Oligodendrocyte Myelination in the Mammalian CNS

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ABSTRACT

Oligodendrocyte myelination is essential for the proper function of the mammalian central nervous system. The generation of myelinating oligodendrocytes is regulated by complex but coordinated signals during CNS development. Recent discoveries of critical transcriptional regulators for oligodendrocyte differentiation and axonal signals for myelin sheath production have significantly advanced our understanding of the molecular mechanisms governing oligodendrocyte myelogenesis. This review highlights current perspectives on the origin of oligodendrocytes and the intrinsic and extrinsic regulation of oligodendrocyte differentiation and myelogenesis. Potential implications of myelin in health and disease are also explored.

Keywords: epigenetic, glial, neuregulin, Olig1, Olig2, origin, Sox, transcriptional regulation

Abbreviations: AEP, anterior entopeduncular area; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; CNS, central nervous system; FGF, fibroblast growth factor; GRP, glial restricted precursors; LGE, lateral ganglion eminence; LIF, leukemia inhibitory factor; MBP, myelin basic protein; MGE, medial ganglion eminence; NGF, nerve growth factor; NRG, neuregulin; NT3, neurotrophin-3; PDGF, platelet-derived growth factor; PDGFRα, platelet-derived growth factor receptor alpha; PLP, proteolipid protein; PSA-NCAM, polysialic acid-neural cell adhesion molecule; Shh, sonic hedgehog; SVZ, subventricular zone; TSA, Trichostatin A

INTRODUCTION

Myelination is the evolutionary conserved process by which myelin sheath spirally wraps around axons to insulate them (Rainé 1984). Axonal insulation by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) enhances saltatory conduction, which is essential for the normal function of the vertebrate nervous system, particularly for motor and cognitive functions. Myelination between exposed nodes of Ranvier allows action potentials to propagate efficiently and maximizes axonal conduction velocity in a spatially-compact manner. Although a few invertebrates possess a myelin-like structure, axonal ensheathment by compact myelin is a unique feature of the vertebrate nervous system (Zalc and Colman 2000). Various pathological insults and nerve injuries may damage CNS myelin and therefore disrupt normal transmission of nerve impulses, eventually leading to myelin-related disorders such as multiple sclerosis and the leukodystrophies (Berger et al. 2001; Trapp et al. 1998). At present, the mechanisms underlying myelinating disease formation and myelin repair are poorly understood.

Oligodendrocytes in rodents are generated during em-
bryonic stages and become mature perinatally after immature oligodendrocytes extend their processes for axonal ensheathment (Raine 1984). In most vertebrates including humans, the process of myelination continues postnatally and into adulthood. It includes process extension, axonal recognition, myelin synthesis, and ensheathment. Recent discoveries of a series of critical transcriptional regulators and extracellular signaling molecules for oligodendrocyte development underscore the complexity of the myelination process, which is controlled by the coordinated action of multiple factors in a spatially and temporally specific manner (Miller 2002; Wegner 2001). In this review we will discuss various aspects of oligodendrocyte specification and maturation, as well as their function in CNS development and disease pathogenesis.

**ORIGINS OF OLIGODENDROCYTES DURING CNS DEVELOPMENT**

**Oligodendrocyte specification in the developing spinal cord**

Oligodendrocyte precursor cells (OPCs) originate from a specialized ventral domain, called the pMN, in the developing spinal cord at an early embryonic stage around embryonic day 12.5 (e12.5) (Miller and Reynolds 2004; Richardson et al. 2000). OPCs generated in the ventral germinal zone migrate laterally and dorsally to populate the entire spinal cord before terminally differentiating into mature myelin-forming oligodendrocytes (Fig. 1A). The expression of PDGFRα is recognized as an indicator of the initial population of OPCs (Pringle and Richardson 1993). In addition to PDGFRα, several transcriptional regulators that specify OPC formation have been used to mark the initial appearance of OPCs (Miller and Reynolds 2004; Richardson et al. 2000). bHLH transcriptional factors Olig1 and Olig2 define the earliest domains of the oligodendrocyte lineage prior to PDGFRα in the developing neural tube (Lu et al. 2000; Takebuyashi et al. 2000; Zhou et al. 2000).

Progenitor cells deriving from the dorsal half of the spinal cord also produce oligodendrocytes during late embryonic stages. OPCs can be detected in Nkx6.1 and Nkx6.2 double mutants lacking the ventral source of oligodendrocytes (Cai et al. 2005; Vallstedt et al. 2005). Fate-mapping of the progeny of dorsally expressing Cre lines (e.g. Dbx2-Cre and Mash1-Cre) suggests that a subset of oligodendrocytes are derived from radial glia in the dorsal spinal cord during late embryogenesis (Battiste et al. 2007; Fogarty et al. 2005). Therefore, dorsal progenitor cells of the spinal cord can generate OPCs in addition to ventral progenitors (Fig. 1B).

**Oligodendrocyte specification in the developing brain**

In a similar fashion to the spinal cord, OPCs in the anterior part of the CNS forebrain initiate from the ventral region of the telencephalon including the medial ganglionic eminence (MGE) and lateral ganglionic eminence (LGE) begin around e14.5 (Woodruff et al. 2001). OPC populations in the thalamus, hypothalamus and developing cerebellum appear to be generated from the ventricular zone of the ventral diencephalon such as anterior entopeduncular area, AEP (Pringle and Richardson 1993; He et al. 2001; Richardson et al. 2006) (Fig. 1C).

