on the contrary, there were more than enough intervening neuroepithelial cells to accommodate two additional nonoverlapping foci of PDGFRa$^+$ cells at the expected distances from the floor plates. The distance between the lateral margins of the floor plate and the foci of PDGFRa$^+$ cells is normally 75 mm whereas, as shown in Fig. 6C for example, the distance between the margins of the natural and ectopic floor plates is approximately 200 mm. Therefore, we tentatively interpret this observation as evidence for the existence of a diffusible ''morphogen'' originating from the floor plate and/or notochord (see Discussion).

Induction of Oligodendrocytes by Notochord and Purified Sonic Hedgehog Protein in Avian Neural Plate Explants

To extend the *in vivo* grafting experiments described above and to confirm that the notochord can induce oligodendrocyte development in naive neural tube tissue, we explanted fragments of E1.5–2 (stage 9–10) quail or chicken ventral, intermediate, or dorsal neural plate and cultured these in collagen gels either alone or in contact with pieces of notochord from the same embryos. After culturing the explants for 12 days, we fixed and immunolabeled them as whole-mount preparations with anti-SMP and/or anti-MBP to visualize oligodendrocytes. Most of our experiments were conducted using quail explants because the time required for appearance of oligodendrocytes *in vitro* was less than with chicken explants and, in addition, we had two independent antibodies (anti-MBP and anti-SMP) with which to score quail oligodendrocytes. However, most of the quail experiments described below were also confirmed qualitatively for chicken explants.

Two independent preliminary experiments were performed to test the inherent capacity of stage 9–10 ventral, intermediate, or dorsal quail neural tube tissue to generate oligodendrocytes *in vitro*. In the absence of notochord, large numbers of (MBP$^+$, SMP$^+$) oligodendrocytes developed in most ventral neural tube explants (13/14), but not in either intermediate (0/7) or dorsal (0/9) explants. However, intermediate neural tube was able to generate hundreds of oligodendrocytes when cultured in direct contact with notochord (17/17 explants; Fig. 7). These results are consistent with those published by Trousse *et al.* (1995), who performed similar experiments with neural tube explants from E4 (stage 23–24) chick embryos.

Sonic hedgehog, a vertebrate homologue of the *Drosophila* patterning gene product hedgehog, is known to be able to induce the development of ventral cell types including floor plate cells, motor neurons, and dopaminergic neurons in neural tube explants. It seemed possible that Shh, which is expressed in the notochord and floor plate during early neurogenesis, might be at least partly responsible for the oligodendrocyte-inducing activity of the notochord identified by our experiments and those of Trousse *et al.* (1995). Therefore, we cultured intermediate neural tube explants in the presence of different concentrations of the autoproteolytic amino-terminal fragment of Shh (Roelink *et al.*, 1995). The results of these experiments are shown in Table 1 and Fig. 7. Whereas floor plate cells were induced by only the highest concentration of Shh tested (7×10^{-9} *M*), both motor neurons and oligodendrocytes were induced by Shh over a range of concentrations from 7×10^{-9} to 7×10^{-10} *M* (Table 1 and Fig. 7). For both motor neurons and oligodendrocytes, there appeared to be a sharp decline in the inducing activity of Shh at lower concentrations than this (Table 1).

DISCUSSION

The experiments reported here show that the influence of the notochord/floor plate extends to the oligodendrocyte lineage in addition to ventral neurons such as motor neurons (Yamada *et al.*, 1991, 1993) and midbrain dopaminergic neurons (Hynes *et al.*, 1995). This suggests that the processes governing development of glial cells and neurons are fundamentally similar. The fact that we can detect the very first PDGFRa$^+$ oligodendrocyte progenitor cells at a precisely defined site at the ventricular surface of the E13 mouse spinal cord (E7.5 chick spinal cord) demonstrates that neuroepithelial precursor cells are not all equivalent at this age and indicates that oligodendrocyte progenitors are prespecified as such before they move away from the central canal. The same might be true for other glial and neuronal lineages; indeed, it has been suggested (Wenger, 1950; Yu *et al.*, 1994) that the entire ventricular zone might consist of a mosaic of predetermined precursor cells, each dedicated to the production of a distinct subset of differentiated neural cell types. The data presented here show that loss of the notochord/floor plate in *Sd* mice results in a

