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Pax6 Influences the Time and Site of Origin of Glial Precursors in the Ventral Neural Tube

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Neuroepithelial precursors in the ventral ventricular zone (VZ) of the spinal cord generate motor neurons (MNs) and interneurons, and then a subset of precursors starts to produce oligodendrocyte progenitors (OLPs). We show that OLPs originate in the ventral-most part of the *Pax6*-positive VZ, which at earlier times generates somatic (*Isl2/Lim3*-positive) MNs. In *Small eye* (Pax6-deficient) mice, the origin of OLPs is shifted dorsally and both OLPs and *Isl2/Lim3* MNs are delayed. We suggest that somatic MNs and OLPs are generated sequentially from a common set of MN-OL precursors whose position in the VZ is influenced by Pax6. Neuron–glia fate switching might be a preprogrammed property of these precursors or a response to feedback from newly generated neurons. OLs developed normally in explants of *Isl1(−/−)* spinal cords, which lack MNs, arguing against feedback control and suggesting that the neuron–glia switch is an intrinsic developmental program in a specific subset of neural precursors.

INTRODUCTION

During development of the vertebrate central nervous system (CNS), distinct neuronal and glial cell types are produced at different dorsoventral levels of the neural tube. The initial dorsoventral polarity of the tube is established through a combination of dorsalizing and ventralizing signals from the dorsal and ventral midlines, respectively. The main ventralizing signal is the product of the *Sonic hedgehog* (*Shh*) gene, the vertebrate homolog of the *Drosophila* patterning gene *hedgehog*. From an early stage of development Shh secretion by the notochord and floor plate generates a ventral-to-dorsal (high–low) concentration gradient in the adjacent neural tube. Initially, this acts to suppress dorsal markers in the ventral neural tube. Then, when a ventral identity has been established, graded Shh signals provide positional cues that direct the development of specific neuronal and glial cell types.

Ventral neurons are derived from dividing neuroepithelial precursors that reside at the ventricular surface of the basal plate. These precursors generate postmitotic progenitor cells that migrate radially and differentiate into motor neurons (MNs) or interneurons (INs), which can be classified by their combinatorial expression of LIM-homeodomain transcription factors and other proteins (Tsuchida *et al.*, 1994; Varela-Echavarría *et al.*, 1996; Burrill *et al.*, 1997; Ericson *et al.*, 1997; Matise and Joyner, 1997). The precursors of these different types of neurons are specified at different positions along the dorsal–ventral axis, defined by the local concentrations of Shh and other factors. In addition, signals from the flanking paraxial and surface mesoderm confer regional specificity of MN subtype along the anterior–posterior axis (Muhr *et al.*, 1997; Ensini *et al.*, 1998).

The intracellular effects of Shh signaling are mediated in part by the transcription factor Pax6 (Goulding *et al.*, 1993; Burrill *et al.*, 1997; Ericson *et al.*, 1997; Osumi *et al.*, 1997). Shh represses *Pax6*, so that Pax6 expression is high where Shh is low and vice versa. As a result, the ventral half of the neural tube is subdivided into a Pax6-negative domain abutting the floor plate and a more dorsal Pax6-positive domain that displays a gradient of Pax6 expression, increasing ventral-to-dorsal (Ericson *et al.*, 1997; Osumi *et al.*, 1997). Pax6 in turn represses expression of the transcription factor Nkx2.2, which is therefore restricted to the Pax6-negative do-

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main (Ericson *et al.,* 1997). In the cervical spinal cord, neuroepithelial precursors in the ventral part of the Pax6-positive domain generate somatic MNs of the median motor column (MMC), which express LIM-domain proteins Isl1, Isl2, Lim3 (also known as Lhx3) and Gsh4 (Lhx4) (Li *et al.,* 1994; Tsuchida *et al.,* 1994; Seidah *et al.,* 1994; Zhadanov *et al.,* 1995; Ericson *et al.,* 1997). Precursors from more dorsal parts of the Pax6-positive domain generate several classes of ventral INs that express different combinations of Pax2, En1, Evx1, Lim1, Lim3, Gsh4 and Chx10 (Burrill *et al.,* 1997; Ericson *et al.,* 1997). Precursors from the Pax6-negative, Nkx2.2-positive domain generate a subset of MNs that express Isl1 but not Isl2 and a population of cells that express *Sim1,* the vertebrate homolog of the *Drosophila* gene *single-minded* (Fan *et al.,* 1996; Ericson *et al.,* 1997).

Inactivating point mutations in *Pax6* underlie multiple eye and brain defects in the *Small eye (Sey)* mouse and *rSey* rat (Hill *et al.,* 1991; Matsuo *et al.,* 1993; Stoykova *et al.,* 1996; Warren and Price, 1997). In homozygous mutants the ventral Nkx2.2-positive domain expands dorsally into what would normally be Pax6 territory. Consequently, Isl2/Lim3-expressing MNs are wholly or partly respecified as more ventral cell types (Varela-Echavarría *et al.,* 1996; Ericson *et al.,* 1997; Osumi *et al.,* 1997). In the brainstem this results in loss of hypoglossal and abducens MNs (and corresponding cranial nerves) and a gain of vagal MNs, while in the spinal cord there seems to be an increase in the number of *Sim1*-expressing cells at the expense of somatic MNs (Tsuchida *et al.,* 1994; Varela-Echavarría *et al.,* 1996; Ericson *et al.,* 1997; Osumi *et al.,* 1997). Pax6-deficient animals also lack En1/Pax2-expressing INs in both brainstem and spinal cord (Burrill *et al.,* 1997; Ericson *et al.,* 1997).

Glial cells are also derived from neuroepithelial precursors in the ventral half of the spinal cord. Oligodendrocytes (OLs), the myelin-forming cells of the CNS, develop from migratory, proliferating progenitor cells (OLPs) that descend from a small number of precursors in the ventral ventricular zone (VZ) near the floor plate (Noll and Miller, 1993; Pringle and Richardson, 1993; Yu *et al.,* 1994; Timsit *et al.,* 1995; Ono *et al.,* 1995; Nishiyama *et al.,* 1996; Pringle *et al.,* 1998: reviewed by Richardson *et al.,* 1995; Miller, 1996). The first OLPs appear in the VZ on Embryonic Day 14 in the rat (E12.5 in the mouse), shortly after the end of MN production (Nornes and Das, 1974; Altman and Bayer, 1984). Shh has been shown to induce the development of oligodendrocytes in explant cultures of dorsal or intermediate neural tube at Shh concentrations that overlap those required for MN induction (Poncet *et al.,* 1996; Pringle *et al.,* 1996). We have suggested, therefore, that the same population of neuroepithelial precursors might give rise first to MNs and then to OLPs (Richardson *et al.,* 1997).

We conducted experiments to compare the requirements of OLPs and ventral neurons for Pax6 in the mouse cervical spinal cord. We established that OLPs arise from the most ventral region of the *Pax6*-positive domain, which also is reported to give rise to Isl2/Lim3 MNs. In *Sey/Sey* mice the origin of OLPs was shifted dorsally and their appearance delayed by up to a day. The appearance of *Isl2/Lim3* MNs was also delayed by about a day. Thus, Pax6 is not absolutely required to specify cell fate but it influences the time and pattern of fate decisions in the VZ. Our results indicate a close developmental relationship between *Isl2/Lim3* MNs and OLPs, suggesting that both cell types descend from the same set of neuroepithelial precursors. Fate switching might depend either on a cell-intrinsic program in the precursors or on feedback control from *Isl2/Lim3* MNs. Against the latter hypothesis, we found that OLs develop normally in explant cultures of *Isl1* (−/−) spinal cords, which do not contain any MNs. Therefore, we propose that a specific subset of neuroepithelial precursors first generates *Isl2/Lim3* MNs and then switches to OLPs and that the neuron–glia fate switch is part of a stereotyped developmental program initiated by Shh together with Pax6.

