

Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFR α signaling

Nathalie Spassky¹, Katharina Heydon¹, Arnaud Mangatal¹, Alexandar Jankovski², Christelle Olivier¹, Françoise Queraud-Lesaux¹, Cécile Goujet-Zalc¹, Jean Léon Thomas* and Bernard Zalc*[†]

¹Laboratoire de Biologie des Interactions Neuron/Glie, INSERM U-495, Université Pierre et Marie Curie, IFR des Neurosciences, Hôpital de la Salpêtrière, 75651 Paris Cedex 13, France

²Neuromorphologie: Développement Evolution, INSERM U-106, Université Pierre et Marie Curie, IFR des Neurosciences, Hôpital de la Salpêtrière, 75651 Paris Cedex 13, France

*These authors contributed equally to this work

[†]Author for correspondence (e-mail: berzalc @ccr.jussieu.fr)

Accepted 26 September 2001

SUMMARY

Most studies on the origin of oligodendrocyte lineage have been performed in the spinal cord. By contrast, molecular mechanisms that regulate the appearance of the oligodendroglial lineage in the brain have not yet attracted much attention. We provide evidence for three distinct sources of oligodendrocytes in the mouse telencephalon. In addition to two subpallial ventricular foci, the anterior entopeduncular area and the medial ganglionic eminence, the rostral telencephalon also gives rise to oligodendrocytes. We show that oligodendrocytes in the olfactory bulb are generated within the rostral pallium from ventricular progenitors characterized by the expression of *Plp*. We provide evidence that these *Plp* oligodendrocyte progenitors do not depend on signal transduction mediated by platelet-derived growth factor receptors (PDGFRs), and therefore propose that they belong to a different lineage than the PDGFR α -expressing

progenitors. Moreover, induction of oligodendrocytes in the telencephalon is dependent on sonic hedgehog signaling, as in the spinal cord. In all these telencephalic ventricular territories, oligodendrocyte progenitors were detected at about the same developmental stage as in the spinal cord. However, both *in vivo* and *in vitro*, the differentiation into O4-positive pre-oligodendrocytes was postponed by 4-5 days in the telencephalon in comparison with the spinal cord. This delay between determination and differentiation appears to be intrinsic to telencephalic oligodendrocytes, as it was not shortened by diffusible or cell-cell contact factors present in the spinal cord.

Key words: Anterior entopeduncular area, Medial ganglionic eminence, Myelin, Olfactory bulb, Olig1/2, Platelet-derived growth factor receptor α , Proteolipid protein, Mouse

INTRODUCTION

The developmental origin of oligodendrocytes, the myelin-forming cells in the central nervous system, has been mostly investigated in the spinal cord. This stems from the initial observation that during development, oligodendrocytes originate from the ventral half of the spinal cord, and the dorsal spinal cord is populated by oligodendrocyte precursors that migrate from ventral to dorsal (Warf et al., 1991). Subsequently, a series of markers have been proposed to identify oligodendrocyte progenitors in the ventricular layer of the ventral spinal cord. In the mouse or rat spinal cord, platelet-derived growth factor receptor α (PDGFR α) (Hall et al., 1996), or myelin genes expressed during embryonic development, such as 2',3'-cyclic nucleotide phosphodiesterase 1 (*Cnp1*) (Yu et al., 1994; Peyron et al., 1997), or proteolipid protein (*Plp*) (Timsit et al., 1995) have been shown to label a discrete

group of ventral ventricular cells appearing around E13-E14. The ventral location of these oligodendrocyte progenitors raised the question of whether development of these cells, like ventral neurons (Roelink et al., 1995; Ericson et al., 1997), depends on inducing signals from the notochord or floor plate. It was subsequently shown that signals from the notochord are indeed necessary and sufficient for the development of spinal cord oligodendrocytes (Trousse et al., 1995; Orentas and Miller, 1996; Pringle et al., 1996) and that sonic hedgehog protein (Shh) is an important component of the signal (Orentas et al., 1999). Recently, two closely related basic helix-loop-helix proteins, Olig1 and Olig2, have been identified and proposed as intracellular mediators of Shh-mediated oligodendrocyte specification (Lu et al., 2000; Zhou et al., 2000).

In the mouse brain, *Plp*⁺ cells are first detected at E9.5 in the basal plate of the diencephalon. In the telencephalon,

ventricular expression of *Plp*⁺ cells occurs at E10 in the alar plate of the anterior entopeduncular area (AEP) (Timsit et al., 1995; Spassky et al., 1998). Expression of *Plp* precedes that of *Pdgfra* and there is precocious segregation of these two cellular populations. In the chick, co-expression of these two transcripts at the cellular level is rarely observed, although *Plp*⁺ and *Pdgfra*⁺ cells are present in the same territories (Perez-Villegas et al., 1999). In the mouse, at E10.5, the patterns of expression of *Plp* and *Pdgfra* appear strikingly different. In the forebrain, *Pdgfra*-expressing cells are detected in territories such as the medial ganglionic eminence (MGE) or the dorsal thalamus, without significant expression of *Plp*. By contrast, *Pdgfra*⁺ cells are scarce in the strongly *Plp*-positive basal plate of prosomeres p1-p2 (Puelles and Rubenstein, 1993), or AEP (Spassky et al., 1998). In the E13.5 rat forebrain, *Pdgfra*⁺ cells are also expressed by ventricular cells in the AEP (Tekki-Kessaris et al., 2001). The cellular segregation of these two early markers of the oligodendrocyte lineage could relate to different stages of differentiation, or indicate the existence of two different oligodendrocyte lineages (Spassky et al., 2000; Richardson et al., 2000).

Using homotopic homochronic quail-chick chimeras, we have recently demonstrated that, in birds, oligodendrocyte progenitors that emerge from the alar AEP migrate tangentially to invade and colonize the entire telencephalon and that all telencephalic oligodendrocytes originate from the AEP. The prominent role of AEP in telencephalic oligodendrogenesis is conserved between birds and mammals as shown by heterospecific mouse-chick chimeras (Olivier et al., 2001). However, taking into account the much larger development of cortical structures in mammals, it is not certain to what extent the AEP contributes to the final oligodendroglial population of the forebrain in mammals, and the existence of additional sites of oligodendrogenesis in the mouse telencephalon cannot be excluded. To address this question, we focused on the olfactory bulb (OB), which is far away from the AEP. As expected from the caudal-to-rostral gradient of myelination, the OB appears to be one of the latest structures to myelinate. The first differentiated postmitotic oligodendrocytes are detected at P2-P3, and the first myelinated fibers at P7 (Monge et al., 1986).

We show that the mouse OB has an intrinsic oligodendroglial potential, and as in the spinal cord, appearance of oligodendrocytes in the caudal telencephalon (AEP, MGE) and rostral telencephalon (OB), is under the control of Shh. Oligodendrocyte progenitors in the OB are characterized by the expression of *Plp* and we provide evidence that these cells do not depend on signal transduction mediated by PDGF receptors, and therefore belong to a different lineage than those expressing PDGFR α .

MATERIALS AND METHODS

Animals

Wild-type outbred OF1 mice were obtained from IFFA-CREDO (Lyon, France), and homozygous transgenic animals mouse lines *plp-sh ble-lacZ* (Spassky et al., 1998), and *1.9mbp-lacZ* (Gow et al., 1992) were bred in our animal room. Heterozygous littermates (transgenic males crossed with non-transgenic OF1 females) were used in this study. The $\beta 2nZ3'1/1.9mbp-lacZ$ (F₁ × F₁) were obtained by crossing $\beta 2nZ3'1$ animals with *1.9mbp-lacZ* mice. In the $\beta 2nZ3'1$ transgenic mouse the *nlsLacZ* reporter is driven by the regulatory sequences of

the $\beta 2$ -microglobulin gene (Cohen-Tannoudji et al., 1992). The midpoint of the dark interval during which mating occurred was designated as day 0 and the embryos were considered to be E0.5 on the morning following fertilization. The average gestation period lasts 19.5-20.5 days. Newborn animals were considered as P1.

Transplantations

$\beta 2nZ3'1/1.9mbp-lacZ$ double transgenic mice pups aged P3-5 or *1.9mbp-lacZ* transgenic embryos aged E17.5 were anesthetized with chloral hydrate (400 mg/kg, ip) and rapidly perfused transcardially with a solution of 0.12 M phosphate buffer (pH 7.3) containing 0.6% glucose to wash out blood cells. Fragments from E17.5 OB or from P3-5 SVZ were transplanted in the SVZ of wild-type pups aged P3-P5 according to Jankovski and Sotelo (Jankovski and Sotelo, 1996). Mice transplanted were fixed after 3 weeks of survival and brains were treated for the histoenzymatic detection of β -galactosidase activity.

Explant cultures

Caudal, rostral and dorsal territories of the telencephalon were carefully dissected from E9.5 and E10.5 mice embryos in Earle's balanced salt solution without Ca²⁺ or Mg²⁺ (EBSS; Life Technologies-BRL). The forebrain was first separated along the midline. At E9.5 the anatomical points of reference used to define the caudal telencephalon were the tip of the lateral ventricle, rostrally, and the optic stalk, caudally. The rostral telencephalon was defined as the most rostral region of the embryo. To avoid contamination of rostral explants by caudal regions, the fragment of tissue comprising the most rostral part of the caudal explant and the most caudal part of the rostral explant was discarded. Similarly, a piece of telencephalic cortex was discarded between the rostral and dorsal explant. At E10.5, the caudal explant was divided into two fragments: the medial ganglionic eminence, rostrally, and the entopeduncular area, caudally. Explants were grown in collagen gels (3 mg/ml) or on laminin-coated (Sigma, St Louis, MO) 14 mm glass coverslips, in Bottenstein and Sato (BS) medium (Bottenstein and Sato, 1979) supplemented with 1% FCS and 1% penicillin-streptomycin (Seromed, Berlin, Germany). Function blocking anti-Shh antibody (mAb 5E1) (Ericson et al., 1996) (Developmental Studies Hybridoma Bank) was added at the time of plating (12.5 μ g/ml) and every 2-3 days.

