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Oligodendrocyte development in the embryonic brain: the contribution of the *plp* lineage

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ABSTRACT Oligodendrocytes are the myelin forming cells of the central nervous system. Over the last decade, their development in the embryonic brain and spinal cord has been documented following the discovery of early oligodendroglial markers. This review highlights the fundamental results obtained on the specification and migration of oligodendroglial cells and illustrates our advances in the knowledge of the cell lineage expressing *plp* (proteolipid protein), one of the early oligodendroglial genes.

KEY WORDS: *embryonic brain, oligodendrocyte, specification, migration, plp/dm-20*

Introduction

The nervous system is constituted of two major cellular families: neurons, which are interconnected in networks to form electrically active circuitry and glial cells, the functions of which still remain in large to be fully elucidated. For long, glia has been assimilated to the connective cell type of the nervous tissue and its function restricted to that of supporting cells, responsible for the nutrition and insulation of neurons. Ongoing observations and experimental arguments, however, provide evidence that glial cells may have a more active role in the structural and functional plasticity of the nervous system. In this respect, it is striking to note that during evolution there is a gradual increase in the proportion of glial cells. In invertebrates, like the fly or nematode, glial cells account for 25-30% of total nervous system cells. This percentage increases to 60-70% in rodents and has been claimed to reach 90% in humans (Pfrieger and Barres, 1995). Glia is subdivided in macroglia (astrocytes, oligodendrocytes and ependymal cells), which like neurons is derived from the neuroectoderm and microglia, which results from the invasion of cerebral tissue by circulating monocytes. Our studies concern the oligodendrocytes which are the myelin forming cells of the central nervous system (CNS). Myelin is specific to vertebrates and facilitates nerve conduction. Consequently, the degree of motor maturation at birth reflects the extent to which myelination has advanced during embryonic development. In the adult, the oligodendrocytes are affected by one main neurological disease: Multiple sclerosis (MS), a frequent and invalidating disease of the young adult. MS is characterised by an inflammatory reaction, probably of an autoimmune type and a demyelination frequently associated with a loss of oligodendrocytes. To date, the available treatments can suppress the inflammation, but have little, if any, efficacy on remyelination. The identification of molecular factors controlling oligodendroglial differentiation, migration and myelination appears thus as a priority to positively influence a remyelination of the lesions. With this perspective in mind, we are investigating the cellular and molecular aspects of oligodendrogenesis and focus our studies on the embryonic brain due to the similarity of mechanisms involved in the differentiation of embryonic neural cells and stem cell populations of the adult brain.

Studies on the origin of oligodendrocytes have long been hampered by the lack of recognized markers of their precursors. We have identified one of the markers of oligodendrocyte precursor cells (OPCs), the *plp/dm-20* transcripts (Timsit *et al.,* 1995; Spassky *et al.,* 1998, 2001a-b; Perez-Villegas *et al.,* 1999). *PLP/dm-20* belongs to the *dm* family of genes whose members have been identified in the shark, the ray (Kitagawa *et al.,* 1993) and the mouse (Yan *et al.,* 1993). *Plp* encodes two alternative spliced products: the proteolipid protein (PLP) and DM-20, which are proteins with four putative transmembrane domains (Popot *et al.,* 1991) and are the major protein components of higher CNS myelin

Abbreviations used in this paper: opc, oligodenrocyte precursor cell; plp, proteolipid protein.

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(Lees and Brostoff, 1984). Although PLP is expressed during the final stages of oligodendrocyte maturation, the corresponding transcripts can be detected much earlier during embryonic development (Timsit *et al.,* 1992). Moreover, the presence of restricted subsets of *plp*$^+$ neuroepithelial cells in the mouse embryonic brain and spinal cord suggested a link between *plp* expression and early oligodendrogenesis (Timsit *et al.,* 1995; Dickinson *et al.,* 1996).

We have thus investigated the cell fate of the *plp* lineage. Their specification and migration have been analyzed both in chick and mouse species and we have searched for molecules guiding *plp*$^+$ OPC migration from the ventricular foci towards the future white matter tracts. The resulting findings were: i) a spatio-temporal description of oligodendrogenesis and OPC migratory pathways in the mouse and chick embryonic brain (Spassky *et al.,* 1998, 2001b; Perez-Villegas *et al.,* 1999; Olivier *et al.,* 2001); ii) the characterization of *plp*$^+$ OPCs as PDGF-A independent precursors, thus distinct from PDGFRα expressing OPCs (Spassky *et al.,* 2001a); iii) the identification of molecules controling OPC migration, some of which act from a distance, like the axonal guidance molecules class 3 semaphorins and netrin-1 (Spassky *et al.,* 2002), while others mediate their effect by a direct cell to cell contact, like the ephrinB2 ligand (Prestoz *et al.,* 2004).

Results

plp/dm-20 *is expressed by restricted progenitor domains generating neurons and oligodendrocytes*

To provide evidence that *plp/dm-20* expressing cells in the germinative neuroepithelium are precursors that give rise to oligodendrocytes, we have generated a transgenic murine line where a zeomycin resistance gene fused to the *lacZ* reporter was expressed under the control of the *plp* - regulatory sequences (Figure 1A). The pattern of β-galactosidase expression was similar and superimposable on the expression pattern of of endogenous *plp/dm-20*. At E12.5, the transgene was expressed predominantly in restricted domains of the ventral neural tube, in the ganglionic eminence, the basal plate of the caudal hypothalamus, diencephalon and rhombencephalon, with the exception of the rhombomeres r3 to r5 (Figure 1B). Both *in vivo* and *in vitro*, the transgene was expressed by O4$^+$ pre-oligodendrocytes and later by differentiated oligodendrocytes, but not by neuronal cells (Figure 1C), astrocytes, or radial glial cells. In cultures derived from E12.5 transgenic embryos, a dramatic enrichment in O4$^+$ pre-oligodendrocytes was observed following zeomycin selection (Figure 1D), whereas in control cultures double labeled cells (O4$^+$/Xgal$^+$) represented only 15-20% of the total population. The enrichment in the O4$^+$ pre-oligodendrocytes, following zeomycin treatment of cultures derived from rostral and caudal territories of the brain anlage, indicate that, in both regions, neural precursors expressing *plp/dm-20* cells give rise to oligodendrocytes.

In the CNS, the *plp* gene was first detected at E9.5 in the basal plate of the diencephalon, the caudal hypothalamus and the entopeduncular area in the ventrocaudal region of the telencephalon. This early *plp* expression coincides with an active period of neurogenesis in the neuroepithelium, which suggested that *plp* progenitors might be pluripotent cells, not restricted to an oligodendroglial fate. To examine the differentiation potential of *plp+* progenitors emerging at early stages of neural tube development (E9.5-10.5), we used transgenic *plp-GFP* mice in

