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Intracellular Signaling Pathway Regulation of Myelination and Remyelination in the CNS

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Abstract

The restoration of myelin sheaths on demyelinated axons remains a major obstacle in the treatment of multiple sclerosis (MS). Currently approved therapies work by modulating the immune system to reduce the number and rate of lesion formation but are only partially effective since they are not able to restore lost myelin. In the healthy CNS, myelin continues to be generated throughout life and spontaneous remyelination occurs readily in response to insults. In patients with MS, however, remyelination eventually fails, at least in part as a result of a failure of oligodendrocyte precursor cell (OPC) differentiation and the subsequent production of new myelin. A better understanding of the molecular mechanisms and signaling pathways that drive the process of myelin sheath formation is therefore important in order to speed the development of novel therapeutics designed to target remyelination. Here we review data supporting critical roles for three highly conserved intracellular signaling pathways: Wnt/ β -catenin, PI3K/AKT/mTOR, and ERK/MAPK in the regulation of OPC differentiation and myelination both during development and in remyelination. Potential points of crosstalk between the three pathways and important areas for future research are also discussed.

Keywords

Wnt/ β -catenin; PI3K/AKT; mTOR; ERK MAPK; oligodendrocyte development; myelination; remyelination; intracellular signaling

Introduction

Oligodendrocytes (OLs) are specialized glial cells responsible for producing myelin sheaths throughout the central nervous system (CNS). OLs generate myelin by concentrically wrapping axons with multi-lamellar sheets of plasma membrane consisting of specific proteins and lipids. Myelin sheaths insulate axons and facilitate saltatory conduction thereby enabling the rapid transmission of action potentials. A number of devastating neurological

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disorders including cerebral palsy (CP) and multiple sclerosis (MS) reflect the loss of myelinating OLs (Chang et al., 2002; Franklin, 2002; Khwaja and Volpe, 2008; Woodward et al., 2006). In MS, CNS myelin is damaged as a result of autoimmune-mediated mechanisms, resulting in many sporadic focal demyelinating lesions. Fortunately, the CNS has a remarkable capacity to regenerate, and in healthy individuals as well as early in the disease course of MS, remyelination occurs readily following a demyelinating event (Crawford et al., 2013). However, with time and recurrent attacks of demyelination, remyelination eventually fails leading to significant clinical disability in affected patients (Fancy et al., 2010; Franklin and Goldman, 2015; Hagemeyer et al., 2012). It is therefore critical to clearly understand the cellular and molecular mechanisms that regulate myelination in order to develop novel therapies to target remyelination (Fancy et al., 2011a; Franklin, 2002; Franklin and Ffrench-Constant, 2008; Kotter et al., 2011; Miller and Bai, 2007; Miron et al., 2011).

OL development, from an oligodendrocyte precursor cell (OPC) to a mature myelinating OL, is controlled by a number of both inhibitory and inductive factors. In areas of white matter injury in human disease OPCs have been found in an arrested state seemingly unable to fully differentiate into myelinating OLs (Back and Rosenberg, 2014; Billiards et al., 2008; Buser et al., 2012; Chang et al., 2002; Fancy et al., 2010; Kuhlmann et al., 2008). These data suggest either a failure of the mechanisms that promote myelination or the presence of strong inhibitory molecular signals that act to suppress OPC differentiation and myelination. Recent studies have elucidated a number of intracellular signaling pathways in OLs that play important roles in the tightly regulated processes of developmental myelination and remyelination following demyelinating injury. In this review, we focus on Wnt/ β -Catenin, PI3K/AKT/mTOR, and ERK/MAPK intracellular signaling and we provide an overview of how each of these pathways affects both developmental myelination and remyelination. Because these signaling pathways are complex and affect many stages of the OL lineage, we have chosen to focus solely on the final steps of OL maturation: OPC differentiation and myelin growth. Finally, we will discuss what is known, and what has been proposed regarding how these intracellular signaling pathways may interact to control myelination.

Canonical Wnt Signaling Pathway

The wingless and integration site (Wnt) intracellular signaling cascade is highly conserved throughout evolution and plays an integral role in animal development, growth, metabolism, and maintenance of stem cells (van Amerongen and Nusse, 2009). In mammals, the canonical Wnt pathway consists of the extracellular Wnt proteins, Frizzled membrane receptors and their co-receptors, low density lipoprotein receptor-related protein 5 and 6 (LRP5/6), intracellular β -catenin, and intranuclear T-cell factors/lymphoid enhancer factors (TCF/LEF), illuminating the complex nature of this pathway (Logan and Nusse, 2004). The intracellular β -catenin destruction complex, consisting of adenomatous polyposis coli (APC), Axin, glycogen synthase kinase 3 β (Gsk3 β), and casein kinase 1 (CK1) mediates the proteasomal degradation of β -catenin and negatively regulates the pathway (Clevers and Nusse, 2012). The canonical Wnt/ β -catenin pathway is activated when extracellular Wnt ligands bind to Frizzled receptors and LRP5/6 co-receptors at the cell surface. The destruction complex is then disassembled, allowing cytosolic β -catenin to accumulate and

translocate to the nucleus, where it interacts with TCF/LEF family members to activate target gene expression (Behrens et al., 1996) (Fig. 1A). The TCF/LEF family consists of four members in mammals, although *Tcf7l2* (also known as *Tcf4*) is most highly expressed by OLs (Fancy et al., 2009; Fu et al., 2009; Fu et al., 2012; Lang et al., 2013; Lurbke et al., 2013; Ye et al., 2009).

The Wnt pathway is a key signaling mechanism that controls multiple aspects of OL development including the specification of OPCs, OPC differentiation, myelination, and remyelination (recently reviewed in detail by (Guo et al., 2015; Xie et al., 2014)). Results obtained from a number of important studies show somewhat paradoxically, that Wnt signaling can function to both inhibit and promote myelination. Here, we describe the effects of canonical Wnt signaling on the final stages of OL development.

Inhibitory effect of the canonical Wnt signaling on myelination

The canonical Wnt pathway was first recognized as an inhibitor of OL development in organotypic cultures derived from embryonic spinal cord (Shimizu et al., 2005). Multiple *in vivo* studies next confirmed an inhibitory role for Wnt signaling in OPC differentiation during early postnatal development (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009). OPC differentiation was delayed following Wnt pathway activation by expressing a dominant-active β -catenin specifically in cells of the OL lineage (*Olig2-Cre/DA-Cat* (Fancy et al., 2009) or *CNP-Cre/DA-Cat* (Feigenson et al., 2009)). These mutant mice had a decreased number of mature proteolipid protein (PLP)-expressing OLs with associated hypomyelination, while the number of OPCs was unaffected (Fancy et al., 2009; Feigenson et al., 2009). Additionally, at postnatal day 15 (P15), *Olig2Cre/DA-Cat* mice showed a significant increase in the proportion of OLs that expressed TCF7L2 (Fancy et al., 2009), an important nuclear binding partner of β -catenin (Clevers, 2006). These data suggest that β -catenin may mediate its negative effect on OPC differentiation at least in part by recruiting TCF7L2 to regulate the transcription of important Wnt pathway target genes (Fancy et al., 2009). Fancy et al. also demonstrated that *Tcf7l2* is normally expressed in OLs during postnatal development but not in adulthood, potentially rendering constitutively active β -catenin ineffectual as the mice age, and providing a potential mechanism for the delay but not complete block in OPC differentiation in the DA-cat mice.

