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The Oligodendrocyte

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INTRODUCTION

The oligodendrocyte is the cellular component of the brain and spinal cord responsible for the production of myelin, a fatty insulation composed of modified plasma membrane that surrounds axons and promotes the rapid and efficient conduction of electrical impulses along myelinated axons (Bunge, 1968). Myelination is essential for the normal functioning of the vertebrate central nervous system (CNS). As failures in myelination are associated with disruptions of normal impulse conduction, such alterations can interfere with neurological function as profoundly as the loss of neurons themselves. Thus, disruption of CNS myelin through injury, pathological degeneration, or genetic reasons leads to severe functional deficits and frequently a reduction in life span. Abnormalities related to myelination may represent the single largest category of neurological dysfunction in the CNS, being seen in multiple developmental syndromes, in traumatic injury of many varieties, and as a result of chronic degenerative processes.

Along with the importance associated with the function of the oligodendrocyte, this cell and its ancestors are of particular interest as a model system for the study of a large range of complex problems in cellular development. The developmental events leading to the generation of oligodendrocytes are currently among the best understood of all such processes (although not without controversy, as will be discussed). For this reason, studies on the precursor cells that give rise to oligodendrocytes have provided a fertile ground for the elucidation of general principles in developmental and cellular biology.

GENERATION OF OLIGODENDROCYTES FROM THEIR IMMEDIATE PRECURSOR CELLS

The cell that is believed to be the direct ancestor of the oligodendrocyte is a precursor cell that, at least *in vitro*, has the capacity to generate both oligodendrocytes and a particular subset of astrocytes (known as type-2 astrocytes). For this reason, these precursor cells originally were named oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells (Raff *et al*., 1983). These cells constitutively differentiate into oligodendrocytes but require exposure to specific environmental signals in order to give rise to astrocytes (Hughes *et al*., 1988; Lillien and Raff, 1990). Whether O-2A progenitors actually generate type-2 astrocytes during normal development has been a matter of considerable controversy and still remains unclear (Knapp, 1991; Espinosa de los Monteros *et al*., 1993). Difficulties in identifying type-2 astrocytes as a derivative of these progenitor cells has led multiple laboratories to refer to this identical population of cells as oligodendrocyte precursor cells (OPCs). In recognition of this still unresolved controversy, the abbreviation O-2A/OPC will be used throughout this chapter.

Although O-2A/OPCs do not require the action of cell-extrinsic signaling molecules to generate oligodendrocytes, they do require the action of such molecules to undergo division. The most extensively characterized mitogen for these precursor cells is the platelet-derived growth factor AA homodimer (PDGF-A) (Noble *et al*., 1988; Richardson *et al*., 1988). In the intact CNS, PDGF-A is ubiquitously distributed, being synthesized by both astrocyte and neuronal populations (Yeh *et al*., 1991; Hutchins and Jefferson, 1992). Overexpression of PDGF-A results in a dramatic increase in the number of spinal cord O-2A/OPCs (Calver *et al*., 1998), while in PDGF-A knockouts, the number of these precursor cells is dramatically reduced (Fruttiger *et al*., 1999).

The responsiveness of O-2A/OPCs to PDGF can be modified by synergistic interactions with a variety of other signaling molecules. For example, the chemokine CXCL1/GRO-α enhances the proliferation of spinal cord-derived O-2A/OPCs exposed to PDGF in a concentration-dependent manner (Robinson *et al*., 1998; Wu *et al*., 2000). Responsiveness to PDGF is also enhanced by co-exposure to neurotrophin-3 (NT-3; Barres *et al*., 1994b; Ibarrola *et al*., 1996) and basic fibroblast growth factor (FGF-2; Bogler *et al*., 1990; McKinnon *et al*., 1990).

In at least some cases, co-exposure to PDGF and other cytokines also alters the balance between self-renewal and differentiation in dividing O-2A/OPCs. Co-exposure to FGF-2, for example, causes these precursor cells to become trapped in a continuous program of self-renewal and appears to almost

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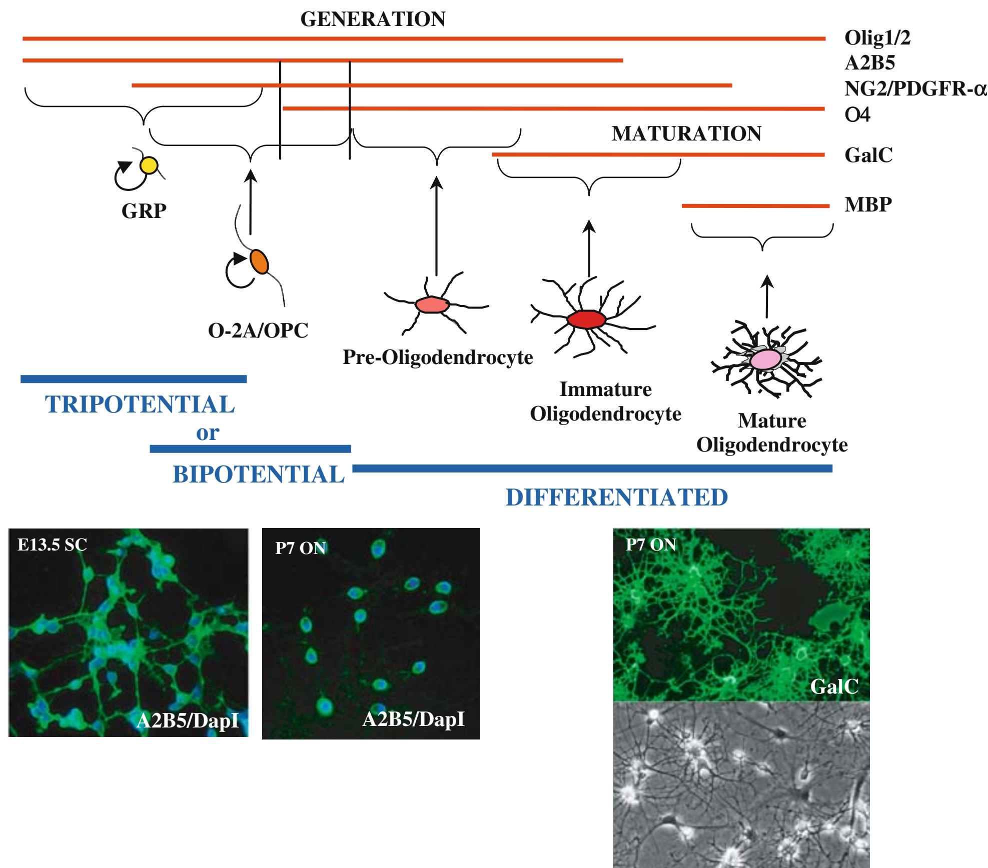

FIGURE 1. Antigenic and morphological development leading to the generation of oligodendrocytes in the spinal cord. The earliest progenitor cell that appears to be specialized for the generation of glial cells is the tripotential glial-restricted precursor (GRP) cell, which can be isolated from the rat spinal cord as early as E12.5. These cells are all A2B5^{+}, and a subset of the ones derived from ventral spinal cord express the *Olig1* and *Olig2* genes. Beginning at ~E14 in the rat, some GRP cells (putatively from the Olig-expressing subset of GRP cells) express the NG2 proteoglycan and the alpha receptor for platelet-derived growth factor. The first cells that can be unambiguously defined as O-2A/OPCs are labeled with the O4 antibody and are seen at E18/19 in the rat spinal cord. O-2A/OPCs express a distinctive bipolar morphology. At E20/21, the first cells appear that express galactocerebroside (GalC), a myelin-specific galactolipid. GalC^{+} oligodendrocytes develop a multipolar morphology very different from that of the O-2A/OPC from which they are derived. These oligodendrocytes continue to mature to express such proteins as myelin basic protein (MBP). For more detailed discussion on the derivation of O-2A/OPCs from GRP cells, the reader is referred to Gregori *et al*. (2002).

It is not yet known if similar developmental progressions pertain outside of the spinal cord. As discussed in Noble *et al*. (2003), the cortex may be more complex than the spinal cord. Whether or not the O-2A/OPCs of the optic nerve are derived from GRP-like cells is also not known.

completely block the generation of oligodendrocytes (Bogler *et al*., 1990). Co-exposure to NT-3 and PDGF also greatly enhances the extent of self-renewal (Ibarrola *et al*., 1996). Such studies have revealed what has proven to be an important general principle in precursor cell biology, to whit, the mitotic capacity of a lineage-restricted precursor cell is regulated in part by the specific combination of signaling molecules to which the cell is exposed. The number of divisions such precursor cells can undergo can be greatly altered depending on exposure to different combinations and concentrations of cell-extrinsic signaling molecules.

The ability of combinations of growth factors to enhance precursor cell self-renewal is counterbalanced by the action of other signaling molecules that promote oligodendrocyte generation. The most extensively studied of such signaling molecules is thyroid hormone (TH), which has been of particular interest due to the severe deficiencies in myelination that occur in children or experimental animals that are hypothyroid for genetic, nutritional, or experimental reasons (Walters and Morell, 1981; Ibarrola *et al*., 1996; Ibarrola and Rodriguez-Pena, 1997; Siragusa *et al*., 1997; Jagannathan *et al*., 1998; Rodriguez-Pena, 1999). Conditioned medium from cultured oligodendrocytes also inhibits the proliferation of O-2A/OPCs and promotes oligodendrocyte generation and this effect may be mediated in part by transforming growth factor-β (Louis *et al*., 1992; McKinnon *et al*., 1993). Still other cytokines, such as leukemia inhibitory effector and ciliary neurotrophic factor (CNTF), also have been found to enhance the generation of oligodendrocytes from O-2A/OPCs (Barres *et al*., 1993; Louis *et al*., 1993; Mayer *et al*., 1994). It is important to note that these same factors, when

applied to other precursor cells, appear to promote the generation of astrocytes (Johe *et al*., 1996; Bonni *et al*., 1997; Rao *et al*., 1998; Mi and Barres, 1999; Park *et al*., 1999; Aberg *et al*., 2001). Moreover, if applied to O-2A/OPCs together with extracellular matrix (ECM) produced by endothelial cells, CNTF enhances astrocyte induction by the as yet unknown inducing factors present in the matrix (Lillien *et al*., 1990; Mayer *et al*., 1994). Thus, it quite clear that the effect of a signaling molecule on glial precursor cell differentiation may be very different for different precursor cells.

Along with soluble signaling molecules, contact-mediated signals also appear to modulate O-2A/OPC proliferation (Zhang and Miller, 1996; Nakatsuji and Miller, 2001). In cultures of embryonic rat spinal cord, oligodendrocyte lineage cells reach a steady-state density independent of the initial number of precursors and the presence of mitogens. This normalization of cell number, which reflects a feedback inhibition of precursor proliferation at high density, is cell-type specific and does not appear to be mediated through the release of a soluble factor (Zhang and Miller, 1996). The signaling pathways mediating density dependent inhibition of O-2A/OPC proliferation are unknown. The concept that cell proliferation may be regulated in a density-dependent fashion is not new (Wieser *et al*., 1990) and some of the cellular mechanisms responsible for the decreases in O-2A/OPC proliferation are beginning to be defined. Increasing cell density of O-2A/OPCs, as in other cell types (Hengst and Reed, 1996; Kato *et al*., 1997), is correlated with an increase in the expression levels of the cell cycle inhibitor $p27^{Kip-1}$, reductions in the expression levels of cyclins (Sherr, 1993), including cyclin A, and changes in the relative phosphorylation levels of Rb (Weinberg, 1995; Nakatsuji and Miller, 2001). Alterations in the expression of these components would all tend to inhibit the progression through the cell cycle and thus reduce proliferation (Nakatsuji and Miller, 2001). Also, it has been reported that increased cell density is associated with increased levels of oxidant production *in vitro*. Shifting of the intracellular redox balance to a more oxidized state is associated with differentiation of O-2A/OPCs (as discussed in more detail later in this chapter), as well as being associated (in at least some cell types) with increased expression of such cell cycle inhibitors as p21 (waf1/cip1) (Esposito *et al*., 1997; Chen *et al*., 1998; Kaneto *et al*., 1999) and p27 (Kip1) (Hannken *et al*., 2000).

In adult animals, O-2A/OPCs are arranged in a regular nonoverlapping distribution, which may be suggestive of the existence of similar regulatory mechanisms *in vivo* (Ong and Levine, 1999). Moreover, contact with CNS myelin prevents differentiation of perinatal O-2A/OPCs, possibly through Notch-1-mediated signaling (Wang *et al*., 1998; Miller, 1999). Thus, the interesting possibility exists that differentiating oligodendrocytes send a signal back to their precursors that prevents the differentiation of the remaining cells. Such a hypothetical mechanism would help to insure development of a sufficient number of oligodendrocytes in addition to allowing for evolutionary (or stochastic) increases in the number of large diameter axons (Levine *et al*., 2001).

Still another contributor to oligodendrocyte generation is the nerve cell itself. It seems likely that both soluble and cell-mediated signals from adjacent axons are integrated into the developmental profile of O-2A/OPCs resulting in cell differentiation, upregulation of myelin gene expression, and formation of the myelin organelle. Candidates for axonally derived soluble factors include FGFs (Bansal *et al*., 1996; Qian *et al*., 1997), while axonal cell surface molecules such as L1, myelin-associated glycoprotein (MAG), NCAM, and *N*-Cadherin may regulate formation of the myelin sheath (Trapp, 1990; Payne and Lemmon, 1993b). Oligodendrocyte development also is influenced by neuregulins that are expressed on many axons. Neuregulin exposure induces morphological changes in cultured oligodendrocytes (Vartanian *et al*., 1994). Furthermore, in the absence of the neuregulin receptor ErbB2, many O-2A/OPCs develop, but few of these cells mature and those that do fail to interact with axons and do not produce myelin (Park *et al*., 2001).

The generation of a complex structure such as the myelin sheath clearly requires a coordinated response in the myelinating cell. The synthesis and assembly of many myelin-specific components such as myelin basic protein (MBP), proteolipid protein (PLP), and the myelin-specific lipid galactocerebroside (GalC) have to be correctly orchestrated to give rise to myelin (Campignoni and Macklin, 1988; Campagnoni, 1995; Madison *et al*., 1999; Campignoni and Skoff, 2001). The functional properties of different myelin proteins are highlighted in relevant mutant animals as discussed later in this chapter, and a detailed understanding of the regulation of assembly of the myelin sheath remains a major goal. Also, as discussed in more detail later, the interactions between the axon and myelinating glial cells are bidirectional and complex. Myelination leads to local changes in the cytoarchitecture of the axon (de Waegh *et al*., 1992) as well as more systemic changes in the biology of myelinated neurons (Brady *et al*., 1999).

THE ORIGIN OF O-2A/OPCs

Generation of Bipotential O-2A/OPCs from Tripotential Glial Restricted Precursors

Identifying the means by which neuroepithelial stem cells of the embryonic CNS give rise to O-2A/OPCs has been a matter of intensive investigation by multiple laboratories. At the time of writing this chapter, there are divergent views on how this occurs.

The only cell that arises earlier in development than O-2A/OPCs and has been shown to be able to give rise to O-2A/OPCs is a tripotential precursor cell that can be isolated from the embryonic spinal cord (Rao *et al*., 1998) and also can be generated directly from neuroepithelial stem cells. This cell, which has been named as the glial-restricted precursor (GRP) cell, is restricted to the generation of glia and can generate oligodendrocytes, type-1 astrocytes and type-2 astrocytes. GRP cells do not give rise to neurons, either when transplanted into neurogenic

zones of the CNS or when grown *in vitro* in conditions that promote generation of neurons from neuroepithelial stem cells or from neuron-restricted precursor (NRP) cells (Rao *et al.*, 1998; Herrera *et al.*, 2001). GRP cells represent one of the first two lineage-restricted populations to arise during differentiation of neuroepithelial stem cells (NSCs) *in vitro* (the other lineage-restricted population being NRP cells [Mayer-Pröschel *et al.*, 1997]). GRP cells arise early *in vivo* and can be isolated directly from the E12.5 rat spinal cord, a stage of development that precedes the appearance of any differentiated glia (Rao *et al.*, 1998; Liu *et al.*, 2002). In contrast, in the rat spinal cord, O-2A/OPCs (defined as cells that undergo bipotential differentiation into oligodendrocytes and type-2 astrocytes when examined in clonal culture) cannot be isolated from the rat spinal cord until at least E17 (Gregori *et al.*, 2002b).

GRP cells differ from O-2A/OPCs in a variety of ways (Fig. 2; Rao *et al.*, 1998; Gregori *et al.*, 2002b). Freshly isolated GRP cells are dependent upon exposure to FGF-2 for both their survival and their division, while division and survival of O-2A/OPCs can be promoted by PDGF and other growth factors. Consistent with this difference in growth factor–response patterns, GRP cells freshly isolated from the E13.5 spinal cord do not express receptors for PDGF, although they do express such receptors with continued growth *in vitro* or *in vivo*. These populations also differ in their response to inducers of differentiation. For example, exposure of GRP cells to the combination of FGF-2 and CNTF induces these cells to differentiate into astrocytes (primarily expressing the antigenic phenotype of type-2 astrocytes) (Rao *et al.*, 1998). In contrast, exposure of O-2A/OPCs to FGF-2 + CNTF promotes the generation of oligodendrocytes (Mayer *et al.*, 1994).

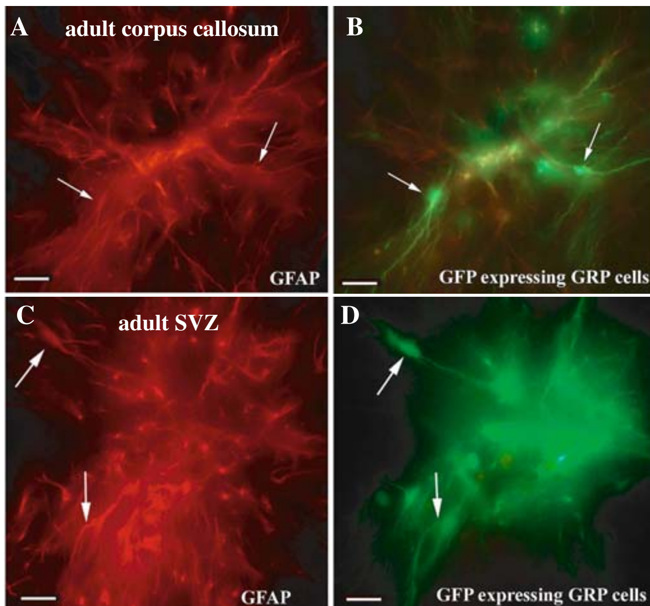

FIGURE 2. GRP cells generate astrocytes when transplanted into the normal CNS. Following isolation from the E13.5 spinal cord, stable green fluorescent protein (GFP) expressing GRP cells were generated *in vitro* using recombinant plnx-retrovirus encoding GFP (Herrera *et al.*, 2001). GRP cells were transplanted into the corpus callosum (A, B) or SVZ (C, D) of adult animals. As shown in this figure (and indicated by arrows), GFP-expressing GRP cells differentiated to yield GFAP$^+$ astrocytes *in vivo*. In other experiments, for which the reader is referred to Herrera *et al.* (2001) for details, transplantation into the neonatal SVZ or into spinal cords of myelin-deficient rats was associated with the generation of oligodendrocytes. In no instance did any of the GFP-labeled GRP cells express type III b-tubulin, a characteristic marker of neurons in the rat CNS.

A further striking difference between GRP cells and O-2A/OPCs is that GRP cells readily generate astrocytes following their transplantation into the adult CNS (Fig. 3; Herrera *et al.*, 2001). This is in striking contrast to primary O-2A/OPCs, which thus far only generate oligodendrocytes in such transplantations (Espinosa de los Monteros *et al.*, 1993) although it has been reported that O-2A/OPC cell lines will generate astrocytes if transplanted in similar circumstances (Franklin and Blakemore, 1995).

Antigenic and *in situ* analysis of development *in vivo* has confirmed that cells with the A2B5$^+$ antigenic phenotype of GRP cells arise in spinal development several days prior to the appearance of GFAP-expressing astrocytes, and also prior to the appearance of cells expressing markers of radial glia (Liu *et al.*, 2002). Thus, these cells can be isolated directly from the developing spinal cord, and cells with the appropriate antigenic phenotype have been found to exist *in vivo* at appropriate ages to play important roles in gliogenesis.

Thus far, analysis of A2B5$^+$ cells isolated from the early embryonic spinal cord reveals a great degree of homogeneity in their ability to generate oligodendrocytes and type-1 and type-2 astrocytes *in vitro* (Rao *et al.*, 1998; Gregori *et al.*, 2002b). In addition, GRP cells have been isolated from multiple species and by multiple means. For example, such cells have been isolated from the rat spinal cord, the mouse spinal cord, and from murine embryonic stem cells (Mujtaba *et al.*, 1999). In addition, A2B5$^+$ precursor cells restricted to the generation of astrocytes and oligodendrocytes have been derived from cultures of human embryonic brain cells (Dietrich *et al.*, 2002). Both mouse and human cells share the ability of rat GRP cells to generate oligodendrocytes and more than one antigenically defined population of astrocytes.

The GRP Cell As an Ancestor of the O-2A/OPC

A number or questions arise from the fact that it is possible to isolate two distinct precursor cell populations (i.e., GRP cells and O2A/OPCs) from the developing animal, each of which can generate oligodendrocytes. Is the relationship between these two populations one of lineage restriction or lineage convergence? If GRP cells and O2A/OPCs are related, what signals promote the generation of one from the other and how can the existence of both populations be integrated with existing studies on the generation of oligodendrocytes during spinal cord development?

In vitro studies have demonstrated that GRP cells can give rise to O-2A/OPCs if exposed to particular signaling molecules. In these experiments, cultures of GRP cells derived from spinal cords of E13.5 rats were grown in conditions known to induce the

generation of oligodendrocytes (Gregori *et al.*, 2002b). At the initiation of these experiments, no cells in the GRP cell cultures were labeled with the O4 antibody, which can be used to recognize O-2A/OPCs at a stage of development at which the generation of both oligodendrocytes and type-2 astrocytes *in vitro* is possible (Trotter and Schachner, 1989; Barnett *et al.*, 1993; Grzenkowski *et al.*, 1999). When GRP cells that originally had been grown in the presence of FGF for several days were exposed to a combination of PDGF and TH, however, $O4^+$ cells were generated in the cultures. Purification of cells that were $O4^+$ but did not express galactocerebroside (GalC, a marker of oligodendrocytes), and subsequent examination of the differentiation potential of these cells at the clonal level, confirmed that they behaved like O-2A/OPCs rather than like GRP cells (Gregori *et al.*, 2002b). When $O4^+GalC^-$ cells were grown in conditions that induced the generation of astrocytes, the resulting clones contained only type-2 astrocytes. In contrast, $O4^-$ cells derived from the GRP cell cultures behaved as did freshly isolated GRP cells in these conditions and generated clones containing both type-1 and type-2 astrocytes. Moreover, experimental analysis suggested that no $GalC^+$ oligodendrocytes were generated in these cultures without prior passage through an $O4^+GalC^-$ stage of development, as has been seen in multiple other analyses.

Unlike $PDGFR^+$ cells, it does not appear that GRP cells are entirely restricted to the ventral spinal cord during early development. Even at E13.5 (up to a full day before the appearance of $PDGFR^+$ cells in the rat ventral cord; Hall *et al.*, 1996), both dorsal and ventral regions of spinal cord contain $A2B5^+$ cells that, when analyzed at the clonal level, were found to be tripotential GRP cells (Gregori *et al.*, 2002b). All clones contained both type-1 and type-2 astrocytes when exposed to fetal calf serum or bone morphogenetic protein (BMP), and all clones were capable of generating both $O4^+GalC^-$ cells and $GalC^+$ oligodendrocytes. Antigenic analysis *in vivo* also confirms that the domain of $A2B5^+$ cells in the spinal cord at E14.5 includes the domain of $PDGFR^+$ cells but extends further laterally, dorsally, and ventrally (Liu *et al.*, 2002).