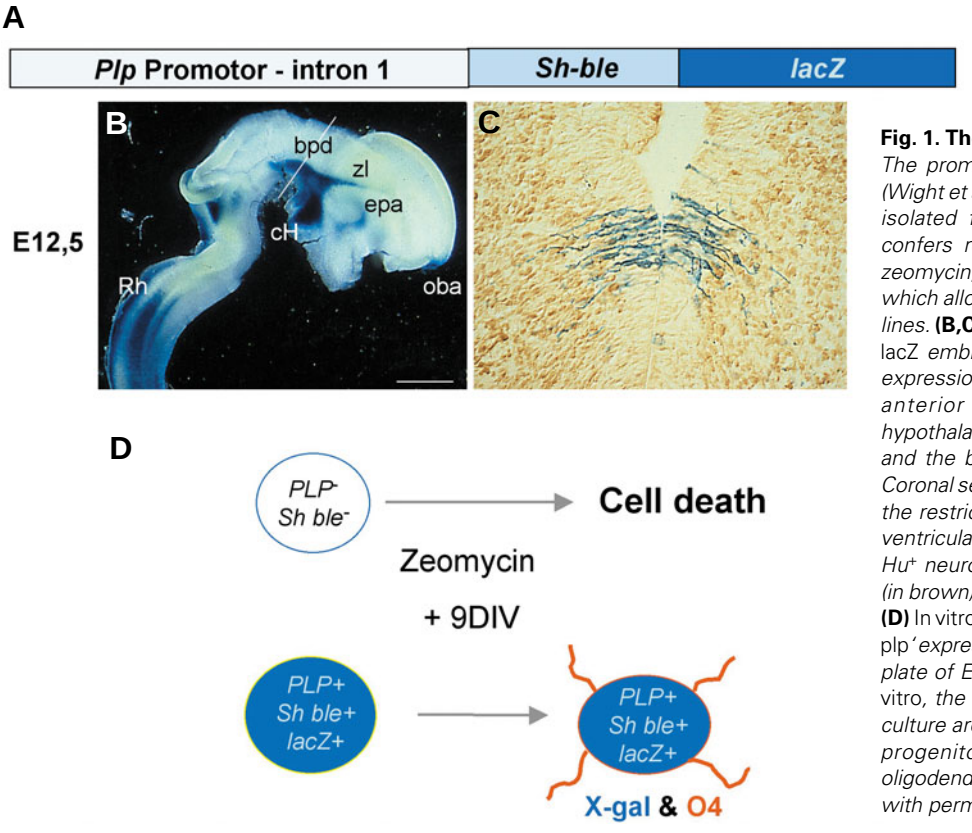

Fig. 1. The *plp-shble-lacZ* murine transgenic line. (A) *The promotor and regulatoty sequences of* plp *gene (Wight et al., 1993) drive the expression of* sh ble*, a gene isolated from Streptoalloteichus hindustanus, which confers resistance to the antibiotics phleomycin and zeomycin, in frame with the E. coli* lacZ *reporter gene, which allows an easy detection of transgene expressing lines.* **(B,C)** *Reporter gene expression in E12.5* plp-sh ble-lacZ *embryonic brain.* **(B)** *From rostral to caudal, note the expression in the prospective olfactory bulb (pob), the anterior entopeduncular area (epa), the caudal hypothalamus (cH), the basal plate of diencephalon (bpd) and the basolateral plate of rhombencehalon (Rh).* **(C)** *Coronal section at the diencephalic level in (B), illustrating the restricted localization of* plp *expressing cells in the ventricular neuroepithelium (in blue), distinct from the Hu*$^+$ *neurons distributed in the surrounding mantle layer (in brown). Scale bar (shown in (B): B, 720 μm; C, 70 μm.* **(D)** In vitro*, zeomycin treatment results in the selection of* plp*'expressing neuroepithelial cells from the diencephalic plate of E12.5* plp-sh ble-lacZ *embryos. After 9 days* in vitro*, the large majority of transgene expressing cells in culture are O4*$^+$ *pre-oligodendrocytes. In vitro, E12.5* plp *progenitors thus show a potentiality to generate oligodendrocytes. Reprinted from Spassky* et al. *(1998), with permission from The Society for Neuroscience.*

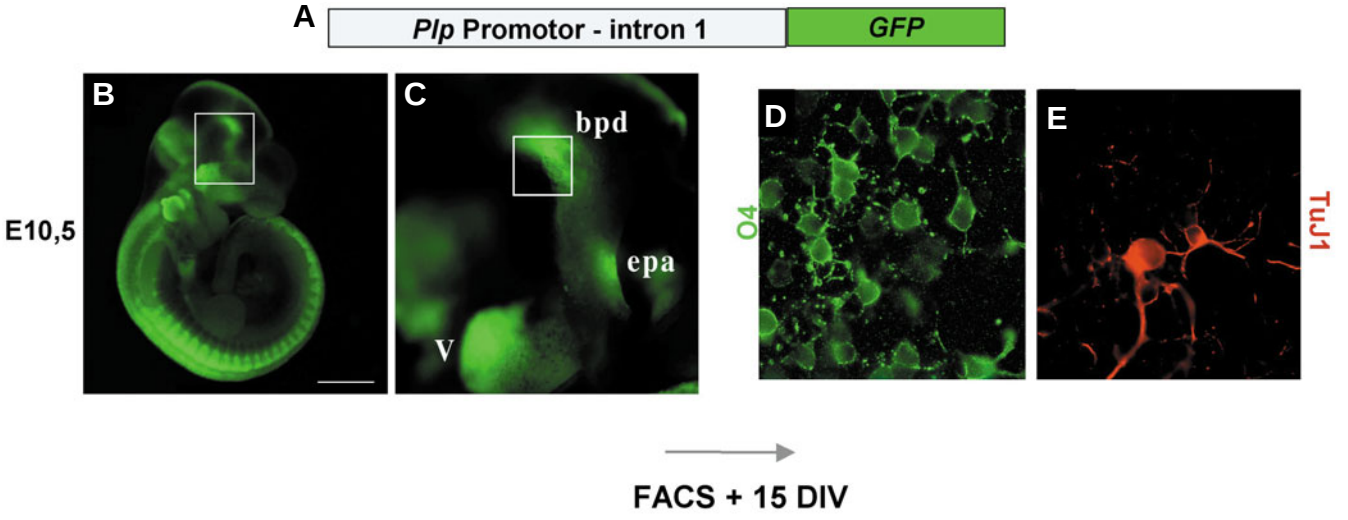

Fig. 2. The *plp-GFP* murine transgenic line. (A) *The* plp *promotor and regulatory sequences are in frame with the reporter gene* EGFP. **(B,C)** *Reporter gene expression in a E10.5* plp-GFP *embryo. Note the expression of transgene in peripheral nervous system derivatives of the neural crest and, in the CNS, in the entopeduncular area (epa) and the basal plate of diencephalon (bpd).* **(C)** *Enlargment of the boxed area in (B). Scale bar (shown in B): B, 380 μm; C, 110 μm; D,E 50 μm.* **(D,E)** *FACS sorted cells from the diencephalic basal plate of E10.5* plp-GFP *embryos, cultured on a feeder layer of astrocytes and 1% fetal calf serum, differentiate into O4⁺ oligodendrocytes* **(D)** *and TuJ1⁺ neurons* **(E)** *after 15 days* in vitro. *Under these culture conditions, E10.5* plp *progenitors are thus specified towards the neuro-oligodendroglial lineages. Reprinted from Spassky* et al. *(2001b), with permission from Karger.*

which expression of the GFP reporter gene is driven by the *plp* regulatory sequences (Figure 2 A,B). Living *plp+* progenitors were isolated from E9.5-10.5 diencephalic basal plate (Figure 2C) by fluorescence-activated cell sorting (FACS) and cultivated on a feeder layer of astrocytes in DMEM enriched with 1% foetal calf serum during 15 days *in vitro*. The presence of neurons and glial cells was then tested by immunohistochemistry. One half of the cells derived from *plp-GFP+* progenitors was composed of differentiated cells: 5% of TuJ1+/GFP negative neurons (Figure 2D) and 45% of O4+/GFP⁺ oligodendrocytes (Figure 2E), but no astrocytes. The other half of the cells in culture were nestin+ and did not express any differentiated phenotype. In contrast, neurons, oligodendrocytes and astrocytes were obtained from the *plp-GFP* negative population sorted by FACS. These results suggest a neuro-oligodendroglial bipotentiality of the population of *plp+* progenitors. They also demonstrate that *plp* expression is downregulated in the neuronal progeny of *plp⁺* progentors while it is maintained in the oligodendroglial progeny.