FIG. 4. Oligodendrocytes do not develop in explant cultures of *Sd*/ / mouse spinal cord in the absence of a floor plate. Spinal cords of E13 *Sd*/ / mice were dissected away from surrounding tissue, split along the dorsal midline, and flattened with the ventricular surface uppermost. The floor plate, where present, could be easily recognized at the ventral midline of such preparations (illustrated in A). In the *Sd*/ / embryos used for these experiments, the region lacking a floor plate was about 1 mm long at the caudal end; unlike the embryos described in Fig. 1, there was no normal spinal cord further posterior to this abnormal region. Transverse sections (0.5 mm thick) of caudal

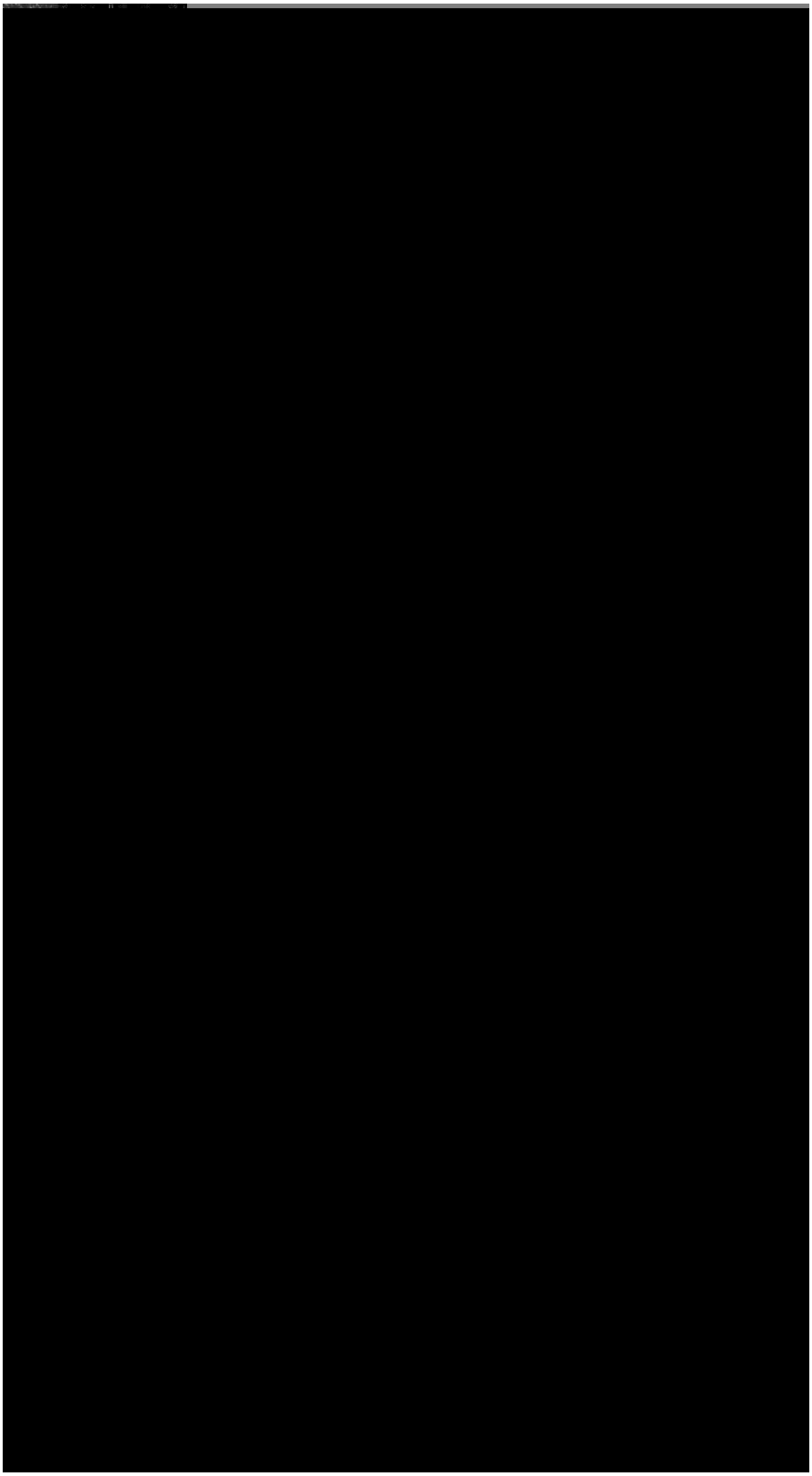

FIG. 5. PDGFRa′

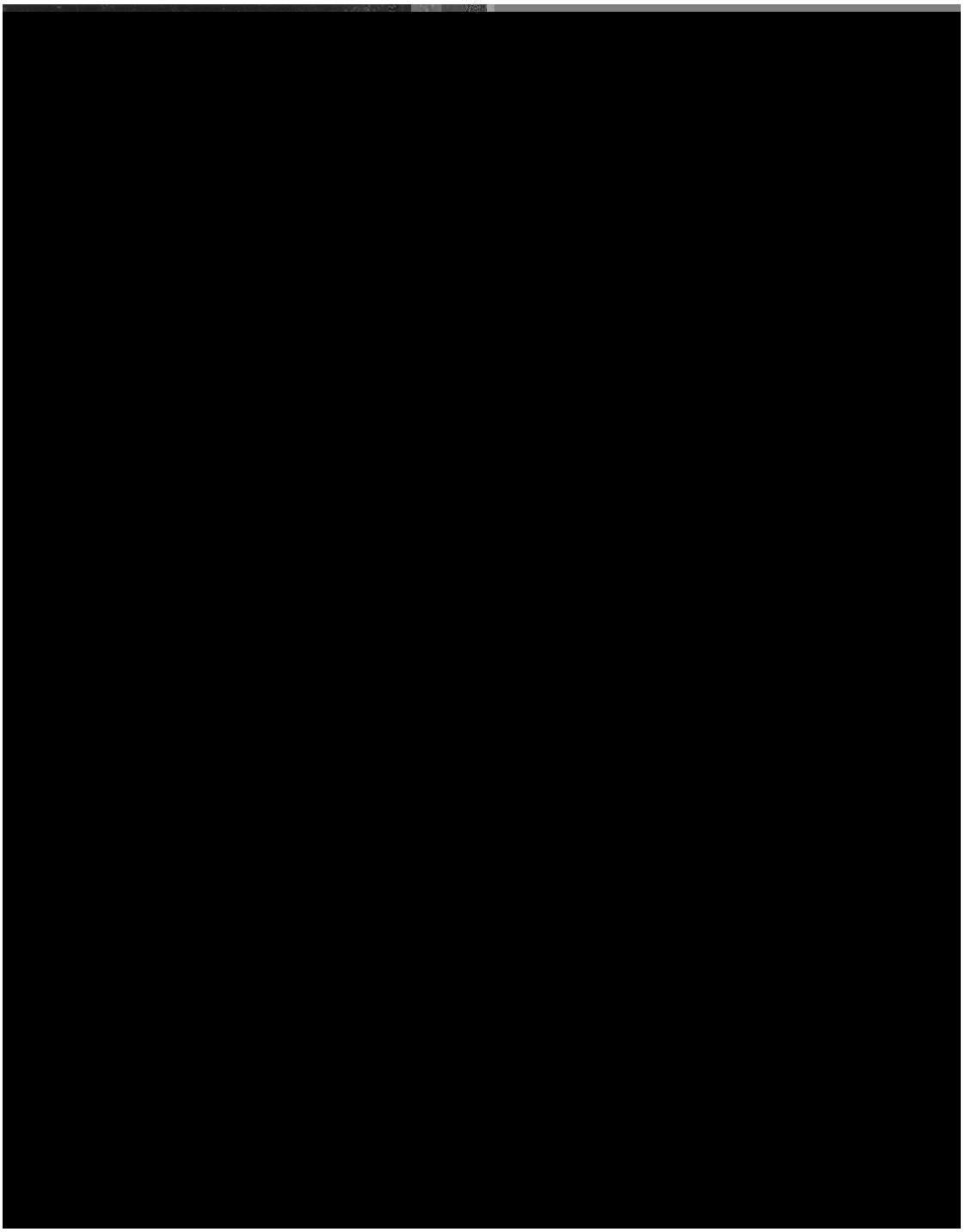

FIG. 7. Induction of floor plate cells, motor neurons, and oligodendrocytes in intermediate neural tube explants by notochord and purified Shh. Intermediate neural tube explants from E1.5–2 quails were cultured in collagen gels on their own or in contact with notochord tissue or in the presence of different concentrations