RESULTS

Oligodendrocyte Progenitors Originate in the Ventral-Most Part of the Pax6-Positive Domain

In transverse sections of E12.5 mouse spinal cord, OLPs first appear as bilateral foci of *PDGFRα*-positive cells in the ventral VZ (Fig. 1B) (Pringle and Richardson, 1993; Yu *et al.,* 1994; Nishiyama *et al.,* 1996; Hardy, 1997; Pringle *et al.,* 1998). We attempted to define the site of origin of OLPs with more precision by mapping the positions of a total of 28 *PDGFRα*$^+$ OLPs in sections of E12.5 spinal cord, relative to the ventral and dorsal midlines (see Materials and Methods). We found that the majority of OLPs clustered in a tightly defined domain of the VZ ranging from about 9% to about 13% of the way from the ventral toward the dorsal midline, measuring along the surface of the spinal cord lumen (Fig. 1D). The mean position was at 11% ± 2% (mean ± SD, $n = 28$)—about 10 to 15 cell diameters from the edge of the floor plate. We also determined the location of the first OLPs relative to the *Pax6*- and *Nkx2.2*-positive domains by comparing adjacent sec-

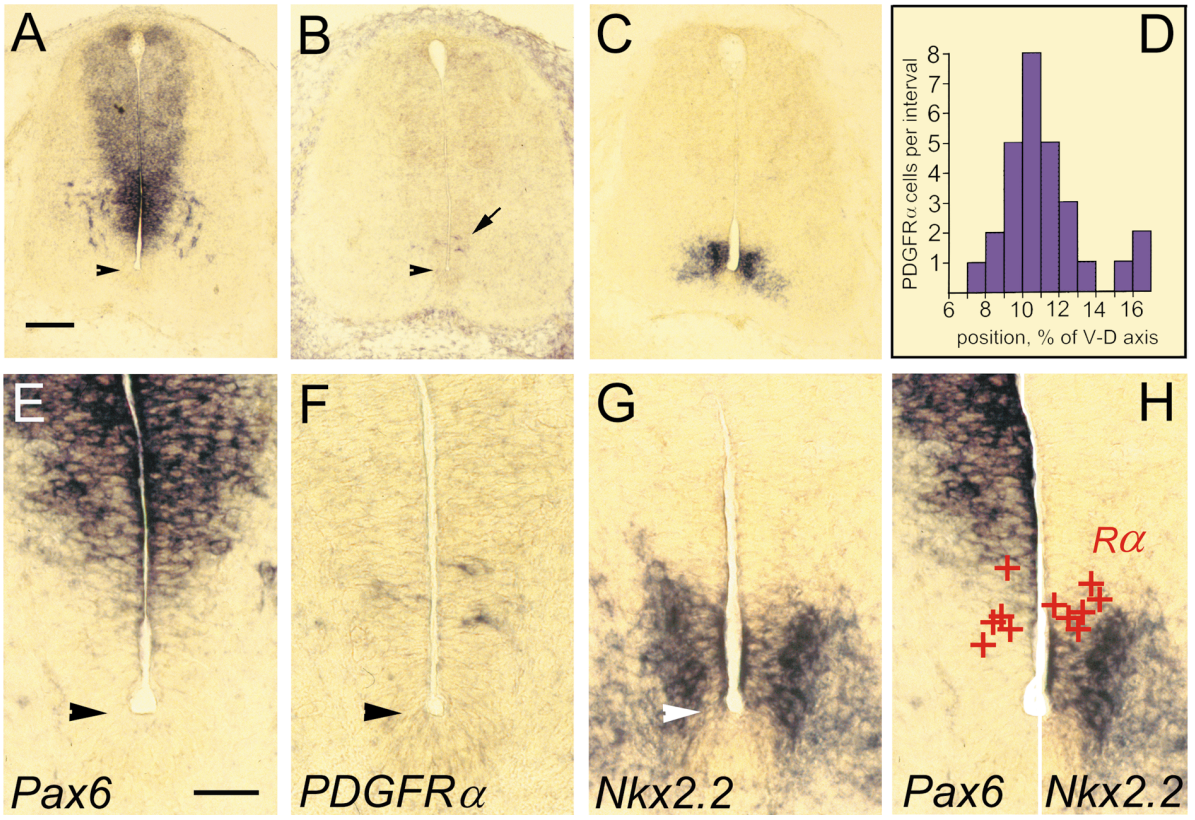

FIG. 1. Site of origin of *PDGFRα*$^+$ OLPs relative to the *Pax6*- and *Nkx2.2*-positive domains of the wild-type spinal cord. Neighboring sections from an E12.5 wild-type spinal cord were hybridized *in situ* with DIG-labeled probes for *Pax6* (A, E), *PDGFRα* (B, F), or *Nkx2.2* (C, G), and hybrids were visualized by alkaline phosphatase. *Pax6* is expressed throughout the VZ except for a small region abutting the floor plate, and also in a population of cells outside the VZ in the ventral part of the cord (A). *Nkx2.2* is expressed in a complementary pattern, restricted to the region adjacent to the floor plate (C). *PDGFRα* is first expressed in a few cells at the ventricular surface (arrow in B). We mapped the positions of 28 *PDGFRα*$^+$ OLPs onto the dorsal–ventral axis (see Materials and Methods) (panel D). The median position was between 10% and 11% (panel D) and the mean position was 11% ± 2% (mean ± SD, $n = 28$). This corresponds to about 10–15 cell diameters from the edge of the floor plate. When higher magnification images of adjacent sections were aligned carefully (E to H), it was clear that the *PDGFRα*$^+$ cells (some of which are signified by red crosses in H) lay close to the boundary of the *Pax6*- and *Nkx2.2* domains. Most *PDGFRα*$^+$ cells mapped to the ventral-most part of the *Pax6*-positive domain (panel H). Some cells whose sites of origin can be defined accurately because they retain visible contact with the ventricular surface are clearly within *Pax6*-positive, *Nkx2.2*-negative territory (e.g., the upper two cells visible in panel F). Others fall in the small area of overlap between *Pax6* and *Nkx2.2*, but whether these cells express both *Pax6* and *Nkx2.2* cannot be determined at this level of analysis. Scale bars, 100 μm (A–C), 20 μm (E–H).

tions of cervical spinal cord (C3–C5) hybridized *in situ* with probes for *PDGFRα*, *Pax6* or *Nkx2.2*.

Pax6 is initially expressed in all regions of the neural tube, with the exception of the ventral midline (Gruss and Walther, 1992). By E9.5, when the first MNs begin to differentiate, its expression is down-regulated and can no longer be detected in the ventral-most region adjacent to the floor plate, while in the remainder of the basal plate *Pax6* transcripts form a dorsal–ventral (high–low) concentration gradient (Ericson *et al.*, 1997). This pattern persists in the E12.5 neural tube (Figs. 1A, 1E).

Nkx2.2 is a homeodomain protein related to the *Drosophila* NK-2 gene product (Price *et al.*, 1992). From before E9.5 to at least E12.5 it is expressed in cells in the most ventral part of the neural tube adjacent to but not including the floor plate (Shimamura *et al.*, 1995; Ericson *et al.*, 1997; Figs. 1C, 1G). The dorsal expression limit of Nkx2.2 approximately coincides with the ventral limit of Pax6 (Ericson *et al.*, 1997). This, together with the observation that Nkx2.2 expression spreads dorsally in the *Sey* mutant mouse, which lacks Pax6 function, suggests that Pax6 acts to repress *Nkx2.2* expression (Ericson *et al.*, 1997).

Direct comparison of *PDGFRα* with *Pax6* and *Nkx2.2* expression in adjacent sections indicates that OLPs are derived from the ventral-most region of the *Pax6*-

expressing domain, possibly in a small region of overlap with *Nkx2.2* (Figs. 1E–1H). Therefore, it is likely that the earliest OLPs express a low level of *Pax6* and possibly also a low level of *Nkx2.2*. *Isl2/Lim3*-positive somatic MNs also are believed to come from the ventral-most part of the *Pax6* domain (Ericson *et al.*, 1997).