plp/dm-20 *progenitors generate PDGFRα independent-OPCs*

Although *plp/dm-20* progenitors generate oligodendrocytes, they do not represent the totality of cells capable of generating oligodendrocytes in the embryonic brain. At E12.5, some *plp/dm-20-* neuroepithelial territories have the potential to generate oligodendrocytes in vitro. Interestingly, these *plp/dm-20-* territories expressed the transcripts of platelet derived growth factor receptor alpha (PDGFRα), another early marker of the oligodendroglial lineage (Pringle *et al.,* 1993; Yu *et al.,* 1994). In the hindbrain and spinal cord, as well as in the medial ganglionic eminence of the telencephalon, both transcripts are present, but co-expression at the cellular level is only detected in very rare cells of the ventricular layer (less than 10% in the entopeduncular area of E5.5 chick embryo). In the diencephalon and midbrain, the expression of *PDGFRα* and *plp/dm-20* alternated. In the olfactory bulb, at E14.5, *PDGFRα* was not detected, in contrast to *plp/dm-20* which was expressed by subsets of ventricular progenitors (Figure 3A). These observations suggested that oligodendrocytes could derive from at least two distinct types of progenitors expressing either *plp/dm-20* or *PDGFRα*. To determine whether *plp/dm-20* and *PDGFRα* progenitors were lineage-linked or not, we followed the development of *plp/dm-20* progenitors in the absence of PDGFRα signalling. Dissociated cell cultures, derived from either the ganglionic eminences or the anlage of the olfactory bulb (E12.5), were performed in the presence of a tyrosine kinase inhibitor STI571 (Gleevec) specific for the PDGFRα (Figure 3B). In olfactory bulb cultures, the survival and proliferation of *plp/dm-20* progenitors, as well as their differentiation into O4⁺/GalC⁺ oligodendrocytes were not affected by blocking the PDGFRα signalling. In contrast, oligodendrogenesis from the ganglionic eminence was reduced by half in the presence of STI571. These data provided strong evidence that *plp/dm-* 20 progenitors do not depend on signal transduction mediated by platelet derived growth factor receptors (PDGFR). Therefore, *plp/dm-20* cells most probably belong to a lineage different from the *PDGFRα* expressing progenitors and oligodendrocytes of the embryonic brain have a multiple origin.

Regional identity of oligodendrocyte migrations in the embryonic brain

To establish the topological relationship between the ventricular foci of oligodendrocyte progenitors and the different populations of brain oligodendrocytes, we have turned to the avian model. The avian embryo is indeed accessible at all stages of development, which allows to generate neural chimeras (LeDouarin, 1969, 1993). In the chick embryonic brain, as in the mouse, *plp/dm-20* was expressed, from the onset of neurogenesis (E2.5), in restricted foci of the ventral neuroepithelium (Figure 4A). From E5-6, the *plp/dm-20* cells expanded in the mantle layer (Figure 4B) and O4⁺ cells delaminated from the ventricular foci of *plp/dm-20* progenitors

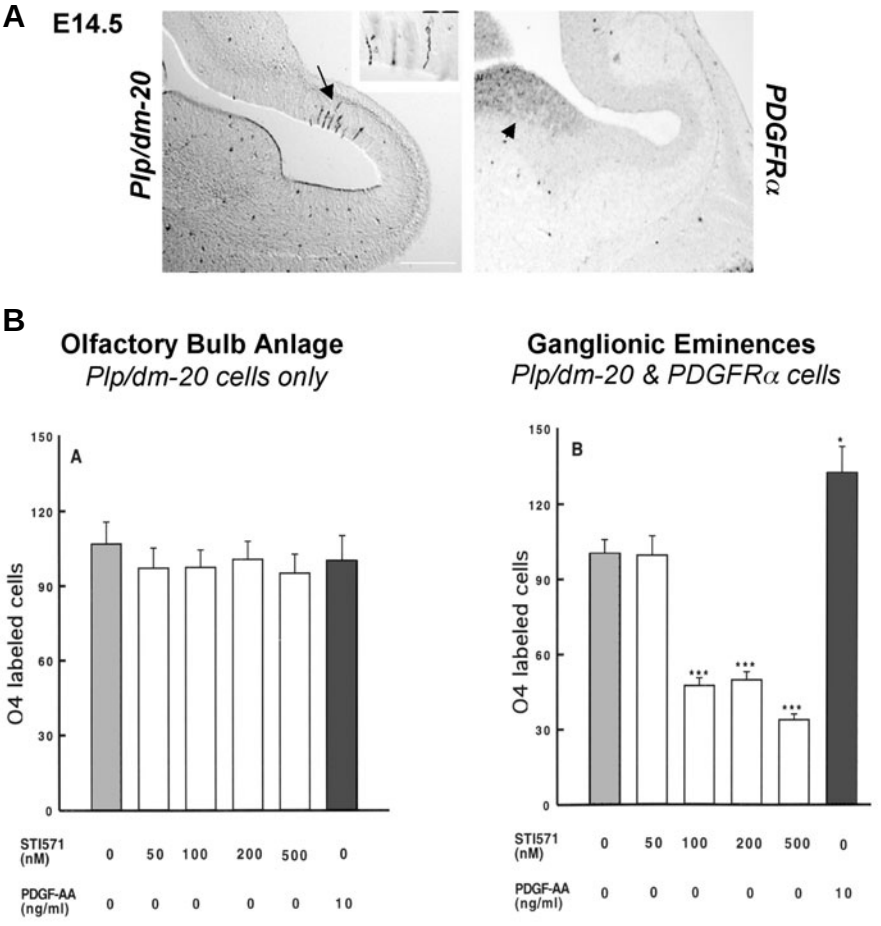

Fig. 3. Heterogeneity among oligodendrocyte precursor cells. (A) *At E14.5, the patterns of distribution of* plp/dm-20 *and* PDGFRα *cells in the rostro-ventral forebrain are distinct. Using a* plp-sh ble-lacZ *embryo,* plp/dm-20 *cells are detected by* lacZ *expression (left panel) and* PDGFRα *cells by* in situ *hybridization (right panel). In the olfactory bulb,* plp/dm-20 *cells (arrow) are restricted to the ventricular layer (magnification in the insert), where* PDGFRα *expression is not detectable. In contrast,* PDGFRα *cells (arrowhead) are abundant in the medial ganglionic eminence (not shown on the left panel). Scale bar: 200 μm; insert in the left panel, 16 μm.* **(B)** *Blocking PDGFR tyrosine kinase does not interfere with development of* plp/dm-20+ *progenitors cells. Olfactory bulb anlage (left panel) or ganglionic eminences (right panel) from E12.5 OF1 wild-type mouse were dissociated, seeded in 96 well dishes and cultivated in BS medium supplemented with 1% FCS. After 2 days* in vitro, *either STI571 (50-500nM) or PDGF-AA (10ng/ml) was added. After 13 days* in vitro, *cultures were immunolabeled with O4 mAb and immunopositive cells were counted. Each column represents the mean + s.e.m of 3 separate experiments representing 14 to 24 different cultures. (*** $p<0.0001$; * $p<0.05$, Student's* t *test). Reprinted from Spassky* et al. *(2001a), with permission from the Society for Neuroscience.*

(Figure 4 C,D). We have established a map of *plp/dm-20* expression sites in the chick embryonic brain (HH26), which predicts the sites of emergence of oligodendrocytes. These foci of oligodendrogenesis are localized in the baso-ventral plate of the hind-, mid- and caudal fore-brain, while in the rostral forebrain oligodendrocytes emerge from alar territories.