Recent studies suggest that forebrain OPCs develop in multiple sequential waves (Kessaris et al. 2006). The first wave of OPCs appears to derive from the ventral domain of the MGE that populates the ventral forebrain. The second wave of OPCs emanates from the dorsal regions of GE (LGE), while the third wave occurs after e18 and is of cortical progenitor origin (Kessaris et al. 2006). In general, oligodendrocytes are progressively generated from ventral to dorsal and from caudal to rostral regions. OPCs arise from dorsal cortical progenitor cells during late embryonic stages and appear to play a major role in myelination of the cortex. Hence, postnatal ablation of an essential oligodendrocyte specification gene Olig2 leads to profound deficits in cortical myelination (Yue et al. 2006); Cai et al. 2007). A population of OPCs generated during earlier waves appears to undergo cell death (Kessaris et al. 2006). This might reflect some local advantages of cortically-derived OPCs in terms of cortical myelination over the ventral OPCs generated during earlier embryonic stages (Kessaris et al. 2007). However, the ventrally-derived oligodendrocytes were found to progressively populate the subcortical white matter in the Olig2 mutant with myelination deficits (Yue et al. 2006), suggesting that ventrally derived oligodendrocytes can contribute to cortical myelination when myelination is limited.

**Fig. 1 Multiple developmental sources of oligodendrocytes.** (A-B) Cross-sections of the spinal cord show ventral and dorsal OPC sources at early (A, e.g. e12.5) and late (B, e.g. e16.5) embryonic stages along with their migration pathways. The oligodendroglial fate of dorsal and ventral progenitors are regulated by a gradient of morphogenetic factors BMP and Shh, respectively. (C) The diagram depicts the regions of OPC production in a coronal section of the developing telencephalon. Arrows show potential OPC migration routes.
Oligodendrocytes in the adult brain

Oligodendrocyte myelination appears to be a continuous event during development and in adulthood (Kaplan and Hinds 1980; McCarthy and Leblond 1988; Yates and Juraska 2007). In the adult forebrain, stem cells from the subventricular zone (SVZ) are a source for new oligodendrocyte generation (Levison and Goldman 1993; Luskin and McDermott 1994; Menn et al. 2006). These cells may potentially participate in myelin repair (Gensert and Goldman 1997; Blakemore and Kearsted 1999; Franklin 2002). It is worth mentioning that OPCs are present in the corpus callosum and other white matter regions and undergo cell division during normal and injured conditions. At present, the extent to which mobilization of adult SVZ stem cells or local endogenous OPCs contributes to myelin repair after injury remains unresolved.

Oligodendrocyte lineage development

The lineage relationship between oligodendrocytes and other neural subtypes has been the subject of intensive study and debate. Although oligodendrocytes and astrocytes have been believed to derive from a common glial progenitor, a fate-mapping study with an Olig1-Cre line indicates that Olig1+ cells are expressed in specific progenitor cell pools that give rise to oligodendrocytes and motor neurons in the developing spinal cord (Lu et al. 2002). Whether motoneurons and oligodendrocytes derive from shared lineage progenitor cells or from separate progenitor cell pools remains a matter of debate (Richardson et al. 2000). However, the current consensus is that Olig1+ cells give rise to motoneuron precursors and OPCs sequentially at different developmental stages (Kessaris et al. 2001; Richardson et al. 1997) (Fig. 2A). This is consistent with a recent lineage tracing study of Ascl1+ (also previously known as Mash1) cells at different developmental time points, revealing that Mash1+ cells generate dorsal horn interneurons at early embryonic stages and subsequently late-born oligodendrocytes (Battiste et al. 2007).

Similarly, Petryniak and colleagues show that Dlx1/2 homeodomain transcription factors act on a common progenitor to determine neuronal versus oligodendroglial cell fate acquisition in the ventral embryonic forebrain (Petryniak et al. 2007). In addition, studies on the origin of oligodendrocytes in the brain indicate that SVZ type B cells can either generate oligodendrocytes alone or oligodendrocytes and neurons (Menn et al. 2006). These observations have led to a paradigm shift in our understanding of oligodendrogenesis. During development, oligodendrocytes may not be derived exclusively from glial restricted progenitors (GRP) as previously thought, they can be also generated from a lineage-restricted progenitor cell pool that gives rise to a subset of early-born neurons in the CNS (Richardson et al. 1997; Kessaris et al. 2001; Noble et al. 2004) (Fig. 2B).

Recently, Wu et al. found that ablation of Olig1-expressing cells results in the elimination of essentially all motoneurons and oligodendrocytes including PDGFRα+ OPCs and differentiated oligodendrocytes (Wu et al. 2006). In the Olig1+ cells-ablated spinal cord, they observed a population of late-appearing Olig2+ cells formed outside of the pMN domain such as in the ventricular zone adjacent to the floor plate and the dorsal region. Based on the assumption that Olig2+ expression represents oligodendrocyte progenitors, they concluded that oligodendrocyte precursors can be generated even after all motoneuron precursors have been eliminated and that motoneuron and oligodendrocytes do not share a common lineage-restricted progenitor. However, several lines of evidence indicate that Olig2+ cells may also identify other cell lineages including astrocytes (Masahira et al. 2006; Cai et al. 2007). It remains unclear whether Olig2+ cells formed after motoneuron elimination strictly represent oligodendrocyte precursors, particularly since there is a complete absence of PDGFRα+ OPCs. An alternative possibility is that these late-born Olig2+ cells may represent a population of astrocytes given that astrocytes were largely spared in their ablation study (Wu et al. 2006). Nonetheless, the loss of both motoneurons and oligodendrocytes in the Olig-_ablated spinal cord is consistent with the derivation of both cell lineages from Olig+ progenitor cell pools.