STI571 treatment of dissociated cultures

Caudal and rostral territories of the telencephalon were dissected from E12.5 OF1 or *plp-sh ble-lacZ* mice and dissociated as previously described (Spassky et al., 1998). After dissociation, cells (5×10^4) were seeded into 96 wells poly-L-lysine and laminin-coated culture dishes and grown in BS medium supplemented with 1% FCS and 1% penicillin streptomycin. After 2 days, STI571 (50 to 500 nM) or PDGF-AA (10 ng/ml) was added. Culture medium was changed every 3 days, and at 13 days in vitro, cultures were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), before being immunostained with either O4 mAb (OF1) or a mixture of O4 mAb and anti- β -galactosidase Ab. Cultures were examined under a Leica DMIRB fluorescent inverted microscope.

Zeomycin treatment

OBs were carefully dissected from E16.5 *plp-sh ble-lacZ* transgenic embryos. Control cultures were maintained in BS medium in order to obtain permanent 2 day-conditioned medium (CM). Zeomycin (Zeocin, Cayla, Toulouse, France) was used at the final concentration of 75 μ g/ml. On the first day, then every other day, culture medium was changed by adding 250 μ l of CM and 250 μ l of fresh BS medium containing 150 μ g/ml of either zeomycin or BS medium alone.

Antibodies and immunolabeling

Mouse monoclonal O4 and O1 antibodies (IgM) (Sommer and Schachner, 1981) were diluted 1:5 and 1:30, respectively, in 10% fetal calf serum (Eurobio, Les Ulis, France) in DMEM. Mouse monoclonal

TuJ1 antibody (IgG2a) (Easter et al., 1993), a gift from A. Frankfurter (University of Virginia, Charlottesville, VA), was diluted 1:1000 in 0.2% gelatin, 0.2% Triton X-100 in PBS. Fluorescein- and rhodamine-conjugated goat antibodies against mouse IgM or IgG2a were from Southern Biotechnology (Birmingham, AL) and were diluted 1:200. Rabbit polyclonal antibody to β -galactosidase (ICM/Cappel) was diluted 1:200 in DMEM, 10% FCS and 0.1% Triton. The corresponding secondary antibodies used were either goat anti-rabbit Cy3-conjugated IgG (ImmunoResearch) or FITC-conjugated swine anti-rabbit IgG (DAKO), diluted 1:800 and 1:400, respectively. Immunoperoxidase staining on sections and immunolabeling of cultures was as described (Spassky et al., 1998).

In situ hybridization and detection of β -galactosidase enzymatic activity

Patterns of gene transcription were determined by in situ hybridization using digoxigenin (DIG)-labeled cRNA antisense probes (Boehringer Mannheim, Mannheim, Germany) transcribed from mouse *Pdgfra* (Pringle and Richardson, 1993), *Shh* (Shimamura et al., 1995), *Olig1* and *Olig2* (Lu et al., 2000) cDNAs. In situ hybridization was performed on cryostat sections according to the protocol of Strähle et al. (Strähle et al., 1994) as modified by Myat et al. (Myat et al., 1996). Detection of reporter β -galactosidase activity in *lacZ* transgenic animals was on freezing microtome cut sections (30 μ m) as detailed elsewhere (Spassky et al., 1998; Jankovski and Sotelo, 1996).

RT-PCR analysis

Messenger RNAs were extracted from freshly dissected explants or after 5 or 10 days in culture using Pharmacia Biotech Quick Prep Micro mRNA Purification Kit. mRNAs were used as a template for first-strand cDNA synthesis using random primers (Pharmacia Biotech, Uppsala, Sweden). M-MLV reverse transcriptase (Life Technologies, Gaithersburg, MD) was used for the extension, according to the manufacturer's protocol. Second-strand cDNA was synthesized during a single cycle of the PCR with a thermostable polymerase (from *Thermus aquaticus*) using oligonucleotide primers (Genset, Paris) specific for mouse *Shh* (Takabatake et al., 1997). The 5' primer (primer I, TCTGTGATGA-ACCAGTGGCC) contained the initiation codon (underlined), whereas the 3' primer (primer II, GCCACGGAGTTGTCTGCTTT) was complementary to the 3' end of the mouse *Shh*-coding region. The following parameters were used for the reaction: denaturation (45 seconds) at 94°C, annealing at 62°C (45 seconds), and elongation at 72°C (45 seconds) for 30 cycles. The PCR mixture was electrophoresed in a 2% agarose gel and stained with Ethidium Bromide.

RESULTS

The rostral SVZ does not contribute to OB oligodendroglial population

During postnatal development, neurons continue to be born in the subventricular zone (SVZ) of the lateral ventricles. These cells migrate as a network of chains through the SVZ and the rostral migratory stream (RMS) into the OB, where they differentiate into mature

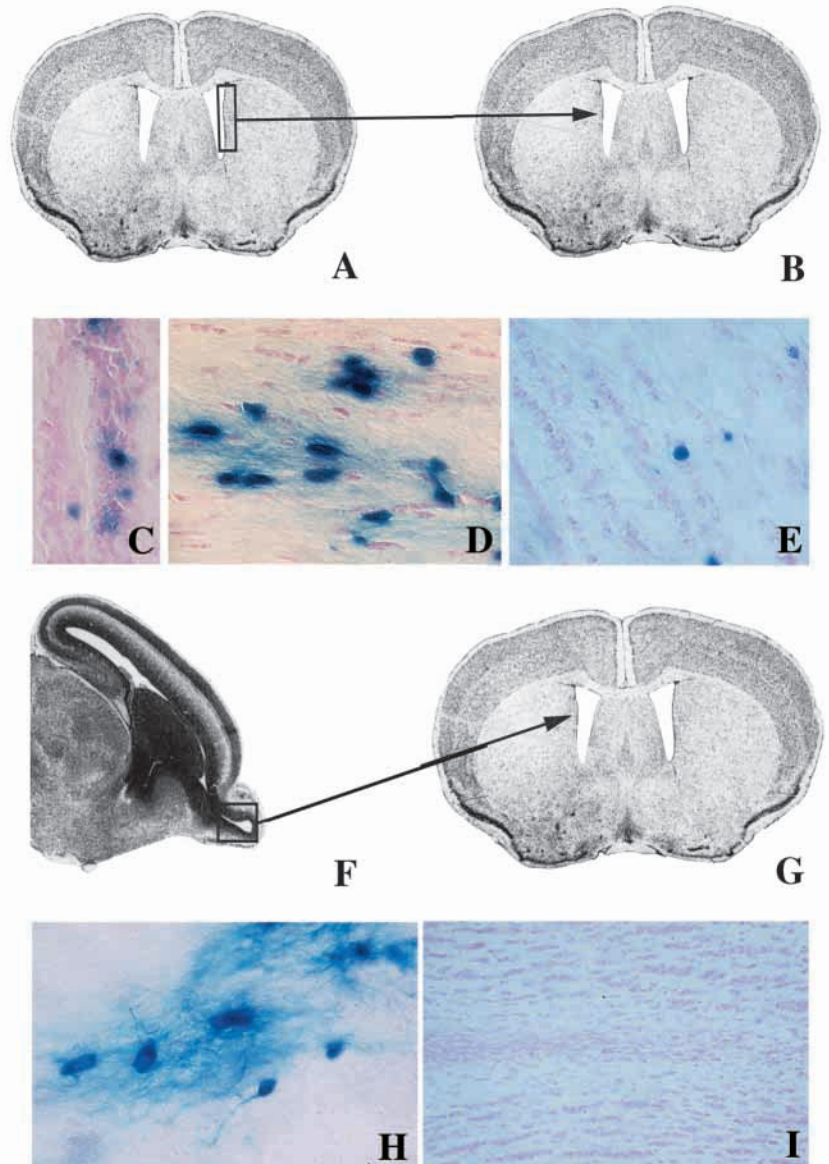


Fig. 1. Grafted postnatal SVZ of the lateral ventricles and embryonic olfactory bulb (OB) generate oligodendrocytes in the corpus callosum. (A-E) Fragments from the SVZ of the lateral ventricles of $\beta 2nZ3'1/1.9 mbp-lacZ$ bigenic mice were transplanted into the SVZ of wild-type mice, as schematized in A,B (A, donor; B, host). (C) Three weeks later, transplanted mice were analyzed after X-gal staining of frozen coronal sections. The presence of β -gal-expressing cells in the SVZ of a transplanted animal indicates the site of the graft. (D,E) Mbp^+ myelinating oligodendrocytes are detectable in the corpus callosum (D), whereas none is seen in the OB (E). In myelinating oligodendrocytes from the donor, the product of X-gal reaction stained the cell body and diffuses along the myelinated fibers. By contrast, $\beta 2nZ3'1^+$ interneurons, which are X-gal positive only in the nucleus, are numerous in the OB (E), illustrating that they have migrated from the graft into the OB. (F-I) Fragments from E17.5 OB from $1.9 mbp-lacZ$ transgenic embryos were transplanted into the SVZ of the lateral ventricles of P3-P5 wild type, as schematized in F,G (F, donor; G, host). Three weeks later, transplanted mice were analyzed after X-gal staining of frozen coronal sections of the corpus callosum (H) and the OB (I). Mbp^+ myelinating oligodendrocytes are seen in the corpus callosum but none is detected in the OB.

neurons (Lois and Alvarez-Buylla, 1994). To determine whether postnatal subventricular precursors could also

contribute to the oligodendrocyte population in the OB, we performed a series of homotopic and isochronic grafts.

