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Review

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Mechanisms Regulating the Development of Oligodendrocytes and
Central Nervous System Myelin

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Abstract

Oligodendrocytes and the myelin they produce are a remarkable vertebrate specialization that enables rapid and efficient nerve conduction within the central nervous system. The generation of myelin during development involves a finely-tuned pathway of oligodendrocyte precursor specification, proliferation and migration followed by differentiation and the subsequent myelination of appropriate axons. In this review we summarize the molecular mechanisms known to regulate each of these processes, including the extracellular ligands that promote or inhibit development of the oligodendrocyte lineage, the intracellular pathways they signal through and the key transcription factors that mediate their effects. Many of these regulatory mechanisms have recurring roles in regulating several transitions during oligodendrocyte development, highlighting their importance. It is also highly likely that many of these developmental mechanisms will also be involved in myelin repair in human neurological disease.

Introduction

The myelination of axons by glial cells is a remarkably specialized cellular interaction specific to vertebrates. This myelination (performed in the central nervous system by oligodendrocytes) allows for the process of saltatory conduction, in which a neuronal action potential is propagated between nodes of Ranvier to increase both the speed and energy efficiency of nerve conduction. In addition to facilitating saltatory conductance, there is increasing evidence that oligodendrocytes provide trophic support to axons and promote their viability. Although the mechanisms for this are not yet fully clear, they may include metabolic coupling, with oligodendrocytes providing axons with lactate as an energy source (Funfschilling et al., 2012).

One of the most striking aspects of CNS myelination is that it is a late-occurring aspect of neural development and occurs over a prolonged time frame. In humans, the majority of CNS myelination occurs throughout the first two decades of life, with late-maturing brain structures such as the prefrontal cortex myelinating last (Yakovlev and Lecours, 1967, Lebel et al., 2008). In mice, the majority of CNS myelination occurs over the first two postnatal months. In both humans and mice, however, there is increasing evidence that myelination occurs throughout life, either to replace lost oligodendrocytes and myelin or to myelinate previously unmyelinated axons (Bartzokis et al., 2012, Young et al., 2013). Given different parts of the CNS myelinate at different stages of development and most regions contain a mix of myelinated and non-myelinated axons, it is clear that exquisite genetic mechanisms must be in place to control the development of oligodendrocytes and their myelination of individual axons. In addition, it is increasingly becoming clear that there is a level of experience-driven plasticity in the myelination process as well. In this review we discuss some of the molecular and cellular mechanisms that allow for this nuanced process to occur.

1. Specification of the oligodendrocyte lineage during development

During development, oligodendrocyte precursor cells (OPCs) are generated in sequential waves from specific germinal regions. In the developing mouse spinal cord, the first wave of OPC production commences in the ventral neuroepithelium within the progenitor domain that also gives rise to motor neurons (pMN) around embryonic day (E) 12.5 (Pringle et al., 1996, Lu et al., 2000). This is followed by a second wave of OPC genesis emanating from more dorsal progenitor domains (dP3-6) starting at E15.5 (Cai et al., 2005, Fogarty et al., 2005). A third wave begins after birth, however its specific origins—whether from progenitor cells around the central canal, or from proliferative NG2⁺ OPCs—remain unclear (Rowitch and Kriegstein, 2010). In the spinal cord, OPC generation is heavily skewed towards ventral origins, with ventrally derived cells accounting for 85-90% of adult oligodendrocytes, while dorsally derived progenitors only contribute 10-15%.

In the developing forebrain, a similar scenario unfolds. At about E12.5 in mice (and E5-6 in chicks), an initial wave of OPC production commences in the medial ganglionic eminence and closely associated enteropeduncular area of the ventral telencephalon (Olivier et al., 2001, Spassky et al., 2001, Tekki-Kessaris et al., 2001). These ventrally derived OPCs quickly spread to populate most of the developing telencephalon by E18 in mice, and account for all oligodendrocytes in the avian cortex. However, unlike the spinal cord, this first wave is largely replaced by more dorsally generated precursors as development progresses. A second and third wave of OPCs emanating from the lateral and caudal ganglionic eminences at E15.5 and from the cortex after birth, respectively, give rise to the majority of adult oligodendrocytes in mice (Kessaris et al., 2006). One potential reason for this disparity between the spinal cord and forebrain may be that the earlier-generated ventral OPCs in the spinal cord outcompete their dorsal counterparts for proliferation and/or survival signals such as platelet derived growth factor (PDGF) and have more time to establish their hegemony (Calver et al., 1998, van Heyningen et al., 2001). Interestingly, genetic ablation of either ventrally or dorsally derived precursors results in a compensatory expansion of the opposing population to give a normal final complement of oligodendrocytes and myelin, signifying a large degree of redundancy (Kessaris et al., 2006, Richardson et al., 2006). Similarly, the ventrally and dorsally generated precursors show similar electrophysiological properties (Tripathi et al., 2011). Whether more subtle differences exist between the oligodendrocytes derived from each source and the relative contributions of each source to processes such as remyelination remains a topic of ongoing research.

Extracellular Signaling in Oligodendroglial Specification

Extrinsic factors with opposing effects act on multipotential neural progenitor cells (NPCs) to specify the oligodendroglial fate both in ventral-dorsal, as well as in rostral-caudal orientations (Figure 1A). During the early stages of oligodendrocyte specification, these extrinsic factors converge on OLIG1 and OLIG2, two transcriptional regulators of the oligodendrocyte lineage (see below). Ventrally, sonic hedgehog (SHH) is secreted by the floor plate and notochord and binds the Notch-1 receptor on the surface of NPCs, activating its co-receptor Smoothened. This induces the expression of *Nkx6* and *Olig2* in ventral NPCs, triggering the first embryonic wave of OPC specification (Pringle et al., 1996, Orentas et al., 1999). Absence of *Shh* precludes the formation of ventrally-derived OPCs in the forebrain or spinal cord (Orentas et al., 1999, Tekki-Kessaris et al., 2001). Furthermore, grafting ventral SHH-expressing tissue adjacent to dorsal neural tube tissue in chick embryos induces the ectopic formation of OPCs (Orentas and Miller, 1996, Poncet et al., 1996), highlighting the importance of spatially segregated SHH signalling in orchestrating OPC development. The effect of SHH is antagonized by the dorsally expressed Wnt/ β -catenin and bone morphogenic protein (BMP) pathways (Mehler et al., 2000, Megason and McMahon, 2002, Mekki-Dauriac et al., 2002, Robertson et al., 2004). Therefore, blockade of either Wnt/ β -catenin signaling *in vivo* (Langseth et al., 2010), or prevention of BMP-mediated inhibition through insulin growth factor (IGF)-1

signaling *in vitro* (Hsieh et al., 2004), results in increased OPC specification from cortical progenitors. It is unclear, however, if IGF-1 plays a direct pro-specification role *in vivo*, in addition to its well characterized pro-survival effect on oligodendrocytes.