INTRINSIC CONTROL OF OLIGODENDROCYTE MATURATION

Myelination in the CNS is a complex process, which is regulated not only by various extracellular signals but also by a network of intrinsic factors. The ability of OPCs to differentiate and synthesize myelin proteins is controlled by intracellular molecules via transcriptional and post-transcriptional mechanisms. Recent studies indicate that a network of DNA-binding transcription factors including the basic helix-loop-helix (bHLH) proteins, homeodomain proteins, HMG-domain proteins and zinc finger proteins play a

Fig. 2 Oligodendrocyte lineage development. Schematic diagram depicts two models for oligodendrocyte lineage development in the CNS. (A) Sequential model. In the developing ventral spinal cord, it is generally believed that Olig+ cells give rise to motoneuron (MN) precursors and OPCs sequentially at different developmental stages. (B) Lineage restricted progenitor model. Oligodendrocytes can be derived directly from NSCs or from lineage restricted progenitors such as intermediate progenitors for neurons and oligodendrocytes or glial restricted progenitor cells (GRP or pO/A). Although lineage restricted neuronal progenitors (pN) have been identified, at present, those lineage restricted progenitors such as pN/O or pO/A (GRP) progenitors are not yet defined in vivo. NSC, neural stem cells; N, neurons; O, oligodendrocytes, A, astrocytes.
Intrinsic factors

- Olig2
- Olig1
- Mash1
- Sox10
- Nkx2.2
- Zfp488

Extrinsic factors

proliferation

- Shh/PDGF/NT3/bFGF/Nrg/IGF

maturation

- Nrg/CNTF/LIF/Thyroid hormone

Embryonic stage

Postnatal stage

**Fig. 3** Intrinsic and extrinsic regulation of the oligodendrocyte maturation process. Schematic diagram depicts the major stages of oligodendrocyte development. Oligodendrocyte maturation involves the progression of cell lineage development as, in a sequential order, OPCs, immature differentiated OLs (dOL), premyelinating OLs (pre-mOL), and mature myelinating OLs (mOL). Several intrinsic transcription factors and extrinsic factors known to regulate oligodendrocyte development in a stage-specific manner (solid lines) are indicated. Oligodendrocyte precursors proliferate at embryonic stages whereas their maturation occurs mainly at postnatal stages and is accompanied by increased myelin component expression followed by myelin sheath assembly.

**Intrinsic control of oligodendrocyte myelination** with an emphasis on transcriptional regulation (Wegner 2001). In this section we will summarize the crucial role in the establishment of myelination in the CNS (Wegner 2001). The function of Olig1/2 in postmitotic OPC maturation remains elusive. Although Olig2 null mutation leads to a complete loss of oligodendrocytes in the developing spinal cord, Olig1 mutation causes a delay in oligodendrocyte maturation. In a recent study, Olig1 mutants produced by the removal of the neomycin selection cassette from the Olig1 targeting locus exhibit severe myelination deficits during postnatal CNS development (Xin et al. 2005). OPCs in Olig1 mutants can develop and migrate throughout the brain and extend their processes to contact axons, but they fail to assemble myelin sheaths around the axon. Thus, the process of myelination in the mutants is arrested prior to the onset of myelin specific gene expression. Furthermore, these data also indicate that axonal recognition and myelination by oligodendrocytes are distinct processes (Xin et al. 2005) (Fig. 3).

Another recent study has indicated that remyelination does not occur in Olig1 mutant adult mice with experimentally induced focal demyelination (Arnett et al. 2004) and highlights the importance of Olig1 during episodes of remyelination. Discrepancies in different loss-of-function studies may be due to different genetic backgrounds and aberrant transcription of neighboring genes of the Olig1 locus (Balabanov and Popko 2005; Xin et al. 2005). The presence of a strong transcriptional enhancer for PGK (phosphoglycerate kinase) in the neomycin selection cassette in the Olig1 targeting locus may influence expression of nearby genes such as the Olig1 homolog, Olig2. In spite of this, all data support the importance of Olig1 in oligodendrocyte myelination (Balabanov and Popko 2005). Moreover, coding variants of OLG1 in demyelinating diseases such as multiple sclerosis were recently identified although their link to the disease susceptibility remains to be established (Goris et al. 2006).

Olig1/2 are expressed in both OPCs and myelinating oligodendrocytes (Lu et al. 2000; Zhou et al. 2000). While their role in oligodendrocyte specification has been elucidated, the function of Olig1/2 in postmitotic OPC maturation remains elusive. Although Olig2 null mutation leads to a complete loss of oligodendrocytes in the developing spinal cord, Olig1 mutation causes a delay in oligodendrocyte maturation. In a recent study, Olig1 mutants produced by the removal of the neomycin selection cassette from the Olig1 targeting locus exhibit severe myelination deficits during postnatal CNS development (Xin et al. 2005). OPCs in Olig1 mutants can develop and migrate throughout the brain and extend their processes to contact axons, but they fail to assemble myelin sheaths around the axon. Thus, the process of myelination in the mutants is arrested prior to the onset of myelin specific gene expression. Furthermore, these data also indicate that axonal recognition and myelination by oligodendrocytes are distinct processes (Xin et al. 2005) (Fig. 3).

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In Mash1 null embryos, the formation of a subset of OPC populations is significantly reduced between E11.5 and E13.5, however, OPCs derived from Mash-negative progenitors are produced to compensate for early deficits in the developing brain and spinal cord (Parras et al. 2004, 2007).