of Shh (see Materials and methods). (a) Oligodendrocytes visualized with anti-SMP in an explant cultured for 12 days in contact with notochord tissue. Most of the oligodendrocytes are localized to a region overlying the original position of the notochord. (b) Control explant cultured in the absence of notochord for 12 days, labeled with anti-SMP. (c) Induced floor plate cells visualized with monoclonal FP1, 48 hr after the addition of Shh (7 1

TABLE 1
Induction of Oligodendrocytes, Motor Neurons, and Floor Plate Cells in Neural Tube Explants by Sonic Hedgehog Protein

[Shh]	7×10^{-9}	2×10^{-9}	7×10^{-10}	2×10^{-10}	7×10^{-11}	Control
Oligodendrocytes	19/21	25/26	14/17	0/16	0/22	1/24
Motor neurons	12/12	16/18	10/13	0/6	0/6	0/13
Floor plate cells	10/10	0/6	ND	ND	ND	0/13

Note. Intermediate neural tube explants from stage 9–10 quail embryos were cultured in the presence of the indicated molar concentrations of Shh (amino terminal fragment, Roelink *et al.*, 1995). Motor neurons were labeled after 36–48 hr in culture with monoclonal 4D5

Guthrie, S., and Lumsden, A. (1994). Collagen gel coculture of neural tissue. *Neuroprotocols* 4, 116–120.

Guthrie, S., and Pini, A. (1995). Chemorepulsion of developing motor axons by the floor plate. *Neuron* 14, 1117–1130.

Hamburger, V., and Hamilton, H. L. (1951). A series of normal changes in the development of the chick embryo. *J. Morphol.* 88, 49–92.

Hynes, M., Poulsen, K., Tessier-Lavigne, M., and Rosenthal, A. (1995). Control of neuronal diversity by the floor plate: Contact-mediated induction of midbrain dopaminergic neurons. *Cell* 80, 95–101.

Johnson, R. L., and Tabin, C. (1995). The long and short of hedgehog signaling. *Cell* 81, 313–316.

Karnovsky, M. J., and Roots, L. (1964). A ''direct-coloring'' thiocholine method for cholinesterases. *J. Histochem. Cytochem.* 12, 219–221.

Kennedy, T. E., Serafini, T., de la Torre, J. R., and Tessier-Levigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 78, 425–435.

Klar, A., Baldassare, M., and Jessel, T. M. (1992). F-Spondin: A gene expressed at high levels in the floor plate encodes a secreted protein that promotes neural cell adhesion and neurite extension. *Cell* 69, 95–110.