We have then investigated the respective territories colonized by oligodendrocyte progenitor cells originating from either the baso-ventral or alar foci and created a series of quail-chick chimeras. Data from homotopic chimeras demonstrated clearly that, during embryonic development, oligodendrocyte progenitors emerging from the alar anterior entopeduncular area migrate tangentially to invade the entire telencephalon, whereas those from the basal rhombomeric foci show a restricted rostro-caudal distribution and colonize only their rhombomere of origin (Figure 4E). Heterotopic chimeras indicated that differences in the migratory properties of oligodendroglial cells did not depend on their baso-ventral or alar ventricular origin. Irrespective of their origin (basal or alar), oligodendrocytes migrated only short distances in the hindbrain and long distances in the prosencephalon. Furthermore, we showed that, in the embryonic chick brain, all telencephalic oligodendrocytes originate from the anterior entopeduncular area and that the prominent role of anterior entopeduncular area in telencephalic oligodendrogenesis is conserved between birds and mammals.

Diffusible class 3 semaphorins and netrin-1 guide the migration of plp *OPCs*

Little was known about the endogenous guidance cues controlling the migration of OPCs from their site of emergence towards their final destination, mainly the future white matter tracts. To investigate the molecular control of OPC migration, we chose the embryonic optic nerve as an expertimental system. The embryonic optic nerve has no intrinsic ability to generate oligodendrocytes (Small *et al.,* 1987), but is populated by OPCs originating in the ventral diencephalon (Figure 5 A,B), which migrate from the chiasm towards the retina (Ono *et al.,* 1997a). In the *plp-lacZ* mouse, we have monitored the oligodendroglial colonisation of the optic nerve (Figure 5C), which started from E14.5 onwards and filled the nerve to its retinal end at E18.5 (Spassky *et al.,* 2002). In addition, the optic nerve does not contain neuronal cell bodies, which allows a selective analysis of oligodendroglial migration *in vitro*, either by collagen gel cultures of optic nerve explants (Figure 5D) or by stripe assays on dissociated cell cultures (Figure 5E).

Since neurons and OPCs share common sites of origin in the embryonic neural tube and further develop with close timing, we first postulated that OPC migration might be influenced by the axonal environment, notably the diffusible factors that contribute to guide the axonal guidance. Chemotactic factors of the semaphorin (Messersmith *et al.,* 1995) and netrin (Serafini *et al.,* 1994) families are expressed in the CNS and control the guidance of axonal growth cones (Goshima *et al.,* 2002; Raper, 2000). Some of them have also been implicated in the migration of neural cells. This is the case for Sema 3A, which has a chemorepulsive effect on neural crest cells (Eickholt *et al.,* 1999), Sema 3A and Sema 3F which repel cortical interneurons (Marìn *et al.,* 2001) and for netrin-1 which guides the migration of precerebellar, cerebellar

and hypothalamic neurons (Manitt and Kennedy, 2002). Therefore, we questioned whether semaphorins and netrin molecules could not only act on neurons, but also on glial cells.

We examined the possible role of signalling molecules, such as class 3 semaphorins and netrin-1, on the migration of OPCs. We showed that OPCs migrating into the embryonic optic nerve express the semaphorin receptors neuropilin-1 and -2, as well as DCC (deleted in colorectal cancer) and, to a lesser extent unc5H1, two of the netrin-1 receptors. Using collagen gel cultures as a functional migration assay, we provided evidence that Sema 3A and netrin-1 exerted opposite chemotactic effects, repulsive or attractive respectively, on embryonic OPCs. In addition, we showed that Sema 3F had a dual, chemoattractive and mitogenic, effect on embryonic OPCs. The localization of cells expressing Sema 3A, Sema 3F and netrin-1 was consistent with a role for these ligands in the migration of OPCs in the embryonic optic nerve (Figure 6). Altogether, these findings suggest that the migration of OPCs in the embryonic optic nerve is modulated by a balance of effects mediated by members of the semaphorin and netrin families.

Eph/ephrin interactions modulate the axonophilic migration of plp *OPCs*

The migration of oligodendrocyte precursors cells (OPCs) is modulated by secreted molecules in their environment (see above) and by cell-cell and matrix-cell interactions. We thus asked whether membrane-anchored guidance proteins such as the ephrin ligands and their Eph receptors could participate in the control of OPC migration in the optic nerve.

The ephrin ligands and their Eph receptors are known as the largest group of receptor tyrosine kinases involved in neural development (Wilkinson, 2001), especially in modulation of axon