Interestingly, neural cultures derived from *Shh* null mice can still generate oligodendrocytes in culture, necessitating a SHH-independent pathway to oligodendrocyte specification (Chandran et al., 2003, Cai et al., 2005). Indeed, fibroblast growth factor (FGF)-2 helps to independently specify OPCs in the dorsal spinal cord and forebrain. Intraventricular microinjection of FGF-2 during embryonic development results in increased staining for OPC specific markers PDGFR α and OLIG2 independently of SHH signaling (Chandran et al., 2003, Naruse et al., 2006). FGF-2 exerts this effect through either FGF receptor (FGFR) 1 or 2, particularly through phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/ERK2) downstream of mitogen-activated protein kinases (MAPK) signaling (Chandran et al., 2003; Furusho et al., 2011). This in turn, helps to suppress SMAD signaling, the main effector of dorsal BMP blockade of oligodendrocytes, thus stimulating *Olig2* expression and OPC specification (Bilican et al., 2008).

OPC fate may also be influenced indirectly by other extracellular factors such as Notch1 and autotaxin by increasing the numbers of NPCs committing to the Olig2+ lineage (Kim et al., 2008, Yuelling et al., 2012). For example, the binding of the ligand Delta to the Notch1 receptors on NPCs, results in a decrease of the cyclin dependent kinase inhibitor (*cdkn1c*), an important factor promoting neuronal development. Increased Notch activity, with decreased activity of *cdkn1c* in NPCs, leads to a block in neuronal differentiation and a resulting increase in the pool of OLIG2+ NPC cells capable of specifying into OPCs (Park et al., 2005, Kim et al., 2008).

Transcription Factors in Oligodendroglial Specification

As with extracellular signals SHH and the BMPs, the transcription factors involved in specification of the oligodendrocyte lineage from NPCs are largely implicated in the definition of the dorso-ventral domains of the developing neural tube. As such, factors such as PAX6, OLIG2, NKX2.2 and NKX6.1/6.2, which are required to define the borders of the pMN domain of the spinal cord during development in response to the SHH and BMP gradients, regulate the formation of the initial ventrally derived wave of OPCs even though they are not necessarily directly required for OPC specification (Liu et al., 2003, Vallstedt et al., 2005). The GLI proteins also have a strong role in mediating the patterning effects of SHH, with SHH inducing the *Gli1/2* activators and repressing the *Gli3* repressor, in turn inducing *Olig2* and *Nkx2.2* and promoting OPC specification (Qi et al., 2003, Tan et al., 2006, Yu et al., 2013a)(Figure 2).

In contrast to these patterning factors, the bHLH factor *Ascl1*/Mash1 appears to have a more fundamental role in oligodendrocyte specification. *Ascl1* is expressed by many OPCs, and *Ascl1* null mice display decreased OPC generation in both the brain and spinal cord at early stages (Parras et al., 2007, Sugimori et

al., 2008). Nevertheless, relatively normal numbers of OPCs are ultimately achieved in these mice. Embryonically, ASCL1 appears to promote OPC formation within the ventral forebrain at the expense of neurogenesis through restricting the expression of pro-neural transcription factor DLX1 and 2 (Petryniak et al., 2007). At later stages, ablation of *Ascl1* gene in the SVZ at P2 results in strong diversion of progenitors to the astrocyte lineage at the expense of oligodendrocytes (Nakatani et al., 2013). This suggests ASCL1 has a general role in promoting specification of neural stem cells towards the oligodendrocyte lineage throughout development. Nevertheless, this role is likely to be contextual, as in the developing spinal cord many ASCL1+ progenitors become interneurons (Battiste et al., 2007).

Perhaps the best-defined transcription factor in oligodendrocyte specification is the basic helix-loop-helix transcription factor *Olig2*, expression of which is induced by ventrally secreted SHH (Lu et al., 2000). The importance of OLIG2 function in the specification of the oligodendrocyte lineage is well established; *Olig2* gene knockouts fail to produce OPCs within most regions of the CNS (Lu et al., 2002, Zhou and Anderson, 2002, Ligon et al., 2006), and inducible expression of *Olig2* in Nestin positive progenitor cells results in an increased generation of OPCs (Maire et al., 2010). Even so, OLIG2 is not an absolute requirement for the production of OPCs; in *Olig2*-null mice some OPCs are still generated in the hindbrain, most likely through compensation by the closely related bHLH *Olig1* (Lu et al., 2002). Moreover, in addition to demonstrating a requirement for OLIG2 in OPC specification, these studies also demonstrate a role for OLIG2 in the generation of astrocytes and motor neurons (Lu et al., 2002, Zhou and Anderson, 2002).

Recent findings have given more mechanistic insight into how OLIG2 determines NPC cell fate. The generation of motoneurons from the pMN domain is favored by homodimerization of OLIG2, which is dependent on phosphorylation at the Serine¹⁴⁷ (S¹⁴⁷) residue (Li et al., 2011) (Figure 3A). In contrast, S¹⁴⁷ dephosphorylation is associated with generation of OPCs (Figure 3B). This residue is a predicted PKA target (and thereby PKC), potentially linking this signaling pathway to the generation of motor neurons. Further research might elucidate which interaction partner acts with unphosphorylated OLIG2 to promote an OPC fate, and whether this is mediated by phosphatases induced by Notch1/HES-5 signaling, as (Li et al., 2011) suggest. An additional level of phosphorylation regulation is mediated by the N-terminus of OLIG2. In NPCs and OPCs the major role of OLIG2 is pro-mitotic and anti-differentiation (Lu et al., 2002, Lee et al., 2005); this function is dependent on the phosphorylation of a triple serine motif on its N-terminus (Sun et al., 2011). Although the pathways that mediate these phosphorylation events have not yet been established, predicted candidates include the CDK5, MAPK, GSK3 and CK1/2 kinases. These findings suggest OLIG2 may serve as a hub in progenitor cells where multiple signaling pathways converge to control both cell proliferation and fate (Figure 3).

2. Proliferation and Migration of OPCs

Once initial glial fate specification has been achieved at the embryonic ventricular zones, OPCs need to migrate to reach the final site of myelination (Figure 1B). In the spinal cord, most ventrally derived OPCs spread out in a ventro-dorsal and medio-lateral trajectory, traversing multiple rostro-caudal levels (Leber and Sanes, 1995, Ono et al., 1995, Miller and Ono, 1998). By contrast, dorsal-origin OPCs remain mostly in dorsal and lateral regions of the spinal cord, particularly the lateral funiculi where they account for more than 50% of all oligodendrocytes (Fogarty et al., 2005). Quail/chick chimera experiments indicate that within the hindbrain, oligodendrocyte progenitors only show limited rostro-caudal migration and largely spread radially to colonize mostly their rhombomere of origin (Olivier et al., 2001). More complex trajectory patterns exist in the cerebellum and diencephalon, with OPCs migrating tangentially and rostrocaudally from the mesencephalic and diencephalic parabasal plates, respectively (Garcia-Lopez and Martinez, 2010, Mecklenburg et al., 2011). In the developing forebrain, precursors migrate from the ventral subpallium to populate the lateral and dorsal portions of the telencephalon, as is the case for medial ganglionic eminence derived OPCs (Kessaris et al., 2006), or to invade the optic chiasm and spread along the optic nerves for pre-optic area derived OPCs (Small et al., 1987, Ono et al., 1997). These diverse and elaborate migratory routes not only highlight the complexity of CNS myelination, but also entail an equally sophisticated system of molecular guidance cues and signals (discussed below) that orchestrate and regulate this crucial step.

