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Title - Canonical Wnt signaling in the oligodendroglial lineage--puzzles remain

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Main Points: (250 characters including spaces, Max. 250)

1. Canonical Wnt/β-catenin signaling has multiple effects on oligodendrogenesis.
2. Forced Wnt signaling inhibits oligodendrogenesis in a context-dependent manner.
3. Endogenous Wnt signaling is dispensable for postnatal oligodendrocyte differentiation.

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Abstract

The straightforward concept that accentuated Wnt signaling via the Wnt-receptor-β-catenin-TCF/LEF cascade (also termed canonical Wnt signaling or Wnt/β-catenin signaling) delays or blocks oligodendrocyte differentiation is very appealing. According to this concept, canonical Wnt signaling is responsible for remyelination failure in multiple sclerosis and for persistent hypomyelination in periventricular leukomalacia. This has given rise to the hope that pharmacologically inhibiting this signaling will be of therapeutic potential in these disabling neurological disorders. But current studies suggest that Wnt/β-catenin signaling plays distinct roles in oligodendrogenesis, oligodendrocyte differentiation and myelination in a context-dependent manner (CNS regions, developmental stages), and that Wnt/β-catenin signaling interplays with, and is subject to regulation by, other central nervous system factors and signaling pathways. On this basis, we propose the more nuanced concept that endogenous Wnt/β-catenin activity is delicately and temporally regulated to ensure the seamless development of oligodendroglial lineage cells in different contexts. In this review, we discuss the role Wnt/β-catenin signaling in oligodendrocyte development, focusing on the interpretation of disparate results, and highlighting areas where important questions remain to be answered about oligodendroglial lineage Wnt/β-catenin signaling.

I: Introduction

Myelination serves important roles in saltatory conduction of axonal signals and in axonal trophic and metabolic support (Nave, 2010; Funfschilling et al., 2012; Lee et al., 2012). CNS myelin is generated by oligodendrocytes (OLs) during development and throughout adult life (Young et al., 2013). Oligodendroglial development encompasses multiple tightly-regulated steps: OPC specification from embryonic neural stem cells (NSCs) (also termed radial glia, RG); OPC proliferation, migration and cell cycle exit; oligodendrocyte differentiation/maturation; and myelination (**Fig. 1**). Adult OPCs and adult NSCs derived from embryonic OPCs and embryonic NSCs, respectively, continue to generate myelinating OLs for adult myelin turn-over (Menn et al., 2006; Young et al., 2013), and respond, with varying success, to demyelinating injury by replenishing damaged OLs to support remyelination (**Fig. 1**) (Picard-Riera et al., 2002; Nait-Oumesmar et al., 2007; Guo et al., 2011; Mecha et al., 2013). Understanding the intrinsic transcriptional regulation of oligodendroglial development may lead to the development of

therapies for dysmyelinating and demyelinating diseases such as periventricular leukomalacia and multiple sclerosis, both of which have a characteristic pathology of deficient myelination/remyelination and differentiation-arrested immature oligodendroglial lineage cells (Chang et al., 2002; Kuhlmann et al., 2008; Buser et al., 2012; Back and Rosenberg, 2014). The oligodendroglial lineage is also subject to extrinsic regulation by Notch (John et al., 2002; Stidworthy et al., 2004; Zhang et al., 2009), LINGO (Mi et al., 2005; Mi et al., 2007), hyaluronan (Back et al., 2005; Buser et al., 2012), and the BMPs (Samanta and Kessler, 2004; See et al., 2007) and Wnts (Kotter et al., 2006; Fancy et al., 2010; Harrison-Uy and Pleasure, 2012; Mitew et al., 2013; Back and Rosenberg, 2014; Xie et al., 2014).

Wnt ligands, developmentally restricted to dorsal domains of the embryonic spinal cord, restrict initial oligodendrogenesis to the ventral spinal cord. Canonical Wnt signaling was first suggested to inhibit OL development a decade ago (Shimizu et al., 2005). Interest in this research topic was ignited in 2009, when several research groups reported that canonical Wnt signaling exerts an inhibitory effect on oligodendroglial differentiation (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009). Since oligodendroglial myelination encompasses multiple stages from OPC genesis to myelin sheath compaction, Wnt/β-catenin signaling regulates multiple events during these developmental stages.

