INTRODUCTION

The myelin-synthesizing oligodendrocyte is compromised in many neuropathological diseases, including demyelinating diseases (e.g. multiple sclerosis), metabolic diseases (e.g. Pelizaeus-Merzbacher), infectious diseases (e.g. progressive multifocal leukoencephalopathy), neurodegenerative diseases (e.g. multisystem degeneration), and possibly neoplasms (e.g. oligodendrogliomas). Understanding the development of the oligodendrocyte has important implications for both the pathogenesis of these diseases and also potential therapy. Over the past 20 yr, research in oligodendrocyte development has delineated a pathway from progenitors to mature oligodendrocytes. In fact, the oligodendrocyte has served as a model for lineage development in part due to the identification of specific phenotypic stages during maturation. From this has come the identification of numerous signaling molecules that instruct oligodendrocyte development. More recently, transgenic and targeted mutagenesis studies have begun to identify new factors involved in oligodendrocyte development and have questioned some of the older observations. This review will attempt to update the current state of research on the progression of the oligodendrocyte lineage.

Oligodendrocytes develop from proliferating precursor cells migrating out of germinal zones in the brain and spinal cord. When the cells reach their final destination in the brain parenchyma, they become postmitotic, extend processes, and begin to synthesize the components of myelin as extensions of their plasma membranes. In most animals, this occurs relatively late in CNS development during late embryonic and early postnatal life, after neurons and astrocytes are formed. This myelin forms an insulating sheath around axons, serving dual functions in the nervous system. Historically, myelin was recognized as critical because it facilitates rapid propagation of nerve impulses through very small spaces, thus permitting axons to be of small caliber (1). More recently, myelin has been found to modulate axonal structure and support axonal integrity, as well (2).

The oligodendrocyte has become a model CNS cell type for the study of lineage development due in large part to the facility with which these cells can be cultured and will proceed through their maturation in culture. This development in vitro has been shown to parallel oligodendrocyte development in vivo in phenotype as well as in timing (3, 4). Work in culture is possible because specific antigens have been identified on the surface of the precursor cells that allow separation of cells in the oligodendrocyte lineage from other cell types, isolation of specific stages within the lineage, and characterization of the response to cell signaling molecules at these stages. Model oligodendrocyte cell lines were generated and refinements of techniques have allowed for the propagation of large numbers of primary oligodendrocyte lineage cells for biochemical and molecular biology studies.

Oligodendrocyte Precursors versus the O-2A Cells

The study of the oligodendrocyte lineage was facilitated by the identification of the oligodendrocyte precursor using the A2B5 antibody that recognizes a surface ganglioside (5, and references contained therein). Although this antibody was first used to label neurons and is notoriously difficult to use in vivo, when used in vitro, it clearly identifies a small, round, process-bearing cell that will become an oligodendrocyte under most conditions. These cells were originally identified in cultures derived from optic nerve but are present throughout the CNS and spinal cord (6). Initial studies of the oligodendrocyte precursors (OP) in vitro found that in serum-free medium, the OP will quickly differentiate and express myelin-associated proteins. When grown in 10%–20% serum or when treated with growth factors, such as bone morphogenetic protein (BMP), the OP will continue to express A2B5 and also express glial fibrillary acidic protein (GFAP), a major internal filament associated with astrocytes (5). Raff and coworkers termed these novel cells “type 2 astrocytes” to distinguish them from the classic type of astrocytes (“type 1 astrocytes”). They called the OP cells “O-2A” to credit their bipotential nature. A recent study shows that following a specific treatment regimen that includes serum and a series of growth factors, OPs express neuronal antigenic determinants, suggesting that they may be even more plastic than previously thought (7).