Extracellular signals regulating OPC proliferation and survival

OPCs remain proliferative throughout their lifespan, both during their developmental migration, in the adult CNS and in response to demyelination or CNS injury (Kang et al., 2010, Clarke et al., 2012). Most signals that promote proliferation of OPCs also inhibit their differentiation into oligodendrocytes, a process that occurs to ensure the proper timing of both the development of the axonal networks, as well as the proper distribution of myelinated white matter (Wang et al., 2007, Cui et al., 2010). Similarly, most proliferative signals tend to promote OPC survival, an intuitive process to ensure non-myelinating cells in pooled OPC populations do not undergo apoptosis (Baron et al., 2005). Indeed, it is estimated that somewhere between 20-50% of OPCs in the developing brain and optic nerve undergo apoptosis at the transition from OPCs to mature OL, highlighting their dependence on limited mitogenic/survival factors (Barres et al., 1992, Trapp et al., 1997).

Perhaps the most important mitogen for OPCs is PDGF-A, signaling through the OPC-expressed receptor PDGFR α (Noble et al., 1988, Richardson et al., 1988, Pringle et al., 1992). Experimental manipulation of PDGF-A levels *in vivo* showed that OPC proliferation and total OPC numbers are tightly coupled to PDGF-A levels (Calver et al., 1998, Fruttiger et al., 1999). Pro-survival signaling through PDGFR α appears to be tightly linked to the $\alpha 6 \beta 1$ integrin receptor as they have been shown to interact at lipid rafts (Baron et al., 2003, Decker and French-Constant, 2004). Moreover, stimulated PDGFR α forms a co-association with the

OPC-expressed $\alpha\text{v}\beta 3$ integrin, activation of which enhances the OPC proliferative response via stimulation of the PI3K/PKC signaling pathways (Baron et al., 2002). Interestingly, axonal signals can also stimulate proliferation and survival via interactions with integrin receptors on OPCs. In this regard neuregulin-1 (NRG1), a member of the NRG superfamily of epidermal growth factor-like ligands that are either axonally-bound or secreted, has been shown to bind the oligodendrocyte ErbB tyrosine kinase receptors and promote OPC survival through the PI3K/Akt pathway (Canoll et al., 1999, Fernandez et al., 2000, Flores et al., 2000). However, in the presence of laminin-2a, ErbB receptors associate with $\alpha 6\beta 1$ integrins on OPCs (much like PDGFR α), switching NRG1's role from a PI3K/Akt-mediated proliferation signal, to a MAPK-driven pro-survival and differentiation signal (Colognato et al., 2002, Colognato et al., 2004). Perhaps surprisingly, genetic abrogation of NRG-1 or its receptors does not seem to substantially impair CNS OPC survival/proliferation as these mice seem to myelinate normally (see below, also Brinkmann et al., 2008), perhaps suggesting other redundant stimulators of the signals downstream of neuregulin signaling.

Other prominent OPC mitogens include the FGF family and IGF-1. The former primarily signal through FGF-2 and FGF-18 acting on FGFR1/2 or FGFR2/3, respectively, to stimulate OPC proliferation and inhibit differentiation, while FGF-8 and FGF-17 primarily inhibit OPC differentiation through FGFR3 alone (Chandran et al., 2003, Fortin et al., 2005, Furusho et al., 2011). FGF-2 also has the ability to promote PDGFR α expression and likewise induces OPC proliferation and inhibits differentiation (McKinnon et al., 1990, Wolswijk and Noble, 1992, Baron et al., 2000). In addition to its potential involvement in OPC specification, IGF-1 also helps to promote OPC proliferation and survival by activating the PI3K/Akt pathway through IGF1R (Pang et al., 2007, Zeger et al., 2007, Bibollet-Bahena and Almazan, 2009, Romanelli et al., 2009). This is achieved through the phosphorylation of GSK3 β , preventing the phosphorylation and degradation of Cyclin-D1 (Frederick et al., 2007). Additionally, FGF2 and IGF-1 work synergistically by activating the ERK1/2 pathway allowing phosphorylated ERK1/2 to activate Cyclin-D1 and OLIG2 within the nucleus, promoting OPC proliferation (Frederick and Wood, 2004, Frederick et al., 2007).

Extracellular signaling regulating OPC migration

There are three main categories of migration effectors; motogenic or chemokinetic molecules that stimulate cell motility, adhesion/contact molecules presented in the extracellular matrices and on OPCs and other CNS cell types that attract, repel, or guide the cells, and the chemotactic cues that coax the OPCs to travel great distances throughout the CNS (see Table 1).

Motogenic factors

The two main motogenic molecules are PDGF-AA and FGF-2, which help to fuel the OPCs' movement throughout the CNS in addition to their mitogenic/survival role. Although these two factors have been shown to cooperate with each other during proliferation, their respective signaling pathways to mediate migration are distinct, with non-synergistic effects (Wolswijk and Noble, 1992, Baron et al., 2000, Miyamoto et al., 2008). FGF-2 exerts its pro-migratory role specifically

through the FGFR1 subtype, resulting in a dose-dependent mitogenic effect (Bansal et al., 1996, Osterhout et al., 1997, Bribián et al., 2006). In the absence of FGF-2, SHH can partially compensate by encouraging OPC migration in the optic nerve (Merchán et al., 2007). In contrast, PDGF acts through a pathway involving Fyn and cdk5 to promote WAVE2 phosphorylation and migration (Miyamoto et al., 2008). Taken together, these mitogenics play a significant role ensuring that OPCs are properly distributed. However, the effects of these mitogenic factors are not absolute as they can interact with molecules in the extracellular matrix (ECM), altering the course of OPC migration. The glycoprotein anosmin-1 in the ECM for example can inhibit the effects of FGF-2 by competing for FGFR1 binding (Bribián et al., 2006).

Adhesion and Contact molecule mediated migration

During migration OPCs must navigate through the ECM and interact with other OPCs, axons, and astrocytes to reach their final destination. The molecules presented in the ECM and on the cell surface of the varying CNS cell types provide a dynamic environment of signals and cues that help guide the OPCs, making the adhesion and contact molecules the most numerous and diverse group of OPC migratory effectors (de Castro et al., 2013). Some of the identified ECM proteins that have been implicated in OPC migration include laminin, fibronectin, merosin, tenascin-C, and anosmin-1 (Frost et al., 1996, Garcion et al., 2001, Chun et al., 2003, Bribián et al., 2006, Bribián et al., 2008, Hu et al., 2009, Relucio et al., 2009, Relucio et al., 2012), although the mechanisms through which they mediate their effects are still being determined. Similarly, numerous cell adhesion molecules such as PSA-NCAM (Decker et al., 2000, Zhang et al., 2004), Eph/ephrins (Prestoz et al., 2004), $\alpha\text{v}\beta\text{1}$ integrins (Milner et al., 1996), claudin-11/OSP (Tiwari-Woodruff et al., 2001, Tiwari-Woodruff et al., 2006), AN2/NG2 (Stegmüller et al., 2002, Biname et al., 2013), and N-cadherin (Payne et al., 1996, Schnadelbach and Fawcett, 2001) have been linked to OPC mitogenesis (see Table 1). By mediating how tightly OPCs stick to the surrounding cells/ECM, this diverse group of factors play a critical role in orchestrating OPC migration over large heterogeneous domains. Intriguingly, it appears OPCs themselves are a potent mediator of their own migration; *in vivo* studies have shown that contact between migrating OPCs is a powerful dispersal cue, causing them to alter their course in the opposite direction (Kirby et al., 2006, Hughes et al., 2013). Although not strictly a directional cue, this seems intuitive if the ultimate goal is a homogenous distribution of OPCs and is likely mediated by membrane surface interactions such as the neurite outgrowth inhibitor, Nogo-A, expressed by OPCs (Chong et al., 2012).