Despite the presence of GRP cells in both dorsal and ventral spinal cord of E13.5 rats, there appear nonetheless to be potentially interesting differences in these two populations. The frequency of $A2B5^+$ cells was greater ventrally than dorsally at E13.5 ($52 \pm 7\%$ vs $19 \pm 8\%$ of all cells, respectively), in agreement with immunohistochemical analysis of spinal cord development (Liu *et al.*, 2002). While both dorsal and ventral GRP cells responded similarly to exposure to PDGF + TH in their ability to generate $O4^+GalC^-$ cells, only ventral-derived cells generated a significant number of oligodendrocytes over a five-day time period (Gregori *et al.*, 2002b). Ventral-derived cells may be generally more inclined to differentiate at this stage, as they also showed a greater tendency to generate astrocytes in response to low concentrations (1 ng/ml) of BMP-4. Strikingly, in the ventral-derived cultures, exposure to BMP-4 was also associated with differentiation of over half of the cells into $O4^+GalC^-$ cells (although not further into $GalC^+$ oligodendrocytes), whereas only 12% of the cells in the dorsal-derived cultures were $O4^+GalC^-$ in these conditions. Thus, it appears in general that although both dorsal- and ventral-derived GRP cells can generate oligodendrocytes, the ventral-derived populations exhibit a greater tendency to readily progress along this pathway.

That the overall population of GRP cells may contain subsets of cells with different properties is also indicated by analysis of patterns of antigen and mRNA expression in the developing spinal cord (Liu *et al.*, 2002). For example, it appears that the domain of Nkx2.2-expressing cells in the E11.5–E13.5 spinal cord forms a subdomain within the population of $A2B5^+$ cells. Whether such heterogeneity in patterns of transcription factor expression is associated with heterogeneity of biological properties may only be revealed by applying techniques of quantitative analysis of what might be subtle differences in clonal properties, as have been developed for analysis of O-2A/OPCs (e.g., Yakovlev *et al.*, 1998a, b; Boucher *et al.*, 1999; Zorin *et al.*, 2000).

At present, the simplest model of oligodendrocyte generation that appears to be consistent with the data discussed thus far would be that production of these cells requires the initial generation of GRP cells from NSCs followed by the generation of O2A/OPCs from GRP cells (Figure 3). Previous studies (Rao and Mayer-Pröschel, 1997; Rao *et al.*, 1998) indicated strongly that GRP cells are a necessary intermediate between NSCs and differentiated glia, and subsequent experiments raise the possibility that O2A/OPCs are a necessary intermediate between GRP cells and oligodendrocytes, at least in the developing spinal cord (Gregori *et al.*, 2002b).

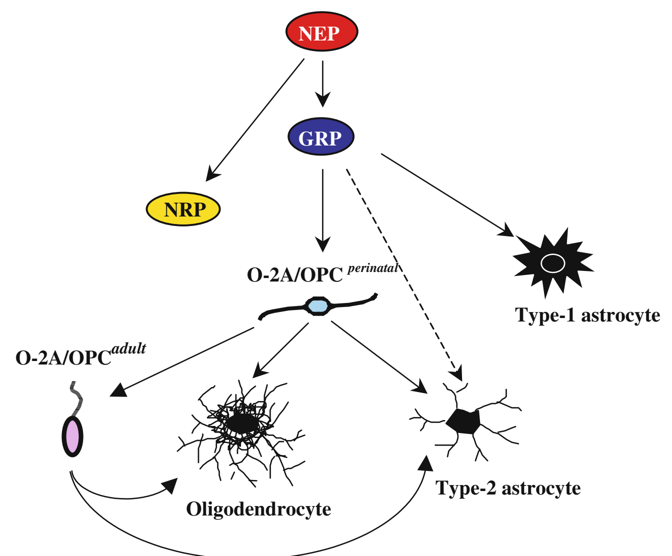

FIGURE 3. Theoretical lineage history of glia in the rat spinal cord. The only immediate ancestor that has thus far been demonstrated for the O-2A/OPC is the tripotential glial-restricted precursor (GRP) cell (Gregori *et al.*, 2002). GRP cells appear to be directly descended from neuroepithelial (NEP) stem cells, which also generate neuron-restricted precursor (NRP) cells. It is possible that the generation of O-2A/OPCs is a necessary intermediate step in the generation of oligodendrocytes from GRP cells. The O-2A/OPC of the perinatal CNS then goes on to generate an adult-specific O-2A/OPC with quite distinct biological properties, as discussed in later sections of this chapter.

It is important to note that there are a number of claims that the GRP cell hypothesis explicitly does *not* make. Critically, this hypothesis does not state that all GRP cells give rise to both oligodendrocytes and astrocytes *in vivo*. It is clear that the differentiation fate of the progeny of a particular founder cell will be modulated by the microenvironment in which those progeny are localized. The generation of astrocytes and oligodendrocytes from a single founder cell requires that the progeny of the founder cell migrate into different microenvironments. What the hypothesis does predict, in contrast, is precisely what has been reported, that is, that A2B5$^+$ cells isolated from ventral regions (where oligodendrocytes will be generated) or dorsal regions (where astrocytes will be generated) of the early spinal cord will all be tripotential cells restricted to the generation of glia.

In addition, the GRP cell hypothesis does not require that all A2B5$^+$ cells derived from the embryonic spinal cord be alike in all ways. As discussed elsewhere in this chapter, O-2A/OPCs from different regions of the developing CNS express profound differences with respect to their tendency to undergo self-renewing division and in their responsiveness to inducers of differentiation (Power *et al*., 2002). It has been suggested that these differences are reflective of the time courses of myelination in the tissues in which these cells are resident. Yet, these cells are all O-2A/OPCs as defined by their apparent restriction to the generation of oligodendrocytes and type-2 astrocytes *in vitro*. Similarly, although it is already clear that GRP cells derived from dorsal and ventral spinal cord of E13.5 rats may express some different properties, they are nonetheless thus far apparently identical with respect to their tripotentiality.

The Motor Neuron/Oligodendrocyte Precursor (MNOP) Cell Hypothesis

At the same time that studies have been ongoing on GRP cells, a wholly separate line of investigation has raised questions about whether oligodendrocytes are developmentally more closely related to motor neurons than they are to astrocytes (Fig. 4). These studies have pursued a hypothesis first suggested by Pringle *et al*. (1996) that motor neurons and oligodendrocytes shared a common precursor cell (i.e., an MNOP). The MNOP hypothesis was based initially on observations that both motor neurons and oligodendrocytes arise in a similar (and possibly identical) discrete zone of the ventral spinal cord (reviewed in Richardson *et al*., 1997, 2000). Moreover, it was found that similar concentrations of Sonic hedgehog (Shh) are required for the induction of both cell types (Pringle *et al*., 1996) and *in vitro* the induction of oligodendrocytes (e.g., by ectopic Shh presentation) is frequently accompanied by the induction of motor neurons (Pringle *et al*., 1996; Orentas *et al*., 1999).

The hypothesis that motor neurons and oligodendrocytes are developmentally related to each other has some intuitive attractiveness arising from the critical importance of this particular cell combination in evolution. The ensheathing of axons with myelin is associated with a large increase in conduction speed. In annelids and crustacea, pseudomyelin is preferentially associated with axons required for rapid escape responses (Roots, 1993; Davis *et al*., 1999). It has been suggested that hagfish, which have no myelin, seem unable to accelerate to avoid capture (Richardson *et al*., 2000). One way to ensure that motor neurons and the cells that ensheath them arise at the same place would be to derive both cells from the same precursor (as suggested in Richardson *et al*., 1997, 2000).

The idea that motor neurons and oligodendrocytes might be developmentally related was given a further boost by the findings, from three separate laboratories, that compromising the function of members of the *Olig* gene family can prevent the generation of both motor neurons and oligodendrocytes (Lu *et al*., 2002; Takebayashi *et al*., 2002; Zhou *et al*., 2002). These studies have been discussed in detail in a number of recent reviews (Richardson *et al*., 2000; Rowitch *et al*., 2002; Sauvageot and Stiles, 2002).

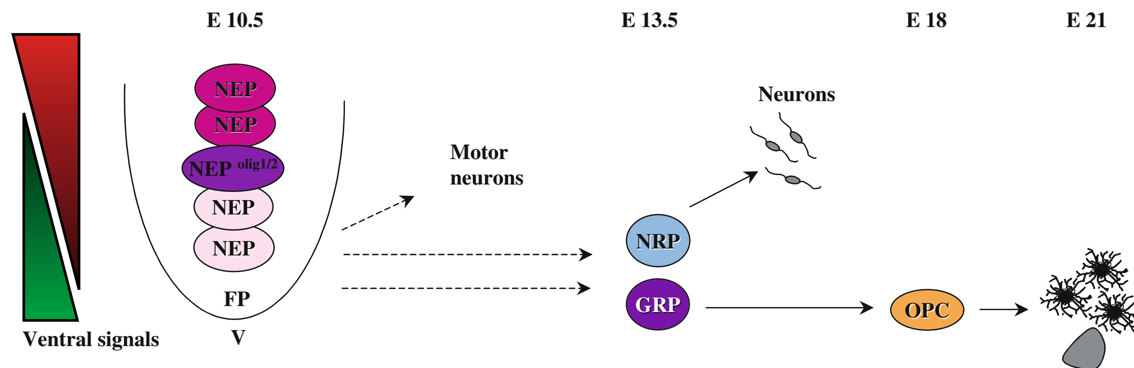

FIGURE 4. Theoretical scheme for generation of GRP and NRP cells in the ventral spinal cord. It appears that ventralizing signals (in particular, Sonic hedgehog) cause the specialization of NEP cells to generate particular cell types. Motor neurons are the first ventral-specific cell type to be generated. Motor neurons can be generated from NRP cells isolated from the spinal cord although, as discussed in the text, there are also arguments being made for the existence of a motor neuron/oligodendrocyte precursor (MNOP) cell. Due to this controversy, motor neuron derivation is indicated separately from GRP cells. Also generated are GRP cells, which go on to generate O-2A/OPCs. There are, however, alternative hypotheses as to how differentiation progresses in the early spinal cord, as summarized in Fig. 5.

Olig1 and *Olig2* genes are expressed in the developing mouse spinal cord within the specific region that appears to give rise to both oligodendrocytes and motor neurons (Lu *et al*., 2000; Takebayashi *et al*., 2000; Zhou *et al*., 2000). Forced expression of *Olig1* or *Olig2* in neuroepithelial stem cells induces expression of early markers of the oligodendrocyte lineage (Lu *et al*., 2000; Zhou *et al*., 2001). Moreover, expression of *Olig2* in conjunction with *neurogenin2* appears to be critical for the generation of motor neurons (Mizuguchi *et al*., 2001; Novitch *et al*., 2001).

Among the most striking experiments that have been suggested to indicate the possible presence of a precursor cell committed to the generation of oligodendrocytes and motor neurons are experiments showing that targeted disruption of *Olig2* prevents oligodendrocyte and motor neuron specification in the spinal cord (Lu *et al*., 2002; Takebayashi *et al*., 2002; Zhou *et al*., 2002). (Disruption of *Olig1*, in contrast, disrupted normal maturation of oligodendrocytes [Lu *et al*., 2002].) Thus, the evidence is quite clear that expression of *Olig2* is required for the generation of both oligodendrocytes and motor neurons. Not all of the regulatory factors, however, that control motor neuron development also affect oligodendrocyte development, and *vice versa*. For example, disruption of function of the *Isl1* gene prevents the generation of motor neurons without apparently affecting oligodendrocyte generation (Pfaff *et al*., 1996). Conversely, disruption of the neuregulin 1 signaling pathway (by knocking out the neuregulin 1 gene itself, or through disruption of the erbB receptors to which this protein binds) appears to disrupt normal oligodendrocyte development without impacting on the generation of motor neurons (Vartanian *et al*., 1999; Park *et al*., 2001).

The Ambiguous Case for a Restricted Motor Neuron/Oligodendrocyte Precursor Cell

Data derived from studies on the expression and function of *Olig1* and *Olig2* genes contain substantial ambiguities. As there is no evidence that has been presented (at the time of writing this chapter) of the derivation of both motor neurons and oligodendrocytes from a single lineage-restricted founder cell, the only conclusion that can be drawn from these experiments is that *Olig1* and *Olig2* genes are expressed in a population of ancestral cells of unknown heterogeneity. This population may well consist of separate *Olig1*$^{+}$/*Olig2*$^{+}$ precursors for neurons and for oligodendrocytes.

Critically, it is not yet possible to draw firm conclusions from existing studies on whether or not astrocytes are ever generated *in vivo* from cells that are at some point induced to express *Olig* genes. Claims that *Olig2*$^{+}$ cells do not generate astrocytes, and that disruption of *Olig* gene function does not alter astrocyte development (Lu *et al*., 2002), are not without problems, as these suggestions have not been tested by determination of whether larger numbers of astrocytes are generated in the developing spinal cord of *Olig*-compromised animals (which would be a predicted consequence of such perturbations). It is also the case that in *Olig2*$^{-/-}$ mice in which the *Olig2* gene was disrupted by targeted replacement with tamoxifen-inducible Cre recombinase reveal at least some of the Cre expressing cells expressed the astrocyte marker S100β (Takebayashi *et al*., 2002). Similarly, in *Olig1*$^{-/-}$ *Olig2*$^{-/-}$ mice in which GFP was expressed in the *Olig2* locus, half of the GFP-expressing cells differentiated into astrocytes *in vivo* (Zhou *et al*., 2002). As these experiments are conducted in animals in which no functional *Olig* genes are expressed, no conclusions regarding *Olig* gene expression and lineage restriction can be drawn. The conclusion can be drawn, however, that the signals that induce *Olig* gene expression are not sufficient to cause restriction of the resultant precursor cells away from astrocytic pathways. If one believes, however, that disruption of the function of *Olig1* and *Olig2* genes *in vivo* is revealing of developmental plasticity, then the interpretation of the experiments of Takebayashi *et al*. (2002) and Zhou *et al*. (2002) would be that cells exposed to signals that induce expression of *Olig1/2* can readily generate astrocytes if *Olig* gene expression is disrupted. Such an interpretation is consistent with the observations that A2B5^{+}NG2^{+}PDGFR^{+} cells derived from the ventral spinal cord of E16 rats readily generate oligodendrocytes and two populations of astrocytes *in vitro* when exposed to appropriate conditions, and thus appear to be GRP cells (Gregori *et al*., 2002b).

Reconciling the GRP and MNOP Hypotheses of Oligodendrocyte Ancestry

One critical question that thus far appears to remain unanswered in studies on early spinal cord development concerns the heterogeneity of the *Olig2* expressing population found in the spinal cord at differing ages. At the time of writing this chapter, however, it is not clear if the population of *Olig1* and *Olig2* expressing cells of the embryonic spinal cord ever comprises an antigenically homogeneous population of cells at any stage beyond the NSC stage. The MNOP and GRP/NRP hypotheses predict very different outcomes of such experiments over a range of developmental stages.

One clear prediction of the GRP/NRP analysis of development is that the cells that express *Olig* genes in the E9.5–E10.5 are pluripotent NSCs. This prediction is made due to the failure to find PSA-N-CAM-expressing NRP or A2B5^{+} GRP cells at this developmental stage (Mayer-Pröschel *et al*., 1997; Liu *et al*., 2002). The GRP/NRP hypothesis also predicts that once motor neuron generation is completed, the *Olig1/2*-expressing population should consist of A2B5^{+} GRP cells, a prediction that appears thus far to be correct. This prediction appears to have been tested as a part of attempts to understand when and how the putative transition from GRP to O-2A/OPCs is regulated *in vivo*. Current data indicates that the “when” component of this transition occurs surprisingly late, and subsequent to the appearance of PDGFR^{+} cells in the ventral region of the embryonic rat spinal cord at E14/14.5. Current data indicates that cells with the bipotential lineage restriction of O-2A/OPCs cannot be isolated from the rat spinal cord until at least E17 (Gregori *et al*., 2002b; MMP *et al*., in progress). Prior to this point, clonal analysis of A2B5^{+} cells isolated from both dorsal and ventral rat spinal cord indicates that all of these cells appear to be tripotential GRP cells, including the ventral-derived ones that are PDGFR^{+}NG2^{+}.

As available data suggests a high level of overlap between *Olig*-expressing cells and PDGFR$^+$ cells (Lu *et al*., 2000; Zhou *et al*., 2000; Tekki-Kessaris *et al*., 2001), it appears that the prediction that such cells are GRP cells is correct.

Nonetheless, it is very important—in the context of the MNOP hypothesis—to ask whether the outcomes of developmental studies *in vivo* are at least consistent with such a hypothesis. This appears not to be the case. The view that *Olig1/2* expression represents restriction to the motor neuron/oligodendrocyte pathways makes a very specific prediction that labeling of a founder cell and its clonal derivatives at stages of spinal cord development after *Olig1/2* expression occurs will reveal that motor neuron-containing clones contain oligodendrocytes but not astrocytes. Potentially relevant experiments appear to have been carried out over a decade ago, using the technique of injecting retroviral particles expressing bacterial β-galactosidase into the developing chick spinal cord, over a range of ages including up to one or two cell cycles before all motor neurons are born (Leber *et al*., 1990). In these experiments, 82% of clones that contained motor neurons also had nonmotor neuron relatives. No evidence was found, however, for the occurrence of cell types in specific combinations. Forty-two percent of the multicellular clones that contained motor neurons contained cells that were clearly glial in both gray and white matter, with many of these glial cells being considered to be astrocytes by morphological criteria. Critically, with respect to the possible longevity of NSCs in the developing spinal cord, injection of retrovirus as late as one or two cell cycles before motor neurons are born revealed clones that contained motor neurons, interneurons, and glia as relatives, with putative astrocytes prominently represented among the glia. Although the focus of this study was on motor neuron development, the authors also noted the existence of other clones that appeared to contain both oligodendrocytes and astrocytes. The results of these studies are subject to multiple interpretations, but the reported frequency of motor neuron/astrocyte clones seems quite divergent from what one would expect were a restricted MNOP a critical contributor to spinal cord development.

While it is not an essential part of the GRP cell hypothesis that single precursor cells give rise to both oligodendrocytes and astrocytes *in vivo* (as this would require dispersion of the progeny of a single precursor cell into different microenvironments), it nonetheless would be of interest to know if such pairing does normally occur. Recent studies by Zerlin et al. (2004) demonstrate that this does indeed occur in the mammalian forebrain. In these experiments, retroviral labeling of cells of the neonatal (P0–P2) rat subventricular zone (SVZ) led to the later appearance of clonally related astrocytes and oligodendrocytes in the cortex. In white matter tracts, in contrast, the majority of progenitors become oligodendrocytes, although some astrocytes are also generated. Critically, as the GRP cell hypothesis predicts, it appears to be necessary for progeny to enter into different microenvironments in order for these clonally related cells to assume different fates. Moreover, if SVZ cells were labeled with retrovirus and then, after a further four or five days of *in vivo* growth, the cells that had migrated into the neocortex were isolated and allowed to develop *in vitro*, clones contained only glia and no neurons. Some clones were composed only of oligodendrocytes, some only of astrocytes, and some contained both kinds of glial cells. As dissection of these cells at earlier stages following retroviral labeling revealed mixed neuronal-glial clones (Levison and Goldman, 1997), it seems that continued development is associated with progressive fate restriction into glioblasts. Whether or not the cells that give rise to oligodendrocytes and astrocytes, but not to neurons, correspond to GRP cells remains to be determined.

There are two interpretations equally consistent with all of the above data, but which are fully compatible with the idea that lineage specialization in the CNS is initiated with the formation of GRP cells and NRP cells, and that do not require the evocation of a precursor cell specialized to generate oligodendrocytes and motor neurons. One interpretation is that the studies that have been discussed are indicative of localized influences on gene expression that cause expression of these genes in NSCs, but that such expression patterns occur before commitment to a particular lineage occurs. According to this hypothesis, *Olig1* and *Olig 2* are already expressed in NSCs prior to the generation of GRP and NRP cells. This hypothesis is consistent with the observations that these genes are expressed at a stage of neural tube development when all cells in the neural tube appear to be pluripotent NSCs able to generate all of the cell types of the CNS. In the ventral regions of the spinal cord, some NSCs coordinately express *neurogenin* genes with *Olig* genes, and such cells differentiate to generate motor neurons. Other NSCs that express *Olig1* and *Olig2*, but do not express neurogenins, differentiate to yield GRP cells and then become further restricted to yield O-2A/OPCs.

Another variant of the above hypothesis is that localized signals in this region cause NRP cells and GRP cells each to express *Olig1* and *Olig2* genes, which play different roles in these two lineages. When expressed in NRP cells in conjunction with neurogenins, they induce the formation of motor neurons. When expressed in GRP cells, they block the action of inducers of astrocytic differentiation (such as BMPs) and promote differentiation of these cells in O-2A/OPCs and, subsequently, into oligodendrocytes.

A common feature of both the above hypotheses is that, if correct, they would indicate that genes thought to be indicative of oligodendrocyte development are in fact expressed prior to terminal commitment to this differentiation path. It is possible, however, that none of the explanations of development that have been suggested thus far are correct. It may also be, as suggested in Fig. 5, that there exist both MNOP cells and GRP cells, each of which plays a contributory role in the generation of oligodendrocytes.

Regional Specialization of the First Appearance of Cells Expressing Oligodendrocyte Lineage Genes Is a Common Feature in CNS Development

Regardless of what emerges as the correct view of lineage specialization during oligodendrocyte development, it is clear that the first stages that can be identified in oligodendrocyte development *in vivo* consistently occur in a regionally specialized manner. As it is not clear whether the cells that have been

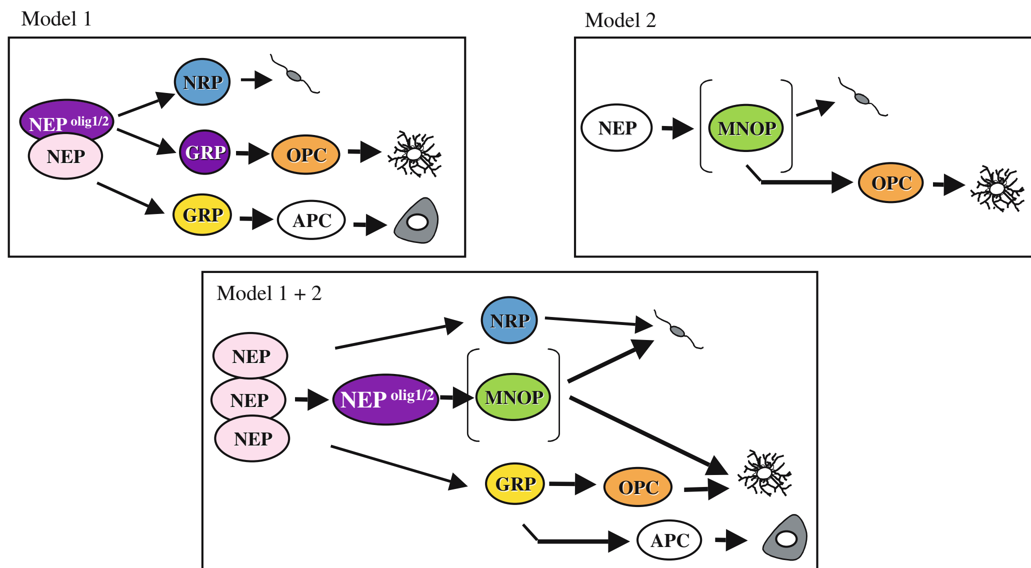

FIGURE 5. Theoretical models of lineage specification in the rat spinal cord between E11.5 and E13.5. The current state of knowledge does not allow any model to be decisively preferred over any other. For example, it could be that Olig1/2+ NEP cells generate NRP cells specialized to make motor neurons and GRP cells specialized to make O-2A/OPCs. It is also possible that NEP cells generate a motor neuron/oligodendrocyte precursor cell (MNOP) that makes only motor neurons and O-2A/OPCs. It is even possible that both of these processes occur, with the generation of GRP cells, NRP cells and MNOP cells.

defined in these studies are O-2A/OPCs, GRP cells, or as yet undefined lineage-restricted precursor cell populations, the generic term "oligodendrocyte precursor" will be used in this section to indicate cells thought to be participating in the eventual generation of oligodendrocytes.