Although classic studies have shown that oligodendrocytes and astrocytes can be generated from the same retroviral labeled cells in the postnatal rat subventricular
zone (8), the formal existence of type 2 astrocytes in vivo has never been proven. Additional evidence against the in vivo existence of type 2 astrocytes includes the observations that astrocytes arise earlier than oligodendrocytes and that OPs generate only oligodendrocytes when transplanted into brain or spinal cord (9). Thus, the use of the term “O-2A” has largely been discontinued in favor of the more general oligodendrocyte precursor or progenitor. Although the bipotential or tripotential nature of the OP remains in doubt, a tight correlation between the antigenic and phenotypic characteristics of cells at that stage has been confirmed in vitro and in vivo.

Several other antibodies are now known to identify the OP. One detects the alpha subunit of the receptor for platelet-derived growth factor (PDGFRα) (10), a potent mitogen, survival and differentiation factor for oligodendrocytes. PDGFRα is present only on cells destined to become oligodendrocytes and is probably the most reliable marker of oligodendrocyte precursors in vivo. Another OP antibody detects chondroitin sulfate proteoglycan NG2 and appears slightly after PDGFRα. The 2 antibodies co-localize both in vitro and in vivo and disappear at differentiation.

NG2+ cells are numerous and ubiquitous in the central nervous system in both the developing and adult CNS (11). This has led some investigators to question whether NG2+ cells are restricted to the oligodendrocyte lineage. However, NG2+ cells do not co-label with cells that express GFAP (astrocytes [12]) or OX-42 (microglia [13]). Some NG2 cells co-label with the O4 antibody, which marks a more mature stage of the oligodendrocyte lineage (see below). Both in vivo and in vitro studies indicate OPs progress from NG2+/O4- to NG2+/O4+ in the cerebral cortex as cells move out toward the pial surface. In summary, the number of NG2-2-positive cells in the developing and adult brain and spinal cord is surprising large, however, there is no clear evidence that NG2+ cells are anything other than oligodendrocyte precursors.

What Precedes the Oligodendrocyte Precursor?

Once the oligodendrocyte precursor was identified and its characterization was underway, it became clear that the OP was a stage in the middle of the oligodendrocyte lineage and oligodendrocyte lineage cell types occurring before and after the OP were identified (Fig. 1). We identified a cell type that appears in the lineage before the OP termed “pre-progenitors” or “pre-O2A cells.” They were originally noted in rat brain cultures that had been depleted of OP and mature oligodendrocytes by complement-mediated cell lysis (6). Treatment of these cultures with PDGF generates large clusters of small, round, process-bearing cells. These cells could be labeled with an antibody to the embryonic form of neural cell adhesion molecule (PSA-NCAM), PDGFRα receptor, and the internal filaments vimentin and nestin (14). A few of these cells double labeled with the A2B5 antibody. These cells have also been recognized in vivo in neonatal rat forebrain (4). In culture, pre-progenitors appear to be multipotential. When treated with growth factors that promote oligodendrogliogenesis or support survival, the pre-progenitors become A2B5+ within 24–48 hours and eventually differentiate into mature oligodendrocytes. When treated with serum or growth factors that promote astrogliogenesis, the cells will express GFAP and resemble type 2 astrocytes although most cells will be A2B5-negative (15). It has been reported that these cells are also capable of becoming neurons under appropriate conditions (16). Pre-progenitors are useful for transplantation studies because of their rapid proliferation and default differentiation to oligodendrocytes within the brain (17). Interestingly, grafting of PSA-NCAM+ cells into a focal demyelinating lesion in the spinal cord resulted in these cells displaying characteristics of Schwann cells (18). The elucidation of the environmental cues that direct these cells to become a specific cell type in vivo remain unknown and will be an important area of investigation.