Chemoattractants and Repellants

OPC migration is also strongly governed by chemotactic cues that serve to attract or repel the highly motile OPCs (Figure 1C). In addition to their mitogenic abilities, both PDGF-AA and FGF-2 act as potent chemoattractants to help direct OPCs (Bribián et al., 2006; Noble et al., 1988; Zhang et al., 2004). SHH signaling also acts as a positive chemotrophic cue, particularly in the optic nerve through binding of the Patched-1 receptor (Merchán et al., 2007). The secreted chemokine CXCL12 acts throughout embryonic and postnatal development as an OPC chemoattractant and survival factor, particularly in the dorsal spinal cord

through binding of its receptor CXCR4 (Dziembowska et al., 2005). Another chemokine, the astrocyte-secreted CXCL1, is a strong chemorepellant antagonizing the pro-migratory effects of PDGF-AA in the developing spinal cord in a rapid and reversible manner (Tsai et al., 2002). The role of CXCL1 as a stop signal was substantiated through knockout of its receptor CXCR2, resulting in spinal cord OPCs migrating all the way to the pial surface, highlighting its importance for proper OPC placement (Tsai et al., 2002, Padovani-Claudio et al., 2006). Other common chemorepellants include the large family of pleiotropic factors, the semaphorins, particularly secreted Semaphorin-3A and membrane bound Semaphorin-4D/F (Spassky et al., 2002, Taniguchi et al., 2009, Armendáriz et al., 2012).

However, the effects of a chemotrophic cue on OPC migration can vary depending on the stage of development, region in the CNS, and the specific receptors expressed on different OPC populations. For example, the secreted netrin-1 acts as a chemoattractant in the first wave of OPC migration in the optic nerve through the deleted in colorectal cancer (DCC) receptor, yet was shown to act as a chemorepellent in the second wave through the Unc5A receptor (Figure 1C; Sugimoto et al., 2001, Spassky et al., 2002). In the developing spinal cord however, floor plate cell-secreted netrin-1 acts purely as a chemorepellent to initiate dispersal of OPCs through activation of either the DCC or UNC5A receptor (Jarjour et al., 2003, Tsai et al., 2003, Tsai et al., 2006). Signaling its effects through the ErbB4 receptor, NRG-1 also has temporally different effects on OPC migration, with NRG-1 having been shown to be a potent chemoattractant during development but completely dispensable for postnatal OPC migration (Ortega et al., 2012).

3. Terminal Differentiation

Once OPCs are correctly positioned, they can terminally differentiate into postmitotic, premyelinating oligodendrocytes – an event that generally proceeds in a caudal-to-rostral gradient in the brain but rostro-caudally in the spinal cord (Brody et al., 1987), and which requires profound changes in both cellular behavior and gene expression. In the mouse the terminal differentiation of OPCs and subsequent myelination are tightly coupled events, with the intermediate premyelinating oligodendrocytes representing a highly transient stage wherein cells either rapidly progress to myelination or undergo apoptosis (Barres et al., 1992, Trapp et al., 1997). In contrast, there is evidence that premyelinating oligodendrocytes may persist for up to several months during human development before finally myelinating (Back et al., 2002). Presumably this delay in humans may give considerably greater potential for axonal selection to be “fine-tuned” to ensure the correct axons are myelinated (see review in this issue by Almeida and Lyons). Although in culture differentiation of OPCs appears to be the “default” pathway when they are deprived of mitogens, *in vivo* the balance between OPC proliferation and differentiation appears to be tightly regulated (Hughes et al., 2013). As such it is not surprising that a number of extracellular signals and intracellular pathways have been implicated in maintaining this balance.

Extracellular Signals in differentiation

Inhibitors of differentiation

Consistent with the differentiation of OPCs into postmitotic oligodendrocytes being a major check-point in the myelination process, oligodendrocyte differentiation is under tight regulation by a number of factors, many of which act to inhibit differentiation. The Leucine rich repeat and immunoglobulin domain containing-1 (LINGO1) is a transmembrane protein expressed on both axons and oligodendrocytes that was first identified to inhibit neurite outgrowth through binding the Nogo-66 receptor/p75 signaling complex (Mi et al., 2004). Further studies elucidated LINGO1 mediated inhibition of oligodendrocyte differentiation through the decreased activity of Fyn kinase and subsequent increases in RhoA signaling (Mi et al., 2005), a pathway implicated in OPC differentiation (Liang et al., 2004). However, the Nogo receptor complex is not expressed on oligodendrocytes, suggesting that another receptor mediates the LINGO1-mediated signal. Recently, researchers have identified that the extracellular domain of LINGO1 binds to the oligodendrocyte expressed LINGO1 in trans, acting as its own receptor, allowing the inhibition of oligodendrocyte differentiation and myelination (Jepson et al., 2012).

The G-protein coupled receptor 17 (GPR17) is an oligodendrocyte specific receptor that is expressed transiently in late stage OPCs/early differentiated oligodendrocytes (Cahoy et al., 2008, Chen et al., 2009, Fumagalli et al., 2011). Through the use of over-expression assays, Chen et al. discovered that GPR17 strongly inhibited OPC differentiation and maturation (Chen et al., 2009). This was associated with an increase in ID2/ID4 expression and nuclear localization, suggesting a mechanism for the GPR17 mediated differentiation block (Chen 2009). Conversely, *Gpr17* knockout mice showed a precocious onset of myelination, confirming that endogenous GPR17 acts as a negative regulator and signaling timer of oligodendrocyte differentiation and myelination (Chen et al., 2009). The relevant endogenous ligands for GPR17 in the context of myelination are yet to be categorically established.

The Notch signaling pathway has been implicated as a regulator of cell differentiation in a number of different cell-types, particularly in oligodendrocytes through Notch-1 (Wang et al., 1998, Kondo and Raff, 2000, Genoud et al., 2002). Jagged-1 and Delta-1 are axonally expressed ligands that bind the transmembrane glycoprotein Notch1 receptor, which upon activation is cleaved and generates the notch intracellular domain (NICD). The NICD then enters the nucleus and increases expression of the *Hes1* and *Hes5* transcription factors that inhibit OPC differentiation (Kondo and Raff, 2000; Wang et al., 1998). Surprisingly, in the presence of the axonally expressed F3/contactin, Notch-1 signaling has been reported to promote OPC differentiation (Hu et al., 2003). In comparison to Jagged1 inhibitory signaling through Notch1, F3/contactin signals cleavage of a Deltex1 associated version of the NICD, leading to expression of differentiation markers such as myelin associated glycoprotein (MAG) (Hu et al., 2003). The developmental change in available extracellular ligands thereby dictates transcriptional targets downstream of Notch.