Appearance of Oligodendrocyte Progenitors Is Delayed in Sey/Sey Mice

Having established that OLPs arise from within the *Pax6*-positive region of the VZ, we investigated the role of *Pax6* in the development of these cells by examining *Sey* mutant mice. In wild-type spinal cord *PDGFRα*$^+$ OLPs are first detected in the ventral VZ at E12.5 (Calver *et al.*, 1998). In *Sey/Sey* spinal cords *PDGFRα*$^+$ OLPs could not be detected until E13.5, 1 day later than normal (see Figs. 4B, 4E). Subsequently, they proliferated and eventually accumulated to normal steady-state numbers, but with a delayed time course relative to wild-type (Fig. 2). By E18.5, wild-type and *Sey* spinal cords were indistinguishable (Fig. 2A, panels G and H, and Fig. 2B).

There was also a delay in OLP development in the caudal hindbrain (R5–R7). At E12.5, when there were significant numbers of *PDGFRα*-positive OLPs in the wild-type brainstem, there were no OLPs in *Sey/Sey* littermates (not shown). However, at E13.5 there were similar numbers of OLPs in both wild-type and mutant brainstem (not shown). We did not investigate OLP development anterior to this.

Oligodendrocyte Progenitor Cell Cycle Dynamics in Sey/Sey Embryos

A distinctive feature of OLP early development is their cell cycle kinetics. Their division cycle is initially

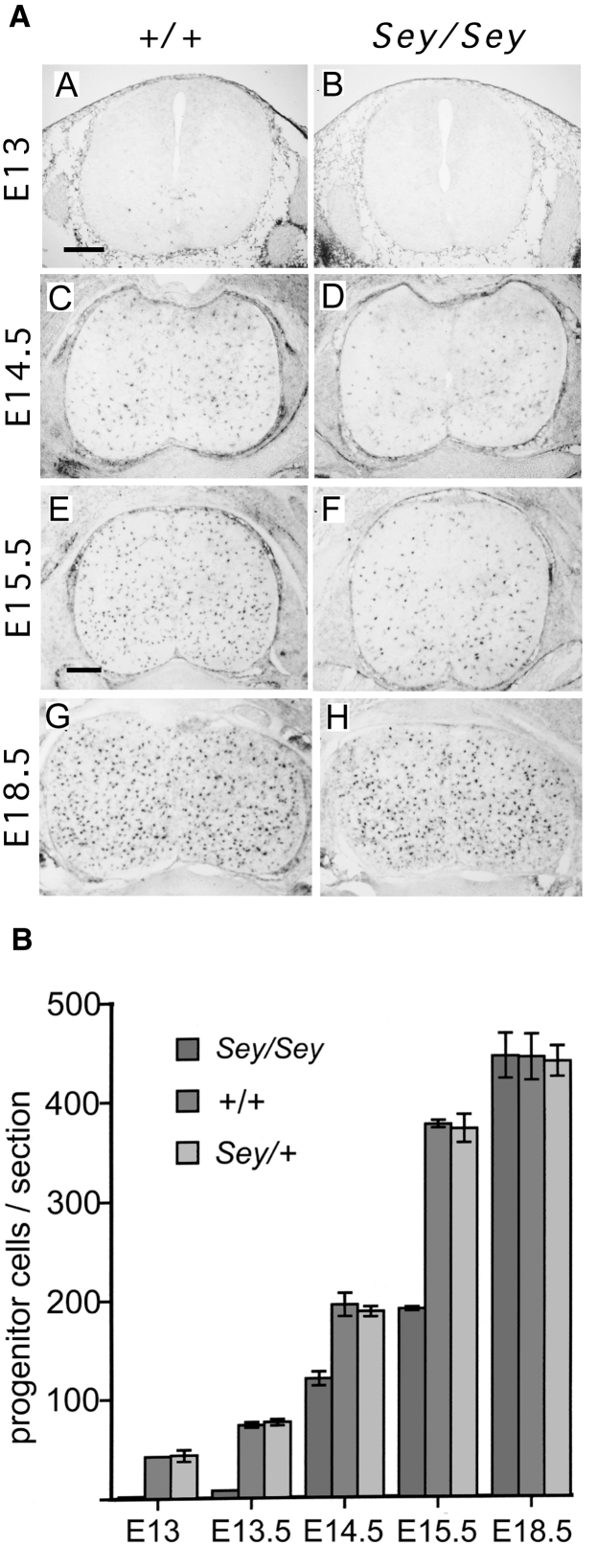

FIG. 2. Development of *PDGFRα*$^+$ OLPs in wild-type and *Sey/Sey* spinal cords. (A) Transverse sections through cervical spinal cords at the indicated ages were hybridized *in situ* with DIG-labeled *PDGFRα* probes. *PDGFRα*$^+$ cells first appeared in the wild-type cord at E12.5 (see Fig. 1). By E13 *PDGFRα*$^+$ cells had already started to spread through the ventral part of the wild-type cord (A) but had not yet appeared in their age-matched *Sey/Sey* littermates (B). *PDGFRα*$^+$ cells first appeared at the ventricular surface of the *Sey/Sey* cord at E13.5 (see Fig. 4). Numbers of *PDGFRα*$^+$ cells in *Sey/Sey* lagged behind those in wild-type until after E15.5 (C–F) but caught up by E18.5 (G, H). Scale bar, 200 μm. (B) *PDGFRα*$^+$ OLPs were counted in transverse sections of wild-type, *Sey/+*, and *Sey/Sey* spinal cords. Data (means ± SD) were obtained from at least six sections from two embryos at each age, either from one (E13 and E13.5) or two litters. The time of first appearance and subsequent proliferation of PDGFRα$^+$ cells were retarded by about a day in *Sey* homozygotes compared to both wild-type and heterozygous *Sey* mice.

very short—6 to 8 h—but slows down to about 24 h over a period of a few days before birth. In other cell populations Pax6 has been implicated in the control of cell proliferation (Warren and Price, 1997), so we asked whether OLP cell cycle dynamics were altered in *Sey/Sey* mice.

We compared cell division rates in mutant and wild-type OLPs by bromodeoxyuridine (BrdU) labeling *in utero.* At different embryonic ages we gave a single injection of BrdU into the mother, fixed the embryos 2 h later, and double-labeled spinal cord sections for BrdU and *PDGFRα* to identify OLPs that had been in S phase during the labeling period. Since we have previously determined that all OLPs are actively engaged in the division cycle (Calver *et al.*, 1998), the BrdU labeling index (proportion of OLPs that incorporates BrdU) correlates inversely with the average cell cycle time.

In wild-type embryos the BrdU labeling index was around 60% at E12.5 but fell rapidly as cell numbers increased, reaching a stable low value of around 15% by E18.5 (Fig. 3). This is in line with our previous *in vivo* labeling experiments (Calver *et al.*, 1998). In *Sey/Sey* littermates the BrdU labeling index also started high (around 60%) and declined to about 15% by E18.5 (Fig. 3). We conclude that the cell cycle kinetics of OLPs are similar in *Sey/Sey* and wild-type embryos, except that the *Sey/Sey* curve is displaced to later ages, as expected, since the first OLPs appear later in the mutant. Pax6 therefore appears to have at most a subtle effect on OLP proliferation.