The presence of the polysialic acid polymer on the NCAM adhesion molecule on the surface of the oligodendrocyte pre-progenitor has been the subject of much interest. The expression of PSA-NCAM on the surface of neonatal precursors coincides with their restriction to the glial fate (16). Expression of the PSA carbohydrate and the polysialtransferases that synthesize it are down-regulated as pre-progenitors, become OPs, and then mature oligodendrocytes (19). This may relate to a loss of mobility as the cells differentiate. Removal of the PSA moiety from NCAM by endonereaminidase has resulted in decreased migration and increased differentiation and myelination in OPs in culture (20, 21). Early precursor cells that can become oligodendrocytes have also been derived from embryonic rat spinal cord as early as E10.5. These cells, termed glial restricted precursors, express A2B5, nestin, and PLP/DM-20 but lack PDGFRα and PSA-NCAM immunoreactivity (22). When cultured first in FGF2 and PDGF, followed by PDGF alone, they are able to differentiate into both type 1 and type 2 astrocytes as well as oligodendrocytes.

The existence of these 2 ostensibly distinct progenitors, which ultimately generate the same mature cell type, is perplexing. However, new in vivo evidence indicates that multiple progenitor cell types exist that give rise to oligodendrocytes with apparently the same mature phenotype (23). Spassky et al have identified 2 types of pre-oligodendrocytes in the brain and spinal cord: one, which expresses PLP/DM20 and does not express or respond to the mitogenic properties of PDGFRα, and the other, which expresses PDGFRα (23).
Fig. 1. Progression of the oligodendrocyte lineage. At the top are photographs of typical cells along the oligodendrocyte lineage for each of the 5 stages identified. These newborn rat cells grown in purified culture are labeled with antibody to PSA-NCAM for pre-progenitors, A2B5 for precursors, 04 for pro-oligodendroblasts, Gal C (R-mAB) for immature oligodendrocytes, and PLP for mature oligodendrocytes. Note the increase in process length and complexity with maturity. Under the diagram of the cell types are markers found at each stage of the lineage, followed by 4 processes oligodendrocyte undergo as they mature with the signaling molecules for these processes noted on the arrows. Putative transcription factors are shown under the processes, located by their main areas of expression or influence.

Pro-Oligodendroblast to Mature Oligodendrocytes: Phenotypes of Oligodendrocyte Differentiation

As rodent OPs begin to differentiate, they express a sulfated surface antigen known as POA (pro-oligodendroblast antigen) that is recognized by the antibodies 04 and A007 (24). Expression of these antigens coincides with the extension of numerous branching processes and a cessation of migration (24). The pro-oligodendroblast is known to represent a transition stage that is characterized by continued proliferation but altered response to mitogens when compared to A2B5+/04− cells (25). In vivo, O4+ cells are seen at the leading edge of myelination in the CNS (26). The O4 antibody continues to recognize mature oligodendrocytes through binding to sulfatide and is thus continuously expressed from the pro-oligodendroblast stage throughout maturation. In the chick, the O4 antibody detects an earlier stage of development that corresponds more closely to the A2B5+ oligodendrocyte precursors or even possibly pre-progenitors (27).
The real beginning of the differentiation of the oligodendrocyte precursors to mature oligodendrocytes is detected by the expression of galactocerebroside on the cell surface (28) accompanied by a dramatic decrease in proliferation and increase in process extension and complexity. Two antibodies are used to detect this stage. The R-mAb (monoclonal antibody) H8H9, (29) detects sulfatide as well as cerebroside and marks a population of cells slightly less differentiated and slightly more responsive to growth factors than is detected with the other antibody, O1 (30, 31).

As oligodendrocytes differentiate, they begin to synthesize the structural proteins of myelin. Each of these is expressed at specific time in the development of the cell on both the RNA and protein level. The first to appear is 2',3'-cyclic nucleotide 3' phosphodiesterase, a basic myelin membrane protein that forms 4% of all myelin. It is present in 2 isoforms, one of which is synthesized before galactocerebroside at the very end of the precursors stage (32). More abundant myelin proteins, proteolipid protein (PLP), and myelin basic protein (MBP) appear several days after galactocerebroside in cultured cells. PLP and its alternatively spliced isoform, DM20, are membrane proteins that constitute 50% of the protein mass of myelin (33). Although PLP was originally thought to merely provide structural support in the myelin membrane, it has recently been shown to be critical to the regulation of differentiation (34). Mutations in PLP are often lethal and result in lack of myelination and premature death of oligodendrocytes by programmed cell death (35). MBP constitutes 30%–40% of CNS myelin proteins and also has multiple isoforms. It plays a major role in myelin compaction and mutations in it result in severe dysmyelination (36).