Several regulators of the TCF7L2/ β -catenin interaction have also been identified. The SRY-Box (Sox) transcription factor 17 forms a Sox17/ β -catenin/TCF7L2 complex, which stimulates the proteasomal degradation of β -catenin and enhances OPC differentiation (Chen et al., 2013; Chew et al., 2011). Additionally, histone deacetylases HDAC1/2 compete with β -catenin for interactions with TCF7L2 and are therefore thought to regulate OPC differentiation, at least in part, by disrupting the TCF7L2/ β -catenin interaction (Ye et al., 2009). Although multiple lines of evidence suggest that β -catenin/TCF7L2 is inhibitory for OPC differentiation and subsequent myelination, other data argue against this interpretation. Two independent studies demonstrated that deletion of *Tcf7l2* inhibited OPC differentiation in the spinal cord, suggesting that TCF7L2 is actually necessary for OPC differentiation (Fu et al., 2009; Ye et al., 2009). To reconcile these conflicting results, Ye *et al.* proposed a model where TCF7L2 is switched “on” by binding to β -catenin, resulting in the inhibition of

OPC differentiation, and switched “off” by binding to HDAC1/2, promoting OPC differentiation (Ye et al., 2009). An alternative hypothesis recently proposed by Guo *et al* suggests that TCF7L2 activation might be uncoupled from the activation of Wnt/ β -catenin signaling, and that the increase in TCF7L2 expression observed in OPCs when they fail to differentiate is not causative but is rather a secondary effect of differentiation failure (Guo et al., 2015; Hammond et al., 2015).

The inhibitory effects of Wnt/ β -catenin signaling on postnatal myelination have been further investigated using additional experimental models. In culture, expression of the extracellular agonist Wnt3a caused an increase in OPC number and a decrease in PLP+ mature OLs (Azim and Butt, 2011; Feigenson et al., 2011). Mice with a conditional deletion of the β -catenin antagonist APC had a decreased number of PLP+ mature OLs as well as decreased levels of several myelin protein transcripts including *Plp*, *Cnp* and *Mbp* compared to wildtype controls (Lang et al., 2013). Interestingly, in contrast to the transient phenotype of the dominant-active β -catenin mice described above, the loss of APC resulted in sustained inhibition of differentiation, causing a hypomyelination phenotype that persisted into adulthood (Lang et al., 2013). β -catenin upregulation through deletion of *Axin2*, another member of the β -catenin degradation complex (Behrens et al., 1998; Jho et al., 2002; Zeng and Nusse, 2010), also demonstrated inhibitory effects on myelination. Genetic deletion of *Axin2* led to fewer mature OLs (Fancy et al., 2011b) and conversely, stabilization of *Axin2* with the small molecule XAV939 (Huang et al., 2009) enhanced the expression of MBP and increased the number of PLP+ OLs (Fancy et al., 2011b). Together, these studies demonstrate that both genetic and pharmacological activation of Wnt/ β -catenin impairs OPC differentiation, thereby preventing cells from maturing and producing myelin sheaths. Studies examining the consequences of endogenous β -catenin inhibition have yielded conflicting results. Conditional deletion of *β -catenin* from cells of the OL-lineage using different Cre drivers (*CNP-Cre*, *NG2-Cre*, or *Olig2-CreER^{T2}*) did not effect OPC differentiation (Lang et al., 2013). In contrast, another group used *Olig1-Cre* to conditionally delete *β -catenin* and showed a significant decrease in the number of *Plp* mRNA+ differentiated OLs from E18.5 to P15 (Dai et al., 2014b). Similarly, a third study found that *Mbp* mRNA+ OLs were diminished after hGFAP-Cre-mediated deletion of *β -catenin* in the forebrain (Gan et al., 2014). The reasons for these conflicting results remain elusive, but may include differences in cellular specificity, the timing of Cre expression and/or deletion efficiency, highlighting the inherent complexity of this signaling pathway. Conditional deletion of *β -catenin* from cells of the OL lineage using the *Olig1-Cre* occurred very early in embryogenesis at ~E10.5, while deletion of *β -catenin* did not occur until later time points either after tamoxifen injections at P6 in mice with *Olig2-CreER*, at ~E12.5 for *CNP-Cre*, or at ~E14 for the *NG2-Cre* mice. It therefore appears that OPCs may be more vulnerable to the loss of *β -catenin* early in embryonic development as opposed to later. The hGFAP promoter drives Cre expression starting at approximately E13.5, a time point comparable to *CNP-Cre* and *NG2-Cre*, however, it is expressed in progenitor cells that go on to become astrocytes, oligodendrocytes and neurons that will then all lack *β -catenin*. Thus, it appears that deletion of *β -catenin* from multiple CNS cell types is more detrimental for OPC differentiation compared to deletion of *β -catenin* solely from OPCs.

Promoting effects of the canonical Wnt signaling pathway on myelination

Although the inhibitory effects of forced activation of canonical Wnt signaling on OPC differentiation are well accepted, numerous other studies have shown that Wnt signaling can positively affect OL maturation. Several studies have demonstrated that addition of extracellular Wnt molecules can increase OPC proliferation and differentiation in culture (Kalani et al., 2008; Ortega et al., 2013; Tawk et al., 2011). Similarly, over-expression of nuclear-localized β -catenin enhanced *Plp* promoter activity (Tawk et al., 2011). Knock down of β -catenin with siRNA or a dominant negative LRP6 decreased myelin gene expression and myelination (Tawk et al., 2011), and knockout of *Tcf7l2* caused a myelin deficient phenotype (Fu et al., 2009). In order to reconcile the seemingly conflicting results obtained from multiple studies, it has been proposed that the levels TCF7L2/ β -catenin must be tightly controlled in order to achieve proper myelination (Fu et al., 2012). During myelin formation, TCF7L2 must be associated with moderate levels of β -catenin and either high or low levels of TCF7L2/ β -catenin are detrimental for myelination. Therefore the extent to which an experimental model increases the level of intranuclear β -catenin correlates with the effect on OL maturation (Fu et al., 2012).

Canonical Wnt signaling during remyelination

A number of studies have examined the role of Wnt signaling during remyelination in the adult CNS. *Olig2Cre; DA-Cat* mice exposed to a lysolecithin (LPC)-induced model of focal demyelination showed a decrease in the number of PLP+ OLs at 14 days post lesion (dpl) resulting in a delay in remyelination (Fancy et al., 2009). The same group further supported an inhibitory role for the Wnt/ β -catenin pathway during remyelination by examining the myelin repair kinetics in mice that lack one copy of APC (APCmin) or in *Axin2* null mice, both of which result in the increased accumulation of β -catenin. APCmin and *Axin2* null mice demonstrated similar deficits in remyelination 14 days after LPC-induced demyelination, each showing a reduction in the number of mature PLP+ OLs despite normal recruitment of OPCs (Fancy et al., 2009; Fancy et al., 2011b). Furthermore, stabilization of the Axin2 protein using XAV939 promoted β -catenin degradation and increased the number of PLP+ OLs compared to controls (Fancy et al., 2011b).