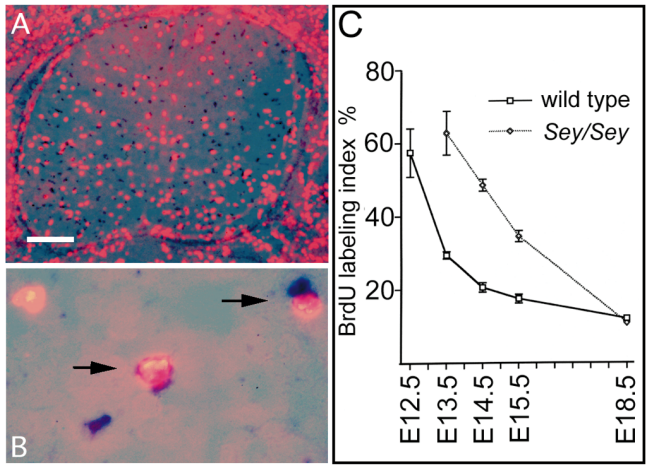

FIG. 3. Cell cycle dynamics of *PDGFRα*$^+$ OLPs analyzed by BrdU incorporation *in vivo.* Pregnant females were given a single intraperitoneal injection of BrdU (50 μg per gram body weight) and their embryos were removed 2 h later and prepared for histochemistry. Transverse sections through cervical spinal cords of *Sey/Sey* embryos and their wild-type littermates were hybridized *in situ* with a DIG-labeled *PDGFRα*$^+$ probe, followed by antibody labeling for BrdU. (A) An immunofluorescence micrograph of an E15.5 *Sey/Sey* spinal cord showing BrdU-labeled cells distributed throughout the cross section of the cord, superimposed on the corresponding *PDGFRα*$^+$ *in situ* hybridization micrograph (scale bar, 200 μm). (B) Part of the same image at higher magnification; in this field there are three *PDGFRα*$^+$ cells, two of which have BrdU-positive nuclei (arrows). Numbers of (*PDGFRα*, BrdU) double-positive cells were plotted as proportions of the total numbers of *PDGFRα*$^+$ cells (BrdU labeling index) for embryos of different ages (C). Data (mean ± SEM) were obtained from at least five sections from two embryos of each age, either from one litter (E12.5 and E13.5) or two litters. In both wild-type and *Sey/Sey* cords, the BrdU labeling index dropped from around 60% at early ages to around 15% at E18.5 and later; however, the curve for *Sey/Sey* was displaced to the right by about a day, consistent with the developmental delay in *Sey* homozygotes.

The Site of Origin of OLPs in the VZ Is Shifted Dorsally in Sey/Sey Embryos

If Pax6 helps relay positional information from Shh to the cell nucleus, one would expect positional values to be altered in *Sey/Sey* mice. The fact that *PDGFRα* marks progenitor cells before they leave their site of origin in the neuroepithelium allowed us to test this prediction. We mapped the positions of emergent *PDGFRα*$^+$ OLPs in the VZ of E13.5 *Sey/Sey* embryos. These cells were clearly displaced dorsally relative to those in wild-type embryos—25% ± 3% (mean ± SD, $n = 11$) of the distance from the ventral toward the dorsal midline, compared to 11% ± 2% in wild-types (Fig. 4C; compare Fig. 1D and Figs. 4E, 4F). Although the cells appear up to a day later in *Sey/Sey* compared to in wild-type, this is unlikely to explain the shift because we found that there was no significant change in the circumference of the spinal cord lumen between E12.5 and E13.5 (data not shown). We conclude that the lack of Pax6 results in a dorsalward shift in specification of OLPs.

Appearance of Specific Neuronal Populations Is Delayed in Sey/Sey Embryos

If the dorsal shift in precursor cell specification were a general consequence of Pax6 deficiency, we would expect ventral neurons to develop in *Sey/Sey* mice, but to originate from more dorsal parts of the VZ. Because the markers commonly used for MNs and INs are not expressed fully until after the cells leave the VZ, it was not possible to test this prediction. However, it seemed possible that a dorsal shift might be accompanied by a delay in their appearance, as for OLPs. We therefore followed the expression of three neuronal markers—*Isl1, Isl2,* and *Lim3*—in cervical spinal cords of wild-type and *Sey* mutant mice from E9.5 to E13.5. Somatic MNs of the MMC express all these marker genes, while one class of ventral INs expresses *Lim3* but neither *Isl1* nor *Isl2*

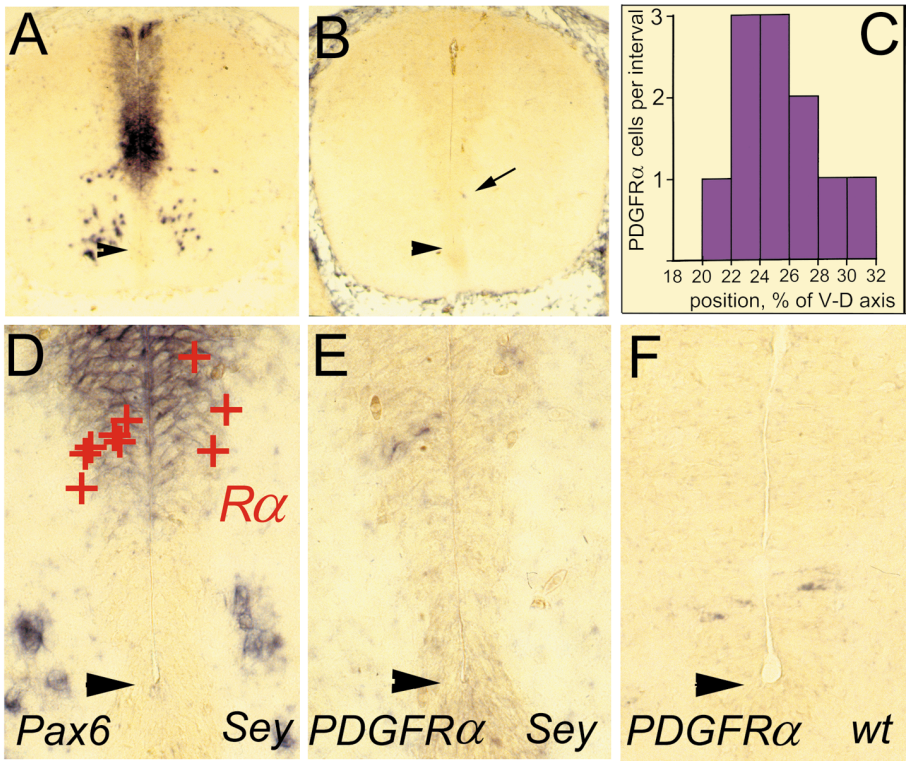

FIG. 4. Site of origin of *PDGFRα*$^+$ OLPs in the ventral VZ of *Sey/Sey* spinal cord shifts dorsally relative to that in wild-type. Sections through the cervical cord of E13.5 *Sey/Sey* embryos were subjected to *in situ* hybridization with DIG-labeled probes for *Pax6* (A, D) or *PDGFRα* (B, E). An E12.5 wild-type cord is shown at the same magnification for comparison (F). The positions of 11 OLPs in the VZ (six sections from one animal) were measured relative to the ventral and dorsal midlines (C) as described under Materials and Methods. All mapped between 20% and 32% on the ventral–dorsal axis (25% ± 3%, mean ± SD). This is shifted dorsally compared to wild-type OLPs (11% ± 2%; see Fig. 1). Arrowheads mark the ventral limit of the spinal cord lumen. The small arrow in B indicates the position of the *PDGFRα*$^+$ OLPs. Red crosses in D mark the positions of *PDGFRα*$^+$ OLPs in four adjacent sections including that shown in E. Magnifications are as in Fig. 1.

(Ericson *et al.*, 1997). Other classes of spinal cord MNs express *Isl1* but not *Isl2*.

In wild-type spinal cord, expression of all three markers was detected at E9.5 (Fig. 5). In *Sey/Sey* embryos at the same morphological stage *Isl1* labeling was similar in intensity to that in wild-type, although it extended further dorsally, but neither *Isl2* nor *Lim3* could be detected (Fig. 5). A day later, at E10.5, *Isl2* and *Lim3* expression was evident in the mutant but fewer cells were labeled than in wild-type, particularly for *Isl2* (Fig. 6). *Isl1* labeling, which was relatively normal at E9.5, marked significantly fewer cells in *Sey/Sey* than in wild-type at E10.5; in particular, there appeared to be a ventromedial population of *Isl1*-positive cells that were absent from the *Sey/Sey* cord (Fig. 6). Also, a group of presumptive sensory neurons in the dorsal cord was missing at E10.5 (Fig. 6). However, by E13.5 expression of all three markers appeared normal in the *Sey/Sey* spinal cord, in both the number and the distribution of labeled cells (Fig. 7).