Early oligodendrocyte development has been most extensively studied in caudal regions of the CNS such as the spinal cord and in the optic nerve. In the rat optic nerve, tissue culture studies suggested that the founder cells of the oligodendrocyte lineage originate in the brain or optic chiasm and migrate along the nerve during subsequent development (Small *et al*., 1987). The source of at least a subset of chick optic nerve oligodendrocytes was subsequently defined as a small foci of cells in the floor of the third ventricle (Ono *et al*., 1997). In the chick spinal cord, oligodendrocyte precursors are located along the entire rostral–caudal extent of the spinal cord as early as neural tube closure (Warf *et al*., 1991). By contrast, separation of dorsal and ventral regions of the spinal cord during early embryogenesis revealed that oligodendrocyte precursors were present only in ventral regions of the spinal cord (Warf *et al*., 1991; Ono *et al*., 1995). The ventral ventricular origin of spinal cord oligodendrocytes appears to be a common feature of vertebrate development and has been demonstrated in a broad range of species including *Xenopus* (Maier and Miller, 1995) and human as well as chick, mouse, and rat (Warf *et al*., 1991; Pringle and Richardson, 1993; Ono *et al*., 1995).

Whether the ventral spinal cord is the only source of oligodendrocytes is unresolved (Spassky *et al*., 1998). A dorsal source of oligodendrocyte precursors in caudal spinal cord regions was supported by initial chick–quail chimera studies in which dorsal portions of the chick neural tube were replaced with equivalent quail tissue and the source of oligodendrocytes determined by species-specific labeling (Cameron-Currey and LeDouarin, 1995). This experimental outcome would be consistent with the observations that GRP cells able to generate oligodendrocytes *in vitro* can be isolated from both the ventral and dorsal spinal cords at times prior to the expression of such supposed markers of oligodendrocyte development as PDGFR-α (Gregori *et al*., 2002b). Nonetheless, subsequent analyses of similar chimeric spinal cords failed to substantiate these studies or provide evidence for a dorsal source for oligodendrocytes, but rather supported the notion that all spinal cord oligodendrocytes were derived from ventral regions (Pringle *et al*., 1998). At this stage, it is uncertain why studies that were theoretically identical in nature gave opposite results.

The spinal cord is not the only region where putative ancestors of oligodendrocytes arise in restricted locations. In more rostral areas of the CNS, the earliest cells thought to be oligodendrocyte precursors appear in defined domains of the ventricular zone and SVZ at particular stages of development (Ono *et al*., 1997). For example, a group of cells in the ventricular mantle zone of the ventral diencephalon of the E13 rat express mRNA for the PDGFR-α (Pringle and Richardson, 1993). During subsequent development, these cells appear to migrate into the developing thalamus and hypothalamus as well as to more dorsal regions including the developing cerebellum (Pringle and Richardson, 1993).

Not all the regions that initially generate oligodendrocyte precursors are ventrally located (Perez Villegas *et al*., 1999). In the chick metencephalon, while the earliest arising population of

progenitors is adjacent to the floor plate in the ventral metencephalon (Ono *et al*., 1997), a second more dorsal source of putative OPCs develops independently (Davies and Miller, 2001). The ancestors to oligodendrocytes that populate the telencephalon appear to be derived from both the medial and lateral ganglion eminence (LGE) and later migrate into the cortex (Spassky *et al*., 1998; He *et al*., 2001), as discussed in more detail later in this chapter. Whether both regions contribute equally to the eventual generation of forebrain oligodendrocytes is not clear. In mutants, in which the medial ganglion eminence (MGE) is converted to the LGE, there is a significant loss of oligodendrocytes suggesting that the MGE is the major source of oligodendrocyte precursors (Sussel *et al*., 1999). Oligodendrocytes that populate the telencephalon also arise from alar regions such as the anterior entopeduncular area (Olivier *et al*., 2001). The exact contribution of each area to the overall population of oligodendrocytes in the forebrain remains to be resolved. For example, it is unclear if individual domains generate oligodendrocytes that populate distinct regions of the forebrain, or if the different domains give rise to morphologically and biochemically distinct types of oligodendrocytes.

The Initial Appearance of Oligodendrocyte Precursors Is Regulated by Local Signals

In principle, two general mechanisms may account for the restricted geographical origin of oligodendrocyte precursors in the spinal cord and other regions of the CNS. Cells in dorsal regions may lack the intrinsic potential to generate oligodendrocytes or ventrally located signals may instruct neighboring cells to assume an oligodendrocyte fate while dorsal signals inhibit oligodendrocyte induction.

Several lines of evidence indicate that the localized appearance of oligodendrocyte precursors is a reflection of local signaling. For example, transplant studies indicate that the initial appearance of spinal cord oligodendrocytes is dependent on local influences from the adjacent notochord (Trousse *et al*., 1995; Orentas and Miller, 1996; Pringle *et al*., 1996). The notochord, a transient mesodermally derived structure, is located ventral to the developing neural tube and signals from the notochord have been shown to be involved in the formation of the dorsal/ventral axis in the developing CNS (van Straaten *et al*., 1988). The establishment of dorsal and ventral polarity results in the subsequent specification of distinct populations of neurons found in the ventral spinal cord (van Straaten *et al*., 1988, 1989; Jessell and Dodd, 1990). Transplantation of an additional notochord adjacent to the dorsal spinal cord resulted in the local induction of ventral neurons (Yamada *et al*., 1991) and an ectopic cluster of oligodendrocyte precursors in chick and *Xenopus* embryos (Orentas and Miller, 1996; Maier and Miller, 1997). Likewise, coculture of dorsal spinal cord explants with isolated notochord is sufficient to induce generation of motor neurons (Yamada *et al*., 1991) and oligodendrocytes in the spinal cord tissue (Orentas and Miller, 1996; Poncet *et al*., 1996; Pringle *et al*., 1996). The ability of the transplanted notochord to induce oligodendrocytes was restricted to a period during early embryonic chick development, which has been suggested to reflect both a change in the signaling capacity of the notochord and a temporally dependent loss of responsiveness of the dorsal spinal cord cells (Orentas and Miller, 1996).

Not only is the notochord competent to induce ectopic oligodendrocytes, but it is also essential for the normal ventral appearance of spinal cord oligodendrocytes. In *Xenopus* embryos UV irradiated at the one-cell stage, oligodendrocytes failed to develop in spinal cord regions lacking a notochord (Maier and Miller, 1997). Likewise, oligodendrocytes did not develop in the spinal cord adjacent to the site of notochord ablation at embryonic or larval stages (Maier and Miller, 1997). Similarly, in the short-tailed Danforth mouse, the notochord is discontinuous along the length of the rostral–caudal axis, and while oligodendrocytes develop normally in regions of the spinal cord adjacent to the notochord, they are absent from those regions lacking a notochord (Pringle *et al*., 1996). Thus, the notochord provides a local signal or signals that result in the subsequent appearance of spinal cord oligodendrocytes.

Many of the inductive properties of the notochord are due to production of the signaling molecule Shh (Echelard *et al*., 1993; Roelink *et al*., 1994). Shh, the vertebrate homologue of the *Drosophila* pattern forming gene *hedgehog*, is localized to the notochord and adjacent floor plate (Roelink *et al*., 1994). *In vitro*, Shh induces the development of floor plate and different classes of motor neurons in a concentration-dependent manner (Roelink *et al*., 1994, 1995), through the activation or repression of a series of homeodomain transcription factors (Jessell, 2000). Oligodendrocytes can be induced *in vitro* at similar concentrations of Shh required for the induction of motor neurons (Pringle *et al*., 1996; Orentas *et al*., 1999), suggesting that the development of these two cell types is closely linked (Richardson *et al*., 1997, 2000). In the chick spinal cord, the generation of oligodendrocyte precursors requires continued Shh signaling after the formation of ventral–dorsal polarity and the generation of motor neurons (Orentas *et al*., 1999). For example, inhibiting Shh signaling immediately prior to the appearance of oligodendrocyte precursors blocks their subsequent appearance, but has little effect on motor neuron pools (Orentas *et al*., 1999). It seems likely that Shh contributes to the initial progression towards the oligodendrocyte lineage, possibly through induction of cell-type specific transcription factors such as the Olig genes (Lu *et al*., 2000; Zhou *et al*., 2000). In addition, recent *in vitro* studies suggest that the continued dependence of oligodendrocyte precursors on Shh signaling reflects a potent survival rather than proliferative influence on these cells (Davies and Miller, 2001).

In more rostral regions of the CNS, the expression of Shh and the appearance of oligodendrocytes also are spatially and temporally closely linked (Davies and Miller, 2001; Nery *et al*., 2001; Tekki-Kessaris *et al*., 2001). Furthermore, ectopic expression of Shh leads to concomitant local development of oligodendrocytes (Nery *et al*., 2001). Whether Shh is essential for the development of all rostral populations of oligodendrocytes is less clear. In cell cultures derived from Shh knockout animals, considerable numbers of oligodendrocytes develop, indicating that oligodendrocytes can arise in the absence of Shh signaling (Nery *et al*., 2001). It seems likely, however, that other members

of the hedgehog family can substitute for Shh in its absence and blocking all hedgehog family member signaling with cyclopamine (Incardona *et al*., 1998) appears to block all oligodendrocyte development (Tekki-Kessaris *et al*., 2001).

In vitro, the development of oligodendrocyte precursors is inhibited by exposure to members of the TGF-β family (Mabie *et al*., 1997). Specifically, BMP-2 and -4 appear to inhibit the development of oligodendrocytes, instead promoting the generation of astrocytes (Mabie *et al*., 1997; Mehler *et al*., 2000). Whether BMP signaling contributes to the spatial patterning of oligodendrocyte precursor induction in the developing intact CNS is currently unknown. For example, the failure of oligodendrocyte development in dorsal spinal cord may reflect active inhibition by BMPs, which is overcome in ventral regions by Shh (Mekki-Dauriac *et al*., 2002). If the source of the BMPs was in dorsal tissue adjacent to the spinal cord, this hypothesis would explain why oligodendrocytes develop in isolated explants of dorsal spinal cord over time (Sussman *et al*., 2000). Additional, as yet uncharacterized, inhibitors of oligodendrocyte precursor development may also exist. Indeed, dorsal spinal cord has been reported to contain an inhibitor of early oligodendrocyte development (Wada *et al*., 2000) that is functionally distinct from any known BMP (Wada *et al*., 2000).

Myelination of Developing White Matter Is Dependent on Oligodendrocyte Precursor Migration

Oligodendrocytes are widely distributed in the adult CNS, even though in early development their precursors arise in highly restricted ventricular domains (as discussed previously). The spatial separation between the location of origin of oligodendrocyte precursors and their final destination means that normal myelination is dependent on the long-distance migration of oligodendrocyte precursors. Although the migratory capacity of oligodendrocyte precursors has been clear for several years, the molecular mechanisms mediating this migration are only now becoming understood.

The earliest indications that oligodendrocyte precursors were capable of long-distance migration came from transplantation studies (Lachapelle *et al*., 1984, 1994). Transplantation of fragments of normal CNS tissue into host animals lacking myelin proteins resulted in the substantial dispersal of normal oligodendrocytes throughout the host CNS (Lachapelle *et al*., 1984), some of which may have occurred by trafficking through the ventricular system. Similar analyses using purified cell populations demonstrated that the capacity for long-distance cell migration through the neuropil of the CNS is predominantly a characteristic of less mature O-2A/OPCs and is lost as the cells mature (Warrington *et al*., 1992, 1993). Likewise, retrospective analyses of the oligodendrocyte precursors that migrated into the cerebellum indicated they were highly immature (Goyne *et al*., 1994), although in these studies (as in most studies of normal development), it is not yet clear if the migrating cell is a GRP cell, an O-2A/OPC, both, or something else entirely. Nonetheless, it seems very likely that the migration of immature oligodendrocyte ancestors of some form is an essential component for normal myelination in the vertebrate CNS.

The extent of oligodendrocyte precursor migration during CNS development has been highlighted by analyses of the optic nerve (Small *et al*., 1987; Ono *et al*., 1997; Sugimoto *et al*., 2001) and spinal cord (Warf *et al*., 1991; Ono *et al*., 1995). The cells that give rise to the oligodendrocytes that populate the optic nerve migrate into the nerve from the brain during late embryonic and early postnatal stages. This migration was first documented using cell culture (Small *et al*., 1987). For example, cell cultures derived from brain or chiasmal regions of the optic nerve acquired the capacity to generate oligodendrocytes several days before cultures isolated from retinal regions of the nerve (Small *et al*., 1987). The source of optic nerve oligodendrocytes and their dispersal along the nerve was directly visualized by labeling the cells at their origin in the floor of the third ventricle in developing chick embryos (Ono *et al*., 1997). In contrast to rat, mouse, and human, where oligodendrocytes and myelin are restricted to the optic nerve, oligodendrocytes migrate into specific regions of the chick (Ono *et al*., 1998) and rabbit retina (ffrench-Constant *et al*., 1988) where they myelinate the proximal region of retinal ganglion cell axons. In the spinal cord, similar approaches have been used to document the migration of oligodendrocyte precursors from ventral to dorsal regions. Isolated cultures of ventral spinal cord generated oligodendrocytes from the time of neural tube closure (E12), while isolated cultures of dorsal spinal cord did not acquire the capacity to generate oligodendrocytes in a physiological time frame until E16 (Warf *et al*., 1991). The acquisition of the capacity to generate oligodendrocytes in dorsal spinal cord correlated directly with the arrival of ventrally derived precursors (Ono *et al*., 1995). Direct evidence of the ventral to dorsal migration of spinal cord oligodendrocyte precursors has been provided by cell tracking experiments using an *in vitro* preparation of rat spinal cord (Warf *et al*., 1991).

In more rostral regions of the CNS, the migration of oligodendrocyte precursors is also pronounced (Fig. 6). During development of the cerebral cortex, immature OPCs have been suggested to migrate from the LGE as well as the MGE into the developing forebrain (Spassky *et al*., 1998; He *et al*., 2001; Marshall and Goldman, 2002). At later stages of development, glial precursor cells, including oligodendrocytes, migrate from the SVZ in radial and tangential directions toward the pial surface (Kakita and Goldman, 1999) to populate all regions of the cortex.

The nature of the cellular substrates utilized and the molecular mechanisms mediating oligodendrocyte precursor migration are not well understood. In the optic nerve, many of the migrating oligodendrocyte precursors are closely associated with retinal ganglion cell axons and it has been suggested that this migration is axophilic (Ono *et al*., 1997). Migration along preexisting axon tracts would also provide a pathway for the ventral to dorsal migration in the spinal cord where migrating cells would utilize the earlier developed circumferential axon tracts. Not all oligodendrocyte precursor migration are dependent upon axons, however. In the rat optic nerve, removal of the retinal ganglion cell axons through eye enucleation or disruption of the neural retina in the postnatal animal (Ueda *et al*., 1999) failed to

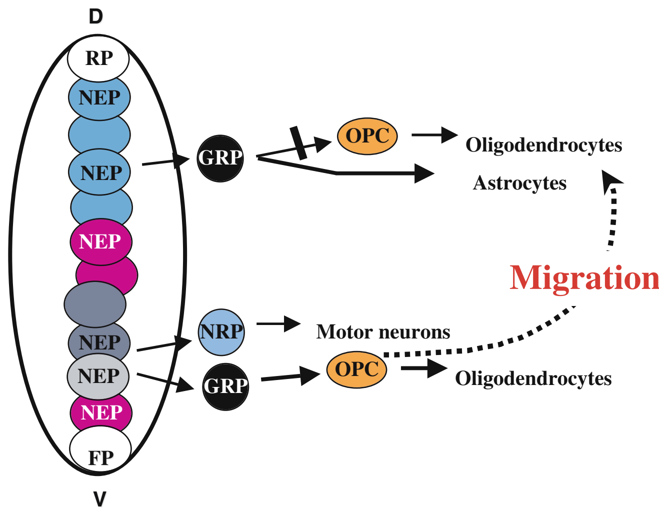

FIGURE 6. Migration plays a key role in the generation of white matter. Following the generation of ancestors of oligodendrocytes in highly specialized regions, these ancestral cells then migrate into regions where oligodendrocyte generation is required. For example, it may be that O-2A/OPCs are normally only generated from the GRP cells of the ventral spinal cord, and that these O-2A/OPCs migrate throughout this tissue to populate both dorsal and ventral white matter.

completely block the population of the nerve by oligodendrocytes although the number of cells was greatly reduced (Ueda *et al.*, 1999). It may be, however, that significant numbers of oligodendrocyte precursors had populated the nerve prior to the perturbation (Small *et al.*, 1987) or residual cues remained after the removal of the axons. Cell surface components such as adhesion molecules (Payne and Lemmon, 1993a; Wang *et al.*, 1994) and ECM receptors have also been proposed to play a role in regulating migration (Kiernan *et al.*, 1996; Garcion *et al.*, 2001). In explant studies, removal of neural cell adhesion molecule (NCAM)-associated polysialic acid (PSA) inhibits the dispersal of oligodendrocyte precursors (Wang *et al.*, 1994, 1996). However, in the developing chick, optic nerve removal of NCAM-associated PSA does not play a pivotal role in regulating precursor migration (Ono *et al.*, 1997). Oligodendrocyte precursors also express an array of integrin receptors that may play important role in regulation of both migration and cell differentiation (Milner and ffrench-Constant, 1994; Garcion *et al.*, 2001).

Guidance of Glial Precursor Migration

The migration of oligodendrocyte precursors throughout the developing CNS is likely to be mediated by specific directional and substrate cues. The immigration of oligodendrocyte precursors into the optic nerve seen in culture and labeling studies (Small *et al.*, 1987; Ono *et al.*, 1997) might simply have reflected the random movement of cells originating from a focal source within the brain. Likewise, the population of the dorsal spinal cord with ventrally derived precursors might occur by nondirectional dispersal (Ono *et al.*, 1995). Such a mechanism is unlikely. It would likely be very slow in dispersing cells and also fails to accommodate the finding that different tracts of the spinal cord become populated with oligodendrocytes at defined times in development. The motility of immature oligodendrocyte precursors is promoted by PDGF, the same growth factor that promotes their proliferation and survival (Armstrong *et al.*, 1990b). In chemotaxis chambers, oligodendrocyte precursors migrate toward higher concentrations of PDGF indicating that this growth factor has chemotactic properties. *In vivo* PDGF appears to be ubiquitously distributed throughout the CNS, being made by populations of astrocytes and neurons (Richardson *et al.*, 1988; Yeh *et al.*, 1991) and so is unlikely to guide glial migration.

Recent studies suggest that the migration of oligodendrocyte precursors is guided by specific cues (Sugimoto *et al.*, 2001). For example, labeling of cells in a section of optic nerve demonstrated that the majority of the cell migration was unidirectional from the chiasm to the retina (Sugimoto *et al.*, 2001). This observation implied that the migration of cells along the nerve is guided by directional cues in the environment. Tissue culture studies indicated that the directional cues were located in the optic chiasm and appeared to be repellent signals produced by the optic chiasm. Without the optic chiasm, the migration of glial precursors was bidirectional with similar numbers of cells moving short distances in both directions along the nerve. By contrast, in the presence of the chiasm, OPCs in the optic nerve exhibited preferential migration away from the chiasm and toward the retina (Sugimoto *et al.*, 2001). Localization studies identified netrin-1 and semaphorin 3a as potential chiasm-derived chemorepellent signals, and consistent with this hypothesis, *in vitro* functional analyses confirmed that both netrin-1 and semaphorin 3a were chemorepellent for optic nerve glial precursors (Sugimoto *et al.*, 2001). It seems likely that the different chemorepellents act on different cell populations, with netrin being chemorepulsive for oligodendrocyte precursors and semaphorin 3a repulsive for astrocyte precursors in the optic nerve although this hypothesis awaits further confirmation.

One question raised by the above studies is how ubiquitous guided glial precursor migration is throughout the developing CNS. Studies in the spinal cord indicate that the dispersal of oligodendrocyte precursors from the ventral ventricular zone is in part mediated by localized expression of netrin-1 consistent with this protein being part of a common mechanism (Tsai *et al.*, 2003). Analyses of the migration paths of glial precursors in other regions of the developing CNS, however, suggest the situation may be more complex (Kakita and Goldman, 1999). For example, in striatum, glial precursors take two pathways that are almost perpendicular to each other. These discrete patterns of migration may reflect utilization of alternative cellular substrates of migration such as radial glial fibers or axon tracts (Ono *et al.*, 1997; Meintanis, 2001) rather than secreted guidance cues.

Stop Signals for Oligodendrocyte Precursors

One striking characteristic of the rodent optic system is the spatial restriction of myelination of retinal ganglion cell axons to the optic nerve, while more proximal portions of the same cell's

axons in the retina are unmyelinated. Myelin is distributed evenly along the optic nerve but stops abruptly at the lamina cribosa of the optic nerve head in a number of species, because this region acts as a barrier for the migration of oligodendrocyte precursors (ffrench Constant *et al*., 1988). The mechanisms that inhibit the migration of oligodendrocyte precursors at the optic nerve head may involve localized expression of the ECM molecules such as Tenascin C in conjunction with other signals (Bartsch *et al*., 1994; Kiernan *et al*., 1996). During development, netrin-1 is transiently expressed at the optic nerve head (Deiner, 1997) and it may be that this localized expression of netrin serves as a repulsive cue to stop the migration of oligodendrocyte precursors into the retina. Another candidate stop signal for migrating oligodendrocyte precursors is the chemokine CXCL1, which appears to regulate spatial and temporal patterning of spinal cord myelination through inhibiting cell motility as well as promoting cell proliferation (Tsai *et al*., 2002). The cellular source of these stop signals is in part astrocytes and other glia.

Oligodendrocyte Development in the Embryonic Cortex: Similarities and Differences from the Spinal Cord

Studies on oligodendrocyte development in the cortex are currently indicating that important similarities and differences are seen from the spinal cord even during the earliest stages of brain formation.

As discussed earlier, in both the brain and spinal cord, it currently appears that the ancestors of oligodendrocytes are generated in discrete locations. Analysis of expression of PDGRα and plp/DM20 suggests the existence of a few localized ventral sites of origin (Spassky *et al*., 2000). In the early mouse forebrain, PDGFR-α expression is seen in the MGE and dorsal thalamus, and plp/DM20 is found in the basal (ventral) plate of the diencephalon, zona limitans intrathalamica, caudal hypothalamus, enteropeduncular area, amygdala, and olfactory bulb (Pringle and Richardson, 1993; Spassky *et al*., 1998; Nery *et al*., 2001), as is expression of the *olig1* and *olig2* genes (Lu *et al*., 2000; Zhou *et al*., 2000; Nery *et al*., 2001).