Interestingly, a number of studies have supported a beneficial role for TCF7L2 during remyelination. Microarray analysis of MS patient tissue uncovered higher expression of *TCF7L2* in active plaques compared to chronic silent plaques and normal-appearing white matter (Lock et al., 2002). TCF7L2 protein was found in remyelinating tissue along the edges of white matter injury in neonates with hypoxic ischemic encephalopathy and in remyelinating tissue from patients with multiple sclerosis (Fancy et al., 2009; Fu et al., 2012). Furthermore, it was demonstrated that TCF7L2 is expressed in early remyelinating plaques but not in actively demyelinating, demyelinated, or chronic lesions in MS patients, suggesting that TCF7L2 expression is limited to specific stages of OL differentiation (Lurbke et al., 2013). In the cuprizone mouse model of demyelination, TCF7L2 expression was detected at 5 weeks after cuprizone treatment when remyelination begins but was not detected at 7 weeks when the remyelination process is mostly complete (Fu et al., 2012). Further support for the role of TCF7L2 in remyelination comes from the observation that remyelination was significantly stalled in *Tcf7l2* null mice (Fu et al., 2009; Ye et al., 2009).

Overall, it appears that over activation of the Wnt pathway in the setting of demyelination is detrimental to OL development, resulting in differentiation failure and a subsequent impairment in remyelination. The expression of TCF7L2, however, appears beneficial for OPC differentiation and remyelination, as long as the β -catenin levels are tightly controlled. Together these data demonstrate that Wnt/ β -catenin signaling must be tightly regulated in order to achieve adequate remyelination.

More recently the effects of proximal aspects of the Wnt/ β -catenin signaling pathway on remyelination have been studied. *Apcdd1*, which is expressed in glial precursors (Kang et al., 2012) and binds to the LRP6 receptor to antagonize Wnt signaling (Shimomura et al., 2010), was shown to promote OPC differentiation and remyelination in both *in vitro* and *in vivo* models (Lee et al., 2015b). A role for Daam2, a protein that interacts with PIP2 to form a signalsome with the LRP6/Frizzled/Dishevelled/PIP5K receptor complex on the intracellular membrane (Lee and Deneen, 2012) to promote Wnt signaling has also been investigated (Lee et al., 2015a). LPC was injected into *Daam2*^{-/-} mice to create a focal demyelinating lesion and address whether Daam2 normally suppresses OPC differentiation during remyelination (Lee et al., 2015a). Increased numbers of MBP and PLP positive cells were found in *Daam2*^{-/-} mice compared to control littermates without any effect on the total number of OLIG2 expressing cells, further supporting an inhibitory role of Wnt/ β -catenin in OPC differentiation during remyelination. Furthermore, gain-of-function experiments revealed a dramatic decrease in mature OLs after spinal cord lesions were injected with a Daam2-expressing virus. These studies support the importance of Wnt signaling inhibition during remyelination to ensure adequate OPC differentiation, although it remains unknown whether the effects described here occurred through a β -catenin dependent (canonical) or β -catenin independent pathway.

AKT/mTOR Signaling Pathway

AKT, also known as protein kinase B, is a serine/threonine kinase that regulates many intracellular molecules involved in basic processes including cell growth, proliferation, and survival (Dudek et al., 1997; Franke et al., 1997; Kennedy et al., 1997). Growth factors such as insulin-like growth factor 1 (IGF-1) stimulate receptor tyrosine kinases (RTKs) to activate this critical intracellular signaling cascade. 3-phosphoinositide 3 kinase (PI3K) then phosphorylates PtdIns(4,5)₂ (PIP2) to generate PtdIns(4,5,3)₃ (PIP3) either directly or indirectly through other proteins such as insulin receptor substrate1 (IRS1). This phosphorylation initiates the recruitment of AKT and 3-phosphoinositide-dependent kinase I (PDK1) to the cell surface where PDK1 phosphorylates and partially activates AKT at Thr308 (Alessi et al., 1997). Mechanistic target of rapamycin complex (mTORC2) enables the complete activation of AKT through phosphorylation of Ser473 (Guertin et al., 2006; Jacinto et al., 2006; Sarbassov et al., 2005). AKT phosphorylates the GTPase-activating protein (GAP) tuberous sclerosis complex 2 (TSC2) resulting in inhibition, which in turn disinhibits the Ras homologue enriched in brain (Rheb). The disinhibition of Rheb then results in the activation of mTORC1 allowing mTORC1 to regulate many critical cellular processes. Additionally, the TSC complex directly activates mTORC2 in a Rheb independent manner (Huang et al., 2008) (Fig. 1B). The AKT/mTOR signaling pathway has been implicated in many aspects of OL development including OPC proliferation, migration,

survival, differentiation, and myelination (reviewed in detail by (Norrmén and Suter, 2013; Wood et al., 2013). Below, we focus on evidence for the importance of AKT/mTOR during OPC differentiation, myelination, and remyelination.

Effects of AKT on myelination

Many *in vitro* and *in vivo* studies have demonstrated the importance of the AKT signaling pathway in CNS myelination. Conditional knockout of the PI3K/AKT upstream inhibitor, *phosphatase and tensin homologue (PTEN)*, using either *Olig2-Cre*, *CNP-Cre* or *PLP-CreERT2* transgenic mice resulted in significant hypermyelination throughout the CNS. The hypermyelination was associated with an increase in PIP3 levels and AKT phosphorylation without an increase in the number of mature OLs, suggesting that PTEN inhibition has a direct effect on myelination without affecting OPC differentiation (Goebbels et al., 2010; Harrington et al., 2010). The effects of up-regulated AKT signaling were further confirmed using the PTEN inhibitor, bisperoxovanadium (phen), in rat and human OPCs co-cultured with dorsal root ganglion neurons. These results demonstrated increased myelination that was further potentiated when the PTEN inhibitor was combined with growth factor administration (De Paula et al., 2014). Further support for the role of AKT in myelination comes from mice that express constitutively active AKT in PLP+ OLs (*Plp-AKT-DD*), where a similar hypermyelinating phenotype occurs (Flores et al., 2008; Narayanan et al., 2009). In addition to thicker myelin, these transgenic mice demonstrated increased myelin protein expression and increased mTORC1 activity (Flores et al., 2008). As the mice aged, myelin protein expression continued to increase and the myelin continued to grow, eventually reaching pathogenic levels (Flores et al., 2008). Treatment with the mTOR inhibitor, rapamycin, was sufficient to inhibit the hypermyelination suggesting that mTOR is a downstream effector of AKT that regulates myelin growth (Narayanan et al., 2009). Another model examined the phenotype of β -site amyloid precursor protein-cleaving enzyme 1 (*Bace-1*) knockout mice (Hu et al., 2013). BACE-1 is an enzyme that cleaves neuregulin-1 type III (NRG1-III) and is an important upstream enzyme that activates AKT signaling (Hu et al., 2006). *Bace-1* knockout mice displayed CNS hypomyelination and decreased phosphorylated AKT (Hu et al., 2006), a phenotype that was rescued when these mice were crossed with *Plp-AKT-DD* mice (Hu et al., 2013). These data suggest that NRG1 is an upstream activator of the AKT pathway and further support the importance of AKT signaling for developmental myelination (Hu et al., 2013).