Transplantation experiments were performed using, as a donor, either the *1.9 mbp-lacZ* transgenic (Gow et al., 1992) or a double transgenic that was generated by crossing *1.9 mbp-lacZ* with $\beta 2nZ3'1$ homozygous animals. We have previously shown that in *1.9 mbp-lacZ* transgenic animals, only myelinating oligodendrocytes express the transgene (Stankoff et al., 1996). In the $\beta 2nZ3'1$ transgenic, only the nuclei of a specific set of developing and mature interneurons are β -gal⁺ (Cohen-Tannoudji et al., 1992; Jankovski and Sotelo, 1996). In bigenic mice, neurons are thus easily distinguished from myelinating oligodendrocytes. Fragments from the SVZ of the lateral ventricles from P3-P5 donors were transplanted into the rostral SVZ of P3-P5 wild-type mice (Fig. 1A,B). Three weeks after transplantation, grafted β -gal-expressing cells were still observed in the SVZ of the recipient (Fig. 1C). β -gal⁺ myelinating oligodendrocytes were observed in the corpus callosum and the anterior commissure, in seven out of 12 grafted animals, regardless of whether *1.9 mbp-lacZ* transgenic or bigenic mice were used as donors (Fig. 1D). No myelinating oligodendrocytes were detected in either the RMS or the OB (Fig. 1E). By contrast, when the double transgenic was used as donor, numerous interneurons (cells that labeled with X-gal only in their nucleus) were present in the granular layer of the OB (Fig. 1E). These results suggest that the SVZ of lateral ventricles does not contribute to the oligodendroglial population of the OB.

Intrinsic potential of embryonic OB to generate oligodendrocytes

In vivo evidence

To investigate a possible intrinsic genesis of oligodendrocytes in the OB, we conducted a series of heterotopic and heterochronic transplantations. OBs from *1.9 mbp-lacZ* transgenic donors at E17.5 were transplanted into the SVZ of the lateral ventricle of P5 wild-type mice (Fig. 1F,G). Three weeks after transplantation, β -gal⁺ myelinating oligodendrocytes were detected in the corpus callosum of three out of six transplanted animals (Fig. 1H), suggesting the

presence of oligodendrocyte progenitors (or precursors) in the grafted tissue at the time of transplantation. However, no β -gal-expressing cells were detected in the OB of the recipient animals (Fig. 1I).

In vitro evidence

To further explore the intrinsic potential of the OB to generate oligodendrocytes, we turned to an in vitro approach. In the mouse embryo, the OB cannot be unambiguously dissected before E12.5. At this developmental stage, however, cells originating in the anterior entopeduncular area (AEP) or medial ganglionic eminence (MGE) have already started to migrate, and the caudorostral migration of *Plp*⁺ oligodendrocyte precursors from the AEP into the OB anlage cannot be excluded. To avoid this pitfall, experiments were conducted at E9.5 and E10.5, i.e. at embryonic stages when, in the AEP, the localization of *Plp*⁺ cells is strictly restricted to the ventricular

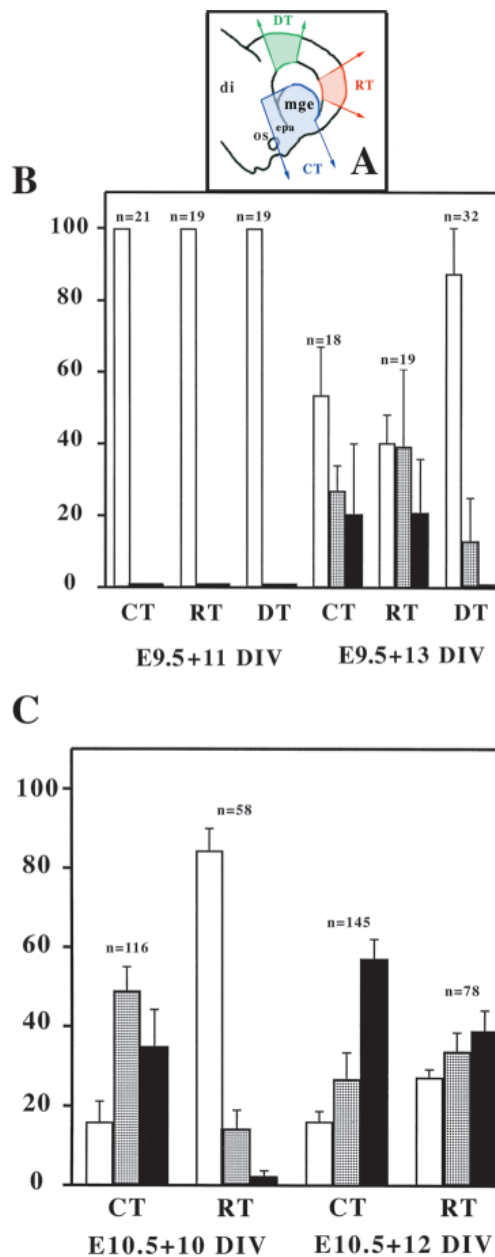
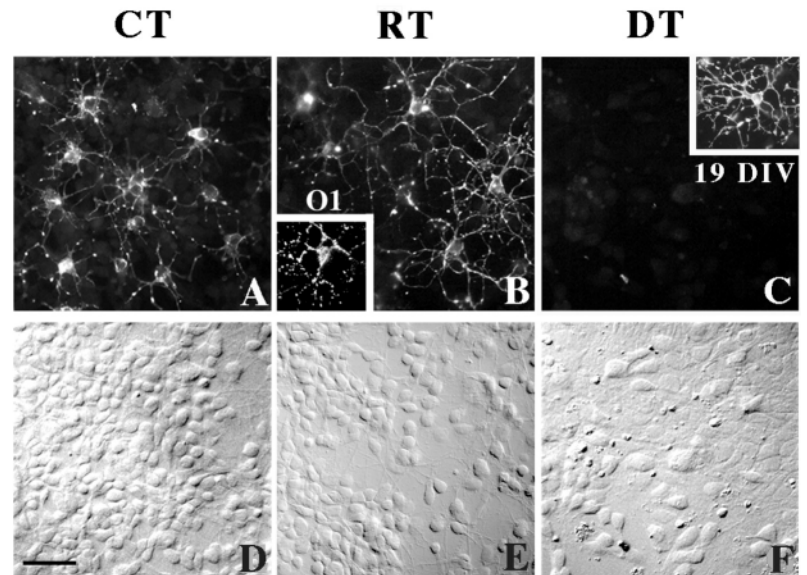


Fig. 2. The development of oligodendrocytes in E9.5- and E10.5-derived telencephalic explants is not restricted to caudal telencephalon. Telencephalon of embryos at E9.5 or E10.5 was carefully split into caudal (CT), rostral (RT) and dorsal (DT) territories. (A) The plane of dissection of an E9.5 embryo used to separate the caudal, rostral and dorsal telencephalon. Explants were cultivated for various periods of time before being analyzed after immunolabeling with O4 mAb. (B) At E9.5, none of the telencephalic explants contained O4⁺ cells after 11 days in vitro (equivalent to E20). After 13 days in vitro (equivalent to P2), 47% and 56% of caudal and rostral telencephalic explants, respectively, contained O4⁺ cells. By contrast, more than 85% of dorsal explants were negative for O4⁺ cells at 13 days in vitro. (C) At E10.5, both caudal and rostral territories were able to generate O4⁺ cells after 10 or 12 days in vitro (P2). The data represent the mean proportion of explants in each category \pm s.e.m. The number of explants analyzed in each category is indicated above the bars. White bars, fewer than 10 O4⁺ cells per explant; hatched bars, 10 to 100 O4⁺ cells per explant; black bars, more than 100 O4⁺ cells per explant. di, diencephalon; epa, entopeduncular area; mge, medial ganglionic eminence; os, optic stalk.

Fig. 3. Cells in caudal (A,D), rostral (B,E) and dorsal (C,F) E9.5-derived telencephalic explants stained with O4 mAb at 13 days in vitro. (A,B) Note that the morphology of O4⁺ cells in caudal and rostral explants is identical. Inset in B is a rostral culture stained at 15 DIV with O1 mAb, showing that cells generated in rostral explants are also O1⁺ and therefore differentiated oligodendrocytes. (C) The vast majority of explants derived from the dorsal telencephalon did not generate O4⁺ cells after 13 days in vitro. This inability was, however, only transient, and O4⁺ cells were observed at 19 days in vitro (inset in C). (A-C) O4 labeling; (D-F) corresponding interferential contrast images. Scale bar: 35 μ m; 50 μ m in the inset.



layer. The telencephalon was divided into three domains (Fig. 2A). The rostral telencephalon was considered as the presumptive territory of the OB (Rubenstein et al., 1998). The caudal telencephalon included two potential foci of oligodendrogenesis: the AEP, a territory with a high density of *Pdgfr α* -expressing cells, and the MGE, which expresses *Pdgfr α* from E10.5 onwards (Spassky et al., 1998). Finally, the dorsal telencephalon was assumed to be a territory that was likely to have little or no oligodendroglial potential. In a first series of experiments, explants were seeded at E9.5 and analyzed by immunolabeling with O4 mAb (a marker of pre-oligodendrocytes) at 11 or 13 days in vitro (equivalent to P1 or P3). After 11 days in vitro, no oligodendrocytes had been generated from any of the territories. By contrast, at 13 days in vitro, O4⁺ cells were observed in 45% and 60% of caudal and rostral explants, respectively (Fig. 2B, Fig. 3A,B,D,E). Similar experiments were then conducted with explants micro-dissected from E10.5 telencephalon. No O4⁺ cells were detected at 9 days in vitro, either in caudal or rostral territories (not shown). However, at 10 days in vitro (P1) O4⁺ cells were present in more than 80% of the caudal explants, and nearly half of them contained more than 100 O4⁺ cells per explant. For the rostral territory, 16% of explants gave rise to O4⁺ cells at 10 days in vitro. At 12 days in vitro, O4⁺ cells were observed in 84% and 71% of explants from caudal and rostral territories, respectively. To prove that O4⁺ cells arising from rostral explants were not possible contaminants from olfactory ensheathing cells (OECs), explants were also immunostained with O1 mAb. It has, indeed, been shown that OECs in culture are O4⁺, but not O1⁺ (Dickinson et al., 1997). At 15 days in vitro, the number of O1⁺ cells was similar in E10.5-derived caudal and rostral explants, and these O1⁺ cells had the typical multi-branched morphology of oligodendrocytes (inset in Fig. 3B). The presence of O1⁺ cells in these explants demonstrates that they belong to the oligodendrocyte lineage. Thus, explants from the rostral and caudal telencephalon of E9.5 or E10.5 mouse have a similar oligodendrogenic potential, which further suggests an intrinsic potential of the OB anlage to generate oligodendrocytes.