Although OPCs are derived from both Mash1-dependent and -independent progenitors in the developing CNS, it is not clear whether Mash1 expression distinguishes distinct oligodendrocyte subtypes. In addition, Mash1 expression is maintained in brain and optic nerve OPCs at postnatal stages and adulthood (Wang et al. 1998; Kondo and Raff 2000; Parras et al. 2004), however, at present, the precise function of Mash1 in oligodendrocyte maturation and myelination is still unknown. Addressing this issue may require the analysis of mice with a conditional ablation of Mash1 at postnatal stages.

A series of inhibitory HLH factors (i.e. Id2 and Id4) with DNA-binding basic domain are found to negatively regulate oligodendrocyte differentiation. Over-expression of Id2 and Id4 in oligodendrocyte progenitors promotes differentiation and consequently decreases myelin gene expression (Kondo and Raff 2000; Gokhan et al. 2005). Id2/4 downregulation and their extra-nuclear translocation coincide with the onset of oligodendrocyte differentiation (Kondo and Raff 2000; Wang et al. 2001). In addition, activation of the Notch pathway by Jagged1 blocks differentiation and maturation of oligodendrocyte progenitors. This inhibition is likely mediated through the induction of inhibitory bHLH Hes5 by Notch signaling (Wang et al. 1998). Collectively, these data suggest that negative HLH factors are part of a network of the intracellular regulators that control the timing of oligodendrocyte precursor differentiation.

HMG domain containing Sox gene family

The Sox family of proteins are transcription factors with a high-mobility-group (HMG) domain, which play an important role during oligodendrocyte development. They can be grouped into ten major groups (A-J) based on their sequence similarity of the HMG domain (Bowles et al. 2000; Wegner 2001). In particular, all members of group E proteins such as Sox 8, Sox9 and Sox10 are known to be expressed in the oligodendrocyte lineage (Stolt et al. 2005).

In Sox10 mutant mice, OPCs can be produced but fail to mature into myelinating oligodendrocytes in the developing neural tube (Stolt et al. 2002). Moreover, overexpression of Sox10 promotes expression of myelin genes, such as Mbp (Stolt et al. 2002). Although Sox8 is co-expressed with Sox10 in developing oligodendrocytes, targeted replacement of Sox10 by Sox8 cannot effectively reverse the deficits of terminal oligodendrocyte differentiation in Sox10-null spinal cords, suggesting a functional disparity between Sox10 and Sox8 in oligodendrocyte maturation (Kellerer et al. 2006).

Another class E member, Sox9 is initially expressed in the ventral neural progenitors of the neural tube and is subsequently restricted to glial cells of the CNS (Stolt et al. 2003). Sox 9, precedes Sox10 and Sox8, is expressed in the early phase of oligodendrocyte-lineage formation, however, it is downregulated in differentiated oligodendrocytes. Targeted mutation of Sox9 indicates that Sox9 is crucial for the proper development of both oligodendrocytes and astrocytes (Stolt et al. 2003). Sox9 may functionally compensate for the absence of Sox10 in order to maintain OPC generation. In the developing spinal cord of Sox9 null mice, the reduction of gliogenesis is accompanied by the increased MN and V2 interneuron production in the ventral ventricular zone (Stolt et al. 2003). This suggests that Sox9 is another major molecular component that controls the fate switch from neurogenesis to gliogenesis in the developing spinal cord.

Group D Sox proteins (Sox D; Sox 5 and Sox 6) were found to compete against group E Sox proteins, Sox9 and Sox10, for DNA binding sites. Even though they are expressed in oligodendrocyte lineage cells, Sox5 and Sox6 negatively regulate oligodendrocyte specification, differentiation, and migration. Sox9D proteins may modulate the activity of Sox E proteins for appropriate timing and progression of OPC differentiation at different stages of oligodendrocyte development (Stolt et al. 2006). The fact that these Sox protein groups interact with each other suggests that a complex regulatory network controls and defines oligodendrocyte fate specification and differentiation.

Homeodomain protein family

The homeodomain transcription factor family plays critical roles in neural fate specification including oligodendrocyte differentiation. Nkx2.2, Nkx6.1, and Nkx6.2 (also known as Gtx2) are homeodomain transcription factors that are expressed in the oligodendrocyte lineage. Although initial expression of Nkx2.2 is restricted to the P3 domain of the ventral ventricular zone adjacent to the floor plate in the developing chick neural tube, it appears subsequently to expand to the prospective oligodendrocyte domain (Kondo et al. 2001). In cooperation with Olig2, Nkx2.2 promotes oligodendrocyte differentiation (Zhou et al. 2001). Although Nkx2.2 is dispensable for OPC formation, it is critical for oligodendrocyte differentiation and maturation (Qi et al. 2001).

Nkx6.1 and Nkx6.2 appear to act upstream of Olig2 and regulate its expression in a dose-dependent manner (Vallstedt et al. 2005). Olig2 expression is delayed and diminished greatly in the developing spinal cord of Nkx6.1 mutant mice. In Nkx6.1/Nkx6.2 double null mutants, the specification of oligodendrocyte precursors and MNs cannot be initiated due to the lack of Olig2 (Cai et al. 2005; Vallstedt et al. 2005). However, in the dorsal region of the spinal cord, oligodendrocyte formation is largely unaffected in the double mutants. This suggests that oligodendrocyte specification from dorsal and ventral precursors is mediated by independent mechanisms. At present, it is not clear whether oligodendrogenesis defects observed in Nkx6 mutants are due to a patterning defect in the ventral spinal cord or to the specific role of Nkx6 in oligodendrocyte lineage development. In contrast to Nkx6.1, Nkx6.2 expression is more restricted to myelinating oligodendrocytes. Loss of function studies indicate that Nkx6.2 regulates axon-glial interactions needed for proper myelin paranode formation (Southwood et al. 2004).

