Development and Differentiation of Oligodendrocytes

Masahisa YAMADA, Tetsushi KAGAWA, and Kazuhiro IKENAKA

National Institute for Physiological Sciences, Okazaki National Research Institutes, Okazaki, 444 Japan

Key words: oligodendrocyte, O-2A progenitor cell, PLP, PDGFR α .

The central nervous system (CNS) is one of the most complicated organ systems in vertebrates. There are many different kinds of neurons and glial cells, which are believed to be developed from the neuroepithelial cells of the embryonic neural tube (Fig. 1). Glial cells constitute the major component of the CNS. Early studies of gliogenesis *in vitro* suggested the generation of two main glial classes, astrocytes and oligodendrocytes, during CNS development.

Type-1 astrocytes first appear in the rodent brain several days before birth, whereas oligodendrocytes first appear after birth. Oligodendrocytes form the myelin of the CNS and differentiate from bipotential (O-2A) progenitor cells, which also have the capacity to differentiate into type-2 astrocytes, at least *in vitro* [1]. However, there is no direct evidence showing its presence *in vivo* in normal rats. O-2A progenitor cells are known to be highly migratory, as shown by transplantation into the brain of myelin-deficient mutants [2]. Therefore, it is not clear yet from which part of the nervous system the O-2A cells originate.

The developmental processes of the oligodendrocyte in the CNS includes the proliferation, migration, differentiation, and survival processes, all of which are supported by a variety of factors [3]. Various environmental signals are considered to control the proliferation and differentiation of glial precursor cells that generate oligodendrocytes. In this paper, recent progress in identifying the origin of oligodendrocytes and factors affecting oligodendrocyte development are reviewed.

I. Origin of Oligodendrocytes

The differentiation of the O-2A progenitor cells into oligodendrocytes and type-2 astrocytes has been well characterized *in vitro* and *in vivo* [4–12]. Furthermore,

several intermediate stages can be identified between the O-2A progenitor and the mature oligodendrocyte (galactocerebroside (GalC)-positive cell). The study on the differentiation of O-2A progenitors into oligodendrocytes depends on the application of sequential immunocytochemical markers (Fig. 2). The ordered and partially overlapping expression of surface antigens recognized by monoclonal antibodies, A2B5, O4, and O1, distinguishes three consecutive oligodendrocyte lineages in the rat. The O-2A progenitors are identified by their bipolar morphology and reaction with A2B5 antibody [1, 13], which binds to one or more of the gangliosides. Other antibodies which recognize the O-2A progenitor cell-specific markers have also been obtained, including anti-GD3 ganglioside [14] and anti-NG2 chondroitin-sulfate proteoglycan antibodies [15]. The oligodendroblast cell is recognized by the mouse monoclonal antibody (mAb) O4 [16], but it does not yet express GalC on its surface. The antibody against O4 recognizes two sulfated glycolipids, sulfatide and seminolipid [17, 18]. The GalC-positive cell is a post-mitotic oligodendrocyte that is recognized by the mouse mAb O1 [16]. Fur-

Fig. 1. Schematic representation of the neuroectoderm lineage. Neurons, astrocytes, and oligodendrocytes are generated from neuroepithelial cell in the CNS.

Received on December 22, 1995

Correspondence should be addressed to: Kazuhiro Ikenaka, National Institute for Physiological Sciences, Okazaki National Research Institutes, 38 Aza-nishigonaka, Myodaiji-cho, Okazaki, 444 Japan. Tel: +81-564-55-7841, Fax: +81-564-55-7843, E-mail: ikenaka@nips.ac.jp

Fig. 2. Schematic representation of three stages of the oligodendrocyte lineage recognized by antibodies against membrane surface components. Studies on the differentiation of O-2A progenitors into oligodendrocytes depends on the application of sequential immunocytochemical markers. The ordered and partially overlapping expression of cell surface antigens recognized by A2B5, O4, and O1 monoclonal antibodies distinguish three consecutive developmental stages of oligodendrocytes.

thermore, oligodendrocyte maturation is defined by the expression of myelin-specific proteins, including 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CN-Pase) [19–22], myelin basic protein (MBP) [23, 24] and myelin proteolipid protein (PLP) [6, 25].