In comparison with caudal or rostral explants, at 13 days in vitro fewer than 15% of E9.5 dorsal explants gave rise to O4⁺ cells, and the number of O4⁺ cells per explant was always below 100 (Fig. 2C, Fig. 3C,F). It is of note that the number of O4⁺ cells generated from E9.5 dorsal explants at 13 days in vitro (P3) was very similar to that of E10.5 rostral explants at 10 days in vitro (P1), suggesting a delayed appearance of oligodendrocytes in dorsal explants. Indeed, the apparent

limited potential of dorsal explants to generate oligodendrocytes was only transient, and in dorsal explants analyzed at 19 days in vitro, O4⁺ cells, some of which had the typical 'sun-like' morphology of mature oligodendrocytes, were detected in all the cases analyzed (inset in Fig. 3C).

Spinal cord environment does not alter the delayed appearance of telencephalic oligodendrocytes

In explants derived from E9.5 rostral or caudal telencephalon, oligodendrocyte differentiation occurred after 13 days in vitro, whereas explants isolated from E9.5 spinal cord gave rise to oligodendrocytes after 9 days in vitro. We therefore examined whether soluble factors from spinal cord cultures could accelerate the onset of production of telencephalic oligodendrocytes. Conditioned medium from E9.5 spinal cord cultures was collected at 3 and 6 days in vitro, and added to telencephalic cultures immediately after plating and subsequently every 2-3 days. Neither the 3 days in vitro nor the 6 days in vitro spinal cord-conditioned medium affected the timing of oligodendrocyte differentiation in telencephalic cultures (Table 1). To determine whether direct cell-cell contact could affect the timing of differentiation of telencephalic oligodendrocytes, we established mixed spinal cord-telencephalon cultures. To distinguish telencephalic and spinal cord oligodendrocytes, telencephalon from E9.5 *plp-sh ble-lacZ* transgenic mice (Spassky et al., 1998) were dissociated and mixed, in a 1 to 1 ratio, with cells dissociated from E9.5 wild-type spinal cord. While O4 cells were observed already at 9 days in vitro, no β -gal⁺/O4⁺ cells were detected before 13 days in vitro, whether or not PDGF-AA was added to the cultures (Table 1). Together, these data suggest that the timing of oligodendrocyte differentiation is driven by an intrinsic developmental clock (Barres et al., 1994; Temple and Raff, 1986), and compared with spinal cord oligodendrocytes, this clock is held up by 4 days in telencephalic oligodendrocytes.

Contribution of *Shh* to rostral telencephalic oligodendrogenesis

The previous experiments strongly suggest an intrinsic potential of the rostral telencephalon to generate

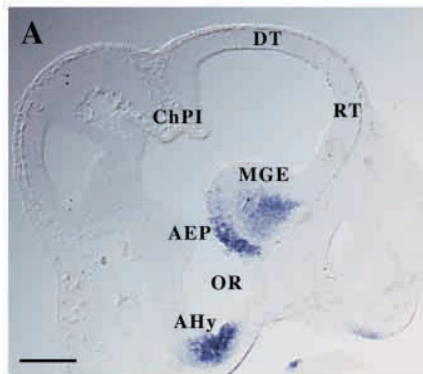
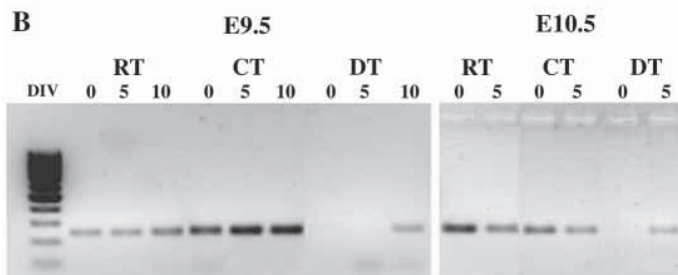


Fig. 4. Low levels of *Shh* are detected in the rostral telencephalon. (A) Sagittal cryosection of E10.5 mouse forebrain hybridized with *Shh* digoxigenin-labeled antisense cRNA probe. The most rostral signal for *Shh* is in the anterior entopeduncular area (AEP), caudal to the medial ganglionic eminence (MGE). No hybridization signal for *Shh* is detected in the rostral (RT) and dorsal (DT) telencephalon. AHy, anterior hypothalamus; ChPI, choroid plexus; OR, optic recess. (B) mRNA was extracted from rostral (RT), caudal (CT) and dorsal (DT) explants derived from E9.5 and E10.5 telencephalon at the time of isolation (lane 0) or after 5 (lane 5) and 10 (lane 10) days in vitro and subjected to RT-PCR with primers specific for *Shh*. In contrast to data obtained by in situ hybridization, a definite positive signal for *Shh* is amplified from E9.5- and E10.5-derived rostral territory explants at the time of isolation. In dorsal explants, *Shh* is detected only after 10 or 5 days in vitro for E9.5- or E10.5-derived explants, respectively. Scale bar: 220 μ m.



oligodendrocytes. To investigate whether *Shh* is required for oligodendrogenesis in the rostral telencephalon, we first examined the expression of *Shh* by in situ hybridization on brain tissue sections, and RT-PCR of mRNAs extracted from explants. As previously described (Shimamura et al., 1995), the most rostral limit of *Shh* expression detected by in situ hybridization at E9.5 and E10.5 corresponded to the AEP, and no signal for *Shh* was detected in the rostral telencephalon (Fig. 4A).

To determine whether *Shh* was expressed at E9.5 and E10.5 in the telencephalon at levels below the lower limit of detection of in situ hybridization, mRNAs extracted from different telencephalic territories were reverse transcribed and amplified. In the caudal and rostral telencephalon, *Shh* transcripts were detected at the time of isolation, as well as after 5 and 10 days in vitro (Fig. 4B). By contrast, *Shh* mRNAs were not detected in freshly isolated dorsal telencephalon explants. However, after 10 days in vitro or 5 days in vitro, a weak, but definite *Shh* signal was detected in explants isolated at E9.5 or E10.5, respectively, suggesting a molecular

reorganization of the dorsal explants in culture (Fig. 4B). This delayed expression of *Shh* might explain the delayed emergence of oligodendrocytes in dorsal, compared with caudal or rostral explants.

To directly address the question of the involvement of *Shh* in the induction of telencephalic oligodendrocytes, rostral, caudal and dorsal telencephalic explants isolated from E10.5 embryos, were treated with a function blocking anti-*Shh* antibody (5E1 mAb) for 12 (caudal and rostral) or 19 (dorsal) days. As illustrated for rostral explants (Fig. 5), in all territories examined, blocking *Shh* signaling strongly inhibited $O4^+$ cells appearance (Fig. 5A,B): no $O4^+$ cells were detected in explants derived from the AEP ($n=16$), the rostral telencephalon ($n=11$) or dorsal telencephalon ($n=7$), and fewer than 20 $O4^+$ cells were detected in only two out of 11 explants derived from the medial ganglionic eminence. Treatment of explants with 5E1 mAb also had a marked effect on the number of TuJ1⁺ neuronal cell bodies and neurites emerging from the cultures (Fig. 5C,D). The effect of anti-*Shh* antibody on the generation of neurons was, however, less drastic than on oligodendrocyte development, as TuJ1 cell bodies and neurites were observed in all the explants examined. Altogether, these results strongly suggest that, as previously demonstrated in the spinal cord, *Shh* is required for the induction of oligodendrocytes in the telencephalon.

Appearance of oligodendrocyte progenitor and precursor cells in the OB

To determine the timing of the appearance of oligodendrocyte progenitors and precursors in the OB, we analyzed the pattern of expression, between E14.5 and P2, of the earliest established markers of oligodendroglial lineage, i.e. *Olig1*

Table 1. Spinal cord environment does not accelerate the process of development of oligodendrocytes in the telencephalon

Type of culture	Timing of appearance of $O4^+$ cells		
	9 days in vitro	11 days in vitro	13 days in vitro
E9.5 spinal cord	+	++	++
E9.5 caudal telencephalon	-	-	+
E9.5 caudal telencephalon + spinal cord CM (3 days in vitro)	-	-	+
E9.5 caudal telencephalon + spinal cord CM (6 days in vitro)	-	-	+
E9.5 caudal telencephalon* + E9.5 spinal cord	-	-	+

E9.5 cells derived from either spinal cord or caudal telencephalon were cultivated for various periods of time before being immunostained with $O4$ mAb. -, no $O4^+$ cells; +, 10 to 100 $O4^+$ cells; ++, more than 100 $O4^+$ cells/explants. Culture medium from spinal cord explants (spinal cord CM) was collected after 3 days in vitro or 6 days in vitro and added to culture medium of caudal telencephalon (1/1, v/v).