Effects of mTOR on OPC differentiation and myelination

A number of excellent studies have provided compelling evidence that the AKT signaling downstream effector, mTOR is critically important for OPC differentiation and myelination. Treatment with the mTOR inhibitor rapamycin in cell culture resulted in deficits in OPC differentiation along with reduced expression of major myelin proteins and mRNAs, although the timing of these defects appears to vary depending on *in vitro* conditions (Guardiola-Diaz et al., 2012; Tyler et al., 2009; Tyler et al., 2011). Wild type mice exposed to rapamycin during development also demonstrated CNS hypomyelination supporting a role for mTOR in myelination *in vivo* (Narayanan et al., 2009). Several recent *in vivo* studies have used Cre-LoxP technology to conditionally delete different components of the mTOR signaling pathway. These studies examined the effects of genetically manipulating the

activity of mTOR itself, the mTORC1 protein subunit Raptor, or the mTORC2 protein subunit Rictor in OLs (Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014; Zou et al., 2014). In the spinal cord, *CNP-Cre;mTOR* (Wahl et al., 2014) and *CNP-Cre;Raptor/Rictor* (Lebrun-Julien et al., 2014) mice both showed an impairment in the initiation of myelination as demonstrated by a decreased percentage of myelinated axons at an early developmental time point. Additionally, both mutants demonstrated significant hypomyelination that persisted until adulthood. Interestingly, although the myelin that was observed was quite thin, it appeared to have normal ultrastructure. To determine whether the hypomyelination was a consequence of impaired differentiation, both groups examined the number of CC1+ mature OLs. Conditional knockout of either *mTOR* or *Raptor/Rictor* resulted in decreased numbers of mature OLs, suggesting that loss of mTOR signaling in the spinal cord leads to significant deficits in OPC differentiation that ultimately result in hypomyelination.

In order to probe the individual roles of the mTORC complexes in the spinal cord, conditional deletion of *Raptor* or *Rheb1*, the immediate early gene that functions to activate mTORC1, was used to assess the function of the mTORC1 complex, while conditional deletion of *Rictor* enabled the assessment of the function of mTORC2 (Bercury et al., 2014; Lebrun-Julien et al., 2014; Zou et al., 2014). *CNP-Cre;Raptor*, *Olig1-Cre;Rheb1*, and *CNP-Cre;Rheb1* mice all demonstrated an impairment in the initiation of myelination and hypomyelination similar to the *mTOR* and *Raptor/Rictor* conditional knockout mice. Two studies found that OPC differentiation was impaired following inactivation of mTORC1 (Bercury et al., 2014; Zou et al., 2014), however, a third study did not report any defects in OPC differentiation following conditional deletion of *Raptor* (Lebrun-Julien et al., 2014). Compared to mTORC1, the effects of the loss of mTORC2 on myelination were much more subtle. Bercury *et al* demonstrated that loss of mTORC2 was not associated with a delay in myelin initiation at P14 or a difference in myelin thickness at P60, but did result in decreased NG2+ cells and increased CC1+ cells at P14, suggesting that precocious OL development may occur in the absence of Rictor. In another study, transient hypomyelination was reported at the slightly earlier time point of P10 in *CNP-Cre; Rictor* mice but no significant changes in OPC differentiation were observed (Lebrun-Julien et al., 2014).

The dynamic nature of myelin sheaths throughout the adult CNS has been supported by recent studies that demonstrate the continued generation of myelin throughout life (Young et al., 2013). A continued role for mTOR signaling was therefore assessed in adult OLs after developmental myelination was complete using tamoxifen-inducible *PLP1-CreRT2* mice to delete *Raptor*, *Rictor*, or *Raptor/Rictor* (Lebrun-Julien et al., 2014). The inducible conditional knockout mice were treated with tamoxifen at 2 months of age to induce recombination. Twelve months later, hypomyelination was observed in *PLP1-CreRT2; Raptor* and *PLP1-CreRT2; Raptor/Rictor* mice but not in *PLP1-CreRT2; Rictor* mice, demonstrating that mTORC1 plays an important role in maintaining myelin within the spinal cord (Lebrun-Julien et al., 2014).

Surprisingly, the effects of *mTOR*, *Raptor*, *Rictor*, or *Raptor/Rictor* deletion on OPC differentiation and myelination in the brain were overall much less severe than in the spinal cord. *CNP-Cre;mTOR* mice showed normal myelination and normal myelin ultrastructure in

the corpus callosum (Wahl et al., 2014). Additionally, OPC differentiation occurred without delay and an increased percentage of OL-lineage cells were found to be CC1+ in the corpus callosum at P14 (Wahl et al., 2014). In contrast, *Olig1-cre;mTOR* mice showed hypomyelination visualized by reduced numbers of Black Gold-positive myelinated fibers in the cortex and corpus callosum that was associated with a decreased number of mature CC1+ OLs in the cortex and corpus callosum and an increase in the number of PDGFR α + progenitor cells in the cortex (Zou et al., 2014). Combined deletion of *Raptor/Rictor* resulted in hypomyelination in the cerebellum at P60, however effects on OPC differentiation were not examined (Lebrun-Julien et al., 2014). In the brain, deletion of mTOR at an earlier time point in the OL lineage by using an *Olig1-Cre* compared to a *CNP-Cre* therefore appears to result in a more severe phenotype. Similar to the spinal cord, the effect of the individual mTOR complexes revealed a more important role for mTORC1 compared to mTORC2 in regulating myelination and OPC differentiation in the brain. In the corpus callosum, *CNP-Cre;Raptor* mice demonstrated normal initiation of myelin, normal myelin ultrastructure, and normal myelin thickness at P29 and P60, although a transient delay in OPC differentiation was observed at P29 (Bercury et al., 2014). In the *CNP-Cre;Raptor* cerebellum, however, hypomyelination was observed at P60 similar to the *Raptor/Rictor* combined conditional knockout mice (Lebrun-Julien et al., 2014). *CNP-Cre;Rheb1* mice did not show defects in the initiation of myelination or the differentiation of OPCs in the cortex or corpus callosum (Zou et al., 2014). Importantly, however, deletion of *Rheb1* using an *Olig1-Cre* or an *Olig2-Cre* resulted in the delayed initiation of myelination in the optic nerve, hypomyelination of the corpus callosum and cortex, and impaired OPC differentiation (Zou et al., 2014). These data suggest that *Rheb1* plays a critical role in myelination of the brain and is important during a specific time window early in the development of OPCs.