Promoters of differentiation

Early *in vitro* work showed that in addition to mitogen withdrawal, OPCs could also be stimulated to stop proliferating and initiate terminal differentiation by thyroid hormone 3 (T3) as part of an intrinsic cell division timer (Barres et al., 1994, Gao et al., 1997, Durand and Raff, 2000). Although T3 and mitogen withdrawal likely work through different cell cycle signaling pathways such as p53 or p27/kip1 mediated signaling, respectively, the two mechanisms probably operate in conjunction *in vivo*, regulating a crucial step in myelin development (Durand et al., 1997, Durand et al., 1998, Tokumoto et al., 2001). The importance of T3 signaling in this step is highlighted by the myelination deficits observed in congenital human hypothyroidism patients (Gupta et al., 1995, Jagannathan et al., 1998) and reduction in myelin gene expression in rodent models of perinatal hypothyroidism (Rodriguez-Pena et al., 1993, Ibarrola and Rodriguez-Pena, 1997, Schoonover et al., 2004). Conversely, hyperthyroidism results in precocious myelination and elevated myelin protein levels (Marta et al., 1998, Pombo et al., 1998). Although these deficits are reversible and T3 is not ultimately essential for OPCs to differentiate, it clearly plays an important role in the timely and efficient production of myelin during development by accelerating OPC differentiation.

In addition to its role in proliferation/survival, studies have shown that overexpressing IGF-1 causes an increase in overall brain growth and myelination, with an increased number of mature oligodendrocytes and myelin gene expression, implicating IGF-1 in promoting differentiation (Carson et al., 1993, Ye et al., 1995, Goddard et al., 1999). Conversely, *Igf1* knockout mice show a decrease in both myelin protein expression and a reduction in the number of mature oligodendrocytes and OPCs, again indicative of its effects in survival, proliferation and myelination (Ye et al., 2002). Moreover, genetic deletion of the oligodendrocyte IGFR1 receptor results in a similar phenotype to the *Igf1* knockout, with decreased oligodendrocyte numbers, reduced proliferation, and increased apoptosis (Zeger et al., 2007).

Transcriptional regulation of terminal differentiation

Transcriptional mediators of the differentiation block

The above extracellular signals converge on a number of well-defined transcription factors that act to block or promote differentiation. The inhibition of OPC differentiation is mediated by several bHLH transcription factors. The anti-differentiation effects of BMP (Cheng et al., 2007) and GPR17 (Chen et al., 2009) signaling are both thought to be mediated by the bHLH molecules ID2 and ID4, which directly bind OLIG1 (and to a lesser extent OLIG2) inhibiting their function (Samanta and Kessler, 2004). Another bHLH protein, HES5, is induced by Delta/Notch signaling (Wang et al., 1998). HES5 can then inhibit differentiation and myelin gene expression (Wang et al., 1998, Kondo and Raff, 2000), at least in part by inhibition of pro-differentiation factors such as SOX10 (Liu et al., 2006).

Changes in mode of Notch and Wnt signaling at the onset of differentiation

The Wnt pathway gives another example of how the transcriptional targets of signaling pathways can change depending on cellular context. Canonical Wnt signaling, in which activation of the receptor Frizzled by Wnt leads to a nuclear localization of β -catenin, strongly inhibits differentiation. This is evident from a block or delay of OPC differentiation under Wnt3A treatment (Shimizu et al., 2005), β -catenin overexpression or release of the inhibitory effects adenomatous polyposis coli (APC) on β -catenin (Fancy et al., 2009). β -catenin mediates this anti-differentiation effect in part by recruiting the transcription factor Tcf712/Tcf4 to β -catenin target genes (Fu et al., 2012). Although the direct targets of Tcf712/ β -catenin in oligodendrocytes are unclear, in other cell types they include ID2 and ID4 (Memezawa et al., 2007). How the occupancy of Tcf712 by β -catenin is later lifted is subject of ongoing research. Possibilities include decreasing Wnt3a concentrations, a down-regulation of Wnt/ β -catenin pathway members by SOX17 (Chew et al., 2011), or sequestration of nuclear β -catenin by N-cadherin, the latter being a factor in the initiation of myelination by Schwann cells (Lewallen et al., 2011). Decrease in β -catenin levels then allow for binding of Tcf712 by the chromatin remodeling proteins Histone Deacetylase Complexes 1 & 2 (HDAC1/2) (Ye et al., 2009). A role for Tcf712 in chromatin remodeling during differentiation is consistent with its tightly controlled, transient expression following cell cycle exit, in postmitotic OPCs/pre-myelinating oligodendrocytes (Fu et al., 2012). Tcf712 would thereby act as a molecular switch, blocking or promoting differentiation depending on available binding partners.

Pro-Differentiation Transcription Factors

Gene knockout studies have highlighted a number of transcription factors required for oligodendrocyte differentiation. In addition to its role in OPC specification, *Ascl1*/*Mash1* has a subsequent role in promoting differentiation, as *Mash1* knockout mice show a deficiency in the generation of postmitotic oligodendrocytes (Parras et al., 2007). This appears to be an autonomous effect; conditional ablation of the *Ascl1* gene in OPCs results in symmetrical division at the expense of asymmetrical divisions that generate oligodendrocytes (Nakatani et al., 2013). Both *in vitro* and *in vivo*, *Ascl1* knockout results in a reduction in the expression of *Nkx2.2* in the oligodendrocyte lineage (Sugimori et al., 2008). This is notable, given *Nkx2.2* knockout mice also display a block in oligodendrocyte differentiation (Qi et al., 2001).

OLIG2 is one of the most consistent markers of the oligodendrocyte lineage, being expressed at all stages and having a clear role in OPC specification in most regions of the CNS (see above). Whether it has additional roles at later stages in the lineage has not been clear, both due to the severe lack of OPCs seen in the null mice and because its expression decreases with differentiation (Kitada and Rowitch, 2006). Using a stage-specific inducible conditional knockout approach, Mei et al. (2013) recently demonstrated that ablation of *Olig2* in OPCs postnatally caused a substantial decrease in oligodendrocyte differentiation. In contrast, ablation in the mature cells had no detrimental effect on myelination, suggesting its role is more to promote differentiation than to mediate myelination long-term. A clear mechanism for OLIG2's role in promoting

differentiation comes from a recent study by Yu et al. (2013b), where they found OLIG2 recruits the chromatin remodeling enzyme BRG1 to regulatory elements of key genes during differentiation (Figure 3C). The genes targeted included *Sox10*, *Sip1/Zhfx1b* as well as pro-myelination transcription factors such as *Myrf* (see below). Interestingly, Yu et al. (2013b) found that Olig2 targeted different genes depending on the stage of the lineage. Whether this specificity is mediated by the phosphorylation state of OLIG2, and whether different cofactors can modulate the regions targeted by OLIG2-BRG1 depending on the differentiation stage will be an important subject of future research.

4. Myelination

The final stage of oligodendrocyte development is myelination. Unlike the PNS where there is a strict size-dependent bias for myelination (only axons >1 μ m diameter enjoy the privilege), in the CNS axons as small as 300nm are sometimes myelinated. Nevertheless, CNS myelination is also influenced by axonal size. Surprisingly, cultured oligodendrocytes will begin myelinating even synthetic axon-like tubes (Lee et al., 2012) lacking the usual neuron-glia signaling cues. In this type of 'blind' myelination, only tubes with a diameter of ≥ 400 nm were myelinated, suggesting this mode of myelination may be particularly important for larger axons. Moreover, once differentiating, it appears that oligodendrocytes only have a very narrow window of opportunity to select which adjacent axons to myelinate (~five hours in the developing zebrafish and ~twelve hours in myelinating cultures of rodent derived cells), irrespective of the total number of sheaths being made (Watkins et al., 2008, Czopka et al., 2013). This implies that the signaling pathways mediating this process are likely to be relatively robust. Although *in vitro* studies have implicated several key pathways, to date no one single molecule has been shown to be indispensable for myelination of CNS axons *in vivo*, highlighting the great degree of redundancy in the control of this vital process. Despite this, recent *in vivo* studies have revealed a great deal about how these various signaling pathways converge to control the extent or timing of myelination, if not overall myelination *per se*.