*Cells were dissociated before being plated (2.5×10^4 cells from each territory) on poly-L-Lysine coated glass coverslips, and *plp-sh ble-lacZ* E9.5 mice were used as a source of caudal telencephalon cells. In addition to $O4$ immunolabeling, mixed dissociated cultures were treated with blue-gal to distinguish telencephalic (β -gal⁺/ $O4^+$) and spinal cord (β -gal⁻/ $O4^+$) oligodendrocytes. For each experimental condition, at least three cultures were analyzed in three separate experiments.

and *Olig2*, *Pdgfra* and *Plp*. Cellular expression of *Olig1*, *Olig2* and *Pdgfra* was examined by in situ hybridization. *Olig1* and *Olig2* had similar patterns of expression. At E14.5, *Olig1/2*-expressing cells were observed in the MGE, mostly in the ventricular layer, although scattered cells were also seen in the marginal layer, but no *Olig1/2*⁺ cells were detected in the OB (Fig. 6A). At E14.5, only a few *Pdgfra*⁺ cells were dispersed in the MGE and none was detected in the OB (Fig. 6D). In the OB, the first *Olig1/2* and *Pdgfra*⁺ cells were observed at E16.5 (Fig. 6B,E). These cells were rare in the ventricular zone, not exceeding two to three cells/section. At P3, cells expressing *Olig1/2* and *Pdgfra* had dramatically increased in number, as illustrated on coronal sections of the OB (Fig. 6C,F). *Pdgfra* and *Olig1/2* cells were abundant mainly in the marginal layer and virtually undetectable in the ventricular layer. To detect *Plp*-expressing cells, we used the *plp-sh ble-lacZ* mouse. In this line, we have previously shown that transgene-expressing cells differentiate into oligodendrocytes upon continuous expression of the transgene (Spassky et al., 1998). The first β -gal-expressing cells were detected in the ventricular layer of the OB at E14.5 (Fig. 6G). Most of these cells were unipolar with a cell body at a variable distance from the lumen of the ventricle, and a long process extending toward the lumen (Fig. 6H). At E17.5, the number of β -gal⁺ cells had dramatically increased. Transgene-expressing cells were detected both in the ventricular and subventricular layers, and most of these cells had a bipolar morphology (Fig. 6I). At P3, β -gal⁺ cells were less numerous and were detected in the ventricular, subventricular and marginal layer (Fig. 6J). The domain of expansion of β -gal⁺ cells in the marginal layer was smaller than for *Pdgfra* and *Olig1/2* (compare Fig. 6K with Fig. 6C,F). At P3, the morphology of β -gal⁺ cells was more complex, some being bipolar, others already extending several processes suggestive of pre-oligodendrocytes.

***Plp*-expressing cells in the OB are oligodendrocyte progenitors**

To determine whether the β -gal⁺/*Plp*⁺ cells are oligodendrocyte progenitors, two types of experiments were performed. First, we took advantage of the presence in the *plp-sh ble-lacZ* line of the *sh ble* gene, which confers resistance to the antibiotic zeomycin to *Plp*-expressing cells. Zeomycin (75 μ g/ml), killed 95 to 100% of control non-transgenic cultures within 10-12

Fig. 6. Developmental expression in the OB of oligodendrocyte lineage early markers. (A-F) Adjacent sagittal cryosections of E14.5 (A,D), E16.5 (B,E) and coronal cryosections of P3 (C,F) mouse OB were hybridized with either *Olig1/2* (A-C) or *Pdgfra* (D-F) DIG-antisense riboprobes. (G-K) Sagittal (G-J) and coronal (K) vibratome sections of E14.5 (G,H), E17.5 (I,J) or P3 (K) *plp-sh ble-lacZ* OBs were treated for histochemical detection of β -gal activity (blue-gal). Higher magnifications show that β -gal⁺ cells have a unipolar morphology at E14.5 (H), and are often bipolar at E17.5 (J). At all ages examined, β -gal⁺ cells are present in the ventricular layer. By contrast, only occasional *Olig1/2*- and *Pdgfra*-expressing cells are seen in the ventricular layer at E16.5, and none at E14.5 or P3. Scale bar: 260 μ m in A-G,I,K; 80 μ m in H; 20 μ m in J.

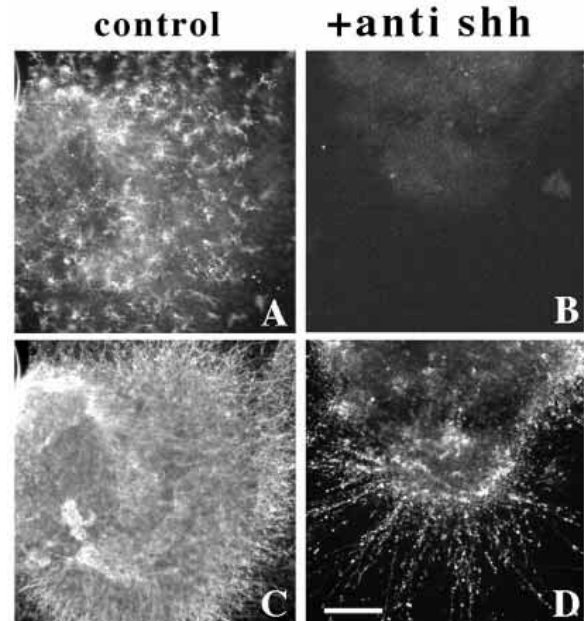


Fig. 5. Development of oligodendrocytes in rostral telencephalon depends on Sonic hedgehog signaling. Explants derived from E10.5 rostral telencephalon were analyzed by double immunostaining with O4 (A,B) and TuJ1 (C,D) mAbs at 12 days in vitro. In control experiments, a large number of O4⁺ (A) and TuJ1⁺ (C) cells developed. When similar explants were grown in the presence of a function blocking anti-Shh antibody for 12 days, no O4⁺ cells were detected (B); however, TuJ1⁺ cells still developed (D), although less efficiently than in control (C). Scale bar: 130 μ m.

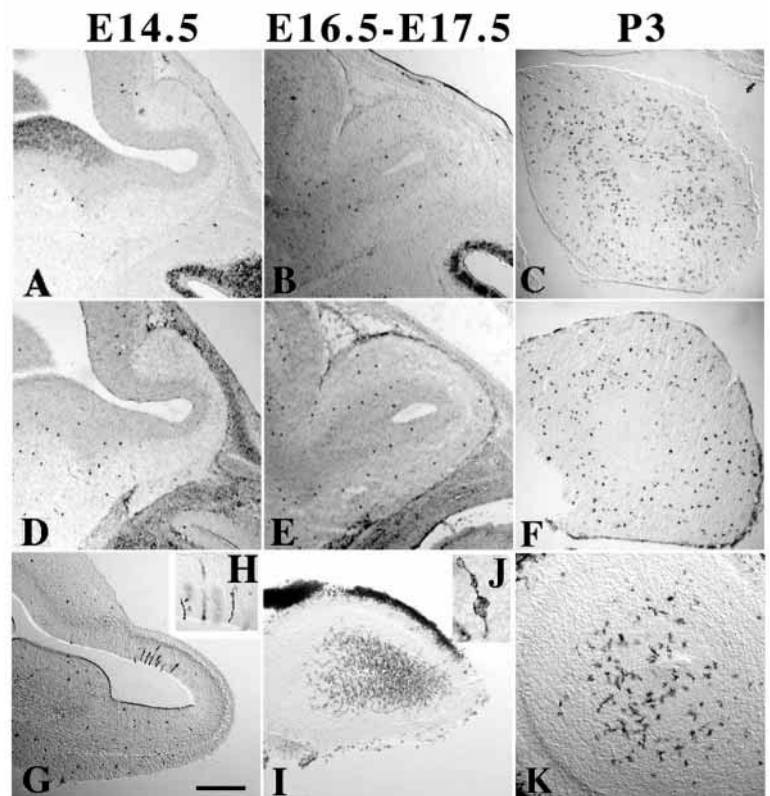
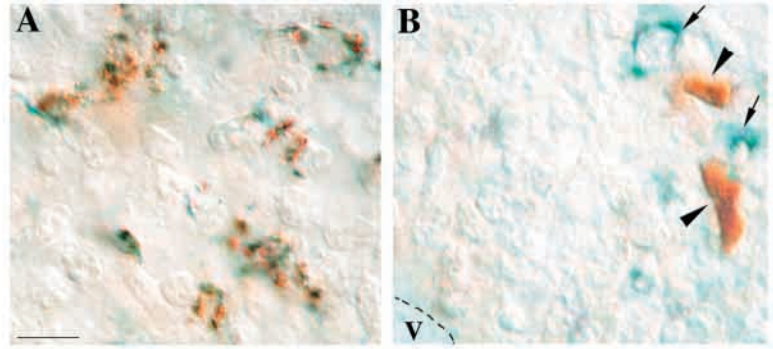


Fig. 7. *Plp*-expressing cells in the OB express the AN2/NG2 marker of oligodendrocyte precursors. Coronal cryosections of E17.5 *plp-sh ble-lacZ* OB were double labeled with blue-gal (A) or X-gal (B) and AN2 polyclonal antibody revealed with peroxidase-conjugated secondary antibody and DAB substrate. (A) Double-labeled β -gal⁺/AN2⁺ cells, some of which (in the upper right corner) have the typical bipolar morphology of oligodendrocyte precursor. (B) In the subventricular layer (the limit of the ventricle (V) is indicated by a broken line), β -gal⁺/AN2⁻ cells (arrows) are *Plp*⁺ cells, which are probably less advanced in the oligodendrocyte lineage than β -gal⁺/AN2⁺ cells. Note that AN2⁺ endothelial cells (with a brown precipitate diffusely distributed in the cell body, arrowheads) are β -gal negative. Scale bar: 13 μ m.



days, but *sh ble*-expressing cells survived for several weeks under these conditions. OBs from E16.5 *plp-sh ble-lacZ* animals were dissociated and cultivated in the presence of zeomycin. After 7 and 15 days in vitro the cell phenotype was analyzed by evaluating the expression of β -gal and immunolabeling with either O4 or O1 mAb. The percentage of X-gal-positive cells increased from 45.7% to 85.6% between 7 and 15 days in vitro. At 7 days in vitro, 95.7% of X-gal⁺ cells were O4⁺, and at 15 days in vitro, 94% of the X-gal⁺ cells were O1⁺. Thus, in the OB the *Plp*⁺ cells differentiate into O1⁺ oligodendrocytes upon continuous expression of the transgene.

Secondly, sections of OB from E17.5 *plp-sh ble-lacZ* mouse, were double labeled with blue-gal and AN2 polyclonal antibody. The AN2 antibody has been raised against a protein recently identified as the NG2 chondroitin-sulfate proteoglycan, an established marker of oligodendrocyte precursor cells (Nishiyama et al., 1996; Niehaus et al., 1999; Dawson et al., 2000). Double-labeled cells were observed in the mantle layer, confirming that the *Plp* cells observed in the OB are oligodendrocyte precursors (Fig. 7A). Not all β -gal⁺ cells were AN2⁺. The β -gal⁺/AN2⁻ cells were mostly observed in the ventricular and subventricular layer, suggesting they were less advanced in their differentiation process (Fig. 7B). AN2/NG2 is also expressed by endothelial cells forming the blood capillaries. None of the NG2⁺ endothelial cells was β -gal⁺ (Fig. 7B). Altogether, these results strongly suggest that *Plp*⁺ cells, first detected at E14.5 in the ventricular layer of the OB, are progenitors of oligodendrocytes.