Zinc finger protein family

Members of zinc finger superfamily have been shown to play an important role in oligodendrocyte differentiation. Among them, Myt1 is cloned by virtue of its binding to cis-regulatory elements of the myelin proteolipid protein (Plp) gene, the most abundantly transcribed CNS myelin gene (Kim and Hudson 1992). Myt1 is a zinc-dependent and DNA-binding protein of the unusual Cys-Cys-His-Cys (C2HC) class. Although it is named Myt1 (myelin transcription factor I), this protein is widely expressed in the developing nervous system (Kim et al. 1997). Myt1 is preferentially expressed in the progenitors of oligodendrocytes. However, the role of Myt1 in oligodendrocyte differentiation remains unclear. Retroviral-mediated expression of a dominant negative form of Myt1 containing four zinc-finger domains inhibits oligodendrocyte differentiation in vitro, although over-expression of intact Myt1 partially suppresses OPC differentiation. These data indicate that Myt1 maintains oligodendrocytes as immature progenitor pools and modulates their transition from progenitors to terminally-differentiated oligodendrocytes (Nielsen et al. 2004).

In addition, a ubiquitously expressed Zn-finger transcription factor Yin Yang 1 (YY1) appears to bind and en-
hance transcription of the Plp promoter (Berndt et al. 2001). By searching for the promoters of genes regulated by HDACs during oligodendrocyte differentiation, He and colleagues identified the consensus DNA binding sequence of YY1 as a common binding motif (He et al. 2007). By specifically ablating YY1 in oligodendrocyte lineage cells, the authors indicated that YY1 is essential for oligodendrocyte progenitor cell differentiation. In addition, YY1 appears to repress optic nerve myelin gene expression (Tcf4 and Id4) by recruiting histone deacetylase-1 (HDAC1) to their promoters during oligodendrocyte differentiation (He et al. 2007).

In a screen for genes down-regulated in the optic nerves of 14-day old Olig1 null mice, Zip488, a previously uncharacterized nuclear zinc-finger transcriptional regulator, was recently identified (Wang et al. 2006). In the developing CNS, Zip488 is restricted to differentiated oligodendrocytes. Its expression increases in parallel with that of major myelin genes such as Mbp and Plp. Furthermore, Zip488 is absent in the spinal cord or the brain of Olig1 null animals, indicating that Zip488 is a direct or indirect downstream target gene for Olig1. Interestingly, Zip488 is a nuclear protein possessing transcriptional repression activity (Wang et al. 2006). In the developing chick neural tube, Zip488 promotes OPC progenitor formation upon Notch activation. In addition, the zinc finger protein Zip488 coregulates with the bHLH transcription factor Olig2 to promote OPC maturation (Wang et al. 2006). Several lines of evidence indicate that the interaction between zinc-finger proteins and proneural bHLH proteins such as X-Myt1/promise bHLH factors is a common theme for terminal neuronal differentiation (Bellefroid et al. 1996; Nakakura et al. 2001 Acar et al. 2006). For example, the zinc finger protein X-Myt1 is able to promote ectopic neuronal differentiation only in cooperation with bHLH transcription factors such as X-NGNR-1 (Bellefroid et al. 1996). Consistent with these observations, the same physical and functional interactions of the zinc finger protein (Zip488) with the bHLH factor (Olig2) that promote oligodendroglial maturation may also reflect a similar mechanism for zinc finger/ bHLH cooperation in regulating oligodendroglial differentiation during development (Wang et al. 2006).

Epigenetic control of oligodendrocyte differentiation

Epigenetic modifications at the histone, DNA methylation, chromatin, and miRNA level control the expression of cell type-specific transcription factors and thereby regulate the establishment and maintenance of oligodendrocyte specific differentiation and maturation (Hsieh and Gage 2004; Cheng et al. 2005). Similarly, myelin specific gene expression is tightly controlled by multiple levels of epigenetic modulation of chromatin components.

Modifications of nucleosomal histones may have a direct influence on chromatin conformations and regulate oligodendrocyte differentiation. Histone deacetylases (HDAC), known as chromatin modifiers, appear to be necessary for oligodendrocyte maturation and myelin gene expression in culture (Marin-Husstege et al. 2002) and in developing animals (Shen et al. 2005). Blocking the activity of classes I and II HDACs by HDAC inhibitors (e.g. valproic acid or TSA) inhibits oligodendrocyte myelination (Shen et al. 2005). These pharmacological inhibition studies suggest that the activity of HDACs controls the timing of oligodendrocyte differentiation. While exogenous histone deacetylation activity at early postnatal stages resulted in significant hypomyelination, no effect was observed on myelin gene expression after initiation of myelogenesis. However, it is not clear whether a single or different members of HDACs act in concert to regulate oligodendrocyte differentiation and myelination during CNS development.

Activation of histone acetylation in isolated oligodendrocyte progenitors seems to not only prevent their differentiation, but also revert these OPCs back into a neural stem cell-like state, which is more receptive to neuronal or astroglial differentiation-inducing signals (Liu et al. 2007). Liu et al. further proposed that plasticity of OPCs is regulated, at least partially, by the activity of histone deacetylases and acetylases, given that “memory” of a cellular phenotype requires epigenetic code establishment. However, it is worth noting that the isolated A2B5+ cells may not be confined to a homogeneous population of OPCs. Neural progenitors present within heterogeneous A2B5+ populations may potentially respond to the HDAC inhibitors and differentiate into neurons and astrocytes.