Two recent studies from the Temple (He *et al*., 2001) and Mehler (Yung *et al*., 2002) laboratories have analyzed several aspects of the cellular biology of early ancestors of oligodendrocytes. At least some of the stem cells that give rise to oligodendrocytes in the cortex appear to arise in the basal (ventral) forebrain and migrate into the overlying dorsal forebrain, including the ventricular zone, SVZ, and intermediate zones (Lavdas *et al*., 1999; Wichterle *et al*., 1999; Anderson *et al*., 2001; Marshall and Goldman, 2002). These basal progeny, which express the members of the *dlx* family of homeodomain transcription factors, migrate dorsally and intermix with other cells to form the dorsolateral SVZ (Marshall and Goldman, 2002).

Prior to the tangential migration of stem/progenitor cells from ventral to dorsal forebrain regions, it appears as if the early stages of specification are regionally biased. For example, when grown in medium supplemented with FGF-2 (He *et al*., 2001), or FGF-2 + Shh (Yung *et al*., 2002), MGE and LGE progenitors of the E13.0 ventral forebrain are biased toward the generation of GABAergic neurons compared to stem cells and progenitors derived from dorsal cortex. In addition, prior to E12.5, few of the progenitor cells from dorsal or basal regions produce glia-only clones: most glia arise from stem cells at this stage, suggesting that divergence of glial lineages with the appearance of glial-restricted progenitors occurs predominantly at later stages. Indeed, it may be that the stem cells that are present in the dorsal forebrain prior to the period of tangential migration are not competent to make oligodendrocytes unless they are exposed to Shh (Yung *et al*., 2002), although this was not found by others (He *et al*., 2001; Qian *et al*., 1997).

The above results raise multiple questions. Are the stem cells or progenitor cells that make oligodendrocytes and/or GABAergic neurons truly migrating from the ventral to the dorsal cortex, or is instead the delayed appearance of such cells dorsally a reflection of a temporally regulated differentiation event that has a different timing in different regions of the CNS? To what extent is this specialization reflective of cell-intrinsic controlling mechanisms, and to what extent do cell-extrinsic signaling molecules contribute to this specification? The association of oligodendrocytes with a particular class of neuron (the GABAergic neuron of the cortex, the motor neuron of the spinal cord) is also of particular interest, particularly in light of the ongoing discussions about whether or not oligodendrocytes and motor neurons are derived from a single lineage-restricted progenitor cell (Pringle *et al*., 1996; Richardson *et al*., 1997, 2000; Orentas *et al*., 1999; Lu *et al*., 2000, 2002; Nery *et al*., 2001; Tekki-Kessaris *et al*., 2001; Mekki-Dauriac *et al*., 2002; Rowitch *et al*., 2002; Sauvageot and Stiles, 2002; Takebayashi *et al*., 2002; Zhou *et al*., 2002). But does this generation of oligodendrocytes within a single clone of cells reflect a lineage restriction of a stem/progenitor cell to the generation of only a limited subset of cell types?

Current evidence suggests strongly that the appearance of cells in the dorsal cortex that are able to generate clones containing both GABAergic neurons and oligodendrocytes is truly reflective of a migration of cells from ventral to dorsal regions. Consistent with these observations, the analysis of *dlx2/tauLacZ* knockin mouse (Corbin *et al*., 2000) also indicates that cells derived from subpallial *dlx2*-expressing progenitors migrate dorsally and intermix with other cells to form the dorsolateral SVZ (Marshall and Goldman, 2002). Moreover, in *dlx1/2* −/− mice, in which there is a generalized defect in tangential migration and a reduction in cortical GABAergic neurons (Anderson *et al*., 2001), there is a failure of such cells to populate the dorsal cortex (He *et al*., 2001; Yung *et al*., 2002).

The consistent association of cell fate with position within a tissue raises the possibility that localized ventral and dorsal signals act on stem cells to make them generate particular, region appropriate, cell types. Hence, basal forebrain stem cells are biased early in development to generate GABAergic neurons that predominate in basal forebrain CNS areas (He *et al*., 2001; Yung *et al*., 2002). It has been suggested that initial ventral forebrain specification and tangential cortical migration would expose these bipotent progenitors to sequential ventral and

dorsal gradient morphogens that normally mediate opposing developmental programs (Zhu *et al*., 1999; Yung *et al*., 2002).

Two of the factors thought to play important roles in inducing ventral cortical stem cells to be biased toward the generation of GABAergic neurons and oligodendrocytes are Shh and BMPs. It appears to be a common principle along the neuraxis that Shh and BMPs are ventral and dorsal gradient morphogens, respectively (Briscoe and Ericson, 1999; Miller *et al*., 1999; Thomas *et al*., 2000), and the role of these signaling molecules in development of the spinal cord has been discussed previously. The concentration of these molecules to which cells are exposed causes elaboration of specific sets of homeodomain and basic helix-loop-helix (bHLH) transcription factors that control the details of cell specification through their combinatorial interactions (Zhou *et al*., 2001, 2002; Rowitch *et al*., 2002). In dorsal domains of the spinal cord, BMP signaling is thought to promote the generation of astrocytes, while Shh promotes the localized generation of motor neurons and oligodendrocytes (Pringle *et al*., 1996; Mabie *et al*., 1997; Richardson *et al*., 1997, 2000; Orentas *et al*., 1999; Lu *et al*., 2000; Mehler *et al*., 2000; Zhou *et al*., 2000; Davies and Miller, 2001; Nery *et al*., 2001; Tekki-Kessaris *et al*., 2001; Mekki-Dauriac *et al*., 2002).

Despite the apparent role of Shh and BMP in directing differentiation of cortical stem/progenitor cells, as well as spinal cord stem/progenitor cells, there are important differences between these two tissues. This difference is already seen at the level of genes induced in cortical stem cells by exposure to Shh. For example, it currently appears that while both cortical and spinal cord stem cells are induced to express *olig2* by exposure to Shh, the cortical cells are induced to express *mash1* while spinal cord stem cells are induced to express *neurogenin2* (Mizuguchi *et al*., 2001; Novitch *et al*., 2001; Sun *et al*., 2001; Zhou *et al*., 2001;Yung *et al*., 2002).

Data reported thus far indicates that the role of BMP may be more complex in the cortex than has thus far been revealed in the spinal cord. Shh promotes generation of GABAergic neurons and oligodendrocytes, but the sequential elaboration of these cells requires spatial and temporal modulation of cortical BMP signaling by BMP and the BMP antagonist, noggin (Yung *et al*., 2002). For example, coincident with the establishment of the cortical SVZ, BMPs from the BMP2/4 factor subgroup now enhance the specification of late-born cortical (GABAergic) neurons. It seems that Shh promotes lineage restriction of ventral forebrain stem cells, in part, by upregulation of Olig2 and Mash1. BMP2 subsequently promotes GABAergic neuronal lineage elaboration by differential modulation of Olig2 and Mash1. Thus, when applied together with Shh, BMP2 potentiates the elaboration of GABAergic neurons from cortical stem/progenitor cells and suppresses oligodendrocyte generation (Mabie *et al*., 1999; Mehler *et al*., 2000), while the BMP antagonist noggin promotes the generation of oligodendrocytes (Li *et al*., 1998; Mehler *et al*., 2000).

How can the above results indicating BMP-promoted generation of neurons be integrated with experiments in the spinal cord (and also on cells derived from the developing brain) indicating that BMPs promote the generation of astrocytes and suppress the generation of oligodendrocytes (Gross *et al*., 1996; Mabie *et al*., 1997; Grinspan *et al*., 2000; Mehler *et al*., 2000; Nakashima *et al*., 2001; Mekki-Dauriac *et al*., 2002; Gregori *et al*., 2002b; Gomes *et al*., 2003)? It is possible that BMP, a potent anti-mitotic agent, is generally able to stimulate differentiation of progenitor cells but that the pathway of differentiation that is promoted is dependent upon as yet poorly understood changes in the target precursor cells themselves. One potentially interesting aspect of the studies of Yung *et al*. (2002), however, that may be relevant to BMP-mediated induction of neuron generation is that these studies address questions about what happens when cells are exposed to more than a single signaling molecule (i.e., Shh + BMP-2), a situation that seems likely to more closely resemble the realities of biology than exposure to a single agent. In this context, an attractive potential solution to this conundrum that needs to be explored is whether the combined exposure of cortical stem cells to BMP and Shh (the conditions applied in the studies of Yung *et al*., 2002) reveals an aspect of BMP signaling different from that which occurs when cells are exposed to BMP alone. Consistent with this possibility, continued Shh exposure also appears to suppress the generation of astrocytes in cortical stem/progenitor cells, which were only seen in cultures of these progenitor cells when expression of *olig2* and *Mash1* was ablated by exposure to antisense oligonucleotide constructs (Yung *et al*., 2002).

It will be of great interest to determine whether the correct paradigm for understanding the interactions between BMP-induced pathways and Shh-induced pathways might be that BMP always suppresses oligodendrocyte generation, but the directionality imposed by BMP is dependent upon the other signals to which the recipient cell is exposed, as well as on the differentiation potential of the target cell itself.

E13.5 Rat Cortex Contains A2B5$^+$ Cells That Can Generate Oligodendrocytes, Two Different Astrocyte Populations and Neurons

As studies conducted by He *et al*. (2001) and Yung *et al*. (2002) indicate the existence of precursor cells that make neurons and glia, and other cells that are restricted to the generation of glia, it is of interest to know the identity of these precursor cells.

Analysis of the precursor cell populations in the cortex, although in their early stages, are revealing a level of complexity not seen in the spinal cord at this age (Noble *et al*., 2003). The E13.5 cortex contains abundant A2B5$^+$ cells that do not express antigens associated with astrocytes or oligodendrocytes. *In vitro* characterization of the differentiation potential of these cells demonstrated that, in contrast with results in the spinal cord (Rao *et al*., 1998), at least some of the cortex-derived A2B5$^+$ cells can generate neurons when grown in the presence of NT-3 and retinoic acid (Noble *et al*., 2003).

A more detailed analysis of the A2B5$^+$ cell population isolated from E13.5 cortex indicates the presence of antigenically distinct subpopulations, only one of which thus far has been found to generate neurons *in vitro*. The subpopulation of cells

that is competent to generate neurons also expresses PSA-NCAM, an antigen that has been found in several instances to be expressed by precursor cells able to generate neurons (Doetsch *et al*., 1997; Mayer-Pröschel *et al*., 1997; Weickert *et al*., 2000). Removal of the PSA-NCAM$^+$ cells from the A2B5$^+$ population was associated with the loss of generation of neurons from this population (Noble *et al*., 2003).

Although the A2B5$^+$/PSA-NCAM$^-$ cells derived from E13.5 cortex appear to be restricted to the generation of glia in their differentiation potential, this population is more heterogeneous than antigenically identical cells isolated from the spinal cord. Unlike the spinal cord, only 44% of clones derived from A2B5$^+$ cells contained both type-1 and type-2 astrocytes when exposed to BMP-4. Many of the cortex-derived clones contained only one astrocyte population, with 16% of clones containing only type-2 astrocytes, and 17% containing type-1 astrocytes only, with no progenitor-like cells found in any of these clones. Virtually all cells appeared to be competent to generate oligodendrocytes, however, as 86% of clones contained at least one oligodendrocyte after being exposed to PDGF$^+$ T3 for five days.

Thus, it appears that the E13.5 rat cortex contains cells with the same antigenic phenotype and differentiation potential of tripotential GRP cells isolated from the embryonic spinal cord. Further investigations are required to determine the degree of identity of these cells with GRP cells of the spinal cord, particularly due to the complexity of the A2B5$^+$ populations isolated from the cortex. In addition, the embryonic cortex contains a further population of A2B5$^+$ cells that co-express PSA-NCAM, an antigen not expressed by GRP cells of the spinal cord. These cells, but not the PSA-NCAM$^-$/A2B5$^+$ cells, are able to generate neurons *in vitro*. Moreover, the observations that ~16% of the clones derived from A2B5$^+$/PSA-NCAM$^-$ cells generated only type-2 astrocytes when exposed to BMP, and ~17% generated clones containing only type-1 astrocytes in these conditions, demonstrates further differences between the A2B5$^+$ population of the E13.5 cortex and the E13.5 spinal cord. In the cord, in contrast with the cortex, this population shows a striking homogeneity with respect to the cell types generated in different conditions (Rao *et al*., 1998; Gregori *et al*., 2002b).

The full differentiation potential of the A2B5/PSA-NCAM double-positive cells that we have identified is still under study. Whether individual cells are capable of generating both neurons and glia is not yet. known. What seems clear, however, is that the embryonic rat cortex contains some A2B5+ precursor cell populations with properties quite different from the A2B5+ populations isolated from the embryonic spinal cord or the developing optic nerve.

Achieving a detailed understanding of the various PSA-NCAM$^+$ populations in the cortex is going to require a considerable research effort. The studies of Noble *et al*. (2003) indicate that, at E13.5, there are both A2B5/PSA-NCAM double-positive cells and other cells that express PSA-NCAM only. It seems clear that the former cells can generate neurons, but it is not yet known whether either group of cells is restricted to the generation of neurons. Multiple previous studies have documented expression of PSA-NCAM on precursors of neurons, including on neuron-restricted precursor cells of the spinal cord (Doetsch and Alvarez-Buylla, 1996; Doetsch *et al*., 1997; Mayer-Pröschel *et al*., 1997; Weickert *et al*., 2000). It has also been previously reported that PSA-NCAM$^+$ cells found in the perinatal SVZ differentiate into astrocytes and oligodendrocytes *in vivo* (Levison *et al*., 1993; Keirstead *et al*., 1999). Still other data suggest that PSA-NCAM$^+$ cells may be able to generate neurons, oligodendrocytes, and astrocytes following transplantation *in vivo* (Vitry *et al*., 2001), while *in vitro* studies have described a PSA-NCAM$^+$/A2B5$^-$ precursor cell that can give rise to A2B5$^+$ O-2A/OPCs (Grinspan *et al*., 1990; Grinspan and Franceschini, 1995; Ben-Hur *et al*., 1998; Grinspan *et al*., 2000).

The heterogeneity of the A2B5$^+$ populations derived from the E13.5 cortex underscores the need for clonal analysis and detailed cell purification protocols in order to analyze successfully the developmental potential of a putative precursor cell population. Any studies on cortical development that do not separate these populations of cells from each other will be impossible to interpret unambiguously. As almost none of the previous studies conducted have combined antigenic characterization of precursor cells with clonal analysis, it is not possible to interpret data contained therein with regard to the lineage potential of particular precursor cell populations. For example, the analysis of purified A2B5$^+$ cells from the E13.5 cortex would lead to the conclusion that cells with this antigenic phenotype can generate neurons. If one were to accept the conclusions of previous studies carried out in the developing rat CNS that A2B5$^+$ cells are glial-restricted progenitor cells (whether O-2A/OPCs, GRP cells, or astrocyte progenitor cells [e.g., Raff *et al*., 1983; Fok-Seang and Miller, 1992, 1994; Rao *et al*., 1998; Mi and Barres, 1999; Power *et al*., 2002]), one might then draw the conclusion that growth *in vitro* is associated with generation of neurons from glial progenitor cells (as in, e.g., Kondo and Raff, 2000). It has been suggested, at least in the case of the studies of Kondo and Raff, that a potential complicating issue in such studies is the presence of a low frequency of true multipotent NSCs in many regions of the perinatal CNS, including the perinatal optic nerve (D. Van der Kooy, unpublished observations). Another possibility is that the failure to distinguish between the PSA-NCAM-positive and negative subsets of A2B5$^+$ cells would lead to a misinterpretation of the behavior of what appears from our analysis thus far to represent two distinct populations of cells.

One of the other potentially intriguing differences between cortical- and optic nerve-derived O-2A/OPCs that has been described is that only the cortical progenitor cells express members of the *dlx* family of transcriptional regulators (He *et al*., 2001). While *dlx1/2* is not required for oligodendrocyte generation (He *et al*., 2001), it is not known if such expression confers different properties on those precursor populations that are expression-positive. It is important to note, however, that just as generation of oligodendrocytes is an ongoing process in the cortex, so also is the generation of progenitor cells. For example, migration of cells from the LGE/MGE may continue after the earliest wave of tangential migration, as retroviral labeling of LGE/MGE cells in slice cultures harvested from E16 mice and grown *in vitro* for up to 72 hr demonstrates migration of cells into

the perinatal SVZ of each slice (Marshall and Goldman, 2002). Nothing is known at this time as to whether O-2A/OPCs express different properties if they are generated from ancestral populations that differ in the spatial *or* their temporal origin, or whether the differences between O-2A/OPCs isolated from cortex and optic nerve discussed in the following section of this chapter are the results of exposure to tissue-specific instructive signals after this stage of lineage restriction has been achieved.

Is There More than One Path to an Oligodendrocyte and Is There More than One Kind of Oligodendrocyte?

All of the above discussions have been formulated as though there was only one path to generating an oligodendrocyte. It may well be that such an idea represents an oversimplification.

One of the striking aspects of CNS development is that different regions of this tissue develop according to different schedules, with great variations seen in the timing of both neurogenesis and gliogenesis. For example, neuron production in the rat spinal cord is largely complete by the time of birth, is still ongoing in the rat cerebellum for at least several days after birth, and continues in the olfactory system and in some regions of the hippocampus of multiple species throughout life. Similarly, myelination has long been known to progress in a rostral–caudal direction, beginning in the spinal cord significantly earlier than in the brain (e.g., Macklin and Weill, 1985; Kinney *et al*., 1988; Foran and Peterson, 1992). Even within a single CNS region, myelination is not synchronous. In the rat optic nerve, for example, myelinogenesis occurs with a retinal-to-chiasmal gradient, with regions of the nerve nearest the retina becoming myelinated first (Skoff *et al*., 1980; Foran and Peterson, 1992). The cortex itself shows the widest range of timing for myelination, both initiating later than many other CNS regions (e.g., Macklin and Weill, 1985; Kinney *et al*., 1988; Foran and Peterson, 1992) and exhibiting an ongoing myelinogenesis that can extend over long periods of time. This latter characteristic is seen perhaps most dramatically in the human brain, for which it has been suggested that myelination may not be complete until after several decades of life (Yakovlev and Lecours, 1967; Benes *et al*., 1994).

Variant time courses of development in different CNS regions could be due to two fundamentally different reasons. One possibility is that precursor cells are sufficiently plastic in their developmental programs that local differences in exposure to modulators of division and differentiation may account for these variances. Alternatively, it may be that the precursor cells, resident in particular tissues, express differing biological properties related to the timing of development in the tissue to which they contribute.

As has been discussed earlier, there is ample evidence for extensive plasticity in the behavior of O-2A/OPCs, which appear to be the direct ancestor of oligodendrocytes. O-2A/OPCs obtained from the optic nerves of seven-day-old (P7) rat pups and grown in the presence of saturating levels of PDGF exhibit an approximately equal probability of undergoing a self-renewing division or exiting the cell cycle and differentiating into an oligodendrocyte (Yakovlev *et al*., 1998b). The tendency of dividing O-2A/OPCs to generate oligodendrocytes is enhanced if cells are co-exposed to such signaling molecules as TH, CNTF, or retinoic acid (e.g., Barres *et al*., 1994a; Mayer *et al*., 1994; Ibarrola *et al*., 1996). In contrast, co-exposure to NT-3 or basic FGF inhibits differentiation and is associated with increased precursor cell division and self-renewal (Bogler *et al*., 1990; Barres *et al*., 1994b; Ibarrola *et al*., 1996). The balance between self-renewal and differentiation in dividing O-2A/OPCs can also be modified by the concentrations of the signaling molecules to which they are exposed, as well as by intracellular redox state (Smith *et al*., 2000). Thus, the effects of the microenvironment could theoretically have considerable effects on the timing and extent of oligodendrocyte generation.

Recent experiments have raised the possibility that the differing timing of oligodendrocyte generation and myelination in different CNS regions is associated with the existence of regionally specialized O-2A/OPCs (Power *et al*., 2002). Characterization of O-2A/OPCs isolated from different regions indicates that these developmental patterns are consistent with properties of the specific O-2A/OPCs resident in each region. In particular, cells isolated from optic nerve, optic chiasm, and cortex of identically aged rats show marked differences in their tendency to undergo self-renewing division and in their sensitivity to known inducers of oligodendrocyte generation. Precursor cells isolated from the cortex, a CNS region where myelination is a more protracted process than in the optic nerve, appear to be intrinsically more likely to begin generating oligodendrocytes at a later stage and over a longer time period than cells isolated from the optic nerve. For example, in conditions where optic nerve-derived O-2A/OPCs generated oligodendrocytes within 2 days, oligodendrocytes arose from chiasm-derived cells after 5 days and from cortical O-2A/OPCs only after 7–10 days. These differences, which appear to be cell-intrinsic, were manifested both in reduced percentages of clones producing oligodendrocytes and in a lesser representation of oligodendrocytes in individual clones. In addition, responsiveness of optic nerve-, chiasm-, and cortex-derived O-2A/OPCs to TH and CNTF, well-characterized inducers of oligodendrocyte generation, was inversely related to the extent of self-renewal observed in basal division conditions.

The above results indicate that the O-2A/OPC population may be more complex than initially envisaged, with the properties of the precursor cells resident in any particular region being reflective of differing physiological requirements of the tissues to which these cell contribute. For example, as discussed earlier, a variety of experiments have indicated that the O-2A/OPC population of the optic nerve arises from a germinal zone located in or near the optic chiasm and enters the nerve by migration (Small *et al*., 1987; Ono *et al*., 1995). Thus, it would not be surprising if the progenitor cells of the optic chiasm expressed properties expected of cells at a potentially earlier developmental stage than those cells that are isolated from optic nerve of the same physiological age. Such properties would be expected to include the capacity to undergo a greater extent of self-renewal, much as has been seen when the properties of O-2A/OPCs from

optic nerves of embryonic rats and postnatal rats have been compared (Gao and Raff, 1997). With respect to the properties of cortical progenitor cells, physiological considerations also appear to be consistent with our observations. The cortex is one of the last regions of the CNS in which myelination is initiated, and the process of myelination can also continue for extended periods in this region (Macklin and Weill, 1985; Kinney *et al.*, 1988; Foran and Peterson, 1992). If the biology of a precursor cell population is reflective of the developmental characteristics of the tissue in which it resides, then one might expect that O-2A/OPCs isolated from this tissue would not initiate oligodendrocyte generation until a later time than it occurs with O-2A/OPCs isolated from structures in which myelination occurs earlier. In addition, cortical O-2A/OPCs might be physiologically required to make oligodendrocytes for a longer time due to the long period of continued development in this tissue, at least as this has been defined in the human CNS (e.g., Yakovlev and Lecours, 1967; Benes *et al.*, 1994).

The observation that O-2A/OPCs from different CNS regions express different levels of responsiveness to inducers of differentiation adds a new level of complexity to attempts to understand how different signaling molecules contribute to the generation of oligodendrocytes. This observation also raises questions about whether cells from different regions also express differing responses to cytotoxic agents, and whether such differences can be biologically dissected so as to yield a better understanding of this currently mysterious form of biological variability.