***Plp*-expressing oligodendrocyte progenitors do not depend on PDGFR α signaling**

In the OB, *Plp*-expressing oligodendrocytes progenitors are detected at E14.5, while the first *Pdgfra*⁺ cells are observed only 2 days later. The delay in the chronology of expression of these two markers of oligodendrocyte progenitors could correspond to successive stages along the differentiation pathway of a single oligodendroglial lineage, or be indicative of the existence of two different lineages characterized by the expression of either *Plp* or *Pdgfra*. In the latter case, oligodendrocytes arising from *Plp*-expressing progenitors would not express PDGFR α and therefore be independent on PDGFR α signaling for their proliferation and survival. To determine whether development of *Plp*⁺ oligodendrocyte progenitors depends on PDGF, we investigated the consequence of blocking PDGFR α signaling on the

population of telencephalic oligodendroglial progenitors. Rostral and caudal telencephalon from E12.5 mice were dissociated and cultivated in BS medium supplemented with 1% FCS and in the presence of increasing concentrations of STI571, an established inhibitor of PDGF receptor tyrosine kinases (Buchdunger et al., 2000). After 13 days in vitro, the cultures were immunolabeled with O4 mAb. In cultures derived from rostral telencephalon, the number of O4 cells was not significantly different when cells were grown in the absence or the presence of STI571 (10–500 nM) (Fig. 8A). To complement the STI571 blocking experiments we also performed a ‘gain-of-function’ experiment by adding PDGF-AA (10 ng/ml) to the culture medium, instead of the inhibitor. Addition of PDGF-AA had no effect on the number of O4-expressing pre-oligodendrocytes (Fig. 8A). By contrast, in cultures derived from E12.5 caudal telencephalon and analyzed at 13 days in vitro, blocking PDGFR α signaling induced a marked decrease in the number of O4⁺ cells (Fig. 8B). This response of STI571 was dose dependent, with no effect at 50 nM, and a maximum 66% inhibition at 500 nM. The effect of PDGF-AA on oligodendrocytes in cultures derived from caudal telencephalon was further supported by the fact that addition of PDGF-AA induced a 32% increase in the number of O4⁺ cells (Fig. 8B). Even at 500 nM, however, STI571 did not completely deplete the O4 population in caudal telencephalon cultures. As this territory includes the AEP and the MGE, the cultures derived from the caudal telencephalon contained a mixture of *Plp* and *Pdgfra*-expressing progenitors. To determine whether the PDGF-AA-independent O4⁺ cells observed in these cultures in the presence of STI571 originated from *Plp*⁺ progenitors, caudal telencephalon cultures derived from E12.5 *plp-sh ble-lacZ* mice were treated with STI571 and analyzed at 12 days in vitro by double immunolabeling with O4 mAb and an anti- β -galactosidase antibody. As expected, the STI571-treated cultures showed a dose-dependent reduction in the number of O4⁺ cells, and a 1.6-fold increase in the presence of PDGF-AA (Fig. 8C). By contrast, the number of β -gal⁺ cells remained constant and was not significantly different from the control, regardless of whether the cultures were treated with STI571 or PDGF-AA (Fig. 8C). Altogether, these results demonstrate that development of *Plp*-expressing oligodendrocyte progenitors occurs independently of PDGFR signaling, and strongly suggest that these progenitor cells constitute an oligodendrocyte lineage that is different from the PDGFR α -expressing cells.

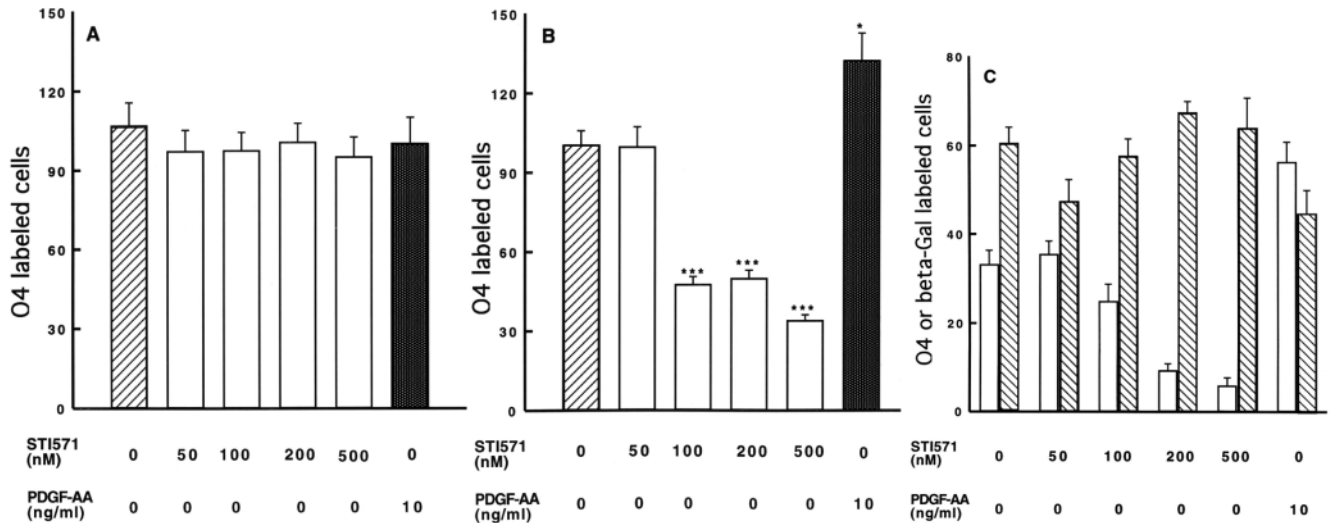


Fig. 8. Blocking PDGFR tyrosine kinase do not interfere with development of *Plp*⁺ progenitors cells. Rostral (A) or caudal (B,C) telencephalon from E12.5 OF1 wild-type (A,B), or *plp-sh ble-lacZ* transgenic (C) mouse were dissociated, seeded in 96 wells dishes and cultivated in BS medium supplemented with 1% FCS. After 2 days in vitro, either STI571 (50-500 nM) or PDGF-AA (10 ng/ml) was added. After 13 (A,B) or 12 (C) days in vitro, cultures were immunolabeled with O4 mAb alone (A,B), or O4 mAb and anti- β -galactosidase Ab (C), and immunopositive cells were counted. In C, the white columns represent the O4⁺ cells and the hatched columns the β -gal⁺ cells. Each column represents the mean \pm s.e.m. of three separate experiments representing 14 to 24 different cultures (A,B) or two separate experiments representing eight to 19 different cultures (C) (***) $P < 0.0001$; * $P < 0.05$, Student's *t*-test).

DISCUSSION

Intrinsic origin of oligodendrocytes in the OB

The aim of our study was to investigate the origin of oligodendrocytes in the OB. Our results strongly suggest that the OB has intrinsic oligodendrogenic potential. Four lines of evidence support this possibility. First, the presence of myelinating oligodendrocytes in the corpus callosum and anterior commissure of host animals transplanted with E17.5 OB shows that oligodendrocyte precursors were present in the territory transplanted at the time of the graft. However, these experiments were performed under heterotopic heterochronic conditions, and do not exclude the possibility that oligodendrocytes are induced by the environment in which the graft was placed. A more convincing demonstration would require transplantation of E9.5-E10.5 rostral telencephalon, the presumptive territory of OB, under homotopic homo-chronic conditions, which is not feasible in the mouse. Second, explants from E9.5 or E10.5 rostral telencephalon generate O4⁺ pre-oligodendrocytes and O1⁺ oligodendrocytes in culture. Explants were isolated at these early developmental stages to avoid a possible contamination by cells from a more caudal origin. It has been shown that rostral migration of cells originating in the MGE or the AEP does not start before E11.5 (Anderson et al., 2001). It is thus very likely that O4⁺ cells observed in the explant are generated by progenitors either already present in the rostral telencephalon at the time of the dissection, or specified in the explant, by oligodendroglial inductive signals present in the rostral telencephalon. Third, the zeomycin selection experiments performed on OB from *plp-sh ble-lacZ* embryos showing that more than 90% of the zeomycin-selected cells differentiate into O1⁺ oligodendrocytes, unambiguously demonstrate that at least part of the oligodendrocytes in the OB arise from the *Plp*-

expressing cells detected in the OB at E14.5. The last piece of evidence that the OB has intrinsic oligodendrogenic potential comes from our observations that there are cells in the OB neuroepithelium that express the NG2 proteoglycan, a marker of oligodendrocyte precursor cells elsewhere in the CNS. In the *plp-sh ble-lacZ* transgenic mouse these NG2⁺ cells are β -gal⁺, providing additional evidence that they are related to the oligodendrocyte lineage.

Consistent with previous work, the SVZ of the lateral ventricles does not contribute to the population of OB oligodendrocytes (Hu and Rutishauser, 1996). We cannot, however, eliminate the possibility that some of the oligodendrocytes in the OB originate from more caudal territories, like the AEP or the MGE. Oligodendrocyte progenitors intrinsic to the OB are most likely to be *Plp*⁺ cells, already present in the ventricular layer at E14.5. Cells expressing *Pdgfra* and *Olig1/2* appear in the OB only at E16.5, and, as discussed below, there is strong evidence that they belong to a different lineage from the *Plp*⁺ cells. In this respect, the *Pdgfra*⁺ cells, which originate in the MGE, are good candidates for a putative population of precursors invading the OB (Tekki-Kessarar et al., 2001).