Extracellular Signals in myelination

The dynamic nature of oligodendrocytes being able to extend processes, sense axons, and myelinate up to 40 different axons necessitates a complex and dynamic system of signaling mechanisms converging on the oligodendrocyte to promote or impede each action. Although these extracellular signals have various roles in the myelination process, they can be grossly categorized into two groups: those that modulate oligodendrocyte process extension and initial axonal contact, and factors that dictate subsequent myelin thickening.

Process extension/Initial axonal contact

To begin myelin formation, immature post-mitotic oligodendrocytes first need to extend numerous cytoplasmic protrusions (filipodia), not unlike the growth cones of developing axons, in order to find suitable myelin-competent axons. As microfilament-rich filipodia extend they are invaded by microtubules, thus further enlarging these processes and converting them to lamellipodia. To

accommodate this rapid, directed growth, the oligodendrocyte cytoskeleton increases microfilament polymerization and branching in response to ECM/axonal signals. The majority of axonal signals identified to date are inhibitory in nature, expressed to prevent the initiation of myelination and/or exuberant over-myelination. Canonical examples of this include axonal PSA-NCAM, which is developmentally down-regulated to coincide with the onset of myelination (Decker et al., 2000, Fewou et al., 2007), Jagged-1, which interacts with Notch-1 receptors to potentially inhibit oligodendrocyte differentiation (Wang et al., 1998, Hu et al., 2003, Watkins et al., 2008), and Lingo1, which associates with the oligodendroglial Nogo receptor to inhibit myelination and process outgrowth (Mi et al., 2005). There are also axonal signals that are pro-myelination. Apart from axonal calibre, which in itself is a potent stimulator of initial myelination, pre-myelinated axons also produce laminin- $\alpha 2$ (Morissette and Carbonetto, 1995, Colognato et al., 2002) which binds to $\beta 1$ integrin receptors on oligodendrocytes, promoting initial process extension (Hu et al., 2009). This results in an activating dephosphorylation of the non-receptor tyrosine kinase Fyn at the inhibitory Tyr531 site, which leads to activation of integrin-linked kinase (ILK), PINCH and α -parvin and initiates actin polymerization (Liang et al., 2004, Bauer et al., 2009). Subsequently, paxillin, the N-WASP/Arp2/3 complex, and WAVE1 are recruited to focal adhesion sites and activated, ultimately mobilizing downstream modulators of actin polymerization and microtubule assembly via focal adhesion kinase (FAK)-mediated activation of RhoGTPases such as Cdc42 and Rac1 (Chun et al., 2003, Bacon et al., 2007, Kim et al., 2008, O'Meara et al., 2013).

Fyn is also activated by other signaling pathways such as binding of axonal L1 ligand to oligodendroglial contactin (Laursen et al., 2009) or the binding of netrin-1 to its Deleted in Colorectal Carcinoma (DCC) receptor (Rajasekharan et al., 2009), highlighting its role as a common downstream effector for multiple pro-myelination pathways. The main effect of Fyn activation is to block the inhibitory actions of RhoA, which it accomplishes via phosphorylating the small GTPase proteins p190/p250 RhoGAP, which encourage the formation of Rho-GDP, and thus RhoA inactivation (Wolf et al., 2001, Taniguchi et al., 2003, Kippert et al., 2007). Consequently, inhibiting RhoA leads to hyperextension of OPC processes and increased branch formation while expressing a constitutively active form of RhoA results in decreased process extension (Liang et al., 2004). Fyn activation also triggers tyrosine phosphorylation of focal adhesion kinase (FAK; also known as protein tyrosine kinase (PTK2)) which is crucial for the activation of Cdc42/Rac1 (Hoshina et al., 2007). Interestingly, FAK has recently been shown to also have an inhibitory effect on process expansion/elaboration in the presence of fibronectin-rich ECM, thus suggesting that FAK is a key regulator of ECM-cytoskeletal responses (Lafrenaye and Fuss, 2010). In migrating OPCs, it mostly signals through the fibronectin pathway helping them to restrain process outgrowth, whereas in post-mitotic immature oligodendrocytes, it signals predominantly through the laminin-2 pathway, driving process expansion.

The importance of the laminin- $\beta 1$ integrin receptor signaling in initial process extension and contact formation is best highlighted by the hypomyelinating

phenotypes exhibited by knocking out specific components of the pathway in mice. Genetic ablation or expression of a constitutively inactive form of Fyn results in significantly fewer myelinated axons with fewer mature oligodendrocytes and thinner myelin sheaths (Umemori et al., 1994, Osterhout et al., 1999, Goto et al., 2008). Likewise, decreasing expression of laminin- $\alpha 2$ (such as in the Dy/Dy mouse) (Chun et al., 2003, Relucio et al., 2009) or knocking out *Ptk2* in oligodendrocytes (Camara et al., 2009), also leads to hypomyelination. Interestingly, interfering with $\beta 1$ integrin signaling has yielded mixed results, with some groups showing decreased numbers of myelinated axons (Lee et al., 2006, Camara et al., 2009), while others showed little effect (Benninger et al., 2006). This discrepancy between the severe phenotype exhibited by laminin- $\alpha 2$ deficient animals and its receptor, $\beta 1$ integrin, knockouts could be explained by the recent discovery that laminins also signal via dystroglycan receptors (Colognato et al., 2007), which likely compensate for lack of $\beta 1$ integrin signaling in these animals. Perturbation of dystroglycan signaling (by using siRNA or antibodies) results in fewer branches and mature oligodendrocytes *in vitro* (Eyermann et al., 2012) however *in vivo* studies need to be conducted to fully appreciate the contribution of this signaling pathway to myelin development.

Myelin thickness

Beyond the initial process outgrowth and contact with axons, laminin- $\alpha 2$ and $\beta 1$ integrin mediated signaling is also required for determining proper myelin thickness and axon selection. Genetic ablation of functional $\beta 1$ integrin subunits or expressing a truncated form without the C-terminus results in thinner myelin sheaths around axons at later stages, however, the downstream signaling pathways that regulate this process, MAPK or Akt, remain unclear (Lee et al., 2006, Barros et al., 2009). Other modulators of myelin thickness include the axonal neuregulins (NRG), particularly NRG-1 (type 3) that bind to ErbB receptors on oligodendrocytes (Taveggia et al., 2007, Brinkmann et al., 2008). Although initial *Nrg1* knock-out studies reported little effect on overall myelination depending on stage and region analyzed (Brinkmann et al., 2008), more recently lack of NRG1 signaling has been shown to result in hypomyelination and thinner myelin sheaths in the prefrontal cortex, a phenotype that is remarkably similar to that following social isolation between post-natal weeks 3-5 (Makinodan et al., 2012). This suggests that although NRG1 signaling via ErbB3 receptors may not be absolutely essential for initial myelination, it may mediate experience-dependent myelination in later-myelinating regions such as the prefrontal cortex. In support of this, overexpression of *Nrg1* in neurons leads to thicker myelination of CNS axons and the myelination of smaller diameter axons that would not normally be myelinated (Brinkmann et al., 2008). Intriguingly, levels of neuronal NRG1 are regulated by activity (Ziskin et al., 2007, Liu et al., 2011), again suggesting this as one of a number of potential mechanisms for linking neuronal activity with myelin production.