II: Canonical Wnt/β-catenin signaling pathway and its regulation

Figure 2 depicts the canonical Wnt/β-catenin/TCF/LEF signaling cascade and regulation. β-Catenin-independent, non-canonical Wnt signaling also plays a critical role during normal development, but is beyond the focus of this review. Activation of the canonical Wnt/β-catenin pathway starts with the binding of extracellular Wnt ligands to their plasma membrane seven-transmembrane domain Frizzled receptors (Fzd) and single-transmembrane domain co-receptor Lrp5/6. The intracellular β-catenin destruction complex, which consists of adenomatous polyposis coli (APC), Axin, glycogen synthase kinase 3β (Gsk3β), and casein kinase 1 (Ck1), is responsible for the proteasome-mediated degradation of β-catenin, but this destruction complex is dissociated, with a consequent rise in cytosolic β-catenin, by recruitment of Axin to Lrp5/6 upon Wnt/receptor binding (Clevers and Nusse, 2012). β-Catenin that accumulates in the cytosol when the destruction complex is not functional can either become bound to a plasma

membrane-associated β-catenin adhesive pool, or translocate to the nucleus, where it interacts with transcription factors of the TCF/LEF family and activates target gene expression.

Canonical Wnt signaling is regulated at multiple levels by physiological and pharmacological mechanisms. Small molecule inhibitors of Porcupine, a membrane-bound acyltransferase, suppress secretion of functional Wnts (Chen et al., 2009). At the cell membrane, formation of the Fzd-Wnt-Lrp5/6 complex (Kawano and Kypta, 2003) is inhibited by secreted frizzled-related proteins (SFRPs), which, by binding Wnts, prevent them from interacting with Fzd receptors (Leyns et al., 1997), and also by members of the Dickkopt (Dkk) class, which prevent Lrp5/6 from binding Wnt (Cruciat and Niehrs, 2013). Molecules that dissociate the plasma membrane E-cadherin/β-catenin/α-catenin adhesive complex (Valenta et al., 2012) may increase the pool of β-catenin available for entry into the nucleus (Nelson and Nusse, 2004). Genetic and pharmacological manipulations of the β-catenin destruction complex also affect the transcriptional pool of β-catenin. For example, XAV939 inhibits Wnt/β-catenin activity through stabilization of Axin via tankyrase inhibition (Huang et al., 2009); pyrvinium inhibits Wnt signaling through activation of Ck1α (Thorne et al., 2010); and Gsk3β inhibitors activate Wnt signaling by "de-activating" the β-catenin destruction complex (Wu and Pan, 2010; Azim and Butt, 2011; Azim et al., 2014a). In the nucleus, β-catenin, itself incapable of DNA binding, must interact with TCF/LEF family transcription factors and recruit other co-activators such as p300 (Rao and Kuhl, 2010) in order to modulate Wnt target gene transcription. ICG-001 and iCRT3,5,14 prevent β-catenin from binding p300 (Emami et al., 2004) and TCF/LEF transcription factors (Lepourcelet et al., 2004), respectively (Watanabe and Dai, 2011). Another level of regulation occurs through negative or positive feedback by Wnt/β-catenin target genes. For example, Dkk (Niida et al., 2004), Axin2 (Jho et al., 2002; Lustig et al., 2002) and Nkd1 (Van Raay et al., 2011) negatively regulate Wnt/β-catenin activity, whereas Lef1 (Hovanes et al., 2001), a TCF/LEF family member, participates in a positive feedback loop to enhance β-catenin-mediated transcriptional activity.