It is widely accepted that oligodendrocytes originate from the neuroepithelial cells. Thus, it should be possible to trace its lineage back to the neuroepithelial cells. However, it is not clear which antibodies recognize the precursor of O-2A cells. GD3 seems to be present in the very early precursor cells of oligodendrocytes [9, 11], however, it is also present in other types of brain cells.

Recently, *in situ* hybridization using probes for the alpha subunit of the receptor for platelet-derived growth factor (PDGFR α) [26, 27], 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) [28, 29] and DM-20 [30], the product of alternative splicing of the myelin proteolipid protein (PLP) gene [31] has yielded evidence that cells of the oligodendrocyte lineage arise from rather restricted regions of the nervous system and then migrate widely into other regions. These observations agree well with the result obtained by Warf *et al.* [32], who showed that GalC-positive oligodendrocytes developed in cultures of ventral spinal cord cells from E14 rat embryos, but did not develop in cultures of dorsal cells from E14 embryos. In contrast, oligodendrocytes developed in cultures of both ventral and dorsal spinal cord cells from E16 embryos. This suggested that oligodendrocyte precursor cells are present in the ventral half but not the dorsal half of the cord at E14, but spread to the

dorsal half by E16. However, there are differences between the localization of PDGFR α mRNA and DM-20 mRNA in the early diencephalon and spinal cord [27–29]. These two populations may be two independent pools of oligodendrocyte precursors, or either one may not be a precursor of oligodendrocytes at an early stage of development. Since evidence is accumulating which indicates that PDGFR α -expressing cells differentiate into oligodendrocytes, we will describe the migration pattern of PDGFR α -positive cells. Most of the results have been obtained by *in situ* hybridization techniques, but similar results have also been obtained by immunohistochemical analysis using antibody against PDGFR α [33].

Direct evidence indicating that PDGFR α -positive cells in the embryonic rat spinal cord are oligodendrocyte precursors came from immunopanning experiments. Cultures of >99% pure PDGFR α -positive cells were established from E17 rat spinal cords by immunoselection with an antibody against the extracellular domain of PDGFR α . When cultured in the presence of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) these cells displayed the characteristic morphology and antigenic phenotype (A2B5 $^{+}$, NG2 $^{+}$) expected for O-2A progenitors. When transferred to defined medium lacking growth factors, they all differentiated into GalC-positive oligodendrocytes [34]. Therefore, PDGFR α -positive cells in the ventral half of the embryonic rat (or mouse) spinal cord are oligodendrocyte precursors; however, it cannot be concluded that all oligodendrocytes originate from PDGFR α -positive cells. It is possible that there are other lineages of oligodendrocytes that differentiate from PDGFR α -negative cells.

PDGFR α -positive cells in the rat spinal cord originate from a very restricted region of the ventricular zone at around E14. These cells are in the ventral half of the spinal cord in two longitudinal columns, one on each side of the central canal. Initially, these columns are only two cells wide but the cells subsequently appear to proliferate and disseminate throughout the spinal cord [27, 28]. This pattern of migration has also been observed in the chick spinal cord. Ono *et al.* [35] showed that monoclonal antibody O4 labels all stages of chick oligodendrocyte lineages from the very early stage. In contrast, as mentioned before, O4 only stains much later stages in rodents. Immunolabelling of transverse sections of the E6 chick spinal cord revealed a focus of O4-positive cells in the ventricular zone in the ventral half of the cord that corresponds closely to the PDGFR α -positive cells in the rat. These O4-positive cells migrated radially from the ventricular zone toward the surface, which is also sim-

ilar to what has been observed in the rat.

It is an interesting issue to ask what the signals are that determine the cells of a particular region of ventricular zone to become oligodendrocytes.