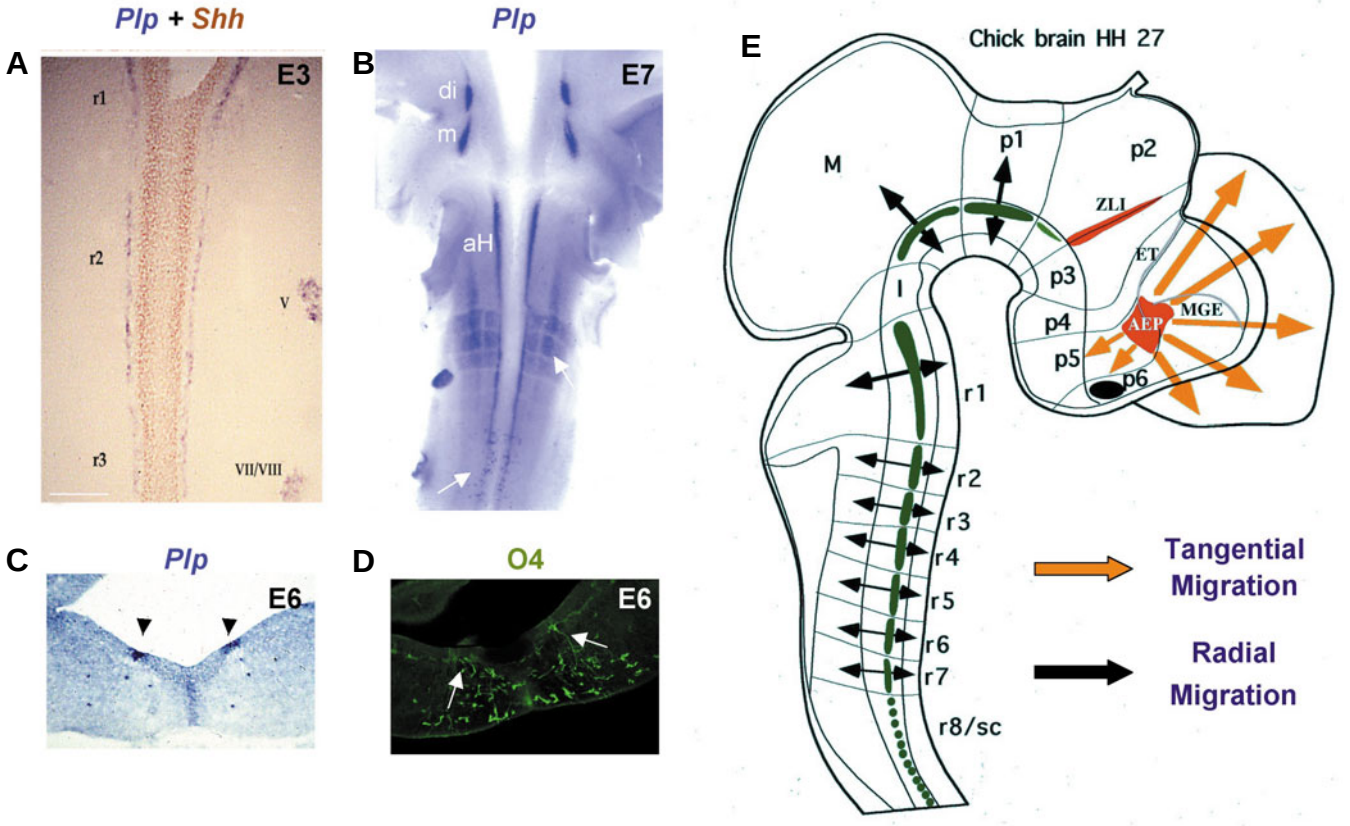

Fig. 4. Regional identity of OPC migrations in the chick embryonic brain. (A-D). *Pattern of* plp *expression of and oligodendroglial development in the embryonic brain.* In situ *hybridizations were performed with* plp *(A,B) and* sonic hedgehog *(A) antisense riboprobes on flat mounts* **(A,B)** *and coronal section* **(C)** *of the brain. Immunolabeling with the pre-oligodendrocyte marker O4 was performed on a coronal section of rhombomere 1* **(D)**, *adjacent to* **(C)**. **(A)** *At E3, restricted foci of* plp *progenitors (in blue) are localized ventrally in the hindbrain neuroepithelium, close to the source of Sonic hedgehog in the floor plate (in red).* **(B)** *At E7, the pattern of* plp *expression is still restricted to the ventral neuroepithelium in the diencephalon (di), mesencephalon (m) and anterior part of the hindbrain (aH), but cells expand laterally in the mantle layer of more caudal rhombomeres (arrows).* **(C-D)** *At E6, in the anterior hindbrain, the foci of* plp/dm-20 *progenitors (arrowheads in c) are facing the O4⁺ pre-oligodendrocytes which migrate and expand in the mantle layer (arrows in D).* **(E)** *Model of spatial development of oligodendrocytes in the avian developing brain. Sagittal representation of the brain at stage HH 27 (E5-6). In the ventricular layer, the basoventral territories of emergence are colored in green, the alar domains in red. Arrows indicate the migratory pathways. Note that oligodendrocyte progenitors emerging from the basoventral foci, in the epicordal domain of the brain, migrate radially (black arrows), while those arising from the alar plate, in the precordal domain of the brain, follow extensive tangential migratory pathways (orange arrows). AEP: anterior entopeduncular area; ET: eminentia thalami; I: Isthmus; M: mesencephalon; MGE: medial ganglionic eminence; p: prosomere (p1 to p6); r: rhombomere (r1 to r7), sc: spinal cord; ZLI: zona limitans intrathalamica. Scale bar (shown in A): A, 30 μm; B, 80 μm; C,D, 270 μm. Reprinted from Perez-Villegas* et al. *(1999), with permission from Elsevier.*

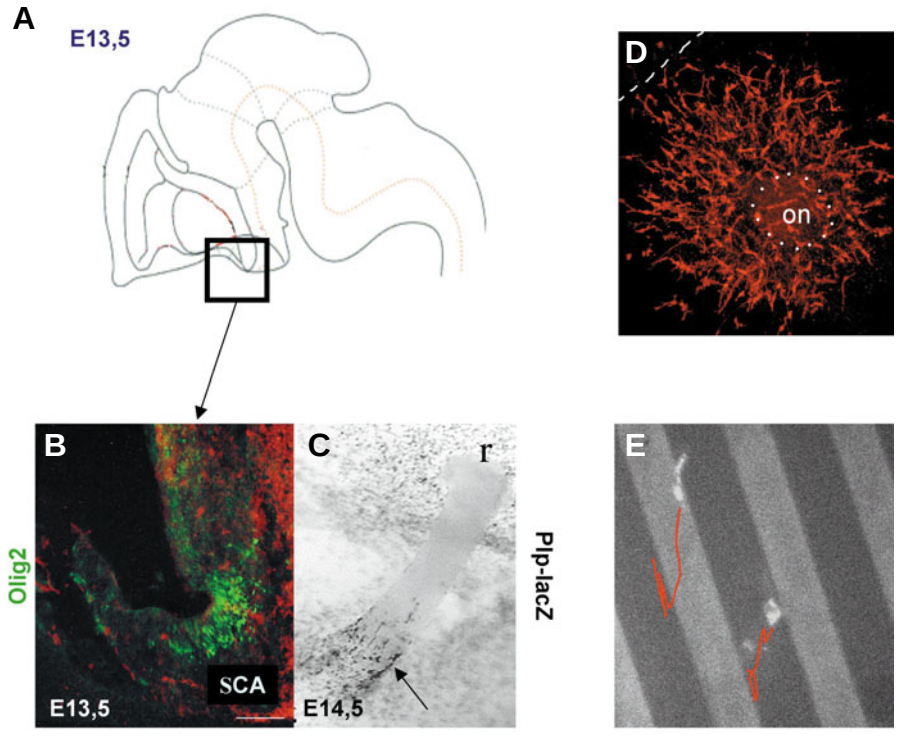

Fig. 5. The embryonic optic nerve: an experimental system for the study of OPC migration. (A) *Schematic representation of the mouse brain at E13.5. The boxed area corresponds to the ventral region of the third ventricule, illustrated in (B).* **(B)** *Coronal section of E13.5 mouse brain at the level of the third ventricle, immunostained with an anti-Olig2 Ab. OPCs are identified by the expression of Olig2 transcription factor (in green) and localized in the ventricular layer of the suprachiasmatic area. Optic nerve OPCs are generated from these progenitors.* **(C)** *Whole-mount X-Gal labeling of the optic nerve of a* plp-sh ble-lacZ *embryo at E14.5. Note the OPCs (arrow) entering the nerve from the chiasmal region and routing towards the retinal end.* **(D,E)** In vitro *systems for analysis of optic nerve OPC migration.* **(D)** *Optic nerve explant isolated at E16.5 and cultured in collagen gel. Immunolabeling with the A2B5 antibody, a marker for optic nerve OPCs, shows a radial migration of OPCs around the explant.* **(E)** *OPCs isolated from the optic nerve E16.5* plp-GFP *embryos were cultured on laminin stripes (in black) versus EphB2-Fc or EphA6-Fc or control -Fc coated stripes (in light gray). The migration of two* GFP+ *OPCs was recorded by time-lapse fluorescent videomicroscopy during 3 to 30 hours (red lines). Scale bar (shown in A): A, 40 μm; B, 110 μm; C, 140 μm; D, 50 μm. Reprinted from Spassky* et al. *(2002), with permission from The Society for Neuroscience.*

pathfinding and control of cell-cell interactions (Himanen and Nikolov, 2003). A specific feature of these molecules is their ability to elicit bi-directional signalling, that is classical «forward signalling» by the Eph receptor via its intrinsic tyrosine kinase activity and «reverse signalling» by the transmembrane ephrinB ligand via its cytoplasmic domain (Mellitzer *et al.,* 2000). Eph receptors are known to participate in several aspects of the visual system development. They are expressed by retinal ganglion cells and their axonal projections and are key regulators of the retinocollicular mapping (Knoll and Drescher, 2002). We thus questioned whether OPCs could express ephrin ligands able to interact with Eph receptors expressed by axons, during their axonophilic migration in the optic nerve.

We showed the expression of ephrins A5, B2 and B3 in the migrating OPCs of the optic nerve as well as in the diencephalic sites from where they originate (Figure 7 A-D). In addition, we provided evidence that activation of these ligands by immobilized EphA6 or EphB2 receptors increases the adhesion of OPCs on the substrate. In addition, we observed by time-lapse videomicroscopy a strong inhibition of OPC migration on EphB2-Fc substrate, suggesting that ephrinB activation provides regulating signals of OPC migration