Oligodendrocytes Develop Normally in Vitro in the Absence of Motor Neurons

The observation that OLPs appear to be generated in the same part of the ventral VZ as somatic MNs (see above) suggests that the same set of neuroepithelial precursors might first generate MNs and then switch to production of OLPs. This neuron–glia switch might be part of an intrinsic developmental program in the neuroepithelial precursors. Alternatively, the switch might be triggered by feedback signals from the newly formed MNs. To test the latter idea, we looked to see if OLs can develop in *Isl1*(−/−) spinal cord, which lacks all classes of MNs and at least one class of ventral (V1) INs (Pfaff *et al.*, 1996). Because the *Isl1*(−/−) mice die *in utero* before the emergence of the OL lineage, we studied OL development in spinal cord explant cultures.

We established explant cultures from the spinal cords of E9.5 embryos. The embryos were genotyped retrospectively by PCR and Isl1/2 immunolabeling as described

in Materials and Methods. After culturing until the equivalent of the day of birth (11 days in culture), the explants were immunolabeled with anti-galactocerebroside (GC) or anti-myelin basic protein (MBP) to visualize differentiated OLs. In five independent experiments on five separate litters, we examined a total of 11 *Isl1*(−/−), 26 *Isl*(+/−), and 6 (+/+) embryos. OLs developed in explants from every one of these embryos (Fig. 8). We conclude that signals from Isl1-expressing MNs are unlikely to be required for normal development of OL lineage cells in the spinal cord.

DISCUSSION

Development of ventral neurons and OLPs depends on Shh signaling *in vivo* and both MNs and OLPs are induced by the same concentrations of Shh *in vitro* (Pringle *et al.*, 1996). This codependence on Shh, together with other evidence (see below), led us to suggest (Pringle *et al.*, 1996; Richardson *et al.*, 1997) that MNs and OLs might descend from a common group of neuroepithelial MN–OL precursors, the specification of which is dependent on Shh. If so, we expect that MNs and OLPs should originate from the same site(s) within the VZ. In support of this idea, we have now established that OLPs arise in the ventral-most region of the *Pax6*-positive domain of the spinal cord VZ, the same region that at earlier times is thought to give rise to somatic MNs of the MMC (Tsuchida *et al.*, 1994; Ericson *et al.*, 1997).

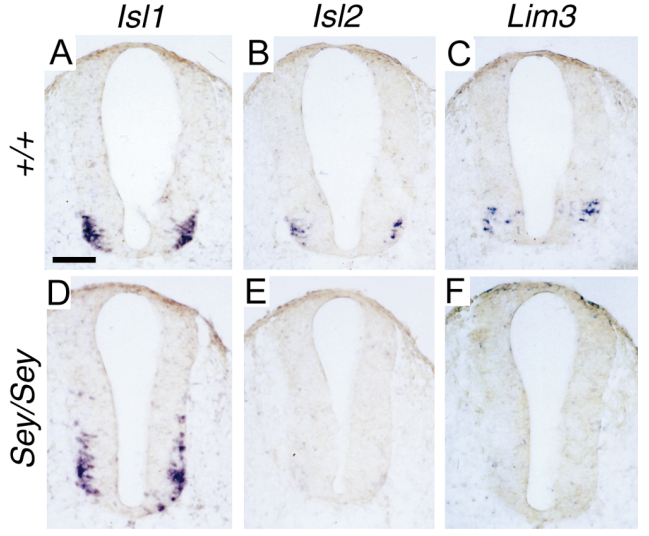

FIG. 5. Expression of LIM-domain transcription factors in E9.5 wild-type and *Sey/Sey* spinal cords. Transverse sections through the cervical region of E9.5 cords (25 somite stage) were hybridized *in situ* with DIG-labeled probes for *Isl1*, *Isl2*, and *Lim3*. *Isl1* expression is relatively normal in *Sey/Sey* embryos at this age, whereas expression of *Isl2* and *Lim3* is delayed in the mutant. Scale bar, 100 μm.

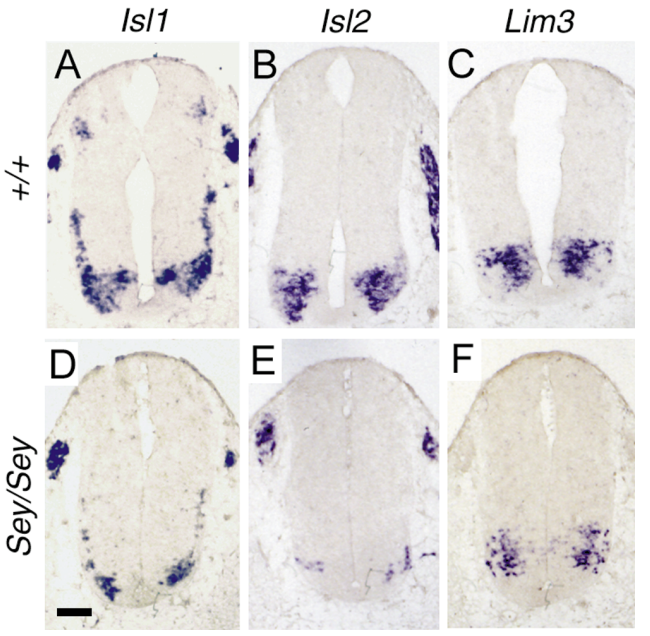

FIG. 6. Expression of LIM-domain transcription factors in E10.5 wild-type and *Sey/Sey* spinal cords. Transverse sections through the cervical regions of E10.5 cords were hybridized *in situ* with probes for *Isl1*, *Isl2*, and *Lim3*. Expression of all three genes seems to be depressed in the *Sey/Sey* cord compared to that in wild-type, but most markedly for *Isl2*. In addition, there is a ventromedial population of *Isl1*-positive cells in the wild-type that is missing from the mutant at this age. Scale bar, 100 μm.

The fact that both MNs and OLPs originate from the same part of the VZ is compatible with a common MN–OL lineage but does not exclude other models. For example, there could be a separate pool of dedicated OLP precursors whose maturation depends on signals generated by previously formed MNs. However, our finding that OLs develop normally in explants of *Isl1*(−/−) spinal cords—in the absence of MNs—tends to argue against a central role for MN-derived signals. It still leaves open the possibility that feedback from other classes of ventral neurons such as the *Lim3/Chx10* (V2) INs might be required. We believe this is unlikely because normal numbers of *Chx10* INs accumulate in the *Sey/Sey* spinal cord before E12, prior to the normal onset of OPL development (Burrill *et al.*, 1997). A lineage relationship between MNs and OLs was previously suggested by retroviral clonal analysis in the embryonic chick spinal cord (Leber *et al.*, 1990; Leber and Sanes, 1995). Common neuron–oligodendrocyte precursors

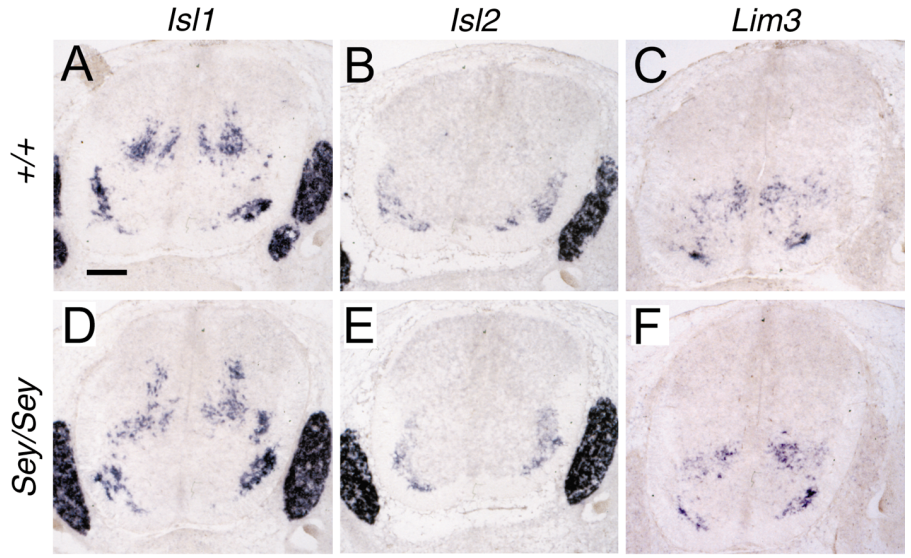

FIG. 7. Expression of LIM-domain transcription factors in E13.5 wild-type and *Sey/Sey* spinal cords. Transverse sections through the cervical regions of E13.5 cords were hybridized *in situ* with probes for *Isl1*, *Isl2*, and *Lim3*. At this age, there appear to be no significant differences between wild-type and *Sey/Sey*. Scale bar, 200 μm.

have also been identified in cell cultures established from developing cerebral cortex (Williams *et al.*, 1991; Davis and Temple, 1994; Qian *et al.*, 1997). Moreover, common neuron–glial precursors are a well-established feature of invertebrate nervous systems (e.g., Udolph *et al.*, 1993).