Several other more minor components of myelin have begun to be characterized. For example, myelin-associated glycoprotein (MAG) constitutes 1% of myelin protein and is involved in neuron:glial interactions (37). Another, myelin/oligodendrocyte glycoprotein (MOG), is a relatively minor membrane protein of myelin and is expressed relatively late in the process of myelination (38) making it a useful marker of very mature oligodendrocytes. These and other myelin proteins have recently been the focus of another review (39).

Recent studies have been able to uncover the function of galactocerebroside. Transgenic animals in which the UDP-galactose:ceramide galactosyltransferase enzyme, which catalyzes the final step in the synthesis of GalC has been eliminated, have normal myelin formation but severe electrophysiological deficits (40). Detailed analysis of these mutants found that oligodendroglial processes are not properly positioned along the length of the axon resulting in abnormal formation of nodes of Ranvier, paranodes, and transverse bands (41). Thus galactocerebroside is necessary for proper axon:glial interactions.

Growth Factors in Oligodendrocyte Development

In the course of development to mature, myelinating oligodendrocyte, the progenitor cell must be specified, proliferate, migrate from the germinal zone out into the white matter, and finally differentiate from the precursor state to the immature and then mature oligodendrocyte. In addition, cells of the oligodendrocyte lineage must avoid programmed cell death, a major regulator of oligodendrocyte number. These 5 processes are controlled by a number of protein growth factors that modulate one or more of these categories (Fig. 1). Studies on the effects of these extracellular signaling molecules have been mostly performed in tissue culture. For some factors, the in vitro effects have been confirmed using transgenics or targeted mutagenesis in mice. In other situations where a combination of factors may be critical in vivo, it has been difficult to assess the in vivo effect of a single factor.

Platelet-Derived Growth Factor

The best studied and perhaps most versatile factor for promoting oligodendrocyte development is platelet-derived growth factor (PDGF). It had been noted that OPs cultured singly quickly differentiated to mature oligodendrocytes, whereas OPs grown on a monolayer of astrocytes would multiply for a finite number of doublings before differentiating. The factor secreted by the astrocytes was subsequently identified as PDGF (42), which is also secreted by neurons (43).

OPs express PDGFα receptors, which can bind all 3 isoforms of PDGF (A, B, and C), and signal as homo- or heterodimers (44). The receptors are slowly down-regulated as the cells begin to mature (45). The density of PDGF receptors seems to be related to the function of the growth factor. In pre-progenitors and OPs, PDGF acts as a mitogen, enhances differentiation, and increases survival (14). As OPs mature, the mitogenic effect of PDGF disappears, however the survival effect is still strong, suggesting that lower levels of PDGF signaling are sufficient for survival but not proliferation.

As a mitogen, PDGF is not particularly strong and works best in combination with other factors, such as basic fibroblast growth factor (bFGF) (46), but may be critical to generating the proper complement of oligodendrocytes. Mice engineered to overexpress PDGF in neurons showed hyper-proliferation and a 7-fold increase in OP numbers (47). This data suggests that existing quantities of PDGF limit OP cell division. This is a function of PDGF A alone because PDGF A null mice have very reduced numbers of progenitors, especially in the spinal cord, optic nerve, and cerebellum. PDGFB null mice show no such effect (48).

As a survival factor, PDGF protects OPs and immature oligodendrocytes from cell death both in vivo and in vitro.
increase in O4 protein and mature oligodendrocytes concomitant with an increase in myelins of perinatal rats demonstrated a decrease in myelin formation in vivo in which bFGF was injected into the ventricles of rats. However, PDGF may not be a long-term survival factor or a survival factor for mature myelinating oligodendrocytes. Injection of PDGF into the CNS of newborn rats resulted in prolonged proliferation of OPs but no ultimate increase in mature oligodendrocytes. In addition, the excess numbers of OPs in PDGF- overexpressing mice appear to be removed by programmed cell death soon after formation so that the final number of myelinating oligodendrocytes is the same as in the wild type animals.