HDAC activity induces the closed conformation of chromatin, which can be further stabilized by methylation on specific lysine residues in the histone tails (Lachner et al. 2001; Nakayama et al. 2001). These modifications may in turn render the DNA inaccessible to specific transcription factors. Apart from histone modification, DNA methylation can be another level of epigenetic regulation that controls oligodendrocyte differentiation. The DNA methylation status of key oligodendrocyte-specific transcription factors such as Sox10 is correlated with reduced expression of myelin genes and oligodendrocyte dysfunction (Iwamoto et al. 2005). However, the precise mechanisms by which specific epigenetic regulators control the development of oligodendrocyte progenitors into functionally myelinating cells remain to be established.

EXTRINSIC CONTROL OF OLIGODENDROCYTE MATURATION

A variety of growth factors and axonal factors have been implicated in proliferation and maturation of OPCs (Miller 2002). For example, growth factors such as Sonic Hedgehog (Shh), BMP, fibroblast growth factors and hormones including thyroid hormones such as triiodothyronine (T3), have primary effects on OPC expansion and differentiation (Barres et al. 1994a; Albert et al. 2001; Younes-Rapozo et al. 2006). There is a growing consensus that oligodendrocyte myelination is regulated by axonal signals, consistent with the fact that oligodendrocytes ensheath axons not dendrites even in culture (Lubetzk et al. 1993). Several lines of evidence indicate that signals from axons regulate OPC development and myelination (Bozzali and Wrabetz 2004; Coman et al. 2005). Myelin gene expression and oligodendrocyte myelination are increased after co-culture with neurons (Macklin et al. 1986; Demerens et al. 1996). Induction of optic nerve OPC formation is dependent on retinal axonal cues (Gao and Miller 2006). Recently, several direct or indirect axonal signals that promote myelination have been identified (Bozzali and Wrabetz 2004). They include neuregulin (Nrg), electrical activity, neurotrophins, extracellular matrix proteins, etc. As discussed below, we focus on the role of axon-glial signaling in CNS myelination (Fig. 3).

Axonal signals in myelination

Neuregulin

An axon-derived molecule, neuregulin plays an important role in myelination of the nervous system. Neuregulin-1 (Nrg1) belongs to a family of membrane associated and secreted factors that have an EGF-like domain at their N-termini. It has three type of isoforms (type I, II and III) derived from alternative splicing. Nrg1 types I and II participate in paracrine signaling, while Nrg type III participates in juxtacrine signaling (Adlkofer and Lai 2000; Falls 2003; Nave and Salzer 2006). Neuregulins activate members of EGF receptor subfamily of protein tyrosine kinases (PTKs) known as ErbB, such as ErbB2, ErbB3 and ErbB4 (Nave and Salzer 2006). Nrg1 signaling plays an essential role in Schwann cell myelination. In Nrg1 or ErbB2 or ErbB4 knockout mice, Schwann cell precursor formation cannot be detected at E10.5 (Meyer and Birchmeier 1995). Mice lacking ErbB3 are completely devoid of precursors and myelinating
Schwann cells (Rietheim et al. 1997). Overexpression of Nrg1 type III in neurons of transgenic mice induces Schwann cell hypermyelination leading to the formation of thicker myelin sheath around the axons (Michailov et al. 2004). In addition, by reducing Nrg1 together with ErbB2/ ErbB3 gene dosages in compound heterozygous mice, Michailov and colleagues suggest that a threshold level of NRG1 type III is essential for the thickness of myelin sheath produced by Schwann cells (Michailov et al. 2004).

Although Nrg1 signaling determines the onset and extent of myelination by Schwann cells in the PNS, it is currently unclear whether or not and to what extent Nrg1 regulates oligodendrocyte myelination in the CNS. Studies on cultured OPCs have shown that Nrg1 has a trophic and mitogenic effect on OPCs but inhibits oligodendrocyte differentiation (Canoll et al. 1996). However, its role in oligodendrocyte differentiation varies considerably between in vitro and in vivo studies. Cells from a spinal cord explant of Nrg1 null animals fail to generate OPCs when cultured at E9.5 (Vartanian et al. 1999). In addition, in a study using spinal cord explants from ErbB2 knockout mice, Park et al. demonstrated that ErbB2-mediated signaling is necessary for the terminal differentiation of OPCs (Park et al. 2001). However, it is not clear the extent to which oligodendrocyte myelination is dependent on Nrg1/erbB signaling in the CNS.

Nrg1 expression is observed in the ventral ventricular zone of the spinal cord and the forebrain (Park et al. 2001). This observation is consistent with the initial sources of oligodendrocyte lineage development. However, it is currently unclear whether or how Nrg1 regulates oligodendrocyte myelination. Recent studies indicate that Bace1 (β-site amyloid precursor protein-cleaving enzyme 1 or β-secretase) regulates myelinogenesis and myelin sheath thickness in the CNS. In Bace1-null mice, there is an accumulation of the unprocessed form of full-length Nrg1 in the absence of β-secretase activity, resulting in a reduction of both myelin gene expression and oligodendrocyte myelination (Hu et al. 2006; Willelm et al. 2006). These observations indicate that the processed form of Nrg1 may function as a ligand to promote axonal ensheathment by oligodendrocytes and Schwann cells (Hu et al. 2006; Willelm et al. 2006). Even though neuregulin signaling between Schwann cells and oligodendrocytes is analogous, the extent to which neuregulin signaling regulates myelination in the CNS and PNS may vary. Further studies with in vivo spatiotemporally specific mutagenesis of Nrg signaling components are required to determine the role of Nrg/ErbB signaling in CNS myelination.