In *Sey/Sey* mice, which lack functional Pax6 protein, the position of the first *PDGFRα*$^+$ OLPs to be specified in the VZ was shifted dorsally relative to that in wild-type. This is in keeping with the idea that Pax6 interprets positional information relayed by Shh (Goulding *et al.*, 1993; Ericson *et al.*, 1997). Shh normally represses *Pax6* in the vicinity of the floor plate and allows other factors such as Nkx2.2, which are repressed by Pax6, to become established there. In the absence of Pax6, the Nkx2.2 expression domain can expand dorsally and "ventralize" more of the VZ. OLPs normally are specified in Pax6 territory just on the border of the ventral domain defined by Nkx2.2; presumably the special conditions that exist there, which are conducive to OLP fate selection, are recreated further dorsal in the *Sey/Sey* spinal cord. Since development generally proceeds in a temporal wave from ventral to dorsal (Nornes and Das, 1974; Altman and Bayer, 1984), this might have a bearing on why specification of OLPs is delayed in *Sey/Sey* animals. A general dorsal shift in cell fates can also explain why certain classes of ventral neurons are absent in *Sey/Sey*—e.g., the *En1/Pax2*-expressing (V1) INs (Burrill *et al.*, 1997; Ericson *et al.*, 1997). Precursors in the most dorsal regions of the basal plate, which normally generate V1 INs, might be respecified to generate more ventral cells (e.g., V2 INs), while precursors more dorsal than that (i.e., within the alar plate) might be unable to generate V1 INs because of the overriding influence of dorsal gene products such as Pax7. This "dorsal shunt" model is illustrated in Fig. 9.

We could not directly test whether neuronal precursors are shunted dorsally in *Sey/Sey* spinal cords because the available markers of these cells are not fully expressed until after the cells leave the VZ. However, our data on OLPs suggested that a dorsal shift might also be accompanied by a developmental delay. To test this, we repeated and extended previous studies of neuronogenesis in *Sey/Sey* spinal cord by following the expression of *Isl1*, *Isl2*, and *Lim3* from earlier to later ages than before. We found a delay of up a day in the onset of expression of *Isl2* and *Lim3*. *Isl2* is a specific marker of MMC–MNs in the cervical spinal cord (Tsuchida *et al.*, 1994), so we can definitely conclude that development of this class of somatic MNs is delayed in *Sey/Sey*. The fact that *Lim3* is also delayed is consistent with this conclusion and also suggests that *Lim3/Chx10* (V2) INs might be delayed as well. A delay in production of V2 INs was already suggested by previous studies; Ericson *et al.* (1997) found reduced numbers of *Chx10* INs in *Sey* homozygotes at E11, yet Burrill *et al.* (1997) found essentially

normal numbers of these cells at E12. Therefore, it appears that development of ventral neurons, like OLPs, is retarded but not blocked by the loss of Pax6.

MATERIALS AND METHODS

Mouse Mutants

Sey mice were propagated by timed heterozygous matings. The pregnant females were killed by cervical dislocation. The embryos were genotyped by Southern blotting and PCR analysis of tail-tip DNA, as described previously (Hill *et al.*, 1991). *Sey/Sey* and *Sey/+* embryos older than E12.5 could be identified by eye abnormalities, but genotypes were confirmed by DNA analysis. Mating was assumed to have occurred at midnight, and noon the following day was designated E0.5. Developmental stage was confirmed and refined by morphology (Theiler, 1972).

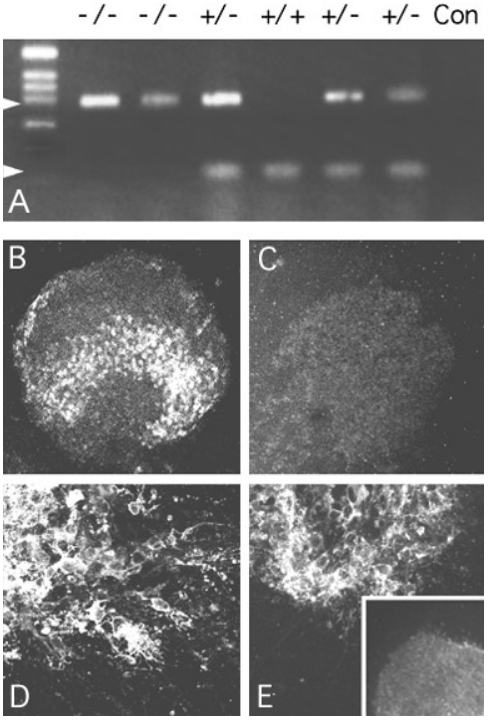

FIG. 8. Oligodendrocytes develop normally in explant cultures of *Isl1*(−/−) spinal cords. Embryonic (E9.5) offspring of heterozygous *Isl1*(−/+) parents were genotyped by PCR as described under Materials and Methods (A). Segments of spinal cord from *Isl1*(−/−), *Isl1*(−/+), and wild-type littermates were cultured in collagen gels on a rocking platform. Some explants were fixed after 2 days and immunolabeled with anti-*Isl1/2* monoclonal antibodies. *Isl1/2*-positive neurons developed in wild-type and *Isl1*(−/+) explants (B) but not in *Isl1*(−/−) explants (C). A majority of explants were cultured for 11 days (until the equivalent of the day of birth) and immunolabeled with monoclonal anti-GC. GC-positive OLs developed in all explants, whether from wild-type (not shown), *Isl1*(−/+), or *Isl1*(−/−) cords (D and E, respectively). The inset in E shows a control immunolabeling in which the primary anti-GC antibody was omitted.

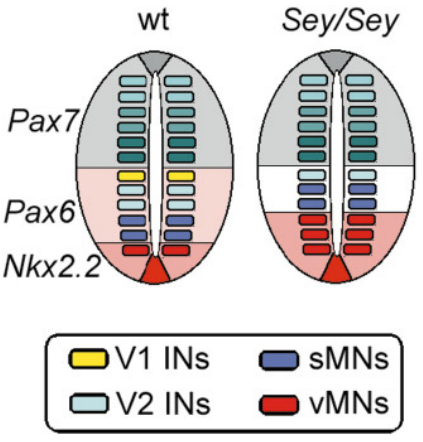

FIG. 9. A "dorsal shunt" model of ventral cell fates in the *Sey/Sey* spinal cord. If the dorsal shift of OLP specification described in the present paper is representative of ventral cell fates generally, one would expect that V1 INs, which descend from precursors (yellow) in the most dorsal part of the basal plate, might be lost in *Sey/Sey* because their precursors are respecified to generate more ventral cell types such as V2 INs (light blue). Precursors in the ventral part of the alar plate (grey/green) might be unable to generate replacement V1 INs because of the overriding dorsalizing influence of Pax7 and other dorsal gene products. Since there is normally a temporal gradient of development ventral-to-dorsal, one might expect a dorsal shift to be accompanied by a delay in development of other cell types from the *Pax6*-positive part of the basal plate—i.e., V2 INs and somatic MNs (dark blue). The diagram also depicts the dorsal expansion of *Nkx2.2* expression and the concomitant increase in production of visceral MNs (vMNs; red) in *Sey/Sey*. This model predicts that sMNs should not be lost without also losing V2 INs. However, in the caudal hindbrain V2 INs seem to be present (though delayed) even though hypoglossal sMNs are lost (Eriscon *et al.*, 1997; Osumi *et al.*, 1997). Assuming that sMNs and OLs share common precursors, and bearing in mind that OLs appear (but are delayed) in the *Sey/Sey* brainstem, we suggest that MN–OL precursors are specified in *Sey/Sey* but are unable to differentiate overtly as hypoglossal MNs because the precursors are both delayed and displaced from their normal position and so fail to receive some necessary environmental signal(s).