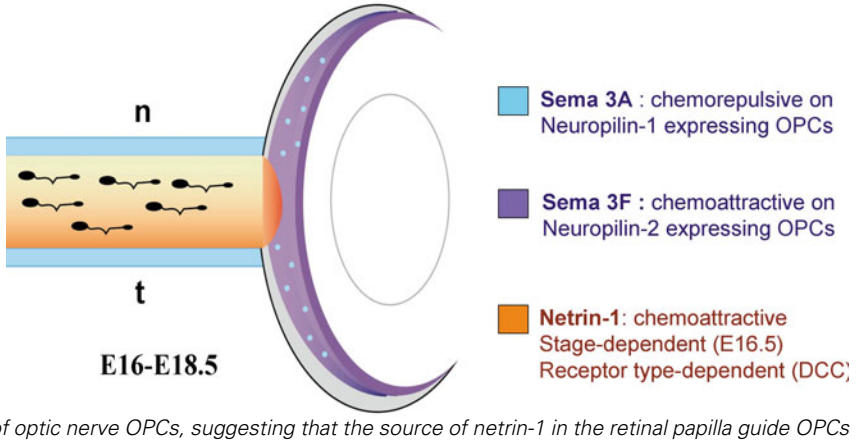

Fig. 6. Role of class3 semaphorins and netrin-1 in the guidance of OPC migration. *A schematic representation of the distribution pattern of cells expressing* Sema 3A *(azur),* netrin-1 *(orange) and* Sema 3F *(purple) in the optic nerve and associated structures at E16.5-18.5. The expression of* Sema 3A, *around the nerve, delineates a clear boundary between the outside and the inside of the nerve, which could force the OPCs to stay within the nerve and migrate along its length.* Sema 3F *is not detected around or inside the ON, but in the retina, including the retinal ganglion cell layer. Sema 3F might thus be synthesized by retinal ganglion cells and transported along the axons to act as a chemoattractant on migrating OPCs.* Netrin-1, *which is expressed all along the temporal quadrant and in the retinal end of the nerve, provides a directional cue and facilitates cellular migration of optic nerve OPCs, suggesting that the source of netrin-1 in the retinal papilla guide OPCs towards the optic nerve end and that in the chiasmal region, the temporal source of netrin-1 helps OPCs to enter the nerve from the extramural stream of the ventral diencephalon. n: nasal; t: temporal. Reprinted from Spassky et al. (2002), with permission from The Society for Neuroscience.*

(Figure 7E). Based on these findings, we proposed that OPCs are characterized by an ephrin code and that Eph/ephrin interactions between axon and OPCs contribute to the control of OPC distribution in the optic axonal tracts as well as the progress and arrest of their migration.

Discussion

Our studies of oligodendrocyte development in the embryonic brain allowed us to identify and define the fate of a population of neural progenitors expressing *plp/dm-20*. The *plp* gene encodes the myelin proteolipid protein in mature oligodendrocytes and, at premyelinating stage, also generates a secreted product promoting oligodendroglial proliferation (Yamada *et al.,* 1999). The *plp/dm-20* progenitors emerge in restricted domains of the ventricular neuroepithelium, localized predominantly ventrally and close to a source of the morphogen Sonic hedgehog. *plp/dm-20* ventricular progenitors sequentially generate neurons and oligodendrocytes, being neurogenic from E9.5 in the mouse and oligodendrogenic from E12.5 in the mouse and E5.5 in the chick. *plp/dm-20* expression is maintained only in the oligodendroglial lineage and *plp* OPCs can develop into oligodendrocytes independent from PDGFRα signalling. The *plp/dm-20* OPCs expand from their ventricular source, predominantly radially in the epicordal regions of the brain and with long range tangential migrations in the forebrain. Notably, the entopeduncalar area, a restricted ventral territory of the caudal telencephalon, appears to be the unique source of OPCs during embryonic development in the chick. The molecular control of *plp* OPCs migration was examined in the optic nerve and shown to implicate the secreted guidance proteins, class 3 semaphorins and netrin-1, as well as cell to cell contact molecules, ephrinB2/EphB, which regulate the adhesion of OPCs to their migration substrate. These findings raise four main questions which are discussed below: i) the positional control of the sites of oligodendrogenesis; ii) the link beween neuronal and oligodendroglial lineages; iii) the heterogeneity among OPCs; iiii) the molecular control of OPC migration.

Sites of origin of oligodendrocytes in the embryonic neural tube

The pioneer experiments on the origin of oligodendrocytes during embryonic development were performed on the rat spinal cord and showed that, before E14, only the ventral portion, but not the dorsal half, can give rise to oligodendrocytes (Warf *et al.,* 1991; Noll and Miller, 1993). This finding has given rise to the concept that oligodendrocytes have a restricted ventral origin. Subsequently, the pattern of expression of a series of markers of oligodendrocyte precursor cells confirmed that the onset of oligodendrogenesis occurred in restricted territories of the ventral neural tube: *PDGFRα* (Pringle *et al.,* 1993), *CNP* (Scherer *et al.,* 1994; Yu *et al.,* 1994), *plp/dm-20* (Timsit *et al.,* 1995; Spassky *et al.,* 1998; Perez-Villegas *et al.,* 1999), the O4-reactive antigen (Ono *et al.,* 1997b), *Sox10* (Kuhlbrodt *et al.,* 1998; Zhou *et al.,* 2000) and *Sox 9* (Stolt *et al.,* 2003), *Olig1/2* (Lu *et al.,* 2000; Zhou *et al.,* 2000), *Nkx2.2* (Xu *et al.,* 2000; Soula *et al.,* 2001; Fu *et al.,* 2002), *Sulfatase 1* (Braquart-Varnier *et al.,* 2004). In the spinal cord, the first oligodendrocyte progenitors are generated in bilateral foci localized dorsally to the ventral midline region. In the brain, oligodendrogenesis also starts in paramedian columns of the basal plate of the caudal diencephalon, mesencephalon and rhombencephalon. Exceptions to this juxtamedial position of oligodendrogenic ventricular foci in the ventral neural tube are the medial ganglionic eminence and the entopeduncular area in the alar territory of the forebrain (Olivier *et al.,* 2001; Tekki-Kessaris *et al.,* 2001) and the rhombic lip of the hindbrain (Ono *et al.,* 1997a). However, in all of these sites, oligodendrogenesis is correlated with the expression of the morphogen Sonic hedgehog (Shh), either in the midline (Figure 4A), or within the neuroepithelium of the entopeduncular area and of the rhombic lip. Shh induces various populations of ventral neurons, notably somatic motoneurons (Briscoe and Ericson, 1999), but is also required for oligodendrocyte specification, as shown by gain- and loss of function experiments in the spinal cord (Poncet *et al.,* 1996; Pringle *et al.,* 1996; Orentas *et al.,* 1999; Soula *et al.,* 2001) and in the telencephalon (Nery *et al.,* 2001; Spassky *et al.,* 2001a; Tekki-Kessaris *et al.,* 2001). In addition to the induction of OPC specification, Shh negatively controls the development of astrocytes (Agius *et al.,* 2004).