**Neurotrophic factors**

The neurotrophin growth factor family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. They have been shown to play an important role in the growth and survival of specific neuronal populations through their mitogenic and/or differentiation effects. For example, NGF to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC) and pan-neurotrophin receptor p75 (Gentry et al. 2004; Rosenberg et al. 2006). It has been proposed that neurotrophins possess dual roles in neural development. They are thought to promote survival and differentiation of neuronal subsets during early neurogenesis while serving as key regulators in glial differentiation at late developmental stages (Rosenberg et al. 2006).

Recent studies have shown that neurotrophins modulate neuron-glia interactions and control myelination by either acting selectively on neurons, or directly on oligodendrocytes. The effects on oligodendrocyte proliferation and differentiation are diverse among members of the neurotrophin family as they either promote or inhibit oligodendrocyte differentiation. For example, NT-3 appears to act directly to promote the proliferation and survival of oligodendrocytes. In conjunction with PDGF, NT-3 stimulates clonal expansion of oligodendrocyte precursor cells (Barres et al. 1994b; Kumar et al. 1998). Loss of NT-3 and its receptor TrkC results in profound deficits in OPC production (Kahn et al. 1999). Neurotrophins may influence oligodendrocyte development in a regionally specific manner. BDNF appears to promote Mbp expression in oligodendrocytes isolated from basal forebrain, but does not affect cortical oligodendrocytes (Du et al. 2003). The selective expression of neurotrophin receptors and the regional distribution of oligodendrocytes may account for the responsiveness to specific neurotrophin-related signals. This functional heterogeneity suggests that oligodendrocytes may be comprised of heterogeneous populations.

Transplantation of genetically modified fibroblasts that secrete NT-3 and/or BDNF, into spinal cord-injured rats, was shown to increase the production of new oligodendrocytes and myelination by Schwann cells (Michailov et al. 2004).

Electrical activity

Electrical activity has been shown to modulate OPCs development at various stages of development. Inhibition of electrical activity by the sodium channel blocker tetrodotoxin (TTX) prevents the initiation of myelination in vitro and in vivo (Demeren et al. 1996). In addition, induction of repetitive electrical activity by a-scorpion toxin enhances myelination (Demeren et al. 1996), suggesting that neuronal signaling is necessary for oligodendrocyte myelination. Recent work indicates that the electrical activity indirectly upregulates the expression of purinergic receptors on the surface of oligodendrocytes and triggers synaptic release of adenosine (Stevens et al. 2002). Adenosine has been shown to act through the purinergic receptor A2 to promote oligodendrocyte myelin formation by inhibiting OPC migration and proliferation (Stevens et al. 2002). To complicate matters, a recent study has revealed that astrocytes are capable of mediating electrically induced myelination. Ishibashi et al. suggest that ATP released by...
axons during electrical stimulation acts on astrocytes and in turn stimulates the release of LIF, which promotes oligodendrocyte myelination (Stankoff et al. 2002; Ishibashi et al. 2006). This finding suggests that astrocytes may have a novel role in axon-oligodendrocyte interactions and demonstrates that the myelination of postmitotic OPCs is activity-dependent, consistent with the notion that cellular microenvironments play a crucial role in normal oligodendrocyte myelination. At present, the precise roles of astrocytes in modulating oligodendrocyte myelination remain to be clarified.

**Extra cellular matrix proteins**

Extra cellular matrix (ECM) proteins like laminin are known to be expressed in neurons and regulate oligodendrocyte migration and maturation (Buttery and ffrench-Constant 1999; ffrench-Constant and Colognato 2004). Integrins are functionally versatile ECM protein receptors. As transmembrane glycoproteins, they consist of α and β heterodimeric chains and are primarily involved in signal transduction processes between extra-cellular and intra-cellular compartments (ffrench-Constant and Colognato 2004). Oligodendrocytes are known to express a limited repertoire of integrins such as αvβ1 for migration, αvβ3 for proliferation, and αvβ5 for differentiation of OPCs (Buttery and ffrench-Constant 1999; Blaschuk et al. 2000; Colognato et al. 2004; Liang et al. 2004). In addition, myelin proteins such as Plp can form a complex with integrins to modulate receptor signaling and regulate oligodendrocyte myelination (Gudz et al. 2002).

Recent study indicates that laminin-2 potentiates the survival and myelination of newly formed OPCs in response to physiological levels of PDGF or NgR1, while α6-integrin null animals exhibit increased OPC apoptosis and a reduction in the number of mature oligodendrocytes compared to wild type animals (Colognato et al. 2002). These data suggest that signals conveyed by integrin-growth factor interactions are important for oligodendrocyte survival and differentiation. In addition, upon axonal contact, integrins appear to mediate a switch from PI(3)K-dependent to MAPK-dependent survival signaling, therefore promote oligodendrocyte differentiation. Thus, Colognato et al. proposed a model that integrins switch growth factor signaling pathways to promote survival of oligodendrocytes upon axonal contact (Colognato et al. 2002).

Although many axonal signals promote myelination, several axonal cell-adhesion molecules (CAMs) such as PSA-NCAM are inhibitory for myelination (Charles et al. 2000). Removal of PSA-NCAM on axonal surface via antibodies or enzymatic cleavage, increases myelination both in vivo and in vitro (Charles et al. 2000). Similarly, adhesion molecule L1, which is predominantly expressed on the axonal surface (Persohn and Schachner 1987), is drastically reduced during myelination (Coman et al. 2005). Thus, the onset of myelogenesis depends on a balance between positive and negative axonal signals during development.

**MYELINATION IN HEALTH AND DISEASE**

Multiple sclerosis (MS) is the most common neurodegenerative disorder resulting from demyelination in the CNS (Franklin 2002). In addition, recent studies indicate that myelination deficits are also indicated in many neuropsychiatric disorders such as Alzheimer’s disease (AD), mood disorders and Schizophrenia.