Fibroblast Growth Factor

Another growth factor that stimulates OP proliferation in vitro is fibroblast growth factor (FGF). FGF works through 4 tyrosine kinase receptors, 3 of which are expressed in OPs at varying levels according to the maturity of the cells. In addition to being a potent mitogen, FGF inhibits OPs from differentiating to immature oligodendrocytes. In fact, OPs can be maintained virtually indefinitely in a combination of PDGF and bFGF. FGF appears to keep OPs in the cell cycle, which has a secondary effect of inhibiting programmed cell death. In vitro, FGF can cause dedifferentiation of GalC+ oligodendrocytes. Studies have shown that treatment of purified cultures of GalC+ cells with bFGF induced them to re-express A2B5 and begin to proliferate. Recent studies in vivo in which bFGF was injected into the ventricles of perinatal rats demonstrated a decrease in myelin protein and mature oligodendrocytes concomitant with an increase in O4+ precursors in accordance with the in vitro observations. However, other studies have suggested that this is not a true dedifferentiation but an assumption of a novel phenotype.

The actual effect of bFGF in vivo remains elusive. Targeted mutagenesis in mice for FGF 1 through 10 are either embryonic lethal or have no obvious oligodendrocyte phenotype. Null mutations from FGFR1 and FGFR2 are also embryonic lethal. Thus conditional mutagenesis will be required to test the role of many of these factors and their receptors in oligodendrocyte development.

Neuregulin

The neuregulin family of growth factors, which includes glial growth factor, neuregulin, neu-differentiation factors, acetylcholine receptor inducing activity, heuregulins, and sensory motor neuron-derived factor (SMDF) has similar effects to FGF. These factors work through the ErbB family of tyrosine kinase receptors and oligodendrocyte precursors express ErbB2, B3, and B4. In culture, neuregulin is a potent mitogen for oligodendrocyte precursors and inhibits differentiation to the mature phenotype. However, some studies failed to find this effect and also failed to find expression of ErbB3, possibly reflecting differences in culture conditions or age of the cells at explant. Neuregulin is necessary for the proper development of oligodendrocytes in vivo although the exact stage of oligodendrocyte development in which it acts is unknown. In explants of ventral spinal cord from mice with a null mutation in the neuregulin gene, oligodendrocytes do not develop unless rescued by the addition of exogenous neuregulin. Conversely, antibodies to neuregulin can inhibit development of oligodendrocytes in wild type mice.

Neuregulin treatment of mature oligodendrocytes in culture also caused a phenotypic reversion to a less mature morphology, loss of myelin protein expression, reexpression of the intermediate filament nestin, and reentry into the cell cycle, again reminiscent of effects of FGF.

Insulin and Insulin-like Growth Factor

Numerous studies indicate that insulin and insulin-like growth factors are potent regulators of oligodendrocyte development. Both insulin and insulin-like growth factor 1 (IGF-1) receptors are present on oligodendrocyte precursors. In culture, IGF-1 increases proliferation of oligodendrocyte precursors and increases numbers of mature GalC+ oligodendrocytes. This is probably largely due to the ability of IGF-1 to inhibit programmed cell death. In addition, exogenous IGF-1 delivered to rat optic nerve can inhibit naturally occurring cell death of immature oligodendrocytes. Furthermore, transgenic mice overexpressing IGF-1 have significantly larger brains, more oligodendrocytes, and more myelin per oligodendrocyte than their wild type littermates. IGF-1 null mice have less myelin than wild type but this reduction is in proportion to a reduction in brain weight and number of axons. Myelination appears to be complete in these animals. These data indicate IGF-1 plays a role in myelination and oligodendrocyte survival in vivo, although how this is mediated is still unknown.