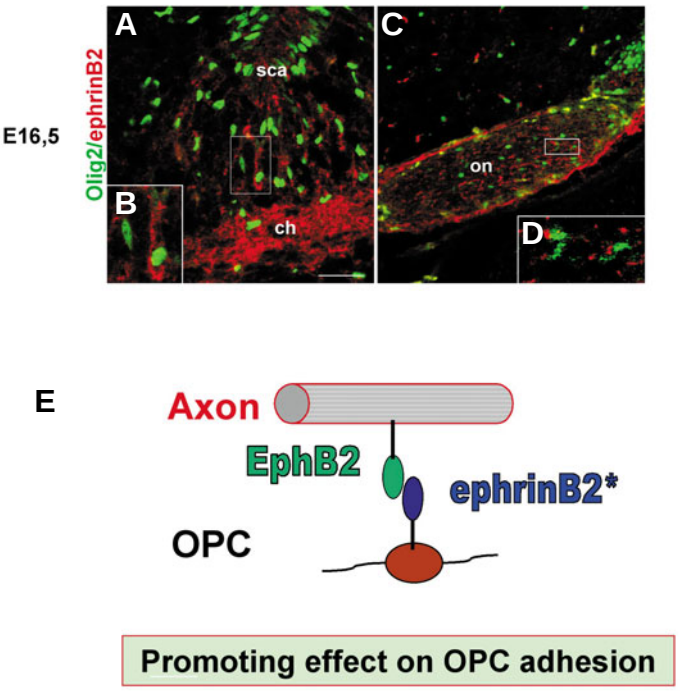

Fig. 7. Role of ephrinB2/EphB interaction in the axonophilic migration of OPCs. (A-D) *Brain coronal sections from an E16.5 mouse embryo doubly labeled with anti-ephrinB2 and anti-Olig2 antibodies. In the suprachiasmatic area* **(A,B)** *the two proteins are co-expressed by OPCs.* **(C,D)** *Semi-coronal section of the optic nerve. The boxed areas in (A,C) are enlarged in (B,D) respectively, to illustrate the co-expression of Olig2 (green, nuclear staining) and ephrinB2 (red) in the same cell.* **(E)** *A schematic model of the contribution of ephrinB2/ephB interactions to the control of axonophilic migration of OPCs in the optic nerve. The ephrinB2 borne at the surface of OPCs is trans-activated by EphB, upon contact with the axons of retinal ganglionic cells. Activation of ephrinB2 has a pro-adhesive effect on OPCs, the migration of which is subsequently modified and can be either promoted or arrested, depending upon the stability of the ligand-receptor complex interaction. Scale bar (shown in A): A, 50 μm; B, 20 μm; C, 70 μm; D, 30 μm. Reprinted from Prestoz* et al. *(2004), with permission from Cambridge University Press.*

Astroglial specification is normally promoted by Bone Morphogenic Proteins (BMPs) (Mehler *et al.,* 2000), which inhibit oligodendrocyte development (Mekki-Dauriac *et al.,* 2002). Therefore, the choice of neuroepithelial progenitors between oligodendroglial and astroglial fate appears to depend upon a balance between the opposite effects of Shh and BMPs.

The possibility that OPCs can derive from other latero-dorsal regions of the brain at later stages of development has also been explored and confirmed by a series of converging data. First, the study of avian chimeras demonstrates a dorsoventral migration of dorsal OPCs, in the thoraco-cervical spinal cord (Cameron-Curry and LeDouarin, 1995). Second, using a Cre-*lox* approach to mark different progenitor populations and their complete progeny within the spinal cord of transgenic mice, a minor population of oligodendrocytes was shown to derive from a mediodorsal domain of progenitors expressing *Dbx1/Dbx2*. These later-born oligodendrocytes undergo a restricted migration to populate mainly the dorsolateral white matter (Fogarty *et al.,* 2005). In mice deficient for both *nkx6.1* and *6.2*, dorsal oligodendrogenesis was also observed, from E14.5, in the absence of *Olig2* progenitors (Cai *et al.,* 2005; Vallstedt *et al.,* 2005; Miller, 2005). Finally, recent work in the mouse spinal cord and telencephalon demonstrates that bFGF can induce, in vitro, the production of oligodendrocytes from *Olig2*$^-$ progenitors (Gabay *et al.,* 2003; Kessaris *et al.,* 2004), suggesting that OPC specification can occur elsewhere than in ventral *Olig2* progenitor domains.

Further studies, especially those using Cre-*lox* transgenic mice, should soon give a definitive picture of how oligodendrogenesis progresses during the course of neuroepithelial development. Although most of the OPCs of the spinal cord derive from *Olig2* progenitors, it is very likely that oligodendrogenesis is not restricted to the ventral *Olig2* progenitor domain, but develops sequentially from ventral to dorsal domains of the neural tube. More ventrally, at the margin of the floor plate, the *nkx2.2* domain has also been proposed to generate OPCs (Soula *et al.,* 2001; Fu *et al.,* 2002) and might be the initial site of oligodendrogenesis, due to the proximity of Shh source. Dorsally to the *Olig2* domain, the oligodendrogenic potential of neuroepithelium could be expressed at later stages of development, from E14.5 onwards in the mouse, in response to new environmental cues such as bFGF.

Similarities between neuronal and oligodendroglial mechanisms of development

According to a classical model of cell specification in the neural tube, stem cells give rise to neuroblasts and glioblasts, the latter cells differentiating into either astrocytes or oligodendrocytes. As an archetype of glioblast, the A2B5$^+$ O2A (Oligodendrocyte-type2 Astrocyte) progenitor, extensively studied by the group of M. Raff (Raff *et al.,* 1983; Barres *et al.,* 1992) generates O4$^+$ pre-oligodendrocytes in vitro, but can also give rise to pure populations of astrocytes in the presence of serum. In contrast, it does not produce neurons under normal conditions. Such a type of glioblast progenitor, common for astrocytes and oligodendrocytes, appeared nevertheless hardly detectable *in vivo*, although recent reports claim that these cells, called GRPs (Glial Restricted Progenitors), could exist in the spinal cord and in the cortex (Noble *et al.,* 2004).

An alternative model of neural cell specification results from a series of converging data obtained during the last five years, which indicates a close relationship between neuronal and oligodendroglial specification. Mechanisms of oligodendrocyte development *in vivo* show striking similarities to those for neuronal subtypes, including the emergence from localized regions of the neural tube, the involvment of common signalling pathways and downstream transcription factors. For instance, neurons and oligodendrocytes, albeit no astrocytes, differentiate from *plp/dm-20* progenitors, as shown *in vitro* (Figure 2) and *in vivo*, using a *plp-Cre/lox-Rosa26-lacZ* transgenic line (Delaunay *et al.,* in preparation). Similarly, somatic motor neurons and oligodendrocytes, but no astrocytes, derive from the progenitor domain expressing the basic Helix-Loop-Helix (bHLH) transcription factors, Olig1 and Olig2. Both neurons and oligodendrocytes are induced by Shh signalling and inhibited by BMP signalling which rather induces astroglial cells. In addition, unsuspected similarities in transcription factor usage between oligodendrocytes and neurons have been shown. The LIM-HomeoDomain (LIM-HD) gene *Nkx2.2* which controls the specification of neuronal identity in the spinal cord and hindbrain (Briscoe *et al.,* 1999; Pattyn *et al.,* 2003) is required for OPC development, at least for the differentiation of OPCs (Fu *et al.,* 2002). The bHLH gene *Mash1*, required for the neurogenesis in the basal ganglia of the telencephalon (Casarosa *et al.,* 1999), is upregulated during OPC differentiation (Kondo and Raff, 2000). Among others bHLH genes acting in parallel for neuronal and OPC development (Ross *et al.,* 2003), *Olig* genes have a key role (Rowitch *et al.,* 2002). *Olig* are required for the development of motor neurons and oligodendrocytes in the developing spinal cord (Lu *et al.,* 2002; Zhou *et al.,* 2002; Takebayachi *et al.,* 2002). Altogether, these similarities in the development of neurons and oligodendrocytes strongly suggest a common intrinsic program of differentiation and the existence of bipotent progenitor cells restricted to a neuronal / oligodendroglial fate. Experimental approaches combining the Cre-lox system and single progenitor cell labeling will probably allow to confirm this hypothesis.