Although mTOR is classically known to regulate translation, several studies reported that levels of both myelin mRNAs and proteins were reduced following inactivation of mTOR signaling (Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014; Zou et al., 2014). These data suggest that mTOR and its associated complexes are regulators of myelination at the transcriptional and translational level. It is possible that mTOR may play a specific role in regulating the transcription of important myelin genes in OLs, or it may regulate the translation of transcription factors that ultimately control the transcription of specific myelin mRNAs (Bercury et al., 2014). Interestingly, the only myelin protein for which translation was specifically effected following the loss of mTOR signaling was MBP. In the spinal cord, mTOR deletion resulted in significantly reduced MBP protein expression at P25 and P60 without a change in mRNA levels (Wahl et al., 2014). Additionally, three independent studies demonstrated significantly decreased MBP protein expression following inactivation of mTORC1 at several developmental time points that were accompanied by minimal or no changes in MBP mRNA levels (Bercury et al., 2014; Lebrun-Julien et al., 2014; Zou et al., 2014). Thus, one important role for mTORC1 during myelination may be to control the trafficking and/or local translation of MBP.

Another mechanism through which mTORC1 appears to regulate myelination is by controlling lipid synthesis via the regulation of SREB1 and SREB2, transcription factors known to regulate genes encoding enzymes required for lipid synthesis. *CNP-Cre;Raptor/Rictor* and *CNP-Cre;Raptor* mice both demonstrated decreased expression of SREB1

downstream targets FA synthase (FASN) and stearoyl-CoA desaturase-1 (SCD1) in the spinal cord (Lebrun-Julien et al., 2014). Furthermore, SREBP2 protein and mRNA expression were reduced along with SREBP2 targets HMG-CoA reductase (HMGCR) and isopentenyl-diphosphate delta isomerase 1 (IDI1). The exact mechanism through which the Akt/mTOR signaling pathway interacts with SREBs to control lipid synthesis in OLs will be an important question to answer in the future.

Overall, data from these recent *in vivo* studies support that mTOR is a key regulator of OPC differentiation and myelination throughout the CNS, consistent with previous *in vitro* data (Guardiola-Diaz et al., 2012; Tyler et al., 2009; Tyler et al., 2011). This regulation occurs by ensuring appropriate OPC differentiation, myelin protein transcription and translation, initiation of myelination and myelin thickness, and lipid biogenesis. Furthermore, mTORC1 and mTORC2 are likely not interchangeable as these complexes seem to have unique functions within the OL developmental program. The differences found between the OPC differentiation and myelination phenotypes observed in the brain and spinal cord, are intriguing and suggest that OLs in the two regions may be heterogeneous, and that in the brain, alternative signaling pathways may be able to compensate more effectively for the absence of mTOR or the individual mTOR complexes to ensure appropriate OL development and myelination.

Additional studies have explored whether activation of mTORC1 might be sufficient to drive OPC differentiation and hypermyelination. In one study, mTORC1 was activated by deleting the mTORC1 inhibitor, Tuberous Sclerosis 1 (*Tsc1*), specifically in CNP expressing OLs, and was confirmed by demonstrating increased activation of the mTORC1 downstream target S6K (Lebrun-Julien et al., 2014). Surprisingly, *Tsc1* conditional knockout mice demonstrated hypomyelination in the spinal cord similar to the phenotype observed in the *Raptor* (mTORC1) conditional knockout mice. The authors then went on to show that although the activation of mTORC1 was clearly increased in the *Tsc1* CKO mice, levels of p4E-BP1 and pAKT at T308 and S473 were decreased. These data suggest that increased mTORC1 activity may result in a negative feedback loop that results in defective PI3K/AKT signaling, a finding that is supported by an earlier study (Zhang et al., 2003). Furthermore, the decreased phosphorylation of AKT at S473 suggests that impaired activation of mTORC2 may also occur in this model and may contribute to the observed hypomyelination (Lebrun-Julien et al., 2014). Finally, mTORC1 over activation also resulted in reductions in SREBP2 as well as SREBP targets similar to what was observed in Raptor mutants, demonstrating that precisely balanced activation of mTORC1 is necessary for lipogenesis. Another study reported a transient increase in the number of mature OLs and precocious myelination in the brain seen early in development in mice where increased mTORC1 activation was achieved in all neural cells by expressing a mutant *Rheb1* (*S16H*) transgene that is relatively resistant to TSC GTPase-activating activity (Zou et al., 2011). In adult mice, however, electron microscopy revealed comparable myelination in the corpus callosum of *Rheb1 S16H* and control littermates demonstrating that increased mTORC1 activity in this context did not result in hypomyelination. A number of factors likely contribute to the different phenotypes observed in these 2 models of mTORC1 activation, perhaps most notably the fact that the *Rheb1* (*S16H*) transgene was expressed in all neural cells instead of OLs alone. Overall, it is clear that mTOR is an important regulator of myelination within a

multifaceted signaling network that likely requires a delicate balance to ensure appropriate and timely OL differentiation and myelination throughout the CNS. Additionally, it is likely that there are other important downstream targets and complex interactions that we have yet to fully understand that undoubtedly play critical roles in this process.

Effect of the AKT signaling pathway on remyelination

Compared to its role in developmental myelination, the AKT pathway has been less intensely studied in experimental models of remyelination. In the *Olig2-Cre; PTENCKO* mouse model, characterized by over activation of the AKT pathway, there was no detectable improvement in remyelination and the hypermyelination seen during development was not recapitulated during recovery from an LPC-mediated focal demyelination in the adult mouse spinal cord (Harrington et al., 2010). To further examine the role of AKT signaling during remyelination, mice with deletion of the PI3-kinase enhancer (PIKE) were studied in an LPC-induced model of demyelination in the corpus callosum. PIKE is a GTPase (Ye and Snyder, 2004) that connects extracellular signals (netrin, glutamate, and neurotrophins) to the intracellular PI3K/AKT signaling cascade (Chan et al., 2012; Hu et al., 2005; Liu et al., 2008). Conditional knockout of *PIKE* in PLP⁺ OLs resulted in impaired OPC proliferation along with decreased differentiation and remyelination (Chan et al., 2014). These results suggest that the AKT pathway plays an important role during remyelination by ensuring the adequate generation of OPCs and the appropriate differentiation of OLs. In an LPC-induced model of focal demyelination, deletion of *Cdk5* from CNP⁺ cells or inhibition of CDK5 with roscovitine resulted in impaired OPC differentiation and delayed remyelination that was accompanied by decreased levels of activated AKT (Luo et al., 2014). These data suggest that CDK5 may be an important regulator of intracellular signaling, at least in part by promoting the activation of AKT following demyelination (Luo et al., 2014). In addition to the LPC model, the experimental autoimmune encephalomyelitis (EAE) model is frequently used to study the processes of demyelination and remyelination. Following induction of EAE, stimulation of the estrogen receptor β has been correlated with increased AKT activity and reduced functional disability, suggesting improved remyelination (Crawford et al., 2010; Kumar et al., 2013; Moore et al., 2014). Important future studies will undoubtedly uncover additional roles for AKT throughout this critical regenerative process.