If there are multiple biologically distinct populations of O-2A/OPCs, it is important to consider whether similar heterogeneity exists among oligodendrocytes themselves. Evidence for morphological heterogeneity among oligodendrocytes is well established. Early silver impregnation studies identified four distinct morphologies of myelinating oligodendrocytes and this was largely confirmed by ultrastructural analyses in a variety of species (Bjartmar *et al.*, 1968; Stensaas and Stensaas, 1968; Remahl and Hildebrand, 1990). Oligodendrocyte morphology is closely correlated with the diameter of the axons with which the cell associates (Butt *et al.*, 1997, 1998). Type I and II oligodendrocytes arise late in development and myelinate many internodes on predominantly small diameter axons while type III and IV oligodendrocytes arise later and myelinate mainly large diameter axons. Such morphological and functional differences between oligodendrocytes are associated with different biochemical characteristics. Oligodendrocytes that myelinate small diameter fibers (type I and II) express higher levels of carbonic anhydrase II (CAII) (Butt *et al.*, 1995, 1998), while those myelinating larger axons (type III and IV) express a specific small isoform of the MAG (Butt *et al.*, 1998). Whether such differences represent the response of homogenous cells to different environments or distinct cell lineages is unclear. Transplant studies demonstrated that presumptive type I and II cells have the capacity to myelinate both small and large diameter axons suggesting that the morphological differences are environmentally induced (Fanarraga *et al.*, 1998). By contrast, some developmental studies have been interpreted to suggest that the different classes of oligodendrocytes may be derived from biochemically distinct precursors (Spassky *et al.*, 2000) that differ in expression of PDGFR-α and PLP/Dm20, although more recent studies are not necessarily supportive of this hypothesis (Mallon *et al.*, 2002).

Just as there is heterogeneity among O-2A/OPCs, it also seems likely that heterogeneity exists among earlier glial precursor cell populations. Separate analysis of GRP cell populations derived from ventral and dorsal spinal cord demonstrates that ventral-derived GRPs may differ from dorsal cells in such a manner as to increase the probability that they will generate O2A/OPCs and/or oligodendrocytes, even in the presence of BMP (Gregori *et al.*, 2002b). Ventral-derived GRP cells yield several-fold larger numbers of oligodendrocytes over the course of several days of *in vitro* growth. When low doses of BMP-4 were applied to dorsal and ventral cultures, the dorsal cultures contained only a few cells with the antigenic characteristics of O-2A/OPCs. In contrast, over half of the cells in ventral-derived GRP cell cultures exposed to low doses of BMP differentiated into cells with the antigenic characteristics of O-2A/OPCs. Whether the O-2A/OPCs or oligodendrocytes derived from dorsal vs ventral GRP cells express different properties is not yet known.

OLIGODENDROCYTE PRECURSORS IN THE ADULT CNS

Once the processes of development ends, there is still a need for a pool of precursor cells for the purposes of tissue homeostasis and repair of injury. It is thus perhaps not surprising to find that the adult CNS also contains O-2A/OPCs. What is rather more remarkable is that current estimates are that these cells (or, at least cells with their antigenic characteristics) may be so abundant in both gray matter and white matter as to comprise 5–8% of all the cells in the adult CNS (Dawson *et al.*, 2000). If such a frequency of these cells turns out to be accurate, then a strong argument can be made that they should be considered the fourth major component of the adult CNS, after astrocytes, neurons, and oligodendrocytes themselves. Moreover, as discussed later, it appears that these cells may represent the major dividing cell population in the adult CNS.

Studies *In Vitro* Reveal Novel Properties of Adult O-2A/OPCs

There are a variety of substantial biological differences between O-2A/OPCs of the adult and perinatal CNS (originally termed O-2A^{perinatal} and O-2A^{adult} progenitor cells, respectively) (Wolswijk and Noble, 1989, 1992; Wolswijk *et al.*, 1990, 1991; Wren *et al.*, 1992). For example, in contrast with the rapid cell cycle times (18 ± 4 hr) and migration (21.4 ± 1.6 μm hr^{-1}) of O-2A/OPCs^{perinatal}, O-2A/OPCs^{adult} exposed to identical concentrations of PDGF divide *in vitro* with cell cycle times of 65 ± 18 hr and migrate at rates of 4.3 ± 0.7 μm hr^{-1}. These cells are also morphologically and antigenically distinct. O-2A/OPCs^{adult} grown *in vitro* are unipolar cells, while O-2A/OPCs^{perinatal}

express predominantly a bipolar morphology. Both progenitor cell populations are labeled by the A2B5 antibody, but *adult* O-2A/OPCs share the peculiar property of oligodendrocytes of expressing no intermediate filament proteins. In addition, it appears thus far that *adult* O-2A/OPCs are always labeled by the O4 antibody, while *perinatal* O-2A/OPCs may be either $O4^-$ or $O4^+$ (although the $O4^+$ cells *perinatal* cells do express different properties than their $O4^-$ ancestors [Gard and Pfeiffer, 1993; Warrington *et al*., 1993]).

One of the particularly interesting features of *adult* O-2A/OPCs is that when these cells are grown in conditions that promote the differentiation into oligodendrocytes of all members of clonal families of O-2A/OPCs^{perinatal}, O-2A/OPCs^{adult} exhibit extensive asymmetric behavior, continuously generating both oligodendrocytes and more progenitor cells (Wren *et al*., 1992). Thus, even though under basal division conditions both *perinatal* and *adult* O-2A/OPCs undergo asymmetric division and differentiation, this tendency is expressed much more strongly in the *adult* cells. Indeed, it is not yet known if there is a condition in which *adult* progenitor cells can be made to undergo the complete clonal differentiation that occurs in *perinatal* O-2A/OPC clones in certain conditions (Ibarrola *et al*., 1996).

Another feature of interest with regard to *adult* O-2A/OPCs is that these cells do have the ability to enter into limited periods of rapid division, which appear to be self-limiting in their extent. This behavior is manifested when cells are exposed to a combination of PDGF + FGF-2, in which conditions the *adult* O-2A/OPCs express a bipolar morphology and begin migrating rapidly (with an average speed of approximately 15 μm hr^{-1}. In addition, their cell cycle time shortens to an average of approximately 30 hr in these conditions (Wolswijk and Noble, 1992). These behaviors continue to be expressed for several days after which, even when maintained in the presence of PDGF + FGF-2, the cells re-express the typical unipolar morphology, slow migration rate and long cell cycle times of freshly isolated *adult* O-2A/OPCs. Other growth conditions, such as exposure to glial growth factor (GGF) can elicit a similar response (Shi *et al*., 1998).

As can be seen from the above, *adult* O-2A/OPCs in fact express many of the characteristics that are normally associated with stem cells in adult animals. They are relatively quiescent, yet have the ability to rapidly divide as transient amplifying populations of the sort generated by many stem cells in response to injury. They also appear to be present throughout the life of the animal, and can even be isolated from elderly rats (which, in the rat, equals about two years of age). In this respect, the definition of a stem cell can be seen to be a complex one, for the *adult* O-2A/OPC would have to be classified as a narrowly lineage-restricted stem cell (in contrast with the pluripotent neuroepithelial stem cell).

The differing phenotypes of *adult* and *perinatal* O-2A/OPCs are strikingly reflective of the physiological requirements of the tissues from which they are isolated. O-2A/OPC^{perinatal} progenitor cells express properties that might be reasonably expected to be required during early CNS development (e.g., rapid division and migration, and the ability to rapidly generate large numbers of oligodendrocytes). In contrast, O-2A/OPC^{adult} progenitor cells express stem cell-like properties that appear to be more consistent with the requirements for the maintenance of a largely stable oligodendrocyte population, and the ability to enter rapid division as might be required for repair of demyelinated lesions (Wolswijk and Noble, 1989, 1992; Wren *et al*., 1992).

It is of particular interest to consider the developmental relationship between *perinatal* and *adult* O-2A/OPCs in light of their fundamentally different properties. One might imagine, for example, that these two distinct populations are derived from different neuroepithelial stem cell populations, which produce lineage-restricted precursor cells with appropriate phenotypes as warranted by the developmental age of the animal. As it has emerged, the actual relationship between these two populations is even more surprising in its nature.

There are multiple indications that the ancestor of the O-2A/OPC^{adult} is in fact the *perinatal* O-2A/OPC itself (Wren *et al*., 1992). This has been shown both by repetitive passaging of *perinatal* O-2A/OPCs, which yields over the course of a few weeks cultures of cells with the characteristics of *adult* O-2A/OPCs. Moreover, time-lapse microscopic observation of clones of *perinatal* O-2A/OPCs provides a direct demonstration of the generation of unipolar, slowly dividing and slowly migrating *adult* cells from bipolar, rapidly dividing and rapidly migrating *perinatal* ones. The processes that modulate this transition remain unknown, but appear to involve a cell-autonomous transition that can be induced to happen more rapidly if *perinatal* cells are exposed to appropriate inducing factors. Intriguingly, one of the inducing factors for this transition appears to be TH, which is also a potent inducer of oligodendrocyte generation (Tang *et al*., 2000). How the choice of a *perinatal* O-2A/OPC to become an oligodendrocyte or an *adult* O-2A/OPC is regulated is wholly unknown.

The generation of *adult* O-2A/OPCs from *perinatal* O-2A/OPCs places the behavior of the *adult* cells exposed to PDGF + FGF-2 in an interesting context. It appears that the underlying genetic and metabolic changes that lead to expression of the *perinatal* phenotype are not irreversibly lost upon generation of the *adult* phenotype. Instead, they are placed under a different control so that very specific combinations of signals are required to elicit them (Wolswijk and Noble, 1992).

Studies *In Vivo*

Based upon the expression of such antigens as NG2 and PDGFR-α, a great deal has been learned regarding the biology of cells *in situ* that are currently thought to be *adult* O-2A/OPCs. Using these antibodies, and the O4 antibody, to label cells, it has been seen that the behavior of putative *adult* O-2A/OPCs *in vivo* is highly consistent with observations made *in vitro*. Adult OPCs do divide *in situ* but, as *in vitro*, they are not rapidly dividing cells in most instances. For example, the labeling index for cells of the adult cerebellar cortex is only 0.2–0.3%. Nonetheless, as there are few other dividing cells in the brain outside of those found in highly specialized germinal zones (such as the SVZ and the

dentate gyrus of the hippocampus), the adult OPC appears to represent the major dividing cell population in the parenchyma of the adult brain (Levine *et al*., 1993; Horner *et al*., 2000). Indeed, of the cells of the uninjured adult brain and spinal cord, it appears that 70% or more of these cells express NG2 (and thus, by current evaluations, might be considered to be adult OPCs) (Horner *et al*., 2000). That these cells are engaged in active division is also confirmed by studies in which retroviruses are injected into the brain parenchyma. As the retroviral genome requires cell division in order to be incorporated into a host cell genome, only dividing cells express the marker gene encoded in the retroviral genome. In these experiments, 35% of all the CNS cells that label with retrovirus are NG2-positive (Levison *et al*., 1999). However, it must be stressed for all of these experiments that it is by no means clear that all of the NG2-expressing (or O4-expressing or PDGFR-α-expressing) cells in the adult CNS are adult O-2A/OPCs. In the hippocampus, for example, such cells may also be able to give rise to neurons (Belachew *et al*., 2003).

One of the most likely functions of *adult* O-2A/OPCs is to provide a reservoir of cells that can respond to injury. As oligodendrocytes themselves do not appear to divide following demyelinating injury (Keirstead and Blakemore, 1997; Carroll *et al*., 1998; Redwine and Armstrong, 1998), the O-2A/OPC^{adult} is of particular interest as a potential source of new oligodendrocytes following demyelinating damage.

Observations made *in vivo* are also consistent with *in vitro* demonstrations that *adult* O-2A/OPCs can be triggered to enter transiently into a period of rapid division. When lesions are created in the adult CNS by injection of anti-oligodendrocyte antibodies (Gensert and Goldman, 1997; Keirstead *et al*., 1998; Redwine and Armstrong, 1998; Cenci di Bello *et al*., 1999), division of NG2^{+} cells is observed in the area adjacent to lesion sites. Rapid increases in the number of *adult* O-2A/OPCs are also seen following creation of demyelinated lesions by injection of ethidium bromide, viral infection, or production of experimental allergic encephalomyelitis (Armstrong *et al*., 1990a; Redwine and Armstrong, 1998; Cenci di Bello *et al*., 1999; Levine and Reynolds, 1999; Watanabe *et al*., 2002). Most of the putative O-2A/OPCs^{adult} in the region of a lesion have the bipolar appearance of immature perinatal glial progenitors rather than the unipolar morphology that appears to be more typical of the *adult* O-2A/OPC, just as is seen *in vitro* when O-2A/OPCs^{adult} are induced to express a rapidly dividing phenotype by exposure to PDGF + FGF-2 (Wolswijk and Noble, 1992). It is also clear that cells that enter into division following injury are responsible for the later generation of oligodendrocytes (Watanabe *et al*., 2002).

A variety of observations indicate that the *adult* O-2A/OPCs react differently depending upon the nature of the CNS injury to which they are exposed. Adult OPCs seems to respond to almost any CNS injury (Armstrong *et al*., 1990a; Levine, 1994; Gensert and Goldman, 1997; Keirstead *et al*., 1998; Redwine and Armstrong, 1998; Cenci di Bello *et al*., 1999; Levine and Reynolds, 1999; Watanabe *et al*., 2002). Response is rapid, and reactive cells (as determined by morphology) can be seen within 24 hr. Kainate lesions of the hippocampus produce the same kinds of changes in NG2+ cells. It appears, however, that the occurrence of demyelination is required to induce *adult* O-2A/OPCs to undergo rapid division *in situ*, even though these cells do show evidence of reaction to other kinds of lesions. For example, *adult* O-2A/OPCs respond to inflammation by undergoing hypertrophy and upregulation of NG2 but, intriguingly, increases in cell division are only seen when inflammation is accompanied by demyelination or more substantial tissue damage (Levine, 1994; Nishiyama *et al*., 1997; Redwine and Armstrong, 1998; Cenci di Bello *et al*., 1999). It also appears that there is a greater increase in response to anti-GalC mediated damage if there is concomitant inflammation (Keirstead *et al*., 1998; Cenci di Bello *et al*., 1999), indicating that the effects of demyelination on these cells are accentuated by the occurrence of concomitant injury. In this respect, the ability of GRO-α to enhance the response of spinal cord–derived *perinatal* O-2A/OPCs to PDGF may be of particular interest (Robinson *et al*., 1998), although it is not yet known if *adult* O-2A/OPCs show any similar responses to Gro-α. Also in agreement with *in vitro* characterizations of *adult* O-2A/OPCs are observations that the progression of remyelination in the adult CNS, however, is considerably slower than is seen in the perinatal CNS (Shields *et al*., 1999).

The wide distribution of O-2A/OPCs *in situ* is also consistent with the idea that these cells are stem cells with a primary role of participating in oligodendrocyte replacement in the normal CNS and in response to injury. It is not clear, however, whether these cells might also express other functions. For example, it is not clear whether *adult* O-2A/OPCs contribute to the astrocytosis that occurs in CNS injury. Glial scars made from astrocytes envelop axons after most types of demyelination (Fok-Seang *et al*., 1995; Schnaedelbach *et al*., 2000). It is known that O-2A/OPCs produce neurocan, phosphacan, NF2, and versican, all of which are present in sites of injury (Asher *et al*., 1999, 2000; Jaworski *et al*., 1999) and can inhibit axonal growth (Dou and Levine, 1994; Fawcett and Asher, 1999; Niederost *et al*., 1999). It is possible that much of the inhibitory chondroitin sulfate proteoglycans found at sites of brain injury are derived from *adult* O-2A/OPCS, or from astrocytes made by *adult* O-2A/OPCs. Whether still other possible functions also need to be considered is a matter of some interest. For example, glutaminergic synapses have been described in the hippocampus on cells thought to be *adult* O-2A/OPCs (Bergles *et al*., 2000). What the cellular function of such synapses might be is not known.

If there are so many O-2A/OPCs in the adult CNS, then why is remyelination not more generally successful? It seems clear that remyelination of initial lesions is well accomplished (at least if they are small enough), but that repeated episodes of myelin destruction eventually result in the formation of chronically demyelinated axons. It seems that after the lesions are resolved, the O-2A/OPCs^{adult} return to pre-lesion levels, consistent with their ability to undergo asymmetric division (Wren *et al*., 1992; Cenci di Bello *et al*., 1999; Levine and Reynolds, 1999). It also seems clear that there are *adult* O-2A/OPCs within chronically demyelinated lesions (Nishiyama *et al*., 1999; Chang *et al*., 2000; Dawson *et al*., 2000; Wolswijk, 2000). Thus, the stock of these does not appear to be completely exhausted.

However, the O-2A/OPCs that are found in such sites as the lesions of individuals with multiple sclerosis (MS) are remarkably quiescent, showing no labeling with antibodies indicative of cells engaged in DNA synthesis (Wolswijk, 2000). The reasons for such quiescent behavior are unknown. There are claims that electrical activity in the axon is involved in regulating survival and differentiation of *perinatal* O-2A/OPCs in development (Barres and Raff, 1993), and it is not known if similar principles apply in demyelinated lesions in which neuronal activity is perhaps compromised. It is also possible that lesion sites produce cytokines, such as TGF-β, that would actively inhibit O-2A/OPC division. At present, however, the reasons why the endogenous precursor pool is not more successful in remyelinating extensive, or repetitive, demyelinating lesions is not known.

The possibility must also be appreciated that there may exist heterogeneity within populations of *adult* O-2A/OPCs (analogous to that seen for *perinatal* O-2A/OPCs; Power *et al*., 2002). Whether such heterogeneity exists, and what its biological relevance might be (e.g., with respect to sensitivity to damage and capacity for repair in the adult CNS), should prove a fruitful ground for continued exploration.

Oligodendrocytes and Their Precursors as Modulators of Neuronal Development and Function

There are multiple indications that oligodendrocytes not only myelinate neurons, but also provide a large variety of signals that modulate axonal function. It has long been known that association of axons with oligodendrocytes has profound physical effects on the axon, and is associated with substantial increases in axonal diameters. Animals in which oligodendrocytes are destroyed (e.g., by radiation) and defective (as in animals lacking PLP) show substantial axonal abnormalities (Colello *et al*., 1994; Griffiths *et al*., 1998). In addition, axonal damage, leading eventually to axonal loss, may also occur in MS (Trapp *et al*., 1998).

One of the dramatic effects of O-2A/OPC lineage cells on axons is to modulate axonal channel properties. During early development, both Na^+ and K^+ channels are distributed uniformly along axons, but become clustered into different axonal domains coincident with the process of myelination (Peles and Salzer, 2000; Rasband and Shrager, 2000). Na^+ channels specifically become clustered into the nodes of Ranvier, the regions of exposed axonal membrane that lay between consecutive myelin sheaths. K^+ channels, in contrast, become clustered in the juxtaparanodal region.

It has become clear from multiple studies that Schwann cells in the peripheral nervous system (PNS), and oligodendrocytes in the CNS, play instructive roles in the clustering of axonal ion channels (Kaplan *et al*., 1997, 2001; Peles and Salzer, 2000; Rasband and Shrager, 2000). These effects are quite specific in their effects on particular channels. Contact with oligodendrocytes, or growth of neurons in oligodendrocyte-conditioned medium, is sufficient to induce axonal clustering of $Na_v1.2$ and β2 subunits, but not of $Na_v1.6$ channels (Kaplan *et al*., 2001). It is not yet known what regulates $Na_v1.6$ clustering, but this may require myelination itself to proceed. Once clustering has occurred, *in vitro* analysis suggests that soluble factors produced by oligodendrocytes are not required to maintain the integrity of the channel clusters.

The ability of oligodendrocytes to modulate axonal channel clustering appears to depend on the age of both the oligodendrocytes and the neurons, with mature oligodendrocytes being more effective and mature axons being more responsive. This age-dependence is in agreement with *in vivo* observations that the increase in Na channel α and β subunit levels and their clustering on the cell surface do not reach the patterns of maturity until two weeks after birth in the rat (Schmidt *et al*., 1985; Wollner *et al*., 1988).

In vivo demonstrations of the importance of oligodendrocytes in the formation and maintenance of axonal nodal specializations come from studies of the jimpy mouse mutant and also of a mouse strain that allows controlled ablation of oligodendrocytes as desired by the experimenter. Jimpy mice have mutations in PLP that are associated with delayed oligodendrocyte damage and death, which occurs spontaneously during the first postnatal weeks (Knapp *et al*., 1986; Vermeesch *et al*., 1990). The timing of oligodendrocyte death in jimpy mice cannot be altered experimentally, as is possible through the study of transgenic mice in which a herpes virus thymidine kinase gene is regulated by the MBP promoter (Mathis *et al*., 2001). Exposure of these animals to the nucleoside analogue FIAU causes specific death of oligodendrocytes; thus, application of FIAU at different time periods allows ablation of cells at any stage of myelination at which MBP is expressed. Killing of oligodendrocytes in the MBP-TK mice is associated with a failure to maintain nodal clusters of ion channels, although the levels of these proteins remained normal. In jimpy mice, a different picture emerges, in which nodal clusters of Na^+ channels remain even in the presence of ongoing oligodendrocyte destruction. K^+ channel clusters were also transiently observed along axons of jimpy mice, but they were in direct contact with nodal markers instead of in the juxtaparanodal regions in which they would normally be found. Thus, it appears that the effect of oligodendrocyte destruction on maintenance of nodal organization is to some extent dependent upon the specific means by which oligodendrocytes are destroyed (Mathis *et al*., 2001).

Oligodendrocytes and O-2A/OPCs as Providers of Growth Factors

There are multiple indications that oligodendrocytes and/or O-2A/OPCs also can provide trophic support for neurons, with some studies indicating that such support may exhibit elements of regional specificity (reviewed in Du and Dreyfuss, 2002). Striatal O-2A/OPC lineage cells have been reported to enhance the survival of substantia nigra neurons through secreted factors (Takeshima *et al*., 1994; Sortwell *et al*., 2000), O-2A/OPC lineage cells from the optic nerve can enhance retinal ganglion cell survival *in vitro* (Meyer-Franke *et al*., 1995), basal forebrain oligodendrocytes enhance the survival of cholinergic

neurons from this same brain region (Dai *et al*., 1998, 2003), and cortical O-2A/OPC lineage cells increase the *in vitro* survival of cortical neurons (Wilkins *et al*., 2001). It is not yet known if the trophic effects that have been reported exhibit stringent regional specificities; if so, this will be indicative of a remarkable degree of specialization in cells of the oligodendrocyte lineage.

While the study of trophic support derived from O-2A/OPCs or oligodendrocytes is still in its infancy, an increasing number of interesting proteins have been observed to be produced by oligodendrocytes. For example, IGF-I, NGF, BDNF, NT-3, and NT-4/5 mRNAs and/or protein have been observed by *in situ* hybridization and via immunocytochemical studies in oligodendrocytes (Dai *et al*., 1997, 2003; Dougherty *et al*., 2000). Consistent with the idea that there might be trophism-related differences in oligodendrocytes from different CNS regions, it does appear that there is regional heterogeneity in the expression of these important proteins (Krenz and Weaver, 2000). Still other proteins that have been suggested to be produced by oligodendrocytes include neuregulin-1 (Vartanian *et al*., 1994; Raabe *et al*., 1997; Cannella *et al*., 1999; Deadwyler *et al*., 2000), GDNF (Strelau and Unsicker, 1999), FGF-9 (Nakamura *et al*., 1999), and members of the TGF family (da Cunha *et al*., 1993; McKinnon *et al*., 1993). Many of the factors that oligodendrocytes appear to produce have been found to influence the development not only of neurons, but also of oligodendrocytes themselves. Thus, it may prove that one of the functions of oligodendrocytes is to produce factors that modulate their own functions. Such a notion is consistent with observations that oligodendrocytes produce factors that feedback to modulate the division and differentiation of O-2A/OPCs in a density-dependent manner (McKinnon *et al*., 1993; Zhang and Miller, 1996).