II. Factors Influencing Development of Oligodendrocytes

O-2A progenitors are stimulated to proliferate by various combinations of environmental growth factors. If these mitogens are absent, O-2A progenitors will stop dividing and begin to differentiate into oligodendrocytes. It has been shown previously that four peptide growth factors promote DNA synthesis in the O-2A progenitors *in vitro*, PDGF, bFGF, neurotrophin-3 (NT-3), and insulin-like growth factor-I (IGF-I), all of which are synthesized by other type of cells [3, 36–44]. PDGF promotes both the proliferation and the survival of O-2A progenitors [40–42], whereas IGF-I or NT-3 alone promote only survival [38]. bFGF alone does not promote survival or stimulate DNA synthesis, whereas the combination of bFGF and IGF-I stimulates DNA synthesis in the O-2A progenitor cells [39, 43, 44]. Thus, multiple extracellular signaling molecules might collaborate to promote proliferation of O-2A progenitors.

III. PLP Gene Products also Affect Development of Oligodendrocytes

One approach to identifying environmental signals that are responsible for neural development is to study the molecular defect in mutant animals which show abnormal development in the nervous system. We have found another humoral factor that affects the development of oligodendrocytes from the analysis of a mutant mouse. PLP is one of the major myelin proteins of CNS myelin and was considered to play a role in stabilization of the myelin membrane (Fig. 3). However, it has been demonstrated that PLP gene expression begins long before the myelin structure is formed (Fig. 4) [45, 46] and mutations within the PLP gene cause abnormalities in the maturation and sur-

Fig. 3. PLP in the CNS myelin. The major CNS myelin proteins, myelin basic protein (MBP), and the proteolipid protein (PLP), represent 70–80% of the total myelin protein content of the membrane. PLP is thought to promote the apposition of extracellular surfaces of myelin lamellae.

Fig. 4. Cell differentiation and PLP gene expression.

A: The differentiation of glial cells has been well characterized *in vitro* and *in vivo*. The reverse transcription-PCR amplified product from DM-20 mRNA was detected throughout the developmental stages investigated. **B:** DM-20 mRNA is formed by an alternative splicing from the PLP gene. PLP mRNA becomes detectable in P2 brain.

vival of oligodendrocytes [47]. These facts indicate that the PLP gene products are somehow involved in oligodendrocyte development. The early PLP gene product is not PLP, but is DM-20, which is the product of alternative splicing of the PLP gene [31].

The *jimpy* mouse is one of the PLP mutants, which shows premature cell death of oligodendrocytes and severe dysmyelination. An A to G conversion at the conserved “AG” residues of the 3′-splice site of the fourth intron of the PLP gene was found in *jimpy* mice [27, 48], resulting in the deletion of the fifth exon of *jimpy* PLP mRNA [49]. Previous studies have reported that *jimpy* oligodendrocytes in culture survive and produce more myelin proteins by the addition of normal astrocyte conditioned medium (Fig. 4) [50, 51], and that partial recovery of *jimpy* oligodendrocyte function was also observed in shiverer brain transplanted with embryonic *jimpy* brain fragments [52]. These results suggest that PLP gene products could induce the secretion of a humoral factor that regulates maturation/survival of oligodendrocytes (oligodendrocyte growth factor, OGF) and this factor is absent from *jimpy* brain. It is hard to think that PLP or DM-20 themselves are secreted into the medium. These proteins are called proteolipid proteins because

they can be extracted by chloroform-methanol, and so are very hydrophobic. OGF may be a processed fragment of PLP/DM-20 or it may be a totally unrelated factor induced by the presence of PLP/DM-20. Identification and characterization of this OGF are important in understanding the development of oligodendrocytes.

Previous studies have reported that conditioned medium from B104 neuroblastoma cell line promotes the growth of O-2A progenitor of neonatal and mature rat brain, and increase the number of oligodendrocytes [53–55]. B104 cells produce mRNA for DM-20 [45]. Moreover, it has also been shown that the relative amounts of the factors secreted is B104>C6>RN22 [52], which correlates well with the amount of DM-20 mRNA found in these cells [44]. These findings prompted us to speculate that the factor(s) secreted by B104 cells is the gliogenic factor(s) (OGF) missing in the *jimpy* brain.