ERK MAPK Signaling Pathway

The extracellular signal-regulated kinases, *Erk1* and *Erk2*, are prototypic members of the mitogen activated protein kinase (MAPK) family (Rubinfeld and Seger, 2005). The ERK MAP Kinase (ERK/MAPK) pathway is a ubiquitous, well-conserved signaling pathway that transduces extracellular signals through an intracellular signal transduction cascade to ultimately control the expression of genes that regulate important processes such as cell proliferation, differentiation, and survival. Growth factors such as PDGF and FGF-2 (Bansal et al., 2003; Bhat and Zhang, 1996; Yim et al., 2001) and neurotrophins such as NGF, NT3, and BDNF (Althaus et al., 1997; Du et al., 2006; Kumar et al., 1998) activate ERK/MAPK signaling by binding to tyrosine kinase receptors at the cell surface. Subsequent activation of the Ras family of GTPases results in the phosphorylation of Raf (MAP3K) (Raman et al., 2007). Raf in turn phosphorylates mitogen/extracellular signal kinases, MEK 1 and MEK 2,

which are the immediate upstream activators of ERK1 and ERK2 (Raman et al., 2007). Once phosphorylated, the ERK proteins move into the nucleus to regulate the expression of a number of different genes (Fig. 1A). ERK1 and ERK2 proteins exhibit >80% sequence homology and have identical substrate specificity (Boulton et al., 1991), but mice deficient for each of these genes are phenotypically very different (Aouadi et al., 2006; Rubinfeld and Seger, 2005). *Erk1* knockout mice are viable (Mazzucchelli et al., 2002), while the *Erk2* knock out mice die during embryogenesis (Hatano et al., 2003; Saba-El-Leil et al., 2003; Yao et al., 2003) suggesting that these two structurally similar proteins may have distinct roles. ERK signaling has been implicated in many different aspects of OL development including proliferation, migration, survival, differentiation and myelination (recently reviewed in detail by (Gonsalvez et al., 2015)). Here, we review evidence for the role of ERK1/2 during OPC differentiation, myelination and remyelination.

Effects of the ERK/MAPK Pathway on OPC Differentiation

A number of *in vitro* studies have pointed to an important role for the ERK/MAPK pathway during OPC differentiation (Baron et al., 2000; Dai et al., 2014a; Fyffe-Maricich et al., 2011; Galabova-Kovacs et al., 2008; Guardiola-Diaz et al., 2012; Stariha et al., 1997; Younes-Rapozo et al., 2009). In these studies, pharmacologic inhibition of ERK/MAPK signaling in primary cultures of mouse or rat OPCs revealed a decrease in the number of OPCs that differentiated to mature myelinating OLs. Additionally, several recent studies have demonstrated a strong correlation between increased ERK activity and OPC differentiation. IL-17A, a cytokine found in active MS lesions was shown to increase both ERK1/2 activity and OPC differentiation when applied to cerebellar slice cultures (Rodgers et al., 2015). Phosphorylation of AKT and I κ B was unchanged after stimulation with IL-17A, suggesting that this cytokine specifically activates the ERK/MAPK pathway. Although the results did not reach significance, there was a clear trend toward a reversal of the enhanced OPC differentiation phenotype after treatment with an ERK inhibitor, supporting that IL-17A is promoting OPC differentiation through the ERK pathway (Rodgers et al., 2015). In another study, TAPP1, a PH domain-containing adapter protein, was knocked down in primary OPC cultures resulting in increased levels of both total and activated ERK1/2 protein along with enhanced OPC differentiation (Chen et al., 2015). Changes in AKT expression were not observed in these experiments suggesting that TAPP1 functions as a negative regulator of OPC differentiation specifically through its inhibition of ERK1/2.

In contrast to experiments performed in culture, *in vivo* studies have yielded mixed results regarding the role of ERK1/2 in OPC differentiation, illuminating the complex nature of this signaling pathway. One study investigated the effects of conditional knockout of *B-Raf*, an upstream kinase that activates MEK, in neural progenitor cells (NPCs) of developing mice (Galabova-Kovacs et al., 2008). This study demonstrated impaired OPC differentiation with an increased number of immature PDGFR α ⁺ OPCs and a decreased number of premyelinating and myelinating OLs at postnatal day 18 (P18) (Galabova-Kovacs et al., 2008). Another study investigated the effects of *Erk2* deletion from hGFAP⁺ radial glial cells and found that O4⁺ OPCs were inhibited from differentiating into O1⁺ OLs when grown in culture and that MBP expression was decreased in the corpus callosum at P10 suggesting an impairment in OPC differentiation (Fyffe-Maricich et al., 2011). Additional

experiments where both *Erk1* and *Erk2* were specifically manipulated in OPCs have failed to demonstrate clear effects on OPC differentiation (Ishii et al., 2012; Xiao et al., 2012). Mice with conditional deletion of *Erk2* from CNP+ cells on an *Erk1* null background (*Erk1/2* dKO) failed to demonstrate any defects in OPC differentiation compared to littermate controls at two different developmental time points (Ishii et al., 2012). Furthermore, sustained activation of ERK1/2 in cells of the OL-lineage was not sufficient to drive increased OPC differentiation (Fyffe-Maricich et al., 2013; Ishii et al., 2013; Xiao et al., 2012). To address the discrepancies between studies done in culture and those done in mice, we hypothesize that when both *Erk1* and *Erk2* are deleted specifically from OPCs, alternative pathways can be activated *in vivo*, but not *in vitro*, and are able to compensate for the function of ERK1/2 during OPC differentiation. Future studies are needed to determine the nature of these potential compensatory pathways. In summary, the abundance of *in vivo* data collected to date supports that at best, ERK/MAPK signaling plays a minimal role in OPC differentiation compared to its main role in controlling myelin growth, which is discussed in detail below.

Effects of ERK/MAPK pathway on myelination

The dominant role of ERK1/2 in myelin sheath expansion was uncovered by analyzing data from both *Erk1/2* dKO mice and mice with sustained activation of ERK1/2. *Erk1/2* dKO mice demonstrated significant hypomyelination with associated decreases in myelin gene mRNA and protein expression that persisted to adulthood (Ishii et al., 2012), in contrast to *Erk2* conditional knockout mice where the hypomyelination phenotype was transient (Fyffe-Maricich et al., 2011). Importantly, since equal numbers of PLP+ mature OLs were found in the *Erk1/2* dKO mice compared to control littermates, but transcripts of the major myelin protein mRNAs *Mbp* and *Plp* were decreased, the reduction in myelin thickness was thought to be a direct result of a decrease in the ability of each individual OL to produce sufficient myelin proteins (Ishii et al., 2012). Importantly, since *Erk1/2* dKO OLs ensheathed all axons with only a few wraps of compact myelin regardless of their size, it appears that ERK1/2 are not essential for axonal contact or the initiation of myelination but that they are required for the proper tailoring of myelin thickness to axon diameter. Similar to *Erk1/2* dKO mice, OL-specific knockout of the neurotrophin receptor (*TrkB*) or growth factor receptors (*Fgfr1/2*), both of which signal through the ERK/MAPK pathway, had no effect on the total number of mature OLs, or the contact and ensheathment of axons, however, significant deficits in myelin thickness were noted (Furusho et al., 2012; Wong et al., 2013).