Neurotrophins

Both oligodendrocyte precursors and mature cells from rat brain express the high affinity neurotrophin receptors TrkA and TrkC and express lower levels of the low affinity neurotrophin receptor p75 and TrkB in a stage-specific and region-specific manner. In culture, the effects of NT-3 and related neurotrophins include an increase in the survival of immature oligodendrocytes and a potentiation of the mitogenic effect of other growth factors.
factors, such as PDGF (66, 67). In vivo, delivery of antibodies to NT-3 into the optic nerve decreased the proliferation of OPs and the nerves were decreased in diameter (66). NT-3 null mice and TrkC null mice have 30% and 15% fewer PDGFRα+ cells, respectively (68). Thus, neurotrophins appear to play a supportive but not essential role in oligodendrocyte development.

Ciliary Neurotrophic Factor and Leukemia Inhibitory Factor

Ciliary neurotrophic factor and leukemia inhibitory factor both appear to enhance the differentiation of OPs. In the presence of the appropriate extracellular matrix, cells treated with either protein will express GFAP and resemble type 2 astrocytes (69, 70). Without the matrix, both proteins enhance oligodendrocyte survival, especially in the presence of other survival factors (70, 71). In transgenic mice lacking ciliary neurotrophic factor, oligodendrocyte production is retarded by myelination but is ultimately normal (72).

Bone Morphogenetic Protein

Bone morphogenetic protein (BMP) treatment of oligodendrocyte precursors or pre-progenitors inhibits oligodendrocyte differentiation and pushes OPs into the astrocyte lineage with the actual phenotype depending on the stage of the oligodendrocyte lineage at treatment (15, 73). BMP 1A and 1B receptors are present on OPs (73). In vivo, the effects of BMPs are unclear. During the development of the CNS, BMP is present in areas of the CNS that do not give rise to oligodendrocytes, such as the dorsal spinal cord. Factors present in the dorsal spinal cord appear to inhibit oligodendrogliogenesis, whether these factors include BMP is the subject of some debate (74).

Other Factors Affecting Oligodendrocyte Development

Oligodendrocyte proliferation and migration can be potentiated by cofactors that work with other growth factors, such as PDGF, to enhance the mitogenic response. One of these factors is the chemokine GRO-α, which is not mitogenic by itself but potentiates the effect of PDGF in spinal cord oligodendrocytes (75). GRO-α may be secreted by astrocytes in vivo.

Extracellular matrix molecules are also known to potentiate proliferation and migration of oligodendrocyte precursors. As oligodendrocytes mature, they express both α and β subunits of integrin cell surface receptors. Extracellular matrix molecules, such as tenascin-C, may interact with these receptors and affect migration and proliferation, as demonstrated both in in vitro assays and in knockout models (76, 77).

Mechanisms of Oligodendrocyte Differentiation

One of the remaining mysteries of oligodendrocyte development is the process by which the cell is able to stop dividing and begin to express myelin proteins. The number of times an OP divides before differentiating has been hypothesized to be controlled by a "biological clock" that counts cell divisions before differentiation (5). Evidence for this includes the observation that 2 daughter cells cultured separately will undergo the same number of cell divisions (usually between 0 and 8) before differentiating (5). The molecular basis of this clock has been investigated using inhibitors of cyclin-dependent kinases, which are essential elements of the mitotic cycle. Inhibition by p27 and p27Kip1 negatively modulates the ability of cyclins to push the cells into S-phase, eventually leading to cell cycle arrest. Both p27 and p27Kip1 accumulate in the OP nucleus with each cell cycle (78). Overexpression of p27 inhibits mitosis but does not lead to increased numbers of immature or mature oligodendrocytes (79). In addition, p27Kip1 null mice have increased numbers of OPs but do not have increased numbers of oligodendrocytes (80). This suggests differentiation in the oligodendrocyte is a 2-step process: one involving cell cycle arrest and the other differentiation.