Culture supernatants from DM-20-producing cell lines (G26, B104, B16) or non-producing cells (NIH3T3) were added to primary glial cell cultures from E17 mouse brain. After 4 d, the number of oligodendrocytes present in cultures with supernatants from DM-20-producing cells was significantly higher than that of control cultures, but not with the NIH3T3-supernatant [56].

To investigate whether PLP gene expression is directly involved in this process, NIH3T3 cells were forced to produce PLP or DM-20. By the addition of the supernatants from the PLP/DM-20 transformants, the number of oligodendrocytes increased [56] (Fig. 5). This activity present in the PLP/DM-20 supernatants was completely inhibited by anti-PLP antibody (AA3), which recognizes the C-terminus of PLP. Thus, PLP/DM-20 themselves (although this possibility is low as mentioned above) or their fragments (containing C-terminal portion) were released into culture medium and increased the number of oligodendrocytes (Yamada *et al.*, manuscript in preparation).

To investigate more directly that the PLP gene products themselves contain the growth activity, purified PLP was added to the same assay system. Purified PLP clearly increased the number of oligodendrocytes in culture, which was inhibited by anti-PLP antibody. Furthermore, the OGF activity present in the conditioned medium from DM-20-producing cell lines, including B104, was also inhibited by anti-PLP antibody. Since the supernatant from B104 is known to promote the growth of O-2A progenitors, PLP/DM-20 themselves or their fragments are suggested to cause the increase in the number of O-2A progenitors (Ya-

Fig. 5. Conditioned media from NIH3T3 cells producing PLP or DM-20 increase GalC-positive cell number. Supernatants from clones of NIH3T3 cells producing PLP or DM-20 were concentrated and dialyzed. Samples (30 µg protein/ml) were added to mix glial cell cultures. Cells were incubated for 4 d in a serum-free chemically defined medium and immunostained for GalC. Ten fields (at ×100 magnification) were counted for GalC-positive oligodendrocytes on the coverslip. Results are shown as mean values from triplicate experiments.

mada *et al.*, manuscript in preparation).

The PLP gene products acting extracellularly increased the number of oligodendrocytes. This function may be modulated by the activity of other growth factor(s). In the assay system of the primary mouse glial cell culture, chemically defined medium contains insulin at the concentration of 10 µg/ml. This concentration of insulin is high enough to activate IGF-I receptors. Thus, it is highly possible that the combination of PLP and IGF-I promotes the proliferation of O-2A progenitor. This possibility is now under investigation.

Previous results from studies on dysmyelinating PLP mutants suggested that a product of the PLP gene may be involved in cell survival and differentiation. This study clearly demonstrated that PLP gene products containing the C-terminal region of PLP can be secreted as a humoral factor that promotes the growth of oligodendrocytes.

IV. Possible Generation of Oligodendrocyte Cell Lineage by Sonic Hedgehog

An interesting and important issue to ask in understanding the development of oligodendrocytes is: what determines a specific region (only one or two cell

widths) of E14 rat spinal cord ventricular zone to express the PDGFR α gene and thus to become oligodendrocyte-lineage. An important factor in determining the dorso-ventral pattern in the spinal cord is the *Sonic hedgehog* (*Shh*). *Shh* is expressed by both the notochord and the floor plate. When ectopic notochord was grafted into a dorso-lateral position relative to the endogenous notochord of a chick embryo, a second floor plate was induced, as expected. It also resulted in the appearance of a new focus of PDGFR α -positive oligodendrocyte precursors in the ventricular zone, the same distance from the induced floor plate as was the original focus from the endogenous floor plate [34]. Thus, *Shh* seems to play a major role in determining neuroepithelial cell fate to oligodendrocyte, as well as to motor neurons [57].

We are grateful to Drs. William D. Richardson and Akiko Nishiyama for informing us of unpublished results.