Appropriate signaling through the ERK/MAPK pathway is clearly necessary for proper myelination, but is sustained activation of this pathway sufficient to drive hypermyelination? To address this question, constitutively active MEK (CA-MEK) was expressed in CNP+ OLs resulting in the sustained activation of ERK1/2. In these transgenic mice, myelin sheath thickness was robustly increased during development and continued to increase slowly over time (Fyffe-Maricich et al., 2013; Ishii et al., 2013). Furthermore, myelinating co-cultures transfected with CA-MEK, showed a robust increase in MAG and MBP expression in addition to an increase in the number of myelinated axonal segments (Xiao et al., 2012). In an additional *in vivo* model, where the basal polarity complex protein Scribble was conditionally deleted in OLs, increased myelin thickness was correlated with a robust

increase in ERK1/2 activation in the optic nerve and spinal cord (Jarjour et al., 2015). Recent data demonstrate that ERK/MAPK signaling is also important for myelin maintenance in the adult and likely functions at least in part by driving expression of *Myrf*, a master transcriptional regulator of critical myelin genes (Ishii et al., 2014). The complete picture of how the ERK proteins regulate myelination is still unclear, highlighting the need for future studies to uncover precisely how this pathway functions to regulate the expansion of myelin sheaths.

Effect of the ERK/MAPK signaling pathway on remyelination

Strong evidence from multiple *in vitro* and *in vivo* models supports a role for the ERKs in remyelination. Mice with sustained activation of ERK1/2 in CNP+ OLs initiated remyelination faster than control littermates starting 7 days after an LPC-induced demyelinating lesion in the adult spinal cord. In this same experimental model, newly generated myelin sheaths were significantly thicker in mutant mice compared to controls 7 weeks after demyelination, a time when remyelination was complete in all mice (Fyffe-Maricich et al., 2013). These data are significant because a pathological hallmark of remyelination is that myelin sheaths generated following demyelinating injury are often thinner than those generated during development (Blakemore, 1974; Ludwin and Maitland, 1984; Suzuki et al., 1969), potentially rendering axons vulnerable to future damage.

Over the last few years, a number of small molecules have been shown to increase remyelination while concurrently increasing ERK1/2 activity. In an LPC-induced model of demyelination in cerebellar slice culture, treatment with the 3'-5'-cyclic adenosine monophosphate (CAMP)-dependent phosphodiesterase 4 (PDE4) inhibitor rolipram, improved remyelination and was associated with increased levels of activated ERK1/2 (Sun et al., 2012). The effects of rolipram on remyelination were further supported in a cuprizone-induced model of demyelination where mice showed improved remyelination after treatment with rolipram during a 10-day recovery period (Sun et al., 2012). A similar effect of accelerated remyelination was also seen following treatment with diosgenin, a plant-derived steroidal sapogenin, in a model of cuprizone-induced demyelination (Xiao et al., 2012). The ERK/MAPK pathway was thought to be the main downstream effector in these experiments as the increases in MBP protein expression seen after diosgenin treatment were blocked by the MEK inhibitor PD98059 (Xiao et al., 2012). Excitingly, the FDA-approved medication, miconazole was recently shown to improve remyelination in adult mice. Twelve days following LPC-induced demyelination in the spinal cord, more than 70% of axons were remyelinated in miconazole-treated mice compared to only 6% in vehicle treated animals, demonstrating a remarkable enhancement of remyelination (Najm et al., 2015). Exploration of the signaling pathways influenced by miconazole using RNA sequencing and phosphoproteomic analyses revealed that proteins in the MAPK pathway were among those most significantly altered. Robust activation of ERK1/2 occurred in response to miconazole treatment in both mouse and human OPCs inducing their differentiation, while miconazole treatment of mouse fibroblasts failed to activate ERK1/2, indicating potential cell-type specificity (Najm et al., 2015).

Although the correlations between ERK/MAPK pathway activation and enhanced remyelination are exciting, and promotion of remyelination following genetic manipulation of this pathway is very promising, the molecular mechanisms and ERK1/2 downstream targets critical for driving the process of remyelination still remain poorly understood. Recently, a role for ERK2 in the translational control of MBP was uncovered during remyelination in the adult mouse brain (Michel et al., 2015). *CNP-Cre; Erk2* conditional knockout mice demonstrated a significant delay in remyelination following LPC-induced demyelination of the corpus callosum. This deficit in remyelination was associated with a specific decrease in MBP protein levels, while *Mbp* mRNA levels were comparable to those of control littermates. This study went on to show that ERK2 influences the rate of remyelination at least in part by controlling the translation of MBP. Future studies are necessary to elucidate additional molecular mechanisms and downstream targets of this pathway in order to accelerate the development of therapeutics aimed to enhance remyelination.

Crosstalk

In an intact animal, in either the context of normal development or following injury, OLs receive multiple signals that are dynamic in nature. These signals are often received simultaneously and may even present conflicting information. All of these signals must be integrated and then transduced in order to elicit appropriate responses such as the production of myelin. It therefore seems reasonable that the many signaling pathways that are each known to be independently critical for myelination may in fact be quite intimately intertwined and that appropriate and efficient myelination occurs only when these interactions are maintained. Recent interest in the role of signaling proteins in myelination has focused on the potential for interactions among several major intracellular signaling pathways. Here, we summarize both what is known and what has been hypothesized regarding interactions between members of the 3 major pathways discussed in this review.

ERK/MAPK and AKT/mTOR

Interactions between the ERK/MAPK and AKT/mTOR signaling pathways seem likely based on the fact that similar myelin phenotypes are observed after sustained activation of either pathway. Augmented signaling through either pathway is sufficient for increased myelin thickness without an accompanying increase in OL proliferation or differentiation (Flores et al., 2008; Fyffe-Maricich et al., 2013; Goebbels et al., 2010; Harrington et al., 2010; Ishii et al., 2013; Xiao et al., 2012). Recent studies have focused on whether these pathways function independently, sequentially, or whether crosstalk between them is important for mediating their effects on OL development and myelination through a common mechanism. Outside of the field of myelination, particularly in the field of cancer research, ERK and AKT pathways are thought to be part of a complex signaling network with multiple points of crosstalk (Aksamitiene et al., 2012; Wood et al., 2013). In tuberous sclerosis, a tumor syndrome caused by mutations in *TSC1* or *TSC2* genes, both ERK1/2 and AKT are thought to contribute to disease progression since they can each independently phosphorylate and inactivate TSC2 at distinct residues, resulting in the activation of mTORC1 (Ma et al., 2005). It is therefore possible that TSC2 represents a similar critical

site of interaction between these two pathways in OLs where they may cooperate to regulate mTOR activation and subsequent myelination. Recently, a number of studies have each demonstrated unique interactions between AKT and ERK1/2 signaling in OLs. One *in vitro* study suggested that these two pathways act sequentially to mediate OPC differentiation, with ERK1/2 mediating the transition of the early OPCs to immature OLs and then AKT/mTOR regulating the maturation from immature to mature oligodendrocytes (Guardiola-Diaz et al., 2012). A second study used both *in vitro* and *in vivo* experiments to demonstrate crosstalk between the two pathways that occurs in a well-coordinated unidirectional manner. AKT/mTOR inhibition was shown to significantly increase the activation of ERK1/2, while inhibition of ERK1/2 had little to no effect on AKT/mTOR activity (Dai et al., 2014a). It was hypothesized that this crosstalk occurs through feedback onto the Insulin Receptor Substrate-1 (IRS-1). This model proposes that under normal conditions, there is a balanced relationship between the AKT/mTOR and ERK1/2 pathways. After inhibition of mTOR, p70S6K activity is reduced such that it no longer feeds back to inhibit IRS-1. IRS-1 is then able to activate the ERK/MAPK pathway, culminating in the activation of ERK1/2 (Dai et al., 2014a). This model suggests a convergence point between the PI3K/AKT and ERK/MAPK pathways on the cell surface through their interactions with IRS-1.