The Raf-MEK-ERK pathway has been identified as a common target for pro-myelination signals, BDNF and FGF-2 in particular (Furusho et al., 2012, Xiao et al., 2012), though it may also mediate signals from the extra-cellular laminins through dystroglycan or the $\beta 1$ subunit of the integrin receptors (Lee et al., 2006,

Galvin et al., 2010). Expression of constitutively active MEK in oligodendrocytes increases myelin basic protein expression myelination *in vitro* (Xiao et al., 2012) and increases myelin thickness *in vivo* (Ishii et al., 2013). Recent data demonstrating that BDNF's promyelinating effect is mediated via ERK1/2 (Xiao et al., 2012) and deletion of the TrkB receptor for BDNF in oligodendrocytes reduces the thickness of the myelin sheath in development (Xiao et al., 2010, Xiao et al., 2012, Wong et al., 2013) also supports the view that ERK1 and 2 act as common downstream effectors of signals that promote myelin thickness. Additional stimulatory signals for myelination appear to be mediated by the PI3K/Akt/mTOR pathway. This pathway appears to mediate a late stage of oligodendrocyte differentiation (Guardiola-Diaz et al., 2012) as well as myelin thickness, with constitutive activation of this pathway in mice resulting in pathological hypermyelination (Flores et al., 2008, Narayanan et al., 2009).

Transcription factors in myelination

Recent findings give surprising insights into what role long known specification factors of the oligodendrocyte lineage and newly found transcription factors play in the terminal differentiation of the cell. Although *Olig1* is expressed from the time of oligodendrocyte specification, it appears to be relatively dispensable for OPC function. In contrast, its importance becomes apparent on the onset of myelination, as *Olig1* knockout mice generate early stage (CNP+, O4+) oligodendrocytes with complex branching morphologies which nevertheless fail to myelinate (Xin et al., 2005). Although the direct targets of OLIG1 are relatively undetermined, it has, in conjunction with SOX10, been shown to drive transcription from the MBP promoter (Xin et al., 2005, Li et al., 2007) (Figure 4). Nevertheless, as oligodendrocytes mature the predominant subcellular localization of OLIG1 changes from nuclear to cytoplasmic (Niu et al., 2012). The change in localization is dependent on its phosphorylation state and is required to promote full membrane extension (Niu et al., 2012). The mechanism by which OLIG1 would act to promote membrane extension when excluded from the nucleus has not been addressed, though it may involve recruiting additional factors from/to the cytoplasm or sequestering inhibitory factors away from the nucleus.

A potential downstream target of OLIG1 is *Zfp488* (Wang et al., 2006). ZFP488 might be involved in myelin gene regulation as increased expression of *Zfp488*, *Mbp* and *Plp1* coincide *in vivo*, and *Zfp488* knockdown results in a decrease of MBP and CNPase levels *in vitro* (Wang et al., 2006). As ZFP488 is thought to be a transcriptional repressor, the exact mechanism by which it upregulates myelin gene expression are not yet clear, however it may hinge on its physical interactions with OLIG2 (Wang et al., 2006). Similarly, the zinc finger protein ZFP191 has also been demonstrated to be required for normal myelin gene expression and myelination (Howng et al., 2010). For both ZFP191 and ZFP488, the direct gene targets during differentiation and myelination are largely yet to be established.

One inhibitory factor known to be downregulated coinciding with the re-expression and cytoplasmic translocation of OLIG1 is NKX2.2. Although expression of *Nkx2.2* is required for the generation of postmitotic oligodendrocytes (Qi et al., 2001), it is transiently down-regulated concurrent with the onset of myelination (Cai et al., 2010). Given that NKX2.2 can inhibit transcription from the MBP promoter (Wei et al., 2005), this transient down-regulation may be necessary for myelin gene expression to be established. During this transient down-regulation of *Nkx2.2*, oligodendrocytes also re-express *Nkx6.2*. The exact downstream targets of NKX2.2 and NKX6.2 are the subject of discussion (Awatramani et al., 1997, Farhadi et al., 2003, Southwood et al., 2004). In *Nkx6.2* knockouts ultrastructural myelin abnormalities and aberrant expression of the paranodal proteins stathmin1, neurofascin and contactin can be seen (Southwood et al., 2004). NKX6.2 and NKX2.2 might therefore act on paranodal gene expression to finely tune the generation of this critical constituent of axo-glial function. Presumably future ChIP-Seq studies will help establish the immediate targets of these genes during differentiation and myelination.

Much like *Olig1*, *Sox10* is vitally required for the generation of myelinating oligodendrocytes, which stall at around the time of differentiation in its absence (Stolt et al., 2002, Takada et al., 2010, Hornig et al., 2013). This most likely reflects a direct and critical role for SOX10 at myelin gene promoters, for instance at the MBP promoter (Li et al., 2007). This is consistent with the known role of SOX10 in the peripheral nervous system, where it directly regulates the expression of myelin gene expression in Schwann cells (LeBlanc et al., 2006, Srinivasan et al., 2012). SOX10 exerts parts of its function by directly binding the mediator subunit 12 (Med12), which links its target transcription factors to the general transcription machinery via the mediator complex (Vogl et al., 2013). Much like *Sox10* null mice, oligodendrocytes in which *Med12* is conditionally ablated stall at the premyelinating stage, prior to expression of *Gpr17* (Vogl et al., 2013).

A recently described key mechanism for SOX10 induction of myelination is its relationship with the pro-myelination factor Myelin Regulatory Factor (*Myrf*). Unlike other oligodendrocyte transcription factors, which are also expressed at the OPC stage, *Myrf* is specifically induced during oligodendrocyte differentiation (Emery et al., 2009). This induction requires the direct activity of SOX10 at an intronic enhancer in the *Myrf* gene, with a near complete loss of MYRF expression in the absence of Sox10 (Hornig et al., 2013). Following its induction, MYRF mediates the progression of the pre-myelinating oligodendrocytes to a mature, myelinating state (Emery et al., 2009). Inducible conditional ablation of *Myrf* in mature oligodendrocytes results in a rapid loss of myelin genes and a subsequent slow demyelination, even in the adult mouse, confirming it has a fundamental role in regulating myelination beyond the differentiation period (Koenning et al., 2012). Consistent with a continued role in the regulation of myelin genes in the mature cells, ChIP-Seq experiments demonstrate that binding of MYRF to DNA is enriched in the vicinity of known myelin genes and other transcripts highly expressed by oligodendrocytes (Cahoy et al., 2008, Bujalka et al., 2013). These include not only many of the protein components of

the myelin sheath, but also key enzymes and cytoskeletal proteins known to underlie the generation of the myelin sheath. In addition, it seems that MYRF and SOX10 target many of the same myelin gene enhancers and promoters (Bujalka et al., 2013, Hornig et al., 2013), at least some in a synergistic manner. Nevertheless, both MYRF and SOX10 appear to be able to target individual enhancers independently of the other to some extent, so the exact functional relationship between the two in promoting myelination still needs to be determined.