REFERENCES

- Raff MC, Miller RH, and Noble M: A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* 303: 390–396, 1983
- Lachapelle F, Gumpel M, Baulac M, Jacque C, Duc P, and Baumann N: Transplantation of CNS fragments into the brain of shiverer mutant mice: Extensive myelination by implanted oligodendrocytes. I. Immunohistochemical studies. *Dev Neurosci* 6: 325–334, 1983
- Barres BA and Raff MC: Control of oligodendrocyte number in the developing rat optic nerve. *Neuron* 12: 935–942, 1994
- Bansal R, Stefansson K, and Pfeiffer SE: Proligodendroblast antigen, a developmental antigen expressed by A007/O4-positive oligodendrocyte progenitors prior to the appearance of sulfatide and galactocerebroside. *J Neurochem* 58: 2221–2229, 1992
- Curtis R, Cohen J, Fok-Seang J, Hanley MR, Gregson NA, Reynolds R, and Wilkin GP: Development of macroglial cells in rat cerebellum. I. Use of antibodies to follow early *in vitro* development and migration of oligodendrocytes. *J Neurocytol* 17: 43–54, 1988
- Dubois-Dalcq M, Behar T, Hudson L, and Lazzarini RA: Emergence of three myelin proteins in oligodendrocyte cultures without neurons. *J Cell Biol* 102: 384–392, 1986
- Gard AL and Pfeiffer SE: Two proliferative stages of the oligodendrocyte lineage (A2B5⁺O4⁻ and O4⁺GalC⁻) under different mitogenic control. *Neuron* 5: 615–625, 1990
- Goldman JE: Regulation of oligodendrocyte differentiation. *Trends Neurosci* 15: 359–362, 1992
- Hardy R and Reynolds R: Proliferation and differentiation potential of rat forebrain oligodendroglial progenitors both *in vitro* and *in vivo*. *Development* 111: 1061–1080, 1991
- Raff MC: Glial cell diversification in the rat optic nerve. *Science* 243: 1450–1455, 1989
- Reynolds R and Wilkin GP: Development of macroglial cell in rat cerebellum II: An *in situ* immunohistochemical study of oligodendroglial lineage from precursor to mature myelinating cell. *Development* 102: 409–425, 1988
- Warrington AE and Pfeiffer SE: Proliferation and differentiation of O4⁺ oligodendrocytes in postnatal rat cerebellum: Analysis in unfixed tissue slice using anti-glycolipid antibodies. *J Neurosci Res* 33: 338–353, 1992
- Behar T, McMorris FA, Novotny EA, Barker JL, and Dubois-Dalcq M: Growth and differentiation properties of O-2A progenitor cells purified from rat cerebral hemispheres. *J Neurosci Res* 21: 168–180, 1988
- Levi G, Aloisi F, and Wilkin GP: Differentiation of cerebellar bipotential glial precursors into oligodendrocytes in primary culture: Developmental profile of surface antigens and mitotic activity. *J Neurosci Res* 18: 407–417, 1987
- Levine JM, Stincone F, and Lee YS: Developmental and differentiation of glial precursor cells in the rat cerebellum. *Glia* 7: 307–321, 1993
- Sommer I and Schachner M: Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: An immunocytological study in the central nervous system. *Develop Biol* 83: 311–327, 1981
- Singh H and Pfeiffer SE: Accumulation of myelin-associated galactolipids by primary cultures from the dissociated fetal brain. *J Neurochem* 45: 1371–1381, 1985
- Bansal R, Warrington AE, Gard AL, Ranscht B, and Pfeiffer SE: Multiple and novel specificities of monoclonal antibodies O1, O4 and R-mAb used in the analysis of oligodendrocyte development. *J Neurosci Res* 24: 548–557, 1989
- Tsukada Y and Kurihara T: 2',3'-Cyclic nucleotide 3'-phosphodiesterase: Molecular characterization and possible functional significance. In: *Myelin: Biology and Chemistry*, ed. Martenson RE, CRC Press, Boca Raton, pp 449–480, 1992