There are 19 Wnt ligand members and 10 Fzd receptor members in mammals, thus subjecting Wnt/β-catenin signaling to complex regulation. A large body of literature has focused on the expression of Wnt ligands and Fzd receptors in neural precursor cells including OPC-generating precursors (Harrison-Uy and Pleasure, 2012). Microarray profiling has demonstrated that panels of Wnts (Wnt2, Wnt 3a, Wnt5a, Wnt7a and Wnt9a) and receptors (Fzd2, Fzd4, Fzd5,

Fzd7 and Fzd9) are upregulated in the lesions of multiple sclerosis and multiple sclerosis animal models (Lock et al., 2002; Fancy et al., 2009). Less is known about expression of these molecules in oligodendroglial lineage cells. Among the 19 Wnts, only Wnt7a, Wnt7b and Wnt4 have been reported to be expressed in primary cultured OPCs (Yuen et al., 2014). Almost all of the 10 Fzd receptors are expressed in the adult spinal cord, amongst which Fzd 5 is expressed by both OPCs and OLs, as well as by astrocytes, neurons and microglia (Gonzalez et al., 2012). It remains unclear whether Wnt signaling modulates OL differentiation via an autocrine mechanism, a paracrine mechanism, or both. Cell type-specific disruption of Wnt production and/or secretion (e.g., by disrupting Wls, a gene required for Wnt secretion) by a Cre-loxP approach may help to clarify this issue.

III: Visualizing the Wnt/β-catenin signaling activity in oligodendroglial lineage cells *in vivo*

Several methods are available for visualizing the *in vivo* activation of Wnt/β-catenin signaling: β-catenin nuclear translocation and accumulation; expression of essential Wnt effectors and target genes; and the use of Wnt reporter transgenic mice. Since almost all the components of Wnt/β-catenin signaling axis are also involved in other biological functions, one must keep in mind that a conclusion drawn from one of these methods should be verified by the others.

β-catenin is often observed immunohistochemically in the nuclei of oligodendroglial lineage cells in which HDAC1 or APC has been conditionally deleted (Ye et al., 2009; Lang et al., 2013; Fancy et al., 2014) or GSK3β has been pharmacologically inhibited (Azim and Butt, 2011). Double immunostaining for β-catenin and pan-oligodendroglial marker Sox10 showed that β-catenin immunoreactivity is rare in $Sox10^{+}$ nuclei during developmental myelination (Azim and Butt, 2011; Lang et al., 2013). β-catenin with non-phosphorylation at Ser33/37 and Thr41 is the stable, active form that is involved in signaling activation, and an exon 3-deleted β-catenin isoform has often been employed to aberrantly activate Wnt/β-catenin signaling (**Table 1**). Western blotting showed that the unphosphorylated active form of β-catenin protein is present between P5 and P30 in mouse subcortical white matter (Chew et al., 2011), but it has not been determined which cell types in white matter are responsible for this expression.

The down-stream effects of canonical Wnt/β-catenin cascade are mediated by the TCF/LEF family, which has four members in mammals (official gene symbols Tcf7, Tcf7l1,

Tcf7l2 and Lef1) (Cadigan and Waterman, 2012), each of which has a β-catenin-binding and an HMG-DNA binding domain. Among the four members, only TCF7l2 is expressed in oligodendroglial lineage cells. However, it is still controversial whether TCF7l2 is more highly expressed in OPCs or in differentiated OLs during developmental myelination and remyelination (Fancy et al., 2009; Fu et al., 2009; Ye et al., 2009; Fancy et al., 2011; Fu et al., 2011; Lang et al., 2013; Lurbke et al., 2013). This issue is important because oligodendroglia-specific TCF7l2 expression has been reported to be linked to the activation of canonical Wnt/β-catenin signaling. Little is known about the role of TCF7l2 in oligodendrocyte differentiation. A recent study showed that TCF7l2 protein was not co-localized with the Wnt target gene Axin2 mRNA under physiological conditions (Fancy et al., 2014), suggesting that TCF7l2 plays roles other than, or in addition to, mediating canonical Wnt signaling in oligodendroglial lineage cells. Our own laboratory has recently found that deletion of TCF7l2 in both OPCs and differentiated OLs, respectively did not alter Axin2 mRNA expression *in vivo* (Hammond et al., unpublished data). It has been established that other transcriptional effectors, Bcl9/pygo2 (de la Roche et al., 2008; Gu et al., 2009; Takada et al., 2012; Gu et al., 2013), can associate with nuclear β-catenin to activate Wnt target gene expression without the need for TCF7l2. It would be very interesting to study the interaction of β-catenin with Bcl9/Pygo2 and the functional significance of this interaction *in vivo* in oligodendroglial lineage cells.

Wnt reporter mice provide a useful tool for detection