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.

Leber, S. M., and Sanes, J. R. (1995). Migratory paths of neurons and glia in the embryonic chick spinal cord. *J. Neurosci.* 15, 1236–1248.

Levison, S. W., Chuang, C., Abramson, B. J., and Goldman, J. E. (1993). The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. *Development* 119, 611–622.

Martí, E., Bumcrot, D. A., Takada, R., and McMahon, A. P. (1995). Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. *Nature* 375, 322–325.

Miller, R. H. (1996). Oligodendrocyte origins. *Trends Neurosci.* 19, 92–96.

Nishiyama, A., Lin, X.-H., Giese, N., Heldin, C.-H., and Stallcup, W. B. (1996). Co-localization of NG2 proteoglycan and PDGF a receptor on O2A progenitor cells in the developing rat brain. *J. Neurosci. Res.* 43, 299–314.

Noll, E., and Miller, R. H. (1993). Oligodendrocyte precursors originate at the ventral ventricular zone dorsal to the ventral midline region in the embryonic rat spinal cord. *Development* 118, 563–573.

Nornes, H. O., and Das, G. D. (1974). Temporal pattern of neurogen-

Porter, J. A., von Kessler, D. P., Ekker, S. C., Young, K. E., Lee, J. J., Moses, K., and Beachy, P. A. (1995). The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature* 374, 363–374.

Pringle, N. P., Mudhar, H. S., Collarini, E. J., and Richardson, W. D. (1992). PDGF receptors in the CNS: During late neurogenesis, expression of PDGF alpha receptors appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* 115, 535–551.

Pringle, N. P., and Richardson, W. D. (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development* 117, 525–533.

Richardson, W. D., Pringle, N. P., Yu, W.-P., Collarini, E. J., and Hall, A. C. (1995). Origin and early development of oligodendrocytes. *In* ''Glial Cell Development'' (K. R. Jessen and W. D. Richardson, Eds.), Bios, Oxford, UK.

Roelink, H., Porter, J. A., Chiang, C., Tanabe, Y., Chang, D. T., Beachy, P. A., and Jessell, T. M. (1995). Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog proteolysis. *Cell* 81, 445–455.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M., and Tessier-Levigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* 78, 409–424.

Small, R. K., Riddle, P., and Noble, M. (1987). Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature* 328, 155–157.

Smith, J. C. (1994). Hedgehog, the floor plate and the zone of polarizing activity. *Cell* 76, 193–196.

Sommer, I., and Schachner, M. (1981). Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: An immunocytological study in the central nervous system. *Dev. Biol.* 83, 311–327.

Stemple, D. L., and Anderson, D. J. (1992). Isolation of a stem cell for neurons and glia from the mammalian neural crest. *Cell* 71, 973–985.

Tamada, A., Shirasaki, R., and Murakami, F. (1995). Floor plate chemoattracts crossed axons and chemorepels uncrossed axons in the vertebrate brain. *Neuron* 14, 1083–1093.

Tanabe, Y., Roelink, H., and Jessell, T. M. (1995). Induction of motor neurons by sonic hedgehog is independent of floor plate differentiation. *Curr. Biol.* 5, 651–658.

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

Trousse, F., Giess, M. C., Soula, C., Ghandour, S., Duprat, A.-M., and Cochard, P. (1995). Notochord and floor plate stimulate oligo-

ventral origin of oligodendrocyte precursors in the rat spinal cord. *J. Neurosci.* 11, 2477–2488.

Wenger, E. L. (1950). An experimental analysis of relations between parts of the brachial spinal cord of the embryonic chick. *J. Exp. Zool.* 114, 51–81.

of cell pattern in the neural tube: Motor neuron induction by diffusible factors from notochord and floor plate. *Cell* 73, 673–686.

Yu, W.-P., Collarini, E. J., Pringle, N. P., and Richardson, W. D.