- Pfeiffer SE, Barbarese E, and Bhat S: Non-coordinate regulation of myelinogenic parameters in primary cultures of dissociated fetal rat brain. *J Neurosci Res* 6: 369–380, 1981
- McMorris FA: Cyclic AMP induction of the myelin enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase in rat oligodendrocytes. *J Neurochem* 14: 506–515, 1983
- Bansal R and Pfeiffer SE: Developmental expression of 2',3'-cyclic nucleotide 3'-phosphohydrolase in dissociated fetal rat brain cultures and rat brain. *J Neurosci Res* 14: 21–34, 1985
- Mirsky R, Winter J, Abney ER, Pruss RM, Gavrilovic J, and Raff MC: Myelin-specific proteins and glycolipids in rat Schwann cells and oligodendrocytes in culture. *J Cell Biol* 84: 483–494, 1980
- Barbarese E and Pfeiffer SE: Developmental regulation of myelin basic protein in dispersed cultures. *Proc Natl Acad Sci USA* 78: 1953–1957, 1981
- Macklin WB, Campagnoni CW, Deininger PL, and Gardiner MV: Structure and expression of the mouse myelin proteolipid protein gene. *J Neurosci Res* 18: 383–394, 1987
- Pringle NP, Mudhar HS, Collarini EJ, and Richardson WD: PDGF receptors in the rat CNS: During late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* 115: 535–551, 1992
- Pringle NP and Richardson WD: 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, 1993
28. Yu WP, Collarini EJ, Pringle NP, and Richardson WD: 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, 1994
 29. Scherer SS, Braun PE, Grinspan J, Collarini E, Wang DY, and Kamholz J: Differential regulation of the 2',3'-cyclic nucleotide 3'-phosphodiesterase gene during oligodendrocyte development. *Neuron* 12: 1363-1375, 1994
 30. Timsit S, Martinez S, Allinquant B, Peyron F, Puelles L, and Zalc B: Oligodendrocytes originate in a restricted zone of the embryonic ventral neural tube defined by DM-20 mRNA expression. *J Neurosci* 15: 1012-1024, 1995
 31. Nave K-A, Lai C, Bloom FE, and Milner RJ: Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. *Proc Natl Acad Sci USA* 84: 5665-5669, 1987
 32. Warf BC, Fok-Seang J, and Miller RH: Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. *J Neurosci* 11: 2477-2488, 1991
 33. Nishiyama A, Lin XH, Giese N, Heldin CH, and Stallcup WB: Colocalization of NG2 proteoglycan and PDGF α receptor on O2A progenitor cells in the developing rat brain. *J Neurosci Res* 43: 299-314, 1996
 34. Richardson WD, Pringle NP, Yu WP, Collarini EJ, and Hall A: Origins and early development of oligodendrocytes. In: *Glial Cell Dev*, ed. Jessen KR and Richardson WD, Bio Scientific Publishers, Oxford (in press)
 35. Ono K, Bansal R, Payne J, Rutishauser U, and Miller RH: Early development and dispersal of oligodendrocyte precursors in the embryonic chick spinal cord. *Development* 121: 1743-1754, 1995
 36. Barres BA, Hart IK, Coles HSR, Burne JF, Voyvodic JT, Richardson WD, and Raff MC: Cell death in the oligodendrocyte lineage. *J Neurobiol* 23: 1221-1230, 1992
 37. Barres BA, Schmid R, Sendtner M, and Raff MC: Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* 118: 283-295, 1993
 38. Barres BA, Raff MC, Gaese F, Bartke I, Dechant G, and Barde YA: A crucial role for neurotrophin-3 in oligodendrocyte development. *Nature* 367: 371-357, 1994
 39. McMorris FA and Dubois-Dalcq M: Insulin-like growth factor promotes cell proliferation and oligodendrocyte commitment in rat glial progenitor cells developing *in vitro*. *J Neurosci Res* 21: 199-209, 1988