A heterogeneity among the OPCs

The proliferation, migration and survival of OPCs have previously been shown to require platelet-derived growth factor A (PDGF-A) and its receptor PDGFR-α (Noble *et al.,* 1988; Richarson *et al.,* 1988; Pringle *et al.,* 1993; Fruttiger *et al.,* 1999; Klinghoffer *et al.,* 2002). However, several observations suggest that oligodendrocyte development *in vivo* requires other growth factors in addition to PDGF-A and that the *PDGFR-*α OPCs do not represent the overall population of OPCs. First, OPCs accumulate in the hindbrain in the absence of PDGF-A or PDGFR-α signalling (Fruttiger *et al.,* 1999; Klinghoffer *et al.,* 2002). Secondly, we have described the existence of a subpopulation of OPCs in the brain, characterized by the expression of *plp/dm-20* (Timsit *et al.,* 1995), which does not express PDGFR-α (Spassky *et al.,* 1998) and does not depend on PDGFR-α signalling for survival and proliferation (Spassky *et al.,* 2001a). These PDGF-α-independent OPCs expressing *plp/dm-20* are detected in several regions of the embryonic brain prior to the emergence of PDGFR-α-expressing cells (Spassky *et al.,* 1998, 2001a, 2002). In addition, after birth, *plp/dm-20* OPCs are also distinct from the population of *PDGFR-*α cells in the subventricular zone of cerebral cortex (Ivanova *et al.,* 2002). This raises the question of the nature of the trophic factor on which the *plp*$^+$ OPCs depend for their survival and proliferation. Recent investigations strongly suggest that a close member of PDGF family, Vascular endothelial growth factor

C (VEGF-C), is specifically required for the survival, proliferation and migration of *plp* OPCs (LeBras, unpublished data). Two closely related signalling pathways, PDGFRα and VEGFR-3, would thus selectively regulate the development of two distinct populations of oligodendrocyte precursor cells. An additional argument for the diversity of OPCs is the divergence in the intrinsic differential timing of OPCs from different brain regions (Spassky *et al.,* 2001b; Power *et al.,* 2002), consistent with the differing time courses of myelination in these regions. Finally, heterogeneity among OPCs has also been suggested in the adult brain by the presence of functional subpopulations of OPCs in MS lesions (Chang *et al.,* 2002).

A heterogeneity among oligodendrocytes has been suggested by Del Rio-Hortega (1928) and Penfield (1932). On the basis of morphological criteria (size of cell body, number of myelinated internodes, diameter of myelinated axons), these authors have distinguished four sub-groups of oligodendrocytes. More recently, other subpopulations have been described based on biochemical criteria, like expression of members of the collapsin response mediator protein (CRMP) family (Ricard *et al.,* 2001). It has also been observed that subsets of oligodendrocytes are not equally resistant to toxic agents such as cuprizone (Komoly *et al.,* 1987). At present, it is impossible to correlate this morphological and biochemical heterogeneity of the oligodendrocyte population with a specific embryonic origin, dorsal or ventral, or *plp/dm-20*$^{+}$ or *PDGFRα*. Nevertheless, the presence of different types of OPCs, requiring distinct signalling to develop, should be kept in mind, considering the possible implication in oligodendrocyte pathologies such as MS, where new signalling molecules might be potential therapeutic agents to restore oligodendrocytes.

Molecular control of OPC migration

During embryonic brain development, the OPCs originate from multiple ventricular foci and migrate along specific pathways (Ono *et al.,* 1997; Olivier *et al.,* 2001). The interaction of OPCs with their environment determines the extent of migration (Olivier *et al.,* 2001), as well as the guidance towards their final position in the brain. Several molecules have already been implicated in the migration and guidance of OPCs. The investigations carried out in the years 90 have demonstrated the role of growth factors and of extra-cellular matrix and integrins signalling molecules in the control of OPC migration. Growth factors, like FGF-2 and PDGF-AA secreted along the migratory pathways, have been reported to act as chemotrophic and kinetic factors for OPCs (Armstrong *et al.,* 1990; Milner *et al.,* 1997). Components of the extra-cellular matrix, like tenascin C (Garcion *et al.,* 2001), have been involved in the migration of optic nerve OPCs, while integrins (Milner and ffrench-Constant, 1994) or PSA-NCAM (Wang *et al.,* 1994), were implicated in the axonophilic migration of OPCs observed in the axonal tracts of the white matter and the optic nerve.

Recent studies have proposed new candidates, either secreted factors or contact proteins, in the molecular control of OPC migration. The role of axonal guidance molecules belonging to class 3 semaphorins and netrins has been investigated in the optic nerve and the spinal cord, during embryonic and neonatal periods. Sema 3A repels OPCs (Sugimoto *et al.,* 2001; Spassky *et al.,* 2002; Tsai *et al.,* 2003), while Sema 3F is attractive for those cells (Spassky *et al.,* 2002). The reports on the effect of netrin-1 appear more contradictory (Jarjour *et al.,* 2004). Netrin-1 was shown to be a repellent for OPCs in the neonatal optic nerve (Sugimoto *et al.,* 2001) and in the embryonic spinal cord (Tsai *et al.,* 2003; Jarjour *et al.,* 2003), wheraes the same factor was attractive for OPCs of the embryonic optic nerve (Spassky *et al.,* 2002). Although it cannot be excluded that differences in the response of OPCs might result from distinct experimental *in vitro* conditions, the effects of netrin-1 depend, at least in part, upon the type of netrin-1 receptor expressed by the oligodendrocyte. In the optic nerve, around birth, OPCs change their expression of netrin-1 receptors, from DCC alone to DCC and Unc5h1. Consequently, the embryonic OPCs are attracted by netrin-1, but after birth OPCs are repelled by this factor (Spassky *et al.,* 2002). Therefore, OPCs express a variety of receptors to class 3 semaphorins and netrin-1 factors, allowing multiple and adaptive responses to the environmental cues found in the course of their migration. This might be related to the presence of different types of OPCs, originating from different ventricular sources. Alternatively, it might indicate that, in the course of ON colonization, OPCs modulate their response to one secreted factor by changing the expression of its specific receptors.

The chemokines, which are secreted signalling molecules that regulate leukocyte migration in a target-specific fashion (Baggiolini, 1998), have also been implicated in the control of OPC migration. Especially, the CXCL1 ligand stimulates the proliferation of spinal cord OPCs in synergy with PDGF-AA (Wu *et al.,* 2000). This chemokine inhibits the migration of OPCs through activation of its receptor CXCR2 and has been proposed as a stop and patterning signal for OPCs in the neonatal spinal cord (Tsai *et al.,* 2002). The participation of others members of this family to the migration of OPCs in the brain is moreover very likely (M. Dubois-Dalcq, personal communication).

The migration of OPCs appears also, to some extent, axonophilic, as best documented by the invasion of the optic nerve, where OPCs migrate in a highly enriched axonal environment. In this context of intercellular contact molecules, the role of ephrins and their Eph receptors had not been examined until our study (Prestoz *et al.,* 2004). We showed that ephrinB2 ligand was expressed by OPCs and induced dramatic changes in the adhesion and migration of OPCs on their substrate, upon contact and activation by EphB receptors. We thus proposed that the Eph/ephrin interactions between axons and migrating OPCs determine a recognition code controlling the progress or the arrest of OPC migration. EphB/ephrinB interaction has been reported to induce cell-cell or cell-matrix repulsion. This effect is associated with a loss of polymerized F-actin structures and a disassembly of focal adhesions (Cowan and Henkemeyer, 2001) and is dependent of endocytosis of EphB/ephrinB complexes at the cell surface. Endocytosis is necessary and sufficient to promote termination of adhesion, cell detachment and further cell repulsion (Zimmer *et al.,* 2003). In particular, blocking endocytosis through C-terminal truncation of either EphB2 or ephrinB1, or both molecules, results in a switch from a repulsive effect of ephrinB1 activation to an attractive effect and a cell detachment. The progress or halt of OPC migration could therefore be linked to the control of EphB-ephrinB bi-directional endocytosis. Such a regulation would explain how migrating OPC stop and fix at the contact of the axon they will myelinate later in development.

Finally, the molecules that influence the migration of OPCs include a combination of short-range attractants and repellents

and long-range chemoattractants and chemorepellents. The role of each of these factors can differ during the course of development and between brain territories, probably due to changes in the receptor repertoire of OPCs and to environmental co-factors. To date, the participation of these ligands and their specific receptors in oligodendrocyte diseases, like MS, has not been reported. Studies on MS plaques and demyelinated animal models have nevertheless started and will most likely confirm the importance of these molecules in the maintenance and repair of oligodendroglial populations in the adult. A synthetic view of the molecular control of oligodendrocyte migrations is therefore a challenging issue for further therapeutic developments against demyelinating pathologies.

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