Isl1(−/−) embryos were obtained from time-mated heterozygous crosses. Pregnant females were killed on E9.5 (homozygous null embryos die soon after E9.5; Pfaff *et al.*, 1996) and the embryos removed. Their heads were used for genotype analysis. Recombinant *Isl1* alleles were identified by PCR using internal *Neo* primers, and wild-type alleles were identified with internal second-LIM-domain-exon primers as previously described (Pfaff *et al.*, 1996). In some experiments the genotypes were confirmed by immunolabeling 48-h

explant cultures of embryonic spinal cord with anti-Isl1/2 antibody 4D5 (see below).

In Situ Hybridization

Embryos were fixed by immersion in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 24 h at 4°C. Embryos aged E14.5 and older were decapitated and their skin was removed before immersion in fixative. After cryoprotecting in 0.5 M sucrose in PBS for 24 h at 4°C, embryos were immersed in OCT embedding compound (BDH), frozen on dry ice, and stored at −70°C until required for sectioning. Frozen sections were cut (10- to 15-μm nominal thickness) on a cryostat and collected on 3-aminopropyltriethoxysilane (APES)-coated glass microscope slides. Sections were air dried for 1–2 h at room temperature and stored at −70°C in a dry box. Digoxygenin (DIG)-labeled antisense RNA probes for *PDGFRα*, *Pax6*, *Nkx2.2*, *Isl1*, *Isl2*, and *Lim3* were prepared by *in vitro* transcription with DIG RNA labeling mix according to the manufacturer's instructions. Hybrids were detected with alkaline phosphatase-conjugated anti-DIG IgG using NBT and BCIP as substrates (all reagents were from Boehringer Mannheim). Each batch of probe was titrated on control sections to discover the optimum dilution for *in situ* hybridization—typically 1:1000 or 1:500. The sections were dehydrated in increasing concentrations of ethanol (30, 60, 80, 95, and 100% for 1 min each) and mounted under coverslips in XAM (BDH).

Mapping PDGFRα⁺ Progenitors in the Dorsal–Ventral Axis

The site of origin of OLPs in the neuroepithelium was estimated from the positions of *PDGFRα*$^+$ cells within the VZ. For each cell, we dropped a perpendicular from the cell body to the ventricular surface, and measured the distance of the intercept from the ventral midline, following the contour of the central canal. This was expressed as a proportion of the half-perimeter of the canal, to minimize the effects of distortions resulting from variable preparation procedures such as fixation, and slight variations in the angle of section relative to the longitudinal axis.

Combined BrdU Immunolabeling and in Situ Hybridization

Pregnant female mice were injected intraperitoneally with 50 μg BrdU (10 mg/ml in PBS) per gram body weight. Animals were sacrificed 2 h after injection and embryos were fixed, sectioned, and subjected to *in situ* hybridization for *PDGFRα* (see above). The *in situ* hybridization reaction was stopped by washing in PBS for 10 min at room temperature. After brief fixation in 4% (w/v) paraformaldehyde in PBS, the sections were fixed in 70% (v/v) ethanol for 20 min at −20°C and then treated at room temperature with 1% (v/v) Triton X-100 for 20 min, 6 M HCl in 1% (v/v) Triton X-100 for 15 min, 0.1 M $Na_2B_4O_7$ (pH 8.5) for 10 min, and 50% normal goat serum in 1% Triton X-100 for 15 min. The sections were then incubated overnight at 4°C in anti-BrdU antibody (monoclonal BU209; Magaud *et al.*, 1989) followed by Texas Red-conjugated goat anti-mouse IgG. After a final postfixation in 4% paraformaldehyde in PBS for 5 min at room temperature, the sections were mounted in Citifluor (City University, London) for microscopy. *In situ* hybridization images were photographed under bright-field illumination and BrdU-labeling under fluorescence illumination. Both images were scanned and superimposed using Adobe Photoshop software.

Spinal Cord Explant Cultures

Embryos were placed in dispase (Boehringer; 1 mg/ml) in Dulbecco's modified Eagle's medium (Gibco-BRL) and the spinal cords were dissected free of surrounding tissues using tungsten needles. Individual spinal cords were further dissected into transverse segments approximately 0.1–0.2 mm in length and cultured in three-dimensional collagen gels as previously described (Tucker *et al.*, 1996) in Bottenstein and Sato (1979) medium with 0.25% fetal calf serum. Collagen gels containing explants were cultured in 6-cm-diameter plastic dishes with 4 ml of medium on a rocking platform (six cycles/minute) so the gels were exposed above the surface of the culture medium for a quarter of each cycle. Half the culture medium was exchanged every other day. Some gels were cultured for 48 h before the explants were removed and immunolabeled with anti-Isl1/2 antibodies to visualize MNs. The remainder was cultured for 11 days, until the equivalent of the day of birth, before staining with antibodies against the OL markers GC or MBP (see below).

Immunohistochemistry

Collagen gels containing spinal cord explants were removed and fixed in 4% (w/v) paraformaldehyde in PBS for 2 h at room temperature and then washed four times for 15 min with PBS. MNs were labeled with monoclonal antibody 4D5 (a gift from T. Jessell) that recognizes both Isl1 and Isl2. The hybridoma superna-

tant was diluted 1:100 in PBS containing 0.1% (v/v) Triton X-100. OLs were labeled with monoclonal anti-GC supernatant (undiluted) or with anti-MBP rabbit serum (a gift from D. Colman, Mount Sinai School of Medicine, New York), diluted 1:1000 in PBS containing 0.1% (v/v) Triton X-100. All primary antibody incubations were overnight at 4°C followed by 4 × 15-min washes with PBS. Secondary antibodies were rhodamine- or fluorescein-conjugated goat anti-rabbit or goat anti-mouse immunoglobulins (all from Pierce). Incubation was for 3 h at room temperature followed by 4 × 15-min washes in PBS before mounting in glycerol for fluorescence microscopy.

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REFERENCES

Altman, J., and Bayer, S. A. (1984). The development of the rat spinal cord. *Adv. Anat. Embryol. Cell Biol.* 85: 1–166.

Bottenstein, J. E., and Sato, G. H. (1979). Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc. Natl. Acad. Sci. USA* 76: 514–517.

Burrill, J. D., Moran, L., Goulding, M. D., and Saueressig, H. (1997). PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development. *Development* 124: 4493–4503.

Calver, A. R., Hall, A. C., Yu, W.-P., Walsh, F. S., Heath, J. K., Betsholtz, C., and Richardson, W. D. (1998). Oligodendrocyte population dynamics and the role of PDGF in vivo. *Neuron* 20: 869–882.

Davis, A. A., and Temple, S. (1994). A self-renewing multipotential stem cell in embryonic rat cerebral cortex. *Nature* 372: 263–266.

Ensini, M., Tsuchida, T. N., Belting, H. G., and Jessell, T. M. (1998). The control of rostrocaudal pattern in the developing spinal cord: Specification of motor neuron subtype identity is initiated by signals from paraxial mesoderm. *Development* 125: 969–982.

Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T. M., and Briscoe, J. (1997). Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* 90: 169–180.