But what signals an OP to differentiate if this is indeed separate from cessation of division? There are currently a number of candidates. One factor that plays a role in brain development and is clearly involved in oligodendrocyte differentiation is thyroid hormone. Thyroid hormone has stage-specific effects in the oligodendrocyte lineage. This may be mediated through 1 of 2 thyroid hormone receptors. The α form is expressed early in brain development, whereas the β form is upregulated around the time of birth, when myelination is beginning in earnest (81). Early on, thyroid hormone aids in proliferation and survival of oligodendrocyte pre-progenitors (16). At later time points, thyroid hormone promotes oligodendrocyte differentiation, although it is not absolutely required (82). Instead, thyroid hormone is thought to be needed to generate appropriate numbers of oligodendrocytes and appears to be involved in the timing of differentiation (83). A lack of thyroid hormone generates fewer oligodendrocytes in vivo. The decrease but not total absence of oligodendrocytes was demonstrated both in hypothyroid animals as well as in a transgenic paradigm in which both maternal and fetal thyroid hormone was missing following gancyclovir treatment of transgenic mice expressing the thymidine kinase gene in thymocytes (82, 84).

Recently, the Id family of helix-loop-helix proteins has been implicated in oligodendrocyte differentiation. The 4 members of this family are known to regulate differentiation in a variety of cell types. The expression of Ids decreases as OPs begin to mature and overexpression of Ids in culture can lead to inhibition of differentiation (85, 86). There is disagreement as to which of the 4 Id proteins is responsible for these effects in the oligodendrocyte lineage. This is an area of active investigation and...
the elucidation of which Ids modulate oligodendrocyte differentiation is likely forthcoming.

The notch signaling pathway may also play a role in the control of the timing or amount of differentiation. Notch1 receptor is expressed by both oligodendrocytes and OPs. In vitro, the notch ligands, Jagged and Delta, can inhibit oligodendrocyte differentiation (87). In vivo, Jagged is expressed in the optic nerve by retinal ganglion cells and the expression is down-regulated as myelination proceeds (87), consistent with the differentiation of OPs. Jagged is also expressed by mature oligodendrocytes (87) and its presence could signal to Notch1 on some OPs, forcing them to remain immature as a source for OPs in the adult.

Regulation of Oligodendrocyte Specification

Research on the development of oligodendrocytes began with the phenotypic characterization of the different cell types and then moved to extrinsic molecules that modulated the developmental processes such as proliferation, migration, differentiation, and survival. Current research efforts are unraveling the intrinsic signals that drive cells of the oligodendrocyte lineage cell through these processes. Several of these factors have been identified during studies to determine the temporal, spatial and cell-type origin of oligodendrocytes. These studies have largely been performed in the ventricular zone of the ventral spinal cord, and have been aided by the relatively simple architecture and a plethora of previous studies describing the regionalization of the developing ventral spinal cord by specific transcription factors.

Oligodendrocyte progenitors in both the spinal cord and brainstem arise in a subdomain of the ventral ventricular zone adjacent to the floor plate (27). This suggested the presence of a particular signal in the region. Given the role of sonic hedgehog (shh), the vertebrate homolog of the Drosophila segment polarity gene hedgehog, in the establishment of the ventral ventricular zone, it seemed likely that this secreted signal would also participate in oligodendrocyte development. Several studies have shown that Shh can induce neuroepithelial cells to become oligodendrocytes. Dorsal or intermediate spinal cord explants that do not themselves give rise to oligodendrocytes will do so under the influence of Shh (88). Likewise, antibodies to Shh will inhibit the generation of oligodendrocytes in ventral cultures (89).