 40. Noble M, Murray K, Stroobant P, Waterfield MD, and Riddle P: Platelet-derived growth factor promotes divisions and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* 333: 560-562, 1988
 41. Raff MC, Lillien LE, Richardson WD, Brune JF, and Noble MD: Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* 333: 562-565, 1988
 42. Richardson WD, Pringle N, Mosley MJ, Westermarck B, and Dubois-Dalcq M: A role for platelet-derived growth factor in normal gliogenesis in the nervous system. *Cell* 53: 309-319, 1988
 43. McKinnon RD, Matsui T, Dubois-Dalcq M, and Aaronson SA: FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* 5: 603-614, 1990
 44. McKinnon RD, Smith C, Behar T, Smith T, and Dubois-Dalcq M: Distinct effects of bFGF and PDGF on oligodendrocyte progenitor cells. *Glia* 7: 245-254, 1993
 45. Ikenaka K, Kagawa T, and Mikoshiba K: Selective expression of DM-20, an alternatively spliced myelin proteolipid protein gene product, in developing nervous system and in nonglial cells. *J Neurochem* 58: 2248-2253, 1992
 46. Timsit S, Bally-Cuif L, Colman DR, and Zalc B: DM-20 mRNA is expressed during the embryonic development of the nervous system of the mouse. *J Neurochem* 58: 1172-1175, 1992
 47. Skoff RP and Knapp PE: Phenotypic expression of X-linked genetic defects affecting myelination. In: *Myelin: Biology and Chemistry*, ed. Martenson RE, CRC Press, Boca Raton, pp 653-676, 1992
 48. Nave K-A, Bloom FE, and Milner RJ: A single nucleotide difference in the gene for myelin proteolipid protein defines the *jimpy* mutation in mouse. *J Neurochem* 49: 1873-1877, 1987
 49. Moriguchi A, Ikenaka K, Furuichi T, Okano H, Iwasaki Y, and Mikoshiba K: The fifth exon of the myelin proteolipid protein-coding gene is not utilized in the brain of *jimpy* mutant mice. *Gene* 55: 333-337, 1987
 50. Bartlett WP, Knapp PE, and Skoff RP: Glial conditioned medium enables *jimpy* oligodendrocyte to express properties of normal oligodendrocytes; Production of myelin antigens and membranes. *Glia* 1: 253-259, 1988
 51. Knapp PE, Skoff RP, Benjamins JA, and Studzinski DM: Oligodendrocytes from hypomyelinated *jimpy* mice synthesize an altered PLP *in vitro*. *Trans Am Soc Neurochem* 23: 134, 1992
 52. Lachapelle F, Lapie P, Campagnoni AT, and Gumpel M: Oligodendrocytes of *jimpy* phenotype can be partially restored by environmental factors *in vivo*. *J Neurosci Res* 29: 235-243, 1991
 53. Bottenstein JE, Hunter SF, and Seidel M: CNS neuronal cell line-derived factors regulated gliogenesis in neonatal rat brain cultures. *J Neurosci Res* 20: 291-303, 1988
 54. Hunter SF and Bottenstein JE: Bipotential glial progenitors are targets of neuronal cell line-derived growth factors. *Dev Brain Res* 49: 33-49, 1991
 55. Hunter SF and Bottenstein JE: O-2A glial progenitors from mature brain respond to CNS neural cell line-derived growth factors. *J Neurosci Res* 28: 574-582, 1991
 56. Nakao J, Yamada M, Kagawa T, Kim SU, Miyao Y, Shimizu K, Mikoshiba K, and Ikenaka K: Expression of proteolipid protein gene is directly associated with secretion of a factor influencing oligodendrocyte development. *J Neurochem* 64: 2396-2403, 1995
 57. Roelink H, Porter JA, Chiang C, Tanabe Y, Chang DT, Beachy PA, and Jessell TM: Floor plate and moter neuron induction by different concentrations of the aminoterminal cleavage product of sonic hedgehog proteolysis. *Cell* 81: 445-455.