Our group recently published data pointing to p70S6K as an additional point of convergence between the two pathways at least in the context of remyelination. OL-specific knockout of *Erk2* resulted in delayed MBP translation and decreased activation of p70S6K and its downstream target S6RP, both important regulators of translation, during a critical window when pre-myelinating OLs were transitioning to mature OLs capable of generating new myelin sheaths (Michel et al., 2015). Importantly, the activation of mTOR, a classical regulator of p70S6K (Lebrun-Julien et al., 2014; Wood et al., 2013) was seemingly unaffected by the loss of ERK2. These data led us to propose a model where the appropriate timing of MBP protein expression during remyelination requires a critical threshold of p70S6K activation that is achieved only after the coordinated activation of both ERK/MAPK and AKT/mTOR pathways.

Together, these studies provide evidence for two critical points of communication between these signaling pathways in OLs. Interestingly one point of crosstalk occurs in the proximal aspect of the signaling pathways at the level of IRS-1, while the second point occurs much further downstream where the 2 pathways appear to converge at the level of p70S6K activation (see Figure 1). It is possible that that these two points of interaction complete a regulatory loop that permits crosstalk between the two pathways by allowing signals from the ERK/MAPK pathway to feedback onto PI3K/AKT/mTOR through the inhibition of IRS-1 by p70S6K. Future studies will undoubtedly uncover additional sites of convergence for these two critically important pathways.

Wnt/ β -Catenin, AKT, and GSK3 β

Early evidence for a potential interaction between the Wnt/ β -catenin pathway and AKT/mTOR signaling in OLs came from an *in vitro* study in which mTOR was inhibited with rapamycin resulting in the increased expression of the Wnt/ β -catenin pathway downstream effector *Tcf712* (Tyler et al., 2009). The results from these *in vitro* studies suggest that the

AKT/mTOR pathway may be a negative regulator of TCF712 expression and as discussed earlier, TCF712 has been reported to have both beneficial and negative effects on OL development. More recent evidence for pathway integration comes from a study by Luo et al where a connection between GSK3 β and the PI3K/AKT pathway was proposed at the level of the atypical cell cycle kinase, *Cdk5*. OL-specific conditional knockout of *Cdk5* resulted in decreased levels of phosphorylated AKT along with increased Gsk3 β activity associated with impaired OPC differentiation and myelination following LPC-induced demyelination (Luo et al., 2014). These data suggest that *Cdk5*, a kinase that is known to be critical for OPC development, is an important integrator of signals from multiple pathways.

ERK/MAPK, Wnt/ β -catenin, and AKT/mTOR

To date, no studies have reported a direct interaction between the ERK/MAPK, Wnt/ β -catenin, and AKT/mTOR pathways in the context of OL development and CNS myelination. Interestingly, GSK3 β is known to communicate with both the ERK/MAPK and the PI3K/AKT pathways in other cell types (McCubrey et al., 2014). In cancer cells, ERK1/2 phosphorylates and inactivates GSK3 β leading to the up-regulation of β -catenin (Ding et al., 2005; McCubrey et al., 2014). The ERK/MAPK pathway is therefore potentially part of a complex regulatory mechanism responsible for the tight control of Wnt/ β -catenin signaling within OLs. Furthermore, GSK3 β increases the activity of p70S6K together with mTOR, in many non-neuronal cell cultures (Shin et al., 2011). Since p70S6K is also a proposed point of convergence for ERK/MAPK and AKT/mTOR in OLs (Michel et al., 2015), it may function as a key node where all three pathways converge to affect myelination, possibly through the regulation of myelin protein translation. Future studies will address these hypotheses and elucidate exactly how these pathways may work together to ensure appropriate and timely myelination and remyelination.

Conclusions

Evidence to date demonstrates important roles for each of the individual signaling pathways discussed here throughout myelination and remyelination, though it is likely that none of them are acting alone. Overall, the data suggest that the Wnt/ β -catenin and Akt/mTOR pathways both play critical roles during OPC differentiation and myelination, while *in vivo* data point to a dominant role for the ERK/MAPK pathway in directly regulating myelin sheath expansion. It is important to consider the large number of factors that contribute to the extent (focal vs. widespread) and timing (acute vs. sustained) of the activation of these pathways. These factors include the specific region of the CNS, the stage of the OL lineage, the nature of the activating signal, and communication between multiple different pathways. Sustained activation of one signaling pathway in OLs in the brain or spinal cord may have different consequences when compared to a short burst of activation of the same pathway in OLs in either the brain or the spinal cord. It is also important to consider that the forced expression of signaling proteins may not truly reflect developmental physiologic states and experiments that use these techniques should be interpreted with caution. The most promising therapeutics for demyelinating disorders will ultimately rely on suppression of the obstacles that inhibit the terminal differentiation of OPCs in combination with other strategies designed to drive the process of new myelin formation. Successful achievement of

these goals will rely on creative experimental design that will enable us to accurately assemble of all the possible highways of communication within this large signaling road map and to compile a comprehensive list of downstream targets essential for myelination.

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Figure 1.

Schematic representation of the (A) Wnt/ β -catenin, (B) AKT/mTOR, (C) ERK/MAPK signaling networks that are important for oligodendrocyte development and myelination. Extracellular ligands are shown binding to their respective receptors to activate downstream signaling pathways. A detailed description of these interactions is provided in the text. Arrows indicate positive interactions and bars indicate inhibitory interactions. Black lines denote pathways described in oligodendrocytes, orange lines denote potential points of communication between the three pathways. Orange dotted lines denote interactions described in other cell types that may be conserved in oligodendrocytes. LRP5/6: Low density lipoprotein receptor-related protein 5 and 6, RTK: receptor tyrosine kinase, APC: adenomatous polyposis coli, CK1: casein kinase 1, GSK3 β : glycogen synthase kinase 3-beta, CDK5: cyclin dependent kinase 5, mTOR: mechanistic target of rapamycin, mTORC: mechanistic target of rapamycin complex, PI3K: phosphoinositide-3-kinase, IRS-1: insulin receptor substrate 1, PIP2: phosphatidylinositol 4,5-bisphosphate, PIP3: phosphatidylinositol 3,4,5 trisphosphate, PTEN: phosphatase and tensin homolog, PDK1: 3-phosphoinositide-dependent protein kinase-1, TSC, tuberous sclerosis complex, Rheb: ras homolog enriched in brain, MEK: mitogen activated kinase kinase, ERK: extracellular signal related kinase, TCF/Lef: T-cell factor/lymphoid enhancer factor, SREB: sterol regulatory element-binding proteins, TF: transcription factors, HDAC: histone deacetylases