O-2A/OPCs and oligodendrocytes also receive trophic support from both astrocytes and neurons. Astrocytes have long been known to produce such modulators of O-2A/OPC division and oligodendrocyte survival as PDGF and IGF-I (Ballotti *et al*., 1987; Noble *et al*., 1988; Raff *et al*., 1988; Richardson *et al*., 1988). Neurons appear to be a another source of PDGF (Yeh *et al*., 1991), but also modulate the behavior of O-2A/OPC lineage cells by other means. For example, it has been reported that injection of tetrodotoxin into the eye, thus eliminating electrical activity of retinal ganglion cells, causes a decrease in proliferation of O-2A/OPCs (Barres and Raff, 1993). O-2A/OPCs and oligodendrocytes express K^+ channels (Barres *et al*., 1990) and also express receptors for a variety of neurotransmitters, including glutamate and acetylcholine (Cohen and Almazan, 1994; Gallo *et al*., 1994; Patneau *et al*., 1994; Rogers *et al*., 2001; Itoh *et al*., 2002), thus enabling them to be responsive to the release of such transmitters in association with neuronal activity. Indeed, exposure to neurotransmitters can profoundly affect the proliferation and differentiation of O-2A/OPCs *in vitro* (Gallo *et al*., 1996). Exposure to neurotransmitters can also alter the expression of neurotrophins (NTs) in oligodendrocytes (Dai *et al*., 2001), raising the possibility that neuronal signaling to oligodendrocytes via neurotransmitter release can alter the trophic support that the oligodendrocyte may provide for the neuron. It is particularly intriguing that there appears to be a great deal of specificity in the effects of different kinds of putative neuron-derived signals on trophic factor expression in oligodendrocytes. KCl has been reported to increase expression of BDNF mRNA, carbachol (an acetylcholine analogue) to increase levels of NGF mRNA, and glutamate specifically to decrease levels of BDNF expression (Dai *et al*., 2001).

Functions of Myelin Components

As one might expect for such a highly specialized biological structure as myelin, there are a large number of proteins and lipids that are specifically produced by myelinating cells. It is therefore of considerable interest to understand the function of these myelin-specific molecules (as reviewed in more detail, e.g., in Campignoni and Macklin, 1988; Yin *et al*., 1998; Campignoni and Skoff, 2001; Pedraza *et al*., 2001; Woodward and Malcolm, 2001).

The two major structural proteins of myelin itself are PLP and MBP. PLP constitutes approximately 50% by weight of myelin proteins (Braun, 1984; Morell *et al*., 1994). It appears to interact homophilically with other PLP chains from the surface of the myelin membrane in the next loop of the spiral (Weimbs and Stoffel, 1992). This ability of PLP to bind to PLP proteins in the next loop of the myelin spiral is thought to play an important role in leading to close apposition of the outer membranes of adjacent myelin spirals. The MBPs are actually a group of proteins that are the next most abundant myelin proteins, comprising 30–40% by weight of the proteins found in myelin (Braun, 1984; Morell *et al*., 1994). In contrast with PLP, MBP is located on the cytoplasmic face of the myelin membrane. It is thought to stabilize the myelin spiral at the major dense line by interacting with negatively charged lipids at the cytoplasmic face of the lipid membrane (Morell *et al*., 1994). Both PLP and MBP are critical in the creation of normal myelin.

The dependency on MBP for normal oligodendrocyte function has long been known due to studies of the shiverer mouse strain. Shiverer (*shi*) mice, which are neurologically mutant and exhibit incomplete myelin sheath formation, lack a large portion of the gene for the MBPs, have virtually no compact myelin in their CNS, and shiver, undergo seizures, and die early. Still another mouse mutant characterized by a deficiency of myelin is the *mld* mutation, which consists of two tandem MBP genes, with the upstream gene containing an inversion of its 3′ region. In these mice, MBP is expressed at low levels and on an abnormal developmental schedule (Popko *et al*., 1988). Still another animal model of defective myelination associated with a mutation in the MBP gene is the Long Evans shaker (*les*) rat. Although scattered myelin sheaths are present in some areas of the CNS, most notably the ventral spinal cord in the young neonatal rat, this myelin is gradually lost, and by 8–12 weeks after birth, little myelin is present throughout the CNS. Despite this severe myelin deficiency, some mutants may live beyond 1 yr of age. Rare, thin myelin sheaths that are present early in development lack MBP. On an ultrastructural examination, these sheaths are poorly compacted and lack a major dense line. Many oligodendrocytes in these animals develop an accumulation of vesicles and membranous bodies, but no abnormal cell death is

observed. Unlike *shi* and its allele, where myelin increases with time and oligodendrocytes become ultrastructurally normal, *les* oligodendrocytes are permanently disabled, continue to demonstrate cytoplasmic abnormalities, and fail to produce myelin beyond the first weeks of life (Kwiecien *et al*., 1998). These various strains of MBP-defective animals also provide an opportunity for analyzing the function of individual MBP splice variants, of which there are at least five. Surprisingly, restoration of just the 17.2 kDa isoform (which is normally one of the minor myelin components) in the germline of transgenic shiverer mice is sufficient to restore myelination and nearly normal behavior (Kimura *et al*., 1998).

Studies on the function of MBP are rendered more complex by the fact that the MBP gene also encodes a novel transcription unit of 105 Kb (called the Golli-mbp gene) (Campagnoni *et al*., 1993). Three unique exons within the Golli gene are alternatively spliced to produce a family of MBP gene-related mRNAs that are under individual developmental regulation. These mRNAs are temporally expressed within cells of the oligodendrocyte lineage at progressive stages of differentiation. Golli proteins show a different developmental pattern than that of MBP, however, with the highest levels of *golli* mRNA expression being in intermediate stages of oligodendrocyte differentiation, and with levels being reduced in mature oligodendrocytes (Givogri *et al*., 2001). Thus, the MBP gene is a part of a more complex gene structure, the products of which may play a role in oligodendrocyte differentiation prior to myelination (Campagnoni *et al*., 1993). For these reasons, compromising the function of the MBP gene actually results in compromised expression of the Golli proteins, and attributing a particular developmental outcome selectively to either MBP transcripts or Golli transcripts is not possible.

Golli expression is also seen in cortical preplate cells, and targeting of herpes simplex thymidine kinase by the *golli* promoter allows selective ablation of preplate cells in the E11-12 embyro, leading to a dyslamination of the cortical plate and a subsequent reduction in short- and long-range cortical projection within the cortex and to subcortical regions (Xie *et al*., 2002). Golli proteins, as well as PLP and DM-20 transcripts of the *plp* gene are also expressed by macrophages in the human thymus, which may be of relevance to the association between MS and immune response to MBP epitopes that are also expressed by *golli* gene products (Pribyl *et al*., 1996).

There are also animal models of mutations in PLP, such as the jimpy mouse strain. In these mice, one sees delayed oligodendrocyte damage and death, which occurs spontaneously during the first postnatal weeks (Knapp *et al*., 1986; Vermeesch *et al*., 1990). PLP does not appear to be required for initial myelination, but is required for maintenance of myelin sheaths. In the absence of PLP, mice assemble compact myelin sheaths but subsequently develop widespread axonal swellings and degeneration (Griffiths *et al*., 1998).

Along with analysis of myelin-specific proteins, it has also been possible to start dissecting the role of specific myelin lipids in oligodendrocyte function by examining CNS development in mice in which key enzymes required in lipid biosynthesis have been genetically disrupted. A particularly interesting demonstration of the importance of the myelin-specific lipids has come from the study of mice that are incapable of synthesizing sulfatide due to disruption of the galactosylceramide sulfotransferase gene (Ishibashi *et al*., 2002). Although compact myelin is itself preserved in these animals, abnormal paranodal junctions are found in both the PNS and CNS. Abnormal nodes are characterized by a decrease in Na^+ and K^+ channel clusters, altered nodal length, abnormal localization of K^+ channel localization, and a diffuse distribution of contactin-associated protein (Caspr) along the internode. This aberrant nodal organization arises despite the fact that the initial timing and number of Na^+ channel clusters are normal during development. The interpretation of these results is that sulfatide plays a critical role in maintaining ion channel organization but is not essential for establishing initial cluster formation. Similar results have been observed in mice lacking GalC (an essential precursor for sulfatide formation; Dupree *et al*., 1998, 1999) and also in mice lacking Caspr (Bhat *et al*., 2001) or contactin (Boyle *et al*., 2001). Interestingly, sulfatide-deficient mice have a milder clinical phenotype than the animals deficient in both GalC and sulfatide, indicating that GalC may itself have other important roles that it plays. Whether the role of these lipids is to participate directly in interactions with components of the axonal membrane, to play a role in organizing oligodendrocyte membrane proteins that are themselves involved in oligodendrocyte–neuron interactions, or have still other unknown roles, is not yet known.

Other means by which oligodendrocyte function is disrupted, and the neurological consequences of such disruption are considered when we examine human genetic diseases that affect myelin.

MYELIN-RELATED DISEASES

Genetic Diseases of Oligodendrocytes and Myelin

A multitude of genetic diseases are associated with myelination defects. Experimental diseases of mice associated with structural mutations in important myelin proteins have been discussed earlier, such as seen in jimpy or shiverer mice, and human diseases associated with defects in myelin proteins are also known. In addition, there are a large number of metabolic diseases in humans in which myelination is abnormal, and white matter damage is even seen in individuals in which the underlying mutation affects proteins involved in RNA translation.

A myelin-related disease associated with a structural protein defect is the X-linked Pelizaeus–Merzbacher disease associated with mutations in the PLP gene (Woodward and Malcolm, 1999). Children with more severe symptoms tend to have severe abnormalities in protein folding in other structural aspects of the myelin, which would cause changes in the physical structure of the myelin. In addition, accumulation of misfolded proteins in the cell may trigger oligodendroglial apoptosis and consequent demyelination (Gow *et al*., 1998). It is interesting that if the gene is completely deleted, affected children have a relatively mild form of the disease, despite the hypomyelination (Raskind *et al*., 1991; Sistermans *et al*., 1996).

Adrenoleukodystrophy is the most commonly occurring leukodystrophy in children. This X-linked disorder, caused by a mutation of the gene encoding a peroxisomal membrane protein, affects one in 20,000 boys (Dubois-Dalcq *et al*., 1999). The mutated protein (called ALD protein) is necessary for transferring very long-chain fatty acids into peroxisomes, where they are metabolized into shorter chain fatty acids for multiple purposes, including incorporation into the myelin membrane. ALD protein is found in all glial cells, but its expression in oligodendrocytes is limited to the locations that correlate well with locations of demyelination in affected children (Fouquet *et al*., 1997), such as corpus callosum, internal capsule, and anterior commissure. While it is not known why myelin breaks down in these children, it appears that the mutation somehow destabilizes the membrane. Then, in conjunction with inflammatory events in putatively dysfunctional microglia (in which the ALD protein is also expressed), this inherent weakness stimulates (or enables) consequent demyelination. MR imaging shows T2 prolongation during the early stages of disease, but whether this is primarily due to myelin breakdown or inflammation is not clear. The inflammation results in localized edema which itself is associated with imaging changes.

Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder caused by deficient activity of the lysosomal enzyme arylsulfatase A. These patients may present at any age, have gait abnormalities, ataxia, nystagmus, hypotonia, diffuse spasticity, and pathologic reflexes (Barkovich, 2000). Myelin is usually formed normally in this condition, but the eventual membrane accumulation of sulfatide associated with this enzymatic defect results in an instability of the myelin membrane with ultimate demyelination. Damage may also occur due to progressive accumulation of sulfatides within oligodendroglial lysosomes, leading to eventual degeneration of the lysosomes themselves. There is extensive demyelination that develops, with complete or nearly complete loss of myelin in the most severely affected regions (van der Knaap and Valk, 1995).

Canavan's disease (CD) is another example of an autosomal recessive early-onset leukodystrophy, caused in this case by mutations in the gene for aspartoacetylase. This is the primary enzyme involved in the catabolic metabolism of *N*-acetylaspartate (NAA), and its deficiency leads to a build-up of NAA in brain with both cellular and extracellular edema, as well as NAA acidemia and NAA aciduria. CD is characterized by loss of the axon's myelin sheath, while leaving the axons intact, and by spongiform degeneration, especially in white matter. The course of the illness can show considerable variation, and can sometimes be protracted. The mechanism by which a defect in NAA metabolism causes myelination deficits remains unknown, although it has been suggested that changes in osmotic balance due to buildup of NAA (which, even in the normal brain, is one of the most abundant single free amino acids detected) may be of importance (Baslow, 2000; Gordon, 2001; Baslow *et al*., 2002). It has also been suggested that NAA supplies acetyl groups for myelin lipid biosynthesis, a possibility consistent with known cellular expression of both NAA and its relevant enzymes (Urenjak *et al*., 1992, 1993; Bhakoo and Pearce, 2000; Bhakoo *et al*., 2001; Chakraborty *et al*., 2001).

Some of the most puzzling of genetic diseases in which myelin is affected are those in which the CNS initially undergoes normal development, and subsequently the individual is afflicted with a chronic and diffuse degenerative attack on the white matter. One of these disorders that has been genetically defined is a syndrome called vanishing white matter (VWM; MIM 603896) (Hanfield *et al*., 1993; van der Knaap *et al*., 1997), also called childhood ataxia with central hypomyelination (CACH; van der Knaap *et al*., 1997). VWM is the most frequent of the unclassified childhood leukoencephalopathies (van der Knaap *et al*., 1999). Onset is most often in late infancy or early childhood, but onset may occur at times ranging from early infancy to adulthood (Hanfield *et al*., 1993; van der Knaap *et al*., 1997, 2001; Francalanci *et al*., 2001; Prass *et al*., 2001). VWM is a chronic progressive disease associated with cerebellar ataxia, spasticity, and an initially, relatively mild mental decline. Death occurs over a very variable period, which may range from a few months to several decades. It has been suggested that oligodendrocyte dysfunction, leading to myelin destruction (and possibly associated with initial hypomyelination in cases with early onset) is the primary pathologic process in VWM (Schiffmann *et al*., 1994; Rodriguez *et al*., 1999; Wong *et al*., 2000).

VWM is an autosomal recessive disease, and it has been recently found that the underlying mutations may be in any of the five subunits of the eukaryotic translation initiation factor (eIF), eIF2B (Leegwater *et al*., 2001; van der Knaap *et al*., 2002). This discovery was quite surprising, as the widespread importance of initiation factors in cellular function makes it difficult to understand why a mutation in one of them should manifest itself so specifically as an abnormality in white matter. Indeed, despite the identification of the genetic basis of VWM, little is known about the biology of this disease, including the answers to such questions as: How can one have a disease in which oligodendrocyte function is apparently normal to begin with, and then at later stages—often after years of normal development and function—a chronic deterioration of myelin begins? And why would such a specific disease result from a mutation in a protein thought to be important in RNA translation throughout the body? Moreover, what function of initiation factors might explain the onset of the chronic white matter degeneration that characterizes this disease?

At the moment, one of the few clues to the underlying pathophysiology of VWM comes from observations that patients with this disease undergo episodes of rapid deterioration following febrile infections and minor head trauma. It has been suggested that mutations in eIF2B might be associated with an inappropriate response by oligodendrocytes to such stress (which would include within it febrile [thermal], oxidative, and chemical perturbations) (van der Knaap *et al*., 2002). Normally, mRNA translation is inhibited in such adverse circumstances, perhaps as a protective response against the capacity of such abnormal metabolic states to compromise normal folding of many proteins. Excessive accumulation of misfolded proteins then could lead to interference with normal cellular function, as has also been suggested earlier for Pelizaeus–Merzbacher disease. Attempts to understand the underlying pathophysiology of this disease remain speculative, however, in the absence of cellular and/or

animal models suitable for detailed analysis. Moreover, it is difficult to reconcile such a hypothesis with observations that VWM disease is inherited as an autosomal recessive, rather than as a dominant trait, as a hypothesis invoking continued mRNA translation would be indicative of a dominant rather than a recessive function. Until such time as appropriate cellular tools (such as precursor cells from a patient with this disease) are available, it will remain unknown as to whether oligodendrocytes are particularly sensitive to alterations in the biology of mRNA translation, whether there is instead a failure in this disease to carry out the normal turning off of injury responses (thus leading to release of glutamate, secretion of tumor necrosis factor [TNF]-α, and other such responses as are associated with oligodendrocyte destruction), or whether other processes are involved in this tragic condition. Given only human autopsy tissue to study, one is limited to such observations as oligodendrocytes in the brains of VWM exhibiting an aberrant foamy cytological structure (Wong *et al*., 2000), but it is wholly unknown whether this is a primary effect of the mutation in eIF2B or a secondary consequent of the extended period of destruction to which they have been subjected.

Studies on VWM also reveal another of the many areas in which our understanding of myelin function is incomplete. It is a striking feature of VWM that magnetic resonance imaging (MRI) reveals diffuse abnormalities of the cerebral white matter prior to the onset of symptoms (van der Knaap *et al*., 1997). MRI and magnetic resonance spectroscopic analysis both indicate that as this disease progresses, increasing amounts of the cerebral white matter vanish and are replaced by cerebrospinal fluid (CSF), as is confirmed by examination of brains at autopsy (van der Knaap *et al*., 1997, 1998; Rodriguez *et al*., 1999). Still, it appears clear that damage to the white matter has already begun before clinical symptoms emerge.

The idea that one can have extensive loss of myelin without evidence of neurological abnormality seems extraordinarily counterintuitive. Yet, it has long been known that extensive demyelination is not always associated with clinical deficits in MS patients. The suggested explanations for this phenomena of "silent lesions" have generally been that they may be located in areas in which a loss of conduction does not manifest itself in a clinically detectable manner and/or that sufficient normally myelinated axons in these regions are spared to enable normal function. Such suggestions are consistent with multiple lines of evidence indicating functional redundancy in axonal pathways. Indeed, in such chronic neurodegenerative diseases as Parkinson's disease and Alzheimer's disease, it is clear that clinical symptoms are not seen until 50–70% of the relevant neurons have been destroyed. Still, it may be that there is a more complex biology that lies behind the situation in which loss of myelin is not associated with clinical manifestations. Such a possibility is indicated by experimental studies in which extensive demyelination was induced by infection of two different strains of mice with Theiler's virus (Rivera-Quinones *et al*., 1998). Normal function was maintained in mice defective for expression of major histocompatibility complex (MHC) class I gene products, despite the presence of a similar distribution and extent of demyelinated lesions as in other mouse strains in which neurological function was compromised. It has been proposed that the maintenance of normal neurological function in class I antigen-deficient mice with extensive demyelination results from increased sodium channel densities and the relative preservation of axons.

Nongenetic Diseases of Myelin

Aberrant myelination is also associated with a wide range of epigenetic physiological insults. Causes of such problems are so diverse as to include various nutritional deficiency disorders, hypothyroidism, fetal alcohol syndrome, treatment of CNS cancers of childhood by radiation, and treatment of even some non-CNS cancers of childhood by chemotherapy.

Hypothyroidism

A major cause of mental retardation and other developmental disorders is hypothyroidism, usually associated with iodine deficiency (e.g., Delange, 1994; Lazarus, 1999; Chan and Kilby, 2000; Thompson and Potter, 2000). It is well established in animal models that perinatal hypothyroidism is associated with defects in myelination and a reduced production of myelin-specific gene products, and that these defects can be at least partially ameliorated if TH therapy is initiated early enough in postnatal life (e.g., Noguchi *et al*., 1985; Munoz *et al*., 1991; Bernal and Nunez, 1995; Ibarrola and Rodriguez-Pena, 1997; Marta *et al*., 1998). As for other deficiency disorders, however, application of hormonal replacement therapy after the appropriate critical period has been completed has relatively little effect.

The actions of TH to promote myelination are several. This hormone has been found to promote the generation of O-2A/ OPCs from GRP cells, as well as promoting the generation of oligodendrocytes from dividing O-2A/OPCs (Barres *et al*., 1994a; Ibarrola *et al*., 1996; Gregori *et al*., 2002a). TH also modulates the expression of multiple myelin genes (e.g., Oppenheimer and Schwartz, 1997; Jeannin *et al*., 1998; Pombo *et al*., 1999; Rodriguez-Pena, 1999). *In vivo*, reduction in TH levels are associated with an 80% reduction in the number of oligodendrocytes, which is the same degree of difference in oligodendrocyte prevalence observed in embryonic brain cultures grown in the presence or absence of TH (Ibarrola *et al*., 1996).

Iron Deficiency

The most prevalent nutrient deficiency in the world is a lack of iron. It has been estimated that 35–58% of healthy women have some degree of iron deficiency (Fairbanks, 1994). Iron deficiency is particularly prevalent during pregnancy. Iron deficiency in children is associated with hypomyelination, changes in fatty acid composition, alterations to the blood brain barrier and behavioral effect (Pollitt and Leibel, 1976; Honig and Oski, 1978; Dobbing, 1990). It has been reported that the prevalence of iron deficiency may be as high as 25% for children under two years of age, as indicated by measurement of auditory brain responses as a measurement of conduction speed (Roncagliolo *et al*., 1998).

That iron deficiency would be particularly important during specific developmental periods has been suggested by observations that there is a temporal correlation between the period in development when most oligodendrocytes are developing and a peak in iron uptake into the brain (Yu *et al*., 1986; Taylor and Morgan, 1990). In iron-deficient animals, where no such peak in iron uptake can occur, there is a relative lack of myelin lipids. The myelin isolated from these iron-deficient animals is normal in the ratios of its myelin components, however, suggesting that the reduced amount of myelin produced in these animals is normal in its biochemical composition.

The Role of Iron in Oligodendrocyte Generation

The role of iron in the myelination process is an emerging area of study in the development of the CNS. It has been noted that when the brains of many different species are histochemically labeled for iron, the cells with the highest iron levels are oligodendrocytes (Hill and Switzer, 1984; Dwork *et al*., 1988; Connor and Menzies, 1990; LeVine and Macklin, 1990; Morris *et al*., 1992; Benkovic and Connor, 1993). While the role of iron in oligodendrocytes is unknown, it has been suggested that a lack of iron might somehow interfere with the function of these cells (Connor and Menzies, 1996). The lack of myelination associated with iron deficiency has been measured in humans using auditory brainstem responses (ABRs). Changes in the latency of the ABRs have been related to the increased nerve conduction velocity that accompanies axonal myelination (Salamy and McKean, 1976; Hecox and Burkard, 1982; Jiang, 1995). A recent study has shown that there are measurable differences in ABR latency between normal and iron-deficient children (Roncagliolo *et al*., 1998), reflecting a myelination disorder.

Iron is taken up by cells predominantly when bound to transferrin, the mammalian iron transporter. Oligodendrocytes have the highest levels of transferrin mRNA and protein, and indeed seem to be responsible for transferrin production in the CNS (Connor and Fine, 1987; Dwork *et al*., 1988; Bartlett *et al*., 1991; Connor *et al*., 1993; Connor, 1994; Dickinson and Connor, 1995). These observations have led to the suggestion that oligodendrocytes are responsible for storing iron and for making it readily available to the environment, as well as suggestions that iron is important in critical—but currently unknown—steps in oligodendrocyte development (Connor and Menzies, 1996).