Delayed differentiation of telencephalic oligodendrocytes

In the rostral telencephalon explants, oligodendrocytes are generated approximately on schedule compared with the in vivo appearance of the first O4 cells in the OB. It is recognized that cells in the oligodendrocyte lineage follow, in vitro, the in vivo developmental timing (Raff, 1989). This appears to be an intrinsic property of oligodendrocyte precursors dictated by their site of origin. In E9.5-derived spinal cord explants, the first O4⁺ cells were detected at 9 days in vitro. This is in agreement with the study by Sussman et al. (Sussman et al.,

2000), who reported that more than 60% of ventral spinal cord explants contain O4⁺ cells after 7 or 5 days in vitro, when derived from E11 or E13 embryos, respectively (equivalent to E18). By contrast, in E9.5 or E10.5 telencephalic explants (whether caudal or rostral), O4⁺ cells are detected after 13 or 11 days in vitro, which is in agreement with a previous report that, in E12.5-derived dissociated telencephalic cultures, O4⁺ cells are first detected after 9 days in vitro (Spassky et al., 1998). As birth in the mouse occurs around E20.5 (P1), this number of days in vitro would correspond with P2-P3 in vivo. Thus emergence of O4⁺ pre-oligodendrocytes is delayed by 4 days in the telencephalon compared with the spinal cord, and this corresponds to the timing of detection of these cells in vivo. The process of differentiation of telencephalic O4⁺ cells was not accelerated by either adding spinal cord conditioned medium, or mixing them with spinal cord cells, thus eliminating the possibility of diffusible or cell-cell contact mediated cues. Because, at E8.5, Shh is already expressed all along the rostrocaudal axis of the neural tube, it is unlikely that this delay reflects earlier priming by Shh of neural stem cells in the spinal cord compared with the brain. Therefore, the difference in the time of appearance of telencephalic versus spinal cord oligodendrocytes is more probably related to cell-intrinsic differences between these two populations (Spassky et al., 2000).

Sonic hedgehog dependence of telencephalic oligodendrocytes

In the spinal cord, there is compelling evidence for the involvement of Shh in the induction of the oligodendrocyte lineage and this process appears to be conserved from birds to humans (Orentas and Miller, 1996; Pringle et al., 1996; Poncet et al., 1996; Hajihosseini et al., 1996; Orentas et al., 1999). Shh also induces motoneurons and interneurons, and these are generated before oligodendrocytes (Roelink et al., 1995; Ericson et al., 1997). It seems unlikely that oligodendrocyte development requires signals from motoneurons. In the chick spinal cord, it has been shown that induction of oligodendrocytes is independent from motoneuron signaling (Soula et al., 2001). In the mouse, oligodendrocyte progenitors develop normally in *Isl1*^{-/-} mutant embryos, which lack motoneurons (Pfaff et al., 1996; Sun et al., 1998). However, it remains possible that signals from motoneuron progenitors, which do not express *Isl1* and are not eliminated in the null mutant, might be involved in oligodendrocyte lineage specification.

There is, as yet, little evidence that the same molecular mechanisms mediate early development of oligodendrocyte progenitors in the forebrain. One potential source of oligodendrocytes in the diencephalon is the zona limitans intrathalamica (ZLI), the P2/P3 interprosomal boundary, which has been shown to be a rich source of *Plp*- and *Olig1/2*-expressing cells by E10.5-E12.5 (Timsit et al., 1995; Lu et al., 2000). Consistent with a role for Shh in the induction of oligodendrocyte lineage in the brain, *Shh* is expressed in the ZLI, and in embryos homozygous for null alleles of *Shh*, *Olig1/2* transcripts are no longer detected in the ZLI (Lu et al., 2000). Based on the distribution of both *Plp* and *Pdgfra*, we have previously proposed that, in the chick, the AEP is the major source of telencephalic oligodendrocytes (Perez-Villegas et al., 1999; Olivier et al., 2001). In the mouse

telencephalon, by contrast, *Pdgfra*⁺ cells are not found in the AEP, but in the adjacent MGE (Spassky et al., 1998), suggesting that telencephalic oligodendrocytes might be generated from both the AEP and the MGE. In both the chick and the mouse, the AEP is the most rostral domain of expression of *Shh* (Fig. 6) (Shimamura et al., 1995). Our data are in agreement with the recent report showing that in the mouse telencephalon expression of the early oligodendrocyte markers *Olig2*, *Plp* and *Pdgfra* corresponds to regions of *Shh* expression (Nery et al., 2001). The finding that blocking of *Shh* inhibits the development of O4⁺ cells from E9.5-derived AEP and MGE explants (referred to as caudal telencephalon), suggests that a common *Shh*-dependent mechanism governs the genesis of oligodendrocytes in the caudal telencephalon and spinal cord. We find that although E9.5 mouse dorsal telencephalon explants do not give rise to oligodendrocytes in short-term cultures (11-13 days in vitro), in long term cultures (19 days in vitro), O4⁺ cells did develop in these explants. A similar observation has been reported for E11 mouse dorsal spinal cord explants, which also generate oligodendrocyte lineage cells after a long delay (Sussman et al., 2000). Consistent with our findings in dorsal telencephalon explants, Sussman et al. (Sussman et al., 2000) have also reported delayed expression of *Shh* in their dorsal spinal cord explants. This delay might reflect molecular reorganization of dorsal territories in vitro, as *Shh* is not normally present during development either in dorsal spinal cord or dorsal telencephalon. Whatever the mechanism, these results emphasize the role of *Shh* in the specification of oligodendrocyte lineage.

Evidence for two different oligodendrocyte lineages

Mainly on the basis of the striking differences in their spatiotemporal pattern of expression, we have previously proposed that *Pdgfra* and *Plp*-expressing cells in the germinative neuroepithelium belong to two different lineages of oligodendrocytes. However, we could not exclude the possibility of a single oligodendrocyte lineage (Spassky et al., 2000; Richardson et al., 2000).

Our experiments using STI571 provide strong evidence that the *Plp*⁺ progenitors belong to a different oligodendrocyte lineage than the PDGFR α -expressing cells. STI571 is a protein-tyrosine kinase inhibitor that has selectivity for the Abl and PDGF receptor tyrosine kinases (Druker et al., 1996; Buchdunger et al., 2000). It has recently been shown that STI571 is a potent inhibitor of Kit and PDGF-mediated signal transduction, but has no effect on closely related kinases such as Fms (Csf1r – Mouse Genome Informatics), Kdr, Flt1, Tek and Flt3 (Buchdunger et al., 2000). Based on its preclinical activity against Bcr-Abl, STI571 is currently in clinical trials for the therapy of chronic myelogenous leukemia (Druker et al., 2001). PDGF-AA has been described as a crucial factor for the proliferation and survival of oligodendrocyte precursor cells (Noble et al., 1988; Raff et al., 1988; Richardson et al., 1988). In the OB, if the *Pdgfra*⁺ cells observed at E16.5 represent a slightly later developmental stage than the *Plp*⁺ neuroepithelial cells already present at E14.5, the number of O4⁺ pre-oligodendrocytes observed after 13 days in culture would be dramatically decreased by the treatment with STI571 and increased by the addition of PDGF-AA. This is not the case: in cultures derived from the caudal telencephalon, the significant

decrease, or increase, in the number of O4⁺ cells after treatment with STI571 or PDGF-AA, respectively, clearly demonstrate the validity of our 'loss- or gain-of-function' experimental strategy. In addition, in cultures derived from caudal telencephalon of *plp-sh ble-lacZ* transgenic mice, treatment with either the inhibitor of PDGFR tyrosine kinase (or PDGF-AA) resulted in a partial depletion (or increase) of the total O4⁺ population, but did not affect the number of transgene-expressing cells, i.e. the *Plp*⁺ cells (Fig. 8C). Because in caudal telencephalon cultures there is a mixture of *Plp*⁺ and *Pdgfr*^α cells, this finding further supports the theory that *Plp*⁺ cells do not depend on PDGFR^α signaling. Defective oligodendrocyte development has been reported in PDGFA knockout mice (Fruttiger et al., 1999). In these animals, however, the reduction in the number of oligodendrocytes is territory-dependent: very severe in the spinal cord, cerebellum and optic nerve, but moderate or minor in the cerebral cortex or brainstem. Interestingly, this uneven depletion of the oligodendroglial population correlates well with the distribution of *Plp*-expressing progenitor cells in the ventricular layer of the developing brain (Spassky et al., 1998; Spassky et al., 2000). In particular, bearing in mind that oligodendrocytes in the cerebral cortex originate from the caudal telencephalon (Olivier et al., 2001; Nery et al., 2001; Tekki-Kessarar et al., 2001), it is worth noting that in STI571-treated caudal telencephalon cultures the 66% reduction in the number of oligodendrocytes induced by inhibition of PDGFR^α signaling (this study), is very similar to the 64% depletion in the oligodendroglial population induced by the loss of PDGFA, as reported in the cerebral cortex of PDGFA null mutant mice (Fruttiger et al., 1999).

Altogether these data demonstrate that *Plp*⁺ progenitors do not depend on PDGF-AA to develop and therefore support the idea of the existence of two different oligodendrocyte lineages. We cannot yet correlate this embryological difference with the situation in the adult. However, because, depending on their site of origin, oligodendrocyte precursors adopt different pattern of migration and invade very selective territories (Olivier et al., 2001), the finding that oligodendrocytes form an heterogeneous population may be of importance in the explanation of territorial differences in the susceptibility to demyelinating insults in diseases such as multiple sclerosis.

We thank Drs C. D. Stiles and D. H. Rowitch for the gift of the *Olig1* and *Olig2* probes and for fruitful discussions, Dr J. Trotter for the gift of AN2 Ab, Dr E. Buchdunger (Novartis Pharma AG, Switzerland) for the gift of STI571, and Dr Bill Richardson for helpful comments on the manuscript. N. S was a fellow of the Ministère de l'Enseignement Supérieur et de la Recherche and Fondation pour la Recherche Médicale. This study was supported by INSERM and by grants from the European Leucodystrophy Association, Association de Recherche sur la Sclérose En Plaques, and Agence Nationale de Recherche sur le SIDA to B. Z., from the European Commission (Contract QLG3-2000-01556) and Association de Recherche sur le Cancer to J. L. T.