Conclusions

The extracellular signals, intracellular pathways and transcription factors regulating oligodendrocyte differentiation are increasingly well-established, perhaps more so than for any other CNS cell type. However, from a therapeutic perspective, we are only just starting to determine how this basic knowledge of oligodendrocyte development, largely obtained during development, can be used to develop strategies to promote remyelination in human demyelinating disease such as Multiple Sclerosis. Identification of neutralization of major inhibitors of remyelination present within MS lesions will be a critical step in this process. Nevertheless, one potential lesson from the extensive studies on inhibition of axonal regrowth following CNS injury is that there may be a great deal of redundancy in inhibitory signals that then converge on common downstream pathways (Yiu and He, 2006). As such, the next challenge will be to identify the common important intracellular and transcriptional mediators of the proliferation, differentiation and myelination pathways and ways that these can be targeted.

	OPC Specification	OPC Survival	OPC Migration	OPC Proliferation	Differentiation	Myelination
Secreted	SHH ¹	SHH	SHH / +	SHH	SHH	
	BMP				BMP	
					WNT	
	Autotaxin					Autotaxin
		PDGF-AA	PDGF-AA / +	PDGF-AA	PDGF-AA	
	FGF-2	FGF-2	FGF-2 / +	FGF-2	FGF-2	FGF-2 / §
		IGF-1		IGF-1	IGF-1	IGF-1 § / *
				BDNF	BDNF ²	BDNF / §
				TH3	TH3	
			HGF / + ³			
			Netrin-1			Netrin-1
			Semaphorin 3A Semaphorin 3F			
			CXCL1	CXCL1	CXCL1 (?) ⁴	
		CXCL12	CXCL12	CXCL12 ⁵	CXCL12 ^{5,6}	
			Slit2 ⁷			
Contact		NRG-1	NRG-1 +	NRG-1	NRG-1	NRG-1 * §
			PSA-NCAM			PSA-NCAM ⁸
	Notch-1				Notch-1 / Notch-1 F3	Notch-1*
		Laminin α2			Laminin α2	Laminin α2 * §
		L1-Contactin				
		α6 Integrin				
		β1 Integrin				β1 Integrin *
					Lingo1	Lingo1*
					Gpr17	
		Fibronectin (via αvβ3)	Fibronectin (via αvβ1)	Fibronectin (via αvβ3)	Fibronectin (via αvβ5)	
			Dystroglycan		Dystroglycan ⁹	Dystroglycan
		Semaphorin 4D ¹⁰	Semaphorin 4F	Semaphorin 4D ¹⁰ Semaphorin 6A ¹¹	Semaphorin 6A ¹¹	
			Ephrins/Eph			
			OSP/Claudin-11	OSP/Claudin-11		OSP/Claudin-11
			N-cadherin ¹²			N-cadherin ¹²
			NG2 +			
			tenascin-C			
			Anosmin-1			

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Figure legends:

Figure 1: A. Sagittal view of an embryonic day E11.5 mouse brain showing the spatial distribution of morphogens sonic hedgehog (Shh, green) in the ventral portion of the neural tube, while bone morphogenic factor (BMP, red) signaling is mostly concentrated along the dorsal domains. Fibroblast growth factor 8 (FGF-8, purple) is secreted mostly from the anterior neural ridge and along the midbrain-hindbrain boundary (MHB). B. Sagittal view of developing chick embryo CNS. Basoventral territories of OPC emergence are colored blue while alar domains are colored red. Arrows indicate their migratory pathways; blue arrows emanating from the basoventral domains of the brain (r, rhombomeres 1-7) migrate radially and seldom leave their area of origin, while those arising from the alar plate, in the precordal domain of the brain, or from the spinal cord, migrate extensively following complex trajectories (green, orange and black arrows) (Figure adapted from Olivier et al., 2001). C. A schematic showing the distribution of cells expressing Netrin-1 (green), Sema3A (orange), Sema3F (purple) and PDGF-AA (blue). Migrating OPCs have to navigate the complex array of chemotactic cues in order to find appropriate retinal ganglion cell (RGC) axons to myelinate. (AEP, anterior entopeduncular area; MGE, medial ganglionic eminence; M, metencephalon; I, isthmus; N, nasal; T, temporal; ZLI, zona limitans intrathalamica)

Figure 2: Schematic representation of oligodendrocyte differentiation stages and relevant transcription factors. After specification of NPCs into OPCs, differentiation progresses via a multi-process, premyelinating stage. During

these stages chromatin remodeling through OLIG2 & BRG1 and TCF7L2 & HDAC1/2 paves the way for future gene expression and cell functions. Upon cues, cells induce the expression of myelin genes, a process driven by promyelinating transcription factors such as SOX10 and MYRF and coinciding with a translocation of Olig1 to the cytoplasm. In terminally differentiated oligodendrocytes, promyelinating transcription factors are coexpressed with transcriptional repressors such as NKX2.2/6.2, potentially to fine-tune myelin homeostasis.

Figure 3: Modes of OLIG2 activity across different cell fates and differentiation stages. A. When phosphorylated at Serine 147 (S147), OLIG2 preferentially forms homodimers, sequestering it from other potential binding partners such as NEUROG2 (NGN2). NGN2 can then homodimerize and drive the expression of genes critical for differentiation into motoneurons. B. When dephosphorylated at S147, OLIG2 can directly bind NGN2 and sequester it from its proneural functions. Via this and potentially other mechanisms, the cell is directed towards an oligodendrocyte precursor cell (OPC) fate. At this stage OLIG2 also has a promotitotic function that requires phosphorylation of three serine residues near its N-terminus. All of these processes may allow for the integration of signaling pathways via kinases and phosphatases. C. OLIG2 also serves an important pro-differentiation function in chromatin remodeling by directing the histone acetylating molecule BRG1 to genes critical for differentiation, thereby enabling their expression.

Figure 4: A model of OLIG1 activity across oligodendrocyte differentiation stages. A. In the OPC, active GPR17 signaling results in an increase in ID2/4 protein levels and nuclear translocation, where it binds to OLIG1 and sequesters it from the nucleus. GPR17 and ID2/4 thereby work towards maintenance of the OPC stage. B. Upon decreased GPR17 signaling OLIG1 can translocate to the nucleus, where it increases the expression of MBP, potentially in concert with other factors. C. Phosphorylation of OLIG1 on residue S138 mediates its translocation to the cytoplasm, which is seen in the mature, myelinating cell stage and shown to be critical for myelin membrane extension.

Table 1: Secreted and contact-mediated extracellular factors that promote (green) or inhibit (red) specific stages of oligodendrocyte development. (+, motogenic; *, involved in size-sensing; §, promotes myelin thickness) (¹Orentas et al., 1999; ²Vondran et al., 2010; ³Yan and Rivkees, 2002; ⁴Kerstetter et al., 2009; ⁵Patel et al., 2012; ⁶Patel et al., 2010; ⁷Liu et al., 2012; ⁸Charles et al., 2000; ⁹Galvin et al., 2010; ¹⁰Yamaguchi et al., 2012; ¹¹Bernard et al., 2012; ¹²Schnadelbach et al., 2000).

Figure 3

A: Motorneuron Fate

B: OPC Fate & Mitosis

C: Pro-Differentiation

A: Pro-Mitotic

B: Differentiation

C: Membrane extension

Mechanisms Regulating the Development of Oligodendrocytes and Central Nervous System Myelin

Highlights:

Extracellular factors and pathways in oligodendrocyte specification and development

Key modulators of oligodendrocyte process extension and myelin thickness

Recent ChIP-Seq findings for key oligodendrocyte transcription factors

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