There is also a temporal correlation between the period in development when most oligodendrocytes are developing and a peak in iron uptake into the brain (Skoff *et al*., 1976a, b; Crowe and Morgan, 1992). In iron-deficient animals, where no such peak in iron uptake can occur, a reduction in myelin lipids can be measured (Connor and Menzies, 1990). The myelin isolated from these iron-deficient animals is normal in the ratios of its myelin components, suggesting that the myelin produced in iron-deficient rats is normal but that overall less myelin is being produced. The suggestion that it might be necessary to have adequate levels of bioavailable iron in order for normal myelination to occur is also supported by the observation that in myelin-deficient rats, in which oligodendrocytes fail to mature due to a genetic defect in the PLP, the levels of transferrin (bioavailable iron) in the brain are well below normal levels (Bartlett *et al*., 1991). Strikingly, exposure of myelin-deficient rats to transferrin can promote the production of myelin (Escobar Cabrera *et al*., 1997).

Despite the considerable evidence linking iron deficiency with defects in myelin production, it is still not clear how a defect in myelination might be established and at what timepoint during gliogenesis iron availability is important. As most data has been provided through descriptive studies *in vivo*, a mechanistic basis for iron-mediated myelin deficiency has not been established.

Cellular biological studies have indicated an importance of iron levels in the generation of oligodendrocytes from GRP cells (presumably through the intermediate generation of O-2A/OPCs, although this has not yet been confirmed) (Morath and Mayer-Proschel, 2001). In contrast, no effects of iron were found on oligodendrocyte maturation or survival *in vitro*, nor did increasing iron availability above basal levels increase oligodendrocyte generation from O-2A/OPCs. These results raise the possibility that iron may affect oligodendrocyte development at stages during early embryogenesis rather than during later development. This possibility is supported by *in vivo* studies demonstrating that iron deficiency during pregnancy affects the iron levels of various brain tissues in the developing fetus, and disrupts not only the proliferation of their glial precursor cells, but also disturbs the generation of oligodendrocytes from these precursor cells (Morath and Mayer-Proschel, 2002).

Selenium Deficiency

Still another syndrome associated with myelination defects is a deficiency in the essential trace element selenium. Selenium deficiency has been postulated to be associated with retarded intellectual development (Foster, 1993) and to neural tube defects (Guvenc *et al*., 1995). It has also been suggested that the incidence of MS is negatively correlated with selenium levels in the soil, suggesting that selenium deficiency may predispose oligodendrocytes to demyelinating injury (Foster, 1993).

In vitro studies have shown that normal selenium levels are required for both the normal morphological development and the survival of oligodendrocytes (Eccleston and Silberberg, 1984; Koper *et al*., 1984). Moreover, exposure to adequate levels of selenium is required for the normal upregulation of genes for PLP, MBP, and MAG. A deficiency of selenium *in vitro* is also associated with a reduction in the generation of oligodendrocytes from their precursor cells (Gu *et al*., 1997).

The mechanisms by which selenium deficiency may alter oligodendrocyte generation are far from clear. *In vivo*, it is known (Kohrle, 1996) that selenium is required for activity of the deiodinase that cleaves one iodine from T4 to make the bioactive T3 (triiodothyronine). Consistent with this role of selenium, deficiency in this trace element is known to cause further impairment of TH metabolism in iodine-deficient rats (Mitchell *et al*., 1998). Selenium also plays a critical role in redox regulation, however, particularly as many of the selenoproteins play critical roles in regulation of intracellular redox balance (Holben and Smith,

1999). In this regard, it may be that a lack of selenium leads to a more oxidized state in O-2A/OPCs, thus leading to their premature transition from dividing progenitor cells to nondividing oligodendrocytes (Smith *et al*., 2000). As this would be associated with a reduction in oligodendrocyte number (secondary to a reduction in progenitor cell number), one would see associated reductions in myelin-specific genes when cultures were examined at the population level.

Nutrition and Oligodendrocyte Generation

We are not yet aware of any studies that have examined nutritional deficiency in a manner directly analogous to studies on TH or iron deficiency. Indeed, developing a model system for studying nutritional deficiency *in vitro* is problematic in a number of respects. Perhaps most importantly, true nutritional deficiency is associated with inadequate supplies of proteins, vitamins, and minerals and can itself lead to reduced production of normal hormonal supplies. This is a considerably more difficult syndrome to reproduce *in vitro* than TH deficiency, for example. Nonetheless, published data, from both *in vivo* and *in vitro* studies, are consistent with the possibility that oligodendrocyte generation is impaired in at least some models of undernourishment. *In vivo*, it is well established that the myelin deficits associated with undernutrition are even observed in animals in which oligodendrocyte number appears to be normal (Sikes *et al*., 1981). In such animals, however, it has been reported (Royland *et al*., 1993) that the mRNAs for three important myelin proteins (MAG, PLP, and MBP) do not undergo the normal increases seen in brains of well-nourished animals. Increases are delayed for several days beyond the normal time (i.e., day 7–9) at which they are observed, and the increases are lower in extent. In addition, still more severe malnutrition regimes have been reported to be associated with a clear reduction in glial cell number *in vivo* (Krigman and Hogan, 1976), although cell type specific markers were not utilized to determine whether this reduction preferentially effected oligodendrocytes rather than astrocytes.

In vitro studies on nutritional deficiency have largely focused on glucose deprivation as a means of mimicking caloric restriction. Such studies have raised the surprising possibility that transient caloric restriction at critical periods may lead to long-term effects on differentiated function (Royland *et al*., 1993). In these experiments, mixed cultures were generated from newborn rat brain and exposed to different glucose concentrations, ranging from 0.55 to 10 mg/ml; the lower doses are within the range that occurs in clinical hypoglycemia. Low glucose concentrations were associated with markedly lower increases in levels of MAG, PLP, and MBP mRNA, and with a subsequent and abnormal downregulation in these mRNA levels. These effects were specific, in that total mRNA levels in the cultures were normal. Most importantly, these effects appeared to be irreversible if the glucose deprivation was applied over a time period that mirrors the critical period for nutritional deprivation *in vivo*. Deprivation coincident with the normal time of myelin gene activation and the period of rapid upregulation (6–14 DIV) was irreversible. Deprivation at a later stage was instead associated with only transient depressing effects. It has also been previously reported that there is a relative reduction in the numbers of oligodendrocytes that are generated in glucose-deprived cultures (Zuppinger *et al*., 1981).

Physiological Insults Associated with Developmental Abnormalities in Myelination

Still another means by which normal developmental processes may be thwarted is through the introduction of toxic substances into the developing organism.

Fetal Alcohol Syndrome

Evidence suggests that abnormal myelination is one factor contributing to the neuropathology associated with fetal alcohol syndrome. Studies on the expression of MBP and MAG, isoforms in experimental animals showed a considerable vulnerability to postnatal (but not prenatal) exposure to ethanol. These studies indicate that ethanol exposure during periods of rapid myelination (postnatal days 4–10) reduced the expression of specific MBP and MAG isoforms (Zoeller *et al*., 1994). *In vitro* studies have also indicated that exposure to ethanol during early stages of oligodendrocyte development is associated with a specific repression of MBP expression, but not of the myelin-specific enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase). Delayed or decreased MBP expression could interfere with normal processes of myelination, as indicated by the adverse consequences of genetic interference with normal MBP expression or function (Bichenkov and Ellingson, 2001). In adult alcoholics, there are changes in expression of as many as 40% of superior frontal cortex-expressed genes (as determined from examination of postmortem samples). In particular, myelin-related genes were significantly downregulated in the brain specimens from alcoholics (Lewohl *et al*., 2000).

Fetal Cocaine Syndrome

Abnormalities in myelination have also been associated with exposure to cocaine. The progeny born to pregnant rats treated daily with oral cocaine during gestation showed a 10% reduction in myelin concentrations in the brain. In contrast with the period of myelin vulnerability for undernourishment, which is thought to be largely postnatal, cross-fostering studies revealed that the fetal period of cocaine exposure presents a greater risk to postnatal myelination than exposure during the suckling period (Wiggins and Ruiz, 1990). As myelination in the human is not complete until the fourth decade (Yakovlev and Lecours, 1967), there has been some concern as to whether the ongoing processes of myelination might be disrupted in cocaine users. Indeed, in normal individuals, there is a continued increase in white matter volume in the frontal and temporal lobes that does not reach a maximum until age 47. In cocaine-dependent subjects, in contrast, this age-related expansion in white matter volume in the frontal and temporal cortex does not appear to occur (Bartzokis *et al*., 2002).

Effects of Organic Mercury Compounds

Exposure to MeHg provides yet another example wherein exposure to toxic substances interferes with normal patterns of development. It is clear from unfortunate experiences with contaminated wheat in Iraq and contaminated fish in Japan that high levels of exposure to MeHg is associated with severe abnormalities in the developing brain, including neuronal migration disorders and diffuse gliosis of the periventricular white matter (Choi, 1989). Studies in the Faroe islands, the Seychelles Island, New Zealand, and the Amazon Basin have further found that children born from mothers exposed during pregnancy to moderate doses of MeHg showed significantly reduced performance on several neuropsychological tests (Crump *et al*., 1998, 2000; Grandjean *et al*., 1998, 1999; Dolbec *et al*., 2000). Children exposed to mercury during development may exhibit a range of neurological problems, including cerebral palsy (which includes failures in normal myelination), developmental delay, and white matter astrocytosis (Castoldi *et al*., 2001; Mendola *et al*., 2002).

The developing nervous system is more sensitive to MeHg neurotoxicity than the adult nervous system (Clarkson, 1997; Myers and Davidson, 1998). MeHg appears to have a wide range of toxic effects on the developing CNS. For example, developmental exposure to MeHg is associated with decreases in cell survival, myelination, and cerebral dysgenesis (Chang *et al*., 1977; Burbacher *et al*., 1990; Barone, Jr., *et al*., 1998), as well as decreased expression and/or activity of proteins involved in neurotrophic factor signaling (Barone, Jr., *et al*., 1998; Haykal-Coates *et al*., 1998; Mundy *et al*., 2000) and changes in neurotrophic factor expression (Lärkfors *et al*., 1991).

An organic mercury compound that has become of considerable recent interest as a potential inducer of developmental abnormalities is Thimerosal, a vaccine preservative that contains 49.6% ethylmercury (by weight) as its active ingredient. Concern has been raised that apparent increases in the prevalence of autism (from 1 in 2000 prior to 1970 up to 1 in 500 in 1996 (Gillberg and Wing, 1999)) have paralleled the increased mercury intake induced by mandatory inoculations. In 1999, the Food and Drug Administration (FDA) recorded Thimerosal usage in over 30 vaccine products (FDA, November 16, 1999). According to the classification of Thimerosal-containing vaccines provided by the Massachusetts Department of Public Health, as of June 2002, Thimerosal was still in use as a preservative in a significant number of vaccines, including diphtheria/tetanus, Hep B, Influenza, Meningococcus, and Rabies vaccines. The World Health Organization (WHO), the American Academy of Pediatrics, and the US Public Health Service have all voiced support for phasing out Thimerosal usage as a vaccine preservative, but the WHO has stressed that this may not be an option for developing countries. While a recent Danish study (Madsen *et al*., 2002) failed to find a link between autism and vaccination with the measles, mumps, rubella (MMR) vaccine, this is not a Thimerosal-containing vaccine and thus did not shed light on controversies related to autism and mercury exposure. The hypothesis that mercury exposure and autism are linked is discussed extensively in Bernard *et al*. (2001), including information on the multiple similarities between the neurological symptoms seen in mercury poisoning and those considered to typify autism.

The amount of mercury that would be delivered to a child born in the 1990s in association with vaccination over the first two years of life is not small, and is delivered in bolus form (as part of a vaccination). The amount of mercury injected at birth is 12.5 μg, followed by 62.5 μg at 2 months, 50 μg at 4 months, another 62.5 μg during the infant's 6-month immunizations, and a final 50 μg at about 15 months (Halsey, 1999). Concerns exist that infants under 6 months may be inefficient at mercury excretion, most likely due to their inability to produce bile, the main excretion route for organic mercury (Koos and Longo, 1976; Clarkson, 1993). More recent studies have challenged these concerns, reporting that blood mercury in Thimerosal-exposed 2-month-olds ranged from less than 3.75 to 20.55 parts per billion; in 6-month-olds, all values were lower than 7.50 parts per billion (Pichichero *et al*., 2002).

Ongoing studies on the effects of MeHg and Thimerosal on cells of the oligodendrocyte lineage have revealed a striking vulnerability of these cells to organic mercury compounds (MN, research in progress). Studies have thus far indicated that exposure of oligodendrocytes and O-2A/OPCs to doses of MeHg or Thimerosal in the ranges of 5–20 parts per billion is associated with significant cell death and inhibition of cell division. These are precisely the ranges of mercury levels that are routinely found in both infant and adult populations. Moreover, exposure to still lower levels of MeHg is sufficient to increase the sensitivity of O-2A/OPCs to killing by glutamate and of oligodendrocytes to killing by TNF. (Such vulnerabilities are discussed in more detail in the following section.) Thus, oligodendrocytes and their precursor cells may also be an important target of action of organic mercury compounds—and perhaps of many other environmental toxicants.

Neurotoxicity of Existing Cancer Treatments

It is becoming increasingly apparent that traditional approaches to cancer therapy are often associated with adverse neurological events, many of which affect the white matter tracts of the CNS. These neurological sequelae are seen in treatment regimes ranging from chemotherapy of primary breast carcinoma to radiation therapy of brain tumors. Even based on the figures available from recent publications (which represent only a beginning appreciation of this general problem), it seems likely that there are significant numbers of individuals for whom such neurotoxicity is a serious concern.

Even though there are still many cancer treatments for which cognitive changes and other neurological sequelae have not been noted in the literature, it appears that these adverse effects may be frequent. The Cancer Statistics Branch of NCI estimates a cancer prevalence in the United States for 1997 of nearly 9 million individuals. If cognitive impairment associated with treatment were to only effect 2.5% of this population, the total number of patients for whom this issue would be a concern is of similar size to the population of individuals with chronic spinal cord injury. As discussed in more detail later, recent

studies raise the specter that such complications may occur in significantly more than 2.5% of individuals treated for cancer. Lowered IQ scores and other evidence of cognitive impairment are relatively frequent in children treated for brain tumors or leukemias, thus presenting survivors and their families with considerable challenges with respect to the ability of these children to achieve normal lives. Data for patients treated for non-CNS tumors are only beginning to emerge, and give grounds for further concern. For example, some studies suggest that as many as 30% of women treated with standard chemotherapy regimes for primary breast carcinoma show significant cognitive impairment 6 months after treatment (van Dam *et al*., 1998; Schagen *et al*., 1999). As the compounds used in the treatment for breast cancer (cyclophosphamide, methotrexate, and 5-fluorouracil) are used fairly widely, it would not be surprising to find problems emerging in other patient populations as more testing is conducted. Thus, current trends support the view that the number of individuals for whom cognitive impairment associated with cancer treatment is a problem may be as great as for many of the more widely recognized neurological syndromes.

Neurological complications have been most extensively studied with respect to radiation therapy to the brain, and these studies indicate the presence of a wide range of potential adverse effects. Radiation-induced neurological complications include radionecrosis, myelopathy, cranial nerve damage, leukoencephalopathy (i.e., white matter damage), and dopa-resistant Parkinsonian syndromes (Keime-Guibert *et al*., 1998). Imaging studies have documented extensive white matter damage in patients receiving radiation to the CNS (Vigliani *et al*., 1999). Cognitive impairment associated with radiotherapy also has been reported in many of these patients. For example, in examination of 31 children, aged 5–15 years, who had received radiotherapy for posterior fossa tumors, and who had been off therapy for at least 1 year, long-term cognitive impairment occurred in most cases (Grill *et al*., 1999). Neurotoxicity also affects older patients, presenting as cognitive dysfunction, ataxia, or dementia as a consequence of leukoencephalopathy and brain atrophy (Schlegel *et al*., 1999). In adults, "subcortical" dementia occurs 3–12 months after cerebral radiotherapy (Vigliani *et al*., 1999).

Potential clues to the biological basis for cognitive impairment have come from studies on the effects of radiation on the brain, for which dose-limiting neurotoxicity has long been recognized (Radcliffe *et al*., 1994; Roman and Sperduto, 1995). On a cellular basis, radiation appears to cause damage to both dividing and nondividing CNS cells. Recent studies have shown that irradiation causes apoptosis in precursor cells of the dentate gyrus subgranular zone of the hippocampus (Peissner *et al*., 1999; Tada *et al*., 2000) and in the subependymal zone (Bellinzona *et al*., 1996), both of which are sites of continuing precursor cell proliferation in the adult CNS. Such damage is also associated with long-term impairment of subependymal repopulation. In addition, it seems to be clear that nondividing cells, such as oligodendrocytes, are killed by irradiation (Li and Wong, 1998). Damage to oligodendrocytes is consistent with clinical evidence, where radiation-induced neurotoxicity has been associated with diffuse myelin and axonal loss in the white matter, with tissue necrosis and diffuse spongiosis of the white matter characterized by the presence of vacuoles that displaced the normally stained myelin sheets and axons (Vigliani *et al*., 1999). Although some damage *in vivo* may well be secondary consequences of vascular damage, evidence also has been provided that radiation is directly damaging to important CNS populations, such as OPCs (Hopewell and van der Kogel, 1999).

Although chemotherapy has been less well studied than radiation in terms of its adverse effects on the CNS, it is becoming increasingly clear that many chemotherapeutic regimens are associated with neurotoxicity. Multiple reports have confirmed cognitive impairment in children and adults after cancer treatment. In particular, improvements in survival for children with leukemias or brain tumors treated with radiotherapy and chemotherapy have led to increasing concerns on quality-of-life issues for long-term survivors, in which neuropsychological testing has revealed a high frequency of cognitive deficits (Appleton *et al*., 1990; Glauser and Packer, 1991; Waber and Tarbell, 1997; Grill *et al*., 1999; Riva and Giorgi, 2000). For example, Cetingul *et al*. recently reported that performance and total IQ scores were significantly reduced in children treated for acute lymphoblastic leukemia who had completed therapy at least a year before and survived more than five years after diagnosis (Cetingul *et al*., 1999). Indeed, it is felt that neurotoxicity of chemotherapy is frequent, and may be particularly hazardous when combined with radiotherapy (Cetingul *et al*., 1999; Schlegel *et al*., 1999). For example, in CT studies of patients receiving both brain radiation and chemotherapy, all patients surviving a malignant glioma for more than 4 yrs developed leukoencephalopathy and brain atrophy (Stylopoulos *et al*., 1988).

Studies on the effects of chemotherapeutic agents on normal CNS cells have revealed a significant vulnerability of oligodendrocytes to BCNU (carmustine, an alkylating agent widely used in the treatment of brain tumors, myeloma, and both Hodgkin and non-Hodgkin lymphoma) (Nutt *et al*., 2000). BCNU was toxic for oligodendrocytes at doses that would be routinely achieved during treatment. More recent studies (MN *et al.*, research in progress) have revealed that such vulnerability extends to such widely used chemotherapeutic agents as cisplatin, and that O-2A/OPCs and GRP cells are as or more vulnerable to the effects of these compounds than are oligodendrocytes. Strikingly, it thus far appears that any dose of chemotherapeutic agents that kill cancer cells is sufficient to kill the cells of the oligodendrocyte lineage.

Myelin Destruction in the Adult

Loss of myelin in the adult is generally associated with chronic degenerative processes or with traumatic injury. As is the case in development, damage to myelin in the adult is a frequent event, associated with virtually all examples of traumatic injury (including spinal cord injury) and most examples of chronic degenerative processes. Even Alzheimer's disease appears to have myelin breakdown as one of its important components (Terry *et al*., 1964; Chia *et al*., 1984; Malone and Szoke, 1985; Englund *et al*., 1988; de la Monte, 1989; Wallin *et al*., 1989;

Svennerholm and Gottfries, 1994; Gottfries *et al*., 1996; Bartzokis *et al*., 2000, 2003; Braak *et al*., 2000; Han *et al*., 2002; Kobayashi *et al*., 2002; Roher *et al*., 2002). It has even been suggested that it is the breakdown of myelin that is the key precipitating event in the initiation of damage to neurons in this syndrome (Bartzokis, 2003).

The most widely known of demyelinating diseases of the adult, and the one that has been studied for the longest time, is that of multiple sclerosis (MS). The demyelination that characterizes the MS lesion, along with the variable amount of axonal destruction and scar formation, was first described in the mid-19th century by Rindfleisch (1863) and Charcot (1868).

Damage to oligodendrocytes in MS is thought to represent the outcome of an autoimmune reaction against myelin antigens. The number of antigens that have been found to be targets of immune attack in MS has continued to grow over the years. In most MS plaques, it is possible to visualize immunoglobulins and deposits of complement at the lesion site (Prineas and Graham, 1981; Gay *et al*., 1997; Barnum, 2002). It has even been suggested that it is possible to observe deposition of antibodies against such specific antigens as myelin oligodendrocyte glycoprotein on dissolving myelin in active lesions (Genain *et al*., 1999), although it is clear that MS patients produce antibodies against a variety of myelin antigens. Indeed, it seems clear that as this disease progresses, the continued destruction of myelin causes an auto-vaccination process that is associated with a phenomenon called epitope spreading, in which the number of antigens recognized continues to increase (Tuohy *et al*., 1998; Goebels *et al*., 2000; Tuohy and Kinkel, 2000; Vanderlugt and Miller, 2002).

The immune reaction that leads to myelin destruction is a complex one, with many components. Along with the clear presence of anti-oligodendrocyte antibodies in the serum and CSF of MS patients, there is also a T-cell mediated immune reaction, which secondarily leads to macrophage activation. Indeed, the range of possible immune-mediated destructive mechanisms that can lead to myelin destruction, and the substantial heterogeneity of the disease process itself, makes it seem likely that MS is more correctly viewed as a constellation of diseases which share certain characteristic features (see, e.g., Lassmann, 1999; Lassmann *et al*., 2001 for review).

Protecting oligodendrocytes against further damage in the MS patient, and restoring the myelin that has been damaged, represent two of the main goals in MS treatment. It is important to note, however, that achieving these goals may be hindered by the presence of inhibitory substances in the MS lesion itself. Such a possibility is indicated by studies showing that MS lesions contain apparent O-2A/OPCs that exist in a condition of stasis, undergoing little or no cell division (Wolswijk, 1998, 2000; Chang *et al*., 2000). In addition, even though there is a relative sparing of axons in MS lesion, there is nonetheless significant axonal loss. This was noted even in the earliest histological descriptions of MS pathology, and has been amply reconfirmed in more recent years (Fromman, 1878; Charcot, 1880; Marburg, 1906; Ferguson *et al*., 1997; Trapp *et al*., 1998; Bjartmar *et al*., 2003). In lesions in which neurons also are lost, replacement of oligodendrocytes (or treatment with 4-AP) is unlikely to provide clinical benefit.

For recent reviews on a variety of aspects of MS, the reader is referred to, for example, Bruck *et al*. (2003), Galetta *et al*. (2002), Hemmer *et al*. (2003), Neuhaus *et al*. (2003), Noseworthy (2003), Waxman (2002).

VULNERABILITIES OF OLIGODENDROCYTES AND THEIR PRECURSOR CELLS

The number of conditions in which oligodendrocytes and their precursors appear to be killed or otherwise compromised makes it of considerable importance to determine what are the mechanisms underlying the death of these cells. A variety of studies are revealing clues regarding such mechanisms.

It is well established that one of the major contributors to CNS damage following traumatic injury is excitotoxic death of neurons caused by exposure to supranormal levels of glutamate. In recent years, it has become apparent that such glutamate toxicity is also seen in cells of the O-2A/OPC lineage, an observation that may be of considerable importance in a variety of pathological conditions (Yoshioka *et al*., 1996; Matute *et al*., 1997; McDonald *et al*., 1998). Glutamate toxicity has been demonstrated *in vitro*, and also has been shown to occur in isolated spinal dorsal columns (Li and Stys, 2000) and *in vivo* following infusion of AMPA/kainate agonists into the optic nerve (Matute *et al*., 1997; Matute, 1998) or subcortical white matter (McDonald *et al*., 1998).