How does Shh signaling establish the appropriate environment for oligodendrocyte development? Shh establishes distinct progenitor domains by a dose dependent mechanism (Fig. 2). Shh is derived from the notochord and floorplate; thus the most ventral neural tube receives the highest concentration and as one proceeds dorsally, lower levels of Shh are received. The result of Shh expression is the induction of specific genes and the repression of others. PDGFRα+ oligodendrocyte precursors arise from a domain which first generates motor neurons and is located on the border of the areas defined by the expression of the transcription factors Olig1, Olig2, and Nkx2.2. In separate studies, both Olig1 and Olig2, basic helix-loop-helix transcription factors, were found to label cells that become oligodendrocyte precursors several days before PDGFRα expression is apparent (90). In addition, many oligodendrocyte precursors express Nkx2.2. Recent studies employing misexpression of Olig2 and/or Nkx2.2 by electroporation of retroviral vectors have shown that expression of Olig2 can induce early oligodendrocyte precursor markers such as PDGFRα, but not later markers such as MBP (91). Nkx2.2 expression appears to be necessary for differentiation from the precursor stage to the mature oligodendrocyte. Nkx2.2 null mice have severely retarded expression of myelin markers such as MBP and PLP. Since the animals die at 8 days postnatal, further development could not be assessed (92). This subject will require further detailed studies to understand the exact role of these factors in the development of oligodendrocytes and their relationship to Shh. However, it appears that specification of
oligodendrocytes in the ventral spinal cord proceeds through the activation of these transcription factors in a dose-dependent manner by Shh. Although most studies have concentrated on the spinal cord, there is evidence for a common mechanism in the forebrain and it seems that at least some oligodendrocytes arise through an Shh-dependent mechanism (93).

Several other transcription factors have been identified in cells along the oligodendrocyte lineage. One of these, Sox 10, is expressed almost exclusively within oligodendrocyte lineage cells in the CNS and appears earlier than other markers, such as PDGFRα and DM-20, but after the expression of Olig1 and Olig2 (94). Its expression remains on in mature cells. Although Sox-10-deficient mice have a severe Schwann cell defect, the generation of oligodendrocytes from Sox-10 null mice appear normal, highlighting the observation that transcription factors in central and peripheral glia can have separate functions. It is possible that other Sox proteins, such as Sox 4 or Sox 11, are redundant for Sox 10 in oligodendrocyte progenitors. Tst-1/Oct6/SCIP is a member of the POU family of homeodomain proteins and is important for Schwann cell development (95). Although SCIP is transiently present in the oligodendrocyte lineage (96), no defects have been detected in SCIP-deficient mice or oligodendrocyte cultures derived from them. Unlike Sox 10 and SCIP, GTX (also known as Nkx6.2) is only expressed in mature oligodendrocytes (97). Since this expression occurs just before expression of the myelin genes MBP and PLP, it was thought that GTX might be involved in activating myelin gene expression (97). This has not yet been confirmed and the exact role of GTX is still unknown. MyT1, a zinc finger transcription factor that binds to an important region in the PLP gene promoter, is also present in oligodendrocyte progenitors (98) and may be involved in myelin gene expression. Additional transcription factors and their roles in oligodendrocyte development are likely to be revealed in the future.

Unanswered Questions and Future Directions

The basic processes involved in the generation of mature oligodendrocytes have been delineated along with a large amount of information on factors which modulate this development and the phenotypic changes the neural cell precursors undergoes as it develops. However, how these processes interact to form a mature oligodendrocyte is still largely unknown. For example, how can progenitor cells generate mature post-mitotic oligodendrocytes at the same time dividing and maintaining an undifferentiated pool of cells? What is the significance of the different types of precursor cells, all of which generate oligodendrocytes with the same general functions? How does programmed cell death really serve to limit the number of myelinating oligodendrocytes given the large number of survival factors present during oligodendrocyte development? These questions are complex and will require in vivo approaches. They will also require an understanding of how neurons and oligodendrocytes interact during development to generate an intact CNS. The answers to these questions may help us better understand the role of the oligodendrocyte in a variety of neuropathological diseases. In addition, understanding the pathways an oligodendrocyte precursor takes to maturity and also the environment necessary to promote this development may enable us to generate functioning oligodendrocytes for repair of these conditions by regeneration of native cells or by transplantation paradigms.

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