REFERENCES

Anderson, S. A., Marin, O., Horn, C., Jennings, K., Rubenstein, J. L. (2001). Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development* **128**, 353-363.

Barres, B. A., Lazar, M. A. and Raff, M. C. (1994). A novel role for thyroid

hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* **120**, 1097-1108.

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.

Buchdunger, E., Cioffi, C. L., Law, N., Stover, D., Ohno-Jones, S., Drucker, B. J. and Lydon, N. B. (2000). Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-Kit and PDGF receptors. *J. Pharmacol. Exp. Ther.* **295**, 139-145.

Cohen-Tannoudji, M., Morello, D. and Babinet, C. (1992). Unexpected position-dependent expression of H-2 and beta 2-microglobulin/lacZ transgenes. *Mol. Reprod. Dev.* **33**, 149-159.

Dawson, M. R., Levine, J. M. and Reynolds, R. (2000). NG2-expressing cells in the central nervous system: Are they oligodendroglial progenitors? *J. Neurosci. Res.* **61**, 471-479.

Dickinson, P. J., Griffiths, I. R., Barrie, J. M., Kyriakides, E., Pollock, G. F. and Barnett, S. C. (1997). Expression of the dm-20 isoform of the *plp* gene in olfactory nerve ensheathing cells: evidence from developmental studies. *J. Neurocytol.* **26**, 181-189.

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J., Lydon, N. B. (1996). Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* **2**, 561-566.

Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., Sawyers, C. L. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *New Engl. J. Med.* **344**, 1031-1037.

Easter, S. S., Jr, Ross, L. S. and Frankfurter, A. (1993). Initial tract formation in the mouse brain. *J. Neurosci.* **13**, 285-299.

Ericson, J., Morton, S., Kawakami, A., Roelink, H. and Jessell, T. M. (1996). Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell* **87**, 661-673.

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.

Fruttiger, M., Karlsson, L., Hall, A. C., Abramsson, A., Calver, A. R., Bostrom, H., Willetts, K., Bertold, C.H., Heath, J. K., Betsholtz, C., Richardson, W. D. (1999). Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice. *Development* **126**, 457-467.

Gow, A., Friedrich, V. L., Jr and Lazzarini, R. A. (1992). Myelin basic protein gene contains separate enhancers for oligodendrocyte and Schwann cell expression. *J. Cell Biol.* **119**, 605-616.

Hajihosseini, M., Tham, T. N. and Dubois-Dalq, M. (1996). Origin of oligodendrocytes within the human spinal cord. *J. Neurosci.* **16**, 7981-7994.

Hall, A., Giese, N. A. and Richardson, W. D. (1996). Spinal cord oligodendrocytes develop from ventrally derived progenitor cells that express PDGF alpha-receptors. *Development* **122**, 4085-4094.

Hu, H. and Rutishauser, U. (1996). A septum-derived chemorepulsive factor for migrating olfactory interneuron precursors. *Neuron* **16**, 933-940.

Jankovskii, A. and Sotelo, C. (1996). Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specificity as determined by heterochronic and heterotopic transplantation. *J. Comp. Neurol.* **371**, 376-396.

Lois, C. and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145-1148.

Lu, Q. R., Yuk, D., Alberta, J. A., Zhu, Z., Pawlitzky, I., Chan, J., McMahon, A. P., Stiles, C. D. and Rowitch, D. H. (2000). Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron* **25**, 317-329.

Monge, M., Kadiiski, D., Jacque, C. and Zalc, B. (1986). Oligodendroglial expression and deposition of four major myelin constituents in the myelin sheath during development. An in vivo study. *Dev. Neurosci.* **8**, 222-235.

Myat, A., Henrique, D., Ish-Horowicz, D. and Lewis, J. (1996). A chick homologue of Serrate and its relationship with Notch and Delta homologues during central neurogenesis. *Dev. Biol.* **174**, 233-247.

Nery, S., Wichterle, H. and Fishell, G. (2001) Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. *Development* **128**, 527-540.

Niehaus, A., Stegmuller, J., Diers-Fenger, M. and Trotter, J. (1999). Cell-surface glycoprotein of oligodendrocyte progenitors involved in migration. *J. Neurosci.* **19**, 4948-4961.

- Nishiyama, A., Lin, X. H., Giese, N., Heldin, C. H. and Stallcup, W. B. (1996). Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. *J. Neurosci. Res.* **43**, 299-314.
- Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562.
- Olivier, C., Cobos, I., Perez Villegas, E. M., Spassky, N., Zalc, B., Martinez, S. and Thomas, J.-L. (2001) Monofocal origin of telencephalic oligodendrocytes in the anterior entopeduncular area of the chick embryo. *Development* **128**, 1757-1769.
- Orentas, D. M. and Miller, R. H. (1996). The origin of spinal cord oligodendrocytes is dependent on local influences from the notochord. *Dev. Biol.* **177**, 43-53.
- Orentas, D. M., Hayes, J. E., Dyer, K. L. and Miller, R. H. (1999). Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. *Development* **126**, 2419-2429.
- Perez-Villegas, E. M., Olivier, C., Spassky, N., Poncet, C., Cochard, P., Zalc, B., Thomas, J.-L. and Martinez, S. (1999). Early specification of oligodendrocytes in the chick embryonic brain. *Dev. Biol.* **216**, 98-113.
- Peyron, F., Timsit, S., Thomas, J. L., Kagawa, T., Ikenaka, K. and Zalc, B. (1997). In situ expression of PLP/DM-20, MBP, and CNP during embryonic and postnatal development of the jimpy mutant and of transgenic mice overexpressing PLP. *J. Neurosci. Res.* **50**, 190-201.
- 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., Pourquie, O., Duprat, A. M. and Cochard, P. (1996). Induction of oligodendrocyte progenitors in the trunk neural tube by ventralizing signals: effects of notochord and floor plate grafts, and of sonic hedgehog. *Mech. Dev.* **60**, 13-32.
- 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.
- Puelles, L. and Rubenstein J. L. R. (1993). Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization. *Trends Neurosci.* **16**, 472-479.
- Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F. and Noble, M. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* **333**, 562-565.
- Raff, M. C. (1989). Glial cell diversification in the rat optic nerve. *Science* **243**, 1450-1455.
- Richardson, W. D., Pringle, N., Mosley, M. J., Westermarck, B. and Dubois-Dalq, M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell* **53**, 309-319.
- Richardson, W. D., Smith, H. K., Sun, T., Pringle, N. P., Hall, A. and Woodruff, R. (2000). Oligodendrocyte lineage and the motor neuron connection. *Glia* **29**, 136-142.
- 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 autoproteolysis. *Cell* **81**, 445-455.
- Rubenstein, J. L., Shimamura, K., Martinez, S. and Puelles, L. (1998). Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.* **21**, 445-477.
- Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.
- 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.
- Spassky, N., Goujet-Zalc, C., Parmantier, E., Olivier, C., Martinez, S., Ivanova, A., Ikenaka, K., Macklin, W., Cerruti, I., Zalc, B. and Thomas, J. L. (1998). Multiple restricted origin of oligodendrocytes. *J. Neurosci.* **18**, 8331-8343.
- Spassky, N., Olivier, C., Perez-Villegas, E., Goujet-Zalc, C., Martinez, S., Thomas, J. and Zalc, B. (2000). Single or multiple oligodendroglial lineages: a controversy. *Glia* **29**, 143-148.
- Stankoff, B., Demerens, C., Goujet-Zalc, C., Monge, M., Peyron, F., Mikoshiba, K., Zalc, B. and Lubetzki, C. (1996). Transcription of myelin basic protein promoted by regulatory elements in the proximal 5' sequence requires myelinogenesis. *Mult. Scler.* **2**, 125-132.
- Soula, C., Danesin, C., Kan, P., Grob, M., Poncet, C. and Cochard, P. (2001). Distinct sites of origin of oligodendrocytes and somatic motoneurons in the chick spinal cord: oligodendrocytes arise from Nkx2.2-expressing progenitors by a Shh-dependent mechanism. *Development* **128**, 1369-1379.
- Strähle, U., Blader, P., Adam, J. and Ingham, P. W. (1994). A simple and efficient procedure for non-isotopic in situ hybridization to sectioned material. *Trends Genet.* **10**, 75-76.
- Sun, T., Pringle, N. P., Hardy, A. P., Richardson, W. D. and Smith, H. K. (1998). Pax6 influences the time and site of origin of glial precursors in the ventral neural tube. *Mol. Cell. Neurosci.* **12**, 228-239.
- Sussman, C. R., Dyer, K. L., Marchionni, M. and Miller, R. H. (2000). Local control of oligodendrocyte development in isolated dorsal mouse spinal cord. *J. Neurosci. Res.* **59**, 413-420.
- Takabatake, T., Ogawa, M., Takahashi, T. C., Mizuno, M., Okamoto, M. and Takeshima, K. (1997). Hedgehog and patched gene expression in adult ocular tissues. *FEBS Lett.* **410**, 485-489.
- Temple, S. and Raff, M. C. (1986). Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. *Cell* **44**, 773-779.
- Tekki-Kessaris, N., Woodruff, R., Hall, A. C., Gaffield, W., Kimura, S. and Richardson, W. D. (2001). HH-dependent oligodendrogenesis in the forebrain. *Development* **128**, 2545-2554.
- 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**, 1012-1024.
- Trousse, F., Giess, M. C., Soula, C., Ghandour, S., Duprat, A. M. and Cochard, P. (1995). Notochord and floor plate stimulate oligodendrocyte differentiation in cultures of the chick dorsal neural tube. *J. Neurosci. Res.* **41**, 552-560.
- Warf, B. C., Fok-Seang, J. and Miller, R. H. (1991). Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. *J. Neurosci.* **11**, 2477-2488.
- 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.
- Zhou, Q., Wang, S. and Anderson, D. J. (2000). Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. *Neuron* **25**, 331-343.