The glutamate receptors expressed by oligodendrocytes and their precursors are of the AMPA-binding subclass, and have some peculiar features. AMPA receptors in differentiated oligodendrocytes lack the GluR2 subunit, thus rendering them permeable to $Ca2^+$ (Burnashev, 1996). Moreover, the GluR6 subunit is edited in such a manner as to also result in receptors that are more permeable to $Ca2^+$ (Burnashev, 1996). These features may be important in the sensitivity of oligodendrocytes to glutamate. Glutamate receptors have also been found in the myelin sheath (Li and Stys, 2000), and it is not known if local stimulation of sheaths with glutamate results in a localized pathology. As would be predicted from the types of glutamate receptors expressed by oligodendrocytes, it appears that AMPA antagonists can protect oligodendrocytes against ischemic damage, at least *in vitro* (Fern and Möller, 2000). Thus, once clinically useful AMPA antagonists become available, it may be that these agents will prove of use in protecting against damage to oligodendrocytes.

Glutamate may not only be intrinsically toxic, but it may also enhance the toxicity of other physiological insults. For example, ischemic injury is characterized by excessive release of glutamate into the extrasynaptic space (Choi, 1988; Lee *et al*., 1999). Ischemia is also characterized by transient deprivation of oxygen and glucose, a physiological insult that is also toxic for oligodendrocytes. Strikingly, the toxicity associated with deprivation of oxygen and glucose is further enhanced by co-exposure to glutamate (Lyons and Kettenmann, 1998; McDonald *et al*., 1998; Fern and Möller, 2000).

Glutamate mediated damage of oligodendrocytes could be of physiological importance in a variety of settings. One dramatic example of oligodendrocyte death in which these pathways have been invoked is that of ischemic injury occurring in birth trauma, which can be associated with periventricular leukomalacia and cerebral palsy (Kinney and Armstrong, 1997). It must also be considered whether glutamate contributes to the demyelination seen in MS, particularly as it has been observed that glutamate levels are increased in the CNS of patients with demyelinating disorders, with levels correlating with disease severity (Stover *et al*., 1997; Barkhatova *et al*., 1998). In this context, it is of potential interest that chronic infusion of kainate (an AMPA receptor agonist) into white matter tracts is associated with the generation of lesions that have many of the characteristics of MS lesions, including extensive regions of demyelination with plaque formation, massive oligodendrocyte death, axonal damage, and inflammation (Matute, 1998). Although acute infusion of kainate produces lesions that are repaired by endogenous cells, lesions induced by chronic kainate infusion are not spontaneously repaired.

Still other potential contributors to oligodendrocyte death are the inflammatory cytokine TNF-α and, surprisingly, the pro-form of nerve growth factor (proNGF). It is known from both *in vitro* and *in vivo* experiments that oligodendrocytes are vulnerable to killing by TNF-α (Louis *et al*., 1993; Butt and Jenkins, 1994; Mayer and Noble, 1994). It has also been shown that glutamate-mediated activation of microglia induces release of TNF-α from these cells. As microglia can themselves release glutamate when they are activated (Piani *et al*., 1991; Noda *et al*., 1999), it is possible that inflammation elicits a set of responses that build upon each other with the eventual result of tissue destruction. The proNGF receptor p75 also is induced by various injuries to the nervous system. Recent studies have shown that p75 is required for the death of oligodendrocytes following spinal cord injury, and its action is mediated mainly by proNGF (Beattie *et al*., 2002). Oligodendrocytes undergoing apoptosis expressed p75, and the absence of p75 resulted in a decrease in the number of apoptotic oligodendrocytes and increased survival of oligodendrocytes. ProNGF is likely responsible for activating p75 *in vivo*, since the proNGF from the injured spinal cord induced apoptosis among p75(+/+), but not among p75(−/−) oligodendrocytes in culture, and its action was blocked by proNGF-specific antibody.

In vivo, it is unlikely to ever be the case that single factors act alone, and in this regard, the interplay between glutamate and TNF-α is of particular interest with regard to induction of demyelination. The combination of glutamate and TNF-α shows a highly lethal synergy when applied together in the thoracic gray matter of the spinal cord (Hermann *et al*., 2001). It is not yet known if similar synergies occur with respect to the killing of oligodendrocytes, either by TNF-α or by proNGF, but such combinatorial effects seem likely.

REPAIR OF DEMYELINATING DAMAGE

The enormous range of clinically important conditions in which myelination is not properly generated, or is destroyed, makes it of paramount importance to understand how to repair this damage. The extensive knowledge regarding myelin biology, and on O-2A/OPCs and other potential ancestors of oligodendrocytes, has made it possible to begin development of a variety of strategies for promoting such repair.

The development of approaches for the repair of demyelinating damage has several components, each of which needs to be successfully addressed to develop a clinically useful strategy. First, there needs to be a means of identifying individuals for whom remyelination therapy might be expected to provide clinical benefit. Second, there needs to be a means of evaluating the success of such therapy. The third and fourth considerations are whether one is going to use transplantation of exogenous precursor cells to generate new oligodendrocytes and myelin, or whether the preferred strategy will be to enhance recruitment of endogenous precursor cells.

Advance identification of individuals who have a high likelihood of benefiting from remyelination therapy is absolutely essential in evaluating the efficacy of the therapy under study. This is because the development of any novel therapy requires a positive outcome to warrant continued devotion of resources and effort to that therapeutic approach. Attempts to restore neurological function in individuals in which repair of abnormal myelination is not sufficient to improve function would fail for reasons that are not germane to evaluating the potential utility of such therapies. For example, the lesions of both spinal cord injury and MS may be associated with substantial axonal loss (Trapp *et al*., 1998; Kakulas, 1999a, b; Dumont *et al*., 2001; Doherty *et al*., 2002), a problem that cannot be solved by remyelination therapies. As destruction of myelin can induce similar failures of impulse conduction as are associated with axonal transection, or with conduction block caused by pressure, a simple clinical examination may not provide unambiguous data regarding the contribution of demyelination to impulse failure. Examination of lesions with standard imaging tools also tends to reveal more information about inflammation and edema than about the local state of myelin.

At present, the most promising tool for identifying individuals who might benefit from remyelination therapy appears to be a blocker of voltage-gated potassium (K^+) channels called 4-aminopyridine (4-AP). Demyelinated axons show increased activity of 4-AP-sensitive K^+ channels (Blight and Gruner, 1987; Blight, 1989; Bunge *et al*., 1993; Fehlings and Nashmi, 1996; Nashmi *et al*., 2000). When myelin is intact, there is only an inward sodium (Na^+) current and little outward K^+ current (Chiu and Ritchie, 1980), but after disruption of the myelin sheath, there is an increased persistent outward K^+ current. 4-AP blocks the leak through the "fast" K^+ channels that are normally located underneath the myelin (Sherratt *et al*., 1980; Bowe *et al*., 1987; Rasband *et al*., 1998). These channels have multiple properties that have been ascribed to them (Nashmi and Fehlings, 2001b), including roles in re-polarization (Kocsis *et al*., 1986), stabilizing the node to prevent re-excitation after a single impulse (Chiu and Ritchie, 1984; Poulter *et al*., 1989; David *et al*., 1993; Poulter and Padjen, 1995), and thereby increasing the security of axonal conduction (Chiu and Ritchie, 1984), and limiting excessive axonal depolarization and inactivation of nodal Na^+ channels (David *et al*., 1992).

A variety of clinical trials have indicated that administration of a sustained release formulation of 4-AP may provide significant benefit to a subset of individuals with MS and also to some individuals with incomplete spinal cord injury (wherein myelin destruction is a frequent event even in the presence of intact axons). Myelin destruction and oligodendrocyte death has been seen in both experimental and clinical injuries (Gledhill and McDonald, 1977; Griffiths and McCulloch, 1983; Bunge *et al*., 1993; Crowe *et al*., 1997; Li *et al*., 1999; Casha *et al*., 2001; Nashmi and Fehlings, 2001a; Koda *et al*., 2002).

If a given individual does not benefit from the utilization of 4-AP, then it may be very difficult to understand underlying reasons for a failure of functional gain associated with testing of a remyelination therapy. Would this be because there was insufficient remyelination to confer benefit, or because the axonal damage was itself sufficiently severe that remyelination was not sufficient to restore conduction? Despite some experimental evidence that 4-AP may also enhance synaptic transmission, separately from any effects on impulse conduction in unmyelinated axons, there thus far appears to be no better approach to the identification of suitable candidates for therapies targeted at enhancing remyelination.

The next critical distinction to be made in the development of remyelination therapies is that of distinguishing between repair by transplantation and repair by recruitment of endogenous precursor cells. As discussed below, these two options themselves segregate further into multiple strategic suboptions.

Attempts to repair demyelinated lesions by cell transplantation will necessarily be focused on instances in which most or all of the damage is found within a discrete lesion site and where there is a reasonable expectation that remyelination will provide functional benefit. There are several conditions that fulfil this requirement, including spinal cord injury, lacunar infarcts, and transverse myelitis. Although lesions in different patients may differ greatly in size, these different conditions nonetheless share the characteristic that successful repair within a single anatomical location has the highest probability of providing clear clinical benefit.

Once a decision is made to attempt to remyelinate lesions by cell transplantation, it is necessary to choose between the multitude of cellular populations that have emerged as candidates for such repair. In experimental animals, remyelination has been successful using O-2A/OPCs (Espinosa de los Monteros *et al*., 1993; Warrington *et al*., 1993; Groves *et al*., 1993a; Utzschneider *et al*., 1994; Duncan, 1996; Jeffery *et al*., 1999), GRP cells (Herrera *et al*., 2001), NSCs (Hammang *et al*., 1997), and embryonic stem cells that have been pretreated to bias differentiation toward a neural cell fate (Brustle *et al*., 1999; Liu *et al*., 2000). It has also been possible to isolate oligodendrocyte-competent glial precursor cells from embryonic stem cells ([Brustle *et al*., 1999; Liu *et al*., 2000], although it is not known whether these precursors are GRP cells, O-2A/OPCs, both, or neither). Precursor cells capable of making oligodendrocytes following transplantation can also be isolated from developing or from adult tissues. Moreover, many of the stem and progenitor cell populations of interest in the generation of new oligodendrocytes can be isolated from human tissues of different ages and sources (Roy *et al*., 1999; Dietrich *et al*., 2002; Windrem *et al*., 2002).

It is not presently known whether any individual population of cells capable of generating oligodendrocytes *in vivo* offers advantages over any other population, but there are reasons to be concerned that different populations may yield divergent outcomes. For example, if properties that cells express *in vitro* are indicative of their behavior *in vivo*, then O-2A/OPCs such as those isolated from the optic nerves of 7-day-old rats might be expected to generate a relatively restricted number of oligodendrocytes quite rapidly (Fig. 7). In contrast, O-2A/OPCs such as those isolated from cortices of the same animals might generate a far larger number of cells but may take a much longer time to generate oligodendrocytes (Power *et al*., 2002). GRP cells could also be used to generate both oligodendrocytes and astrocytes (Herrera *et al*., 2001), which may be beneficial. In contrast, O-2A/OPCs could be used to more selectively generate oligodendrocytes (Espinosa de los Monteros *et al*., 1993; Groves *et al*., 1993b; Warrington *et al*., 1993).

At this point in time, very little is known about the comparative utility of different precursor cell populations in lesion repair. Thus, an essential component of the development of remyelination therapies will be the determination of whether specific precursor populations are generally advantageous, or whether repair of different types of lesions will require transplantation of different types of cells.

In contrast with repair of focal lesions, the repair of the distributed lesions like those seen in MS patients seems more likely to be initially attempted by the application of strategies that recruit endogenous precursor cells. The most theoretically attractive strategy in this regard would be systemic administration of a therapeutic compound that specifically promotes division of glial precursor cells capable of generating oligodendrocytes.

At the time of writing this chapter, the only published approach to enhancing function of endogenous cells that seems

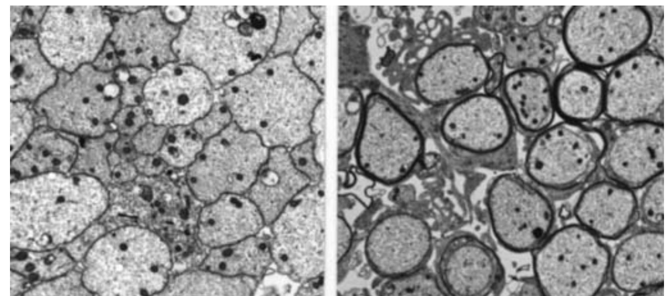

FIGURE 7. Remyelination by transplantation of O-2A/OPCs. In these experiments, O-2A/OPCs isolated from optic nerves of P7 rat pups and expanded *in vitro* for 3–4 weeks by being grown in the presence of PDGF + FGF-2. These cells were then transplanted into the spinal cord of rats that received a local injection of ethidium bromide to kill all cells with DNA in the injection site. Such an injection kills all glial cells while sparing the axons. In addition, the animals are irradiated so that host precursor cells cannot repair this damage. In the absence of cell transplantation, the tissue contains only axons running in a glial-free space (as shown in the left-hand electron micrograph). Following transplantation of O-2A/OPCs, >90% of the axons are remyelinated. For greater detail, the reader is referred to Groves *et al*. (1993a).

close to clinical evaluation is the application of antibodies that have been reported to promote remyelination. These antibodies were first identified in paradoxical studies indicating that monoclonal antibodies directed against myelin antigens could promote remyelination in a number of different circumstances (Asakura and Rodriguez, 1998; Warrington *et al*., 2000). Effectiveness of these antibodies has been observed in the immune-mediated demyelination model of infection with Theiler's virus (Asakura and Rodriguez, 1998) as well as in the case of demyelination induced by injection of lysolecithin into white matter tracts (Pavelko *et al*., 1998). Remyelination-promoting monoclonal antibodies also reduce relapse rates and prolong relapse onset in the autoimmune model of experimental allergic autoencephalomyelitis, an experimental model of MS (Miller *et al*., 1997). The fact that many of the antibodies that have been found to be effective in this paradigm bind specifically to oligodendrocytes and/or their precursors provides an important potential for specificity of action of this strategy.

Antibodies that promote remyelination appear to work by physiologic stimulation of reparative systems. Intraperitoneal injection of remyelination-promoting antibodies labeled with radioactive amino acids has shown that these antibodies enter the CNS and bind primarily to cells in the demyelinated lesion (Hunter *et al*., 1997). While the mechanism by which these antibodies promote remyelination remains uncertain, it is of potential interest that all remyelination-promoting antibodies tested evoke Ca^{++} transients in mixed glial cultures while isotype- and species-matched control antibodies do not. Thus, it may be that the ability of these antibodies to stimulate Ca^{++} fluxes activates a signal transduction cascade critical for myelinogenesis (Soldán *et al*., 2003).

It is possible that growth factors will also be found that have the ability to beneficially stimulate specific precursor cell populations *in vivo* (McTigue *et al*., 1998), but the ability of growth factors to modulate the biology of multiple cell types will make the careful elucidation of potential side effects of particular importance. Achieving adequate growth factor delivery is also a matter of concern. Although it is possible to infuse growth factors into CSF, many studies have shown that the extent to which such molecules can distribute into the CNS parenchyma due to diffusion is very limited (Bobo *et al*., 1994; Lieberman *et al*., 1995). Normal diffusion processes are intrinsically limited, with reductions in growth factor concentration being reduced according to the inverse square law that governs diffusion from a point source. Diffusion in the real setting of the CNS, moreover, is even more compromised. The fact that growth factors bind to cells and matrix in the diffusion path means that the distance of diffusion is reduced to an even greater extent than in a free diffusion system, and the reduction in growth factor concentration falls more sharply than in a simple inverse square relationship. Thus, successful growth factor delivery may require the utilization of convective delivery strategies (Bobo *et al*., 1994; Lieberman *et al*., 1995; Lonser *et al*., 1999, 2002).

Successful application of strategies to recruit endogenous precursor cells will be dependent upon there being sufficient numbers of cells available to carry out repair and on the physiological condition of the patient being conducive to repair. At this point in time, little is known about whether there are limitations in precursor cell production that preclude extensive or repetitive repair, or whether the environment itself is refractory to repair. On the one hand, there are indications that there are such large numbers of putative *adult* O-2A/OPCs in the normal CNS as to potentially represent 5–8% of the total cells in the normal CNS (Nishiyama *et al*., 1999; Dawson *et al*., 2000; Levine *et al*., 2001). On the other hand, we know little about the biological heterogeneity of this $NG2^{+}$ cell population, about the prevalence of cells following a lesion, or about the functional competence of those cells that are found in the post-lesioned CNS.

If it is the case that endogenous precursor cells are too depleted, or otherwise compromised, to allow effective repair, then usage of growth-promoting strategies in conjunction with cellular transplantation might provide an optimal approach to enhancing remyelination. If the CNS has become refractory to repair, for example, by generation of glial scar tissue that might inhibit O-2A/OPC migration into lesion sites (ffrench Constant *et al*., 1988; Groves *et al*., 1993b), then it will be essential to develop means of overcoming such inhibitory signals. That some form of refractory phenomena might occur is indicated by the apparent presence of nondividing O-2A/OPCs in lesions of MS patients (Chang *et al*., 2000; Wolswijk, 2000). Moreover, it appears that although transplanted oligodendrocyte progenitor cells survive and remyelinate in acute lesion areas, normal white matter is inhibitory to the migration of these cells (O'Leary and Blakemore, 1997). Thus, there may well be *in vivo* constraints that limit the effectiveness of transplanted cells.

One of the most important and challenging ventures will be repair of myelination abnormalities that are diffusely distributed—or even globally distributed—throughout the CNS. Such a distributed failure of normal myelination occurs in many children with a variety of CNS diseases.

As indicated earlier in this chapter, the three general causes of diffuse, or global, abnormalities in myelination are (a) genetic disorders, (b) nutritional and hormonal deficiency disorders, and (c) exposure to any of a large variety of physiological insults. Different approaches may be required for each of these conditions.

A number of the genetic diseases that result in failures of normal myelination have been discussed previously in this chapter. They share the problem that recruitment of endogenous precursor cells is not a viable strategy in the absence of repair of the underlying genetic lesion, as it is clear that the genetically defective cells are themselves not capable of normal myelination. Thus, it is of paramount importance to develop strategies that allow the genetic lesion to be directly repaired, or for its effects to be overridden.

Two potential approaches to repair in the case of genetic diseases are to repair the genetic damage so that endogenous precursor cells can carry out repair or to transplant normal cells into the genetically abnormal environment. Promising progress has been made for both of these approaches. An example of the former approach has been the use of lentivirus vectors to obtain clear clinical improvement in adult beta-glucuronidase deficient

(mucopolysaccharidosis type VII {MPS VII}) mice, an animal model of lysosomal storage disease (Brooks *et al*., 2002). Lysosomal accumulation of glycosaminoglycans occurs in the brain and other tissues of individuals with this disease, causing a fatal progressive degenerative disorder, including mental retardation as one of its outcomes. Treatments are designed to provide a source of normal enzyme for uptake by diseased cells and thus can theoretically be treated by introduction of cells that express beta-glucuronidase. Improvement in this mouse model has also been obtained by transplantation of beta-glucuronidase-expressing neural stem cells into the cerebral ventricles of newborn animals. When these animals were examined at maturity, donor-derived cells were found to be present as normal constituents of diverse brain regions. β-Glucuronidase activity was expressed along the entire neuraxis, resulting in widespread correction of lysosomal storage in neurons and glia (Snyder *et al*., 1995). A similar approach also has been applied in attempts to repair the global dysmyelination found in shiverer mice, in which myelin is not produced due to a genetic defect in the oligodendrocytes themselves. Transplantation of genetically normal NSCs in the ventricles of newborn shiverer mice was associated with widespread engraftment and generation of normal myelin in the shiverer brain (Yandava *et al*., 1999).

Nutritional and hormonal deficiency disorders that compromise myelination may offer somewhat easier targets for repair than genetic myelination disorders in that there is a hope that existing cells are not compromised in their function. There is some reason to be optimistic about this possibility, due to the well-documented ability of myelination to return to normal levels in hypothyroid, or nutritionally-deprived, animals in which the underlying metabolic defect is corrected sufficiently early in development (Wiggins *et al*., 1976; Wiggins, 1979, 1982; Wiggins and Fuller, 1979; Noguchi *et al*., 1985; Munoz *et al*., 1991; Bernal and Nunez, 1995; Ibarrola and Rodriguez-Pena, 1997; Marta *et al*., 1998).

Despite the ability of endogenous precursor cells to correct myelination deficiencies if metabolic defects are corrected early enough in development, studies on nutritional and hormonal deficiency disorders have also demonstrated the critical importance of restoring normal metabolic function by an early enough time if one is going to achieve repair. For example, repair of dysmyelination associated with nutritional deprivation requires restoration of normal nutritional intake in order to achieve normal levels of myelination (Wiggins *et al*., 1976; Wiggins, 1979, 1982; Wiggins and Fuller, 1979). Similarly, restoration of TH in the case of hypothyroidism only is associated with repair of dysmyelination if hormonal replacement therapy is initiated early enough in life (Noguchi *et al*., 1985; Munoz *et al*., 1991; Bernal and Nunez, 1995; Ibarrola and Rodriguez-Pena, 1997; Marta *et al*., 1998). The existence of these critical developmental periods for enabling remaining CNS precursor cells to generate normal levels of myelination *in vivo* raises questions as to what is the underlying biology of such critical periods. One possible component of these periods of opportunity for successful repair could be the observed transition from the presence in the CNS by O-2A/OPCs of a *perinatal* phenotype to those with an *adult*-specific phenotype, a transition that occurs in the rat optic nerve largely during the period of 2–3 weeks after birth (Wolswijk *et al*., 1990).

The existence of critical periods after which restoration of normal metabolism is no longer associated with an equivalent restoration of normal myelination suggests that it will also be necessary to apply strategies of enhancing function of endogenous precursor cells and/or transplanting additional precursor cells to achieve repair of these syndromes. It is important to stress, however, how little is known about the reasons for the failure of repair if metabolic repair is delayed too long. For example, it is not even known whether the CNS itself of older animals with metabolic disorders expresses properties that make it refractory to repair. This is a critical area for further study.

A further question that needs to be considered is whether there is a need to utilize more than one cell type for repair of tissue. For example, in global disorders of myelination, there may be value in transplanting O-2A/OPCs to achieve more rapid generation of oligodendrocytes, as well as transplanting NSCs in order to populate the germinal zones of the brain with cells capable of contributing glial precursor cells for a prolonged period. Or, in spinal cord injury or other forms of traumatic injury, there may be value in transplanting GRP cells to generate normal astrocytes together with O-2A/OPCs to increase the yield of oligodendrocytes. It is also not known whether successful remyelination will require multiple transplantations. And if so, then how many? With what interval between them? Will they need to be spread over particular physical distances?

While many questions remain to be answered to enable the application of our increasing knowledge about oligodendrocyte biology to the treatment of important medical problems, it is nonetheless extraordinary to consider the advances that have been made in a relatively short time. With such a rate of progress, it cannot be long before we are able to accomplish the remarkable feat of repairing damage to this vital component of the CNS. Moreover, it seems certain that the ongoing study of these fascinating cells will continue to provide insights relevant to a range of biological problems that extend far beyond the questions of how myelin is formed, maintained, and replaced.

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