Fan, C. M., Kuwana, E., Bulfone, A., Fletcher, C. F., Copeland, N. G., Jenkins, N. A., Crews, S., Martinez, S., Puelles, L., Rubenstein, L. R., and Tessier-Lavigne, M. (1996). Expression patterns of two murine homologs of *Drosophila* single-minded suggest possible roles in embryonic patterning and in the pathogenesis of Down's syndrome. *Mol. Cell. Neurosci.* 7: 519.

Goulding, M. D., Lumsden, A., and Gruss, P. (1993). Signals from the notochord and floor plate regulate the region-specific expression of two Pax genes in the developing spinal cord. *Development* 117: 1001–1016.

Gruss, P., and Walther, C. (1992). Pax in development. *Cell* 69: 719–722.

Hardy, R. J. (1997). Dorsoventral patterning and oligodendroglial specification in the developing central nervous system. *J. Neurosci.* 50: 139–145.

Hill, R. E., Hogan, B. L., Ton, C. C., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D., and van Heyningen, V. (1991). Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* 354: 522–525. [Erratum in *Nature* 355: 750. (1992)].

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.

Li, H., Witte, D. P., Branford, W. W., Aronow, B. J., Weinstein, M., Kaur, S., Wert, S., Singh, G., Schreiner, C. M., Whitsett, J. A., Scott, W. J., Jr., and Potter, S. (1994). *Gsh-4* encodes a LIM-type homeodomain, is expressed in the developing central nervous system and is required for early postnatal survival. *EMBO J.* 13: 2876–2885.

Magaud, J. P., Sargent, I., Clarke, P. J., Ffrench, M., Rimokh, R., and Mason, D. Y. (1989). Double immunocytochemistry labeling of cell and tissue samples with monoclonal anti-bromodeoxyuridine. *J. Histochem. Cytochem.* 37: 1517–1527.

Matise, M. P., and Joyner, A. L. (1997). Expression patterns of developmental control genes in normal and Engrailed-1 mutant mouse spinal cord reveal early diversity in developing interneurons. *J. Neurosci.* 17: 7805–7816.

Matsuo, T., Osumi-Yamashita, N., Noji, S., Ohuchi, H., Kowama, E., Myokai, F., Matsuo, N., Taniguchi, S., Doi, H., and Iseki, S. (1993). A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. *Nat. Genet.* 4: 299–304.

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

Muhr, J., Jessell, T. M., and Edlund, T. (1997). Assignment of early caudal identity to neural plate cells by a signal from caudal paraxial mesoderm. *Neuron* 19: 487–502.

Nishiyama, A., Lin, X.-H., Giese, N., Heldin, C.-H., and Stallcup, W. B. (1996). Co-localization of NG2 proteoglycan and PDGF α 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 neurogenesis in spinal cord of rat. I. An autoradiographic study—Time and sites of origin and migration and settling patterns of neuroblasts. *Brain Res.* 73: 121–138.

Ono, K., Bansal, R., Payne, J., Rutishauser, U., and Miller, R. H. (1995). Early development and dispersal of oligodendrocyte precursors in the embryonic chick spinal cord. *Development* 121: 1743–1754.

Osumi, N., Hirota, A., Ohuchi, H., Nakafuku, M., Iimura, T., Kuratani, S., Fujiwara, M., Noji, S., and Eto, K. (1997). Pax-6 is involved in the specification of hindbrain motor neuron subtype. *Development* 124: 2961–2972.

Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T., and Jessell, T. M. (1996). Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* 84: 309–320.

Poncet, C., Soula, C., Trousse, F., Kan, P., Hirsinger, E., Pourquié, O., Duprat, A.-M., and Cochard, P. (1996). Induction of oligodendrocyte

precursors in the trunk neural tube by ventralizing signals: Effects of notochord and floor plate grafts, and of sonic hedgehog. *Mech. Dev.* 60: 13–32.

Price, M., Lazzaro, D., Pohl, T., Mattei, M. G., Ruther, U., Olivo, J. C., Duboule, D., and Di Lauro, R. (1992). Regional expression of the homeobox gene Nkx-2.2 in the developing mammalian forebrain. *Neuron* 8: 241–255.

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.

Pringle, N. P., Yu, W.-P., Guthrie, S., Roelink, H., Lumsden, A., Peterson, A. C., and Richardson, W. D. (1996). Determination of neuroepithelial cell fate: Induction of the oligodendrocyte lineage by ventral midline cells and Sonic hedgehog. *Dev. Biol.* 177: 30–42.

Pringle, N. P., Guthrie, S., Lumsden, A., and Richardson, W. D. (1998). Dorsal spinal cord neuroepithelium generates astrocytes but not oligodendrocytes. *Neuron* 20: 883–893.

Qian, X., Davis, A. A., Goderie, S. K., and Temple, S. (1997). FGF2 concentration regulates the generation of neurons and glia from multipotent cortical stem cells. *Neuron* 18: 81–93.

Richardson, W. D., Pringle, N. P., Yu, W.-P., Collarini, E. J., and Hall, A. C. (1995). Origins and early development of oligodendrocytes. In *Glial Cell Development* (K. R. Jessen and W. D. Richardson, Eds.), pp. 53–70. Oxford Univ. Press, Oxford., New York.

Richardson, W. D., Pringle, N. P., Yu, W.-P., and Hall, A. C. (1997). Origins of spinal cord oligodendrocytes: Possible developmental and evolutionary relationships with motor neurons. *Dev. Neurosci* 19: 54–64.

Seidah, N. G., Barale, J. C., Marcinkiewicz, M., Mattei, M. G., Day, R., and Chretien, M. (1994). The mouse homeoprotein mLIM-3 is expressed early in cells derived from the neuroepithelium and persists in adult pituitary. *DNA Cell Biol* 13: 1163–1180.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L., and Rubinstein, L. R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* 121: 3923–3933.

Stoykova, A., Fritsch, R., Walther, C., and Gruss, P. (1996). Forebrain patterning defects in Small eye mutant mice. *Development* 122: 3453–3465.

Theiler, K. (1972). *The House Mouse.* Springer-Verlag, Berlin / Heidelberg / New York.

Timsit, S., Martinez, S., Allinquant, B., Peyron, F., Puelles, L., and Zalc, B. (1995). Oligodendrocytes originate in a restricted zone of the embryonic ventral neural tube defined by DM-20 mRNA expression. *J. Neurosci.* 15: 1012–1124.

Tsuchida, T., Ensisi, M., Morton, S. B., Baldassare, M., Edlund, T., Jessell, T. M., and Pfaff, S. L. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* 79: 957–970.

Tucker, A., Lumsden, A., and Guthrie, S. (1996). Cranial motor axons respond differently to the floor plate and sensory ganglia in collagen gel co-cultures. *Eur. J. Neurosci.* 8: 906–916.

Udolph, G., Prokop, A., Bossing, T., and Technau, G. M. (1993). A common precursor for glia and neurons in the embryonic CNS of *Drosophila* gives rise to segment-specific lineage variants. *Development* 118: 765–775.

Varela-Echavarrìa, A., Pfaff, S. L., and Guthrie, S. (1996). Differential expression of LIM homeobox genes among motor neuron subpopulations in the developing chick brain stem. *Mol. Cell Neurosci.* 8: 242–257.

Warren, N., and Price, D. J. (1997). Roles of Pax-6 in murine diencephalic development. *Development* 124: 1573–1582.

Williams, B. P., Read, J., and Price, J. (1991). The generation of neurons and oligodendrocytes from a common precursor cell. *Neuron* 7: 685–693.

Yu, W.-P., Collarini, E. J., Pringle, N. P., and Richardson, W. D. (1994). Embryonic expression of myelin genes: Evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube. *Neuron* 12: 1353–1362.

Zhadanov, A. B., Bertuzzi, S., Taira, M., Dawid, I. B., and Westphal, H. (1995). Expression pattern of the murine LIM class homeobox gene Lhx3 in subsets of neural and neuroendocrine tissues. *Dev. Dyn.* 202: 354–364.

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