Although in Alzheimer’s disease major emphasis has been placed on neuronal death and synaptic impairment induced by β-amyloid peptide (Selkoe and Schenk 2003; Selkoe 2004), substantial white matter damage and its consequences for brain function point to an important role of oligodendrocytes in AD pathogenesis and progression. The observations of oligodendrocytes and myelin impairment in AD are supported by neurochemical, electrophysiological and imaging studies (Malone and Szoke 1985; Roher et al. 2002; Bartozok 2004; de Leeuw et al. 2004).

The presence of Aβ proteins (β-amyloid protein) is observed in the white matter of AD patients (Roher et al. 2002). While Aβ aggregates have been generally considered to be non-toxic and responsible for neuronal dysfunction and neurodegeneration in AD, they are also detrimental to cholesterol rich membranes, such as myelin sheaths (Bartozok 2004). It has been reported that exposure of mature oligodendrocytes to Aβ causes oligodendrocyte death and this cytotoxic effect can be minimized by co-culture with astrocytes or in the presence of anti-inflammatory cytokines (Ramirez et al. 2005; Roth et al. 2005). Aβ senile plaques stimulate production of cytotoxic reactive oxygen species in neurons and this process is further enhanced in the presence of iron released from dying oligodendrocytes (Varadarajan et al. 2000). In general, these studies suggest that oligodendrocyte damage occurs in the AD brain and contributes to AD formation, but it is not clear whether this effect is secondary to disease progression or a primary event occurring at the onset of disease progression.

Since Aβ is primarily responsible for plaque formation in AD, Aβ precursors and revealing their distinct pathways have been highlighted as potential drug targets in the treatment of AD. One of the cleavage enzymes called Bace1 has been considered to be a drug target based on transgenic animal studies (Cai et al. 2001; Luo et al. 2001). However, recent studies indicate that Bace-1 not only targets Aβ proteins, but also is crucial for myelination in both PNS and CNS (Hu et al. 2006; Willem et al. 2006). These studies raise some concerns over the use of Bace-1 as a pharmacological target to treat AD.

Multiple lines of evidence now indicate that oligodendrocyte morphology and myelination are critical for neuronal connectivity, dysfunction of which is the central abnormality of schizophrenia (Davis et al. 2003; Segal et al. 2007; Urano et al. 2007). Disconnection between prefrontal and posterior areas, altered connectivity and synaptic protein expression have been demonstrated in schizophrenia (McGlashan and Hoffman 2000; Winterer et al. 2003; Stephan et al. 2006). There has been a considerable overlap between the cognitive deficits of schizophrenia and demyelinating diseases such as metachromatic leukodystrophy (MLD) and multiple sclerosis (Stevens 1988; Baumann et al. 2002; Haroutunian and Davis 2007; Kumperscak et al. 2007). Post-mortem studies of schizophrenic patients have shown myelin and oligodendroglial abnormalities in the prefrontal cortex and caudate nucleus (Flynn et al. 2003; Uranova et al. 2003). From these immunohistological studies, microarray analysis of prefrontal cortex of schizophrenic patients has identified significant down-regulation of major oligodendrocyte related gene products (Hakak et al. 2001). Interestingly, some of the ultrastructural changes observed in MAG–deficient mice resemble the structural features of schizophrenic brains (Segal et al. 2007; Urano et al. 2007). Furthermore, recent genetic interaction studies have linked Olig2 and its interacting genes such as the neuregulin receptor ErbB4 to schizophrenia susceptibility (Georgeiva et al. 2006). In addition, hypermethylation of the Sox10 promoter, a key regulator for oligodendrocyte differentiation, results in Sox10 downregulation which is correlated with schizophrenia (Iwamoto et al. 2005). Together, these data point to the possibility that abnormal oligodendrocyte function could be a primary etiological factor in schizophrenia.

In addition to the involvement in myelinating diseases and neuropsychiatric disorders, a recent study suggests that oligodendrocytes are also involved in the development of neural plasticity, specifically in the establishment of ocular dominance columns of the visual cortex (McGee et al. 2005). Nogo-66/NgR receptor is required for the myelin derived Nogo, MAG and Ompg signaling, which is responsible for limiting axonal regeneration after pathological insult (McGee et al. 2005). By using Nogo-66 receptor mutant
mice. Macgee et al. studied the experience-driven visual cortex plasticity during and after the critical period. Whereas ocular dominance plasticity in wild type mice is limited after the critical period, Nogo-66 receptor mutant mice exhibit the ability to undergo experience-driven ocular dominance plasticity even after the critical period. This study highlights the importance of the Nogo receptor and its myelin derived ligands in delimiting neural plasticity in the developing visual cortex.

CONCLUDING REMARKS

Recent molecular, genetic, and biochemical studies have significantly advanced our knowledge regarding the mechanisms of oligodendrocyte formation and function. Identification of multiple sources for oligodendrocyte generation can potentially enhance our capacity to promote myelin repair after injury. Great progress has been made in the identification of intracellular molecules, such as transcriptional and epigenetic factors, as well as extracellular molecules such as axonal signals. These intrinsic and extrinsic factors control and modulate different phases of oligodendrocytes development and myelination. Although curative treatments for demyelinating diseases elude our grasp at present, such knowledge has increased our understanding of oligodendrocyte biology and etiology of myelin related diseases. Advances in human and mouse genetics highlight the importance of oligodendrocytes in the regulation of neuronal plasticity and connectivity as well as neurological pathogenesis. Thus, an understanding of oligodendroglial myelination in its entirety will eventually aid us in the identification of potential drug targets for various neurological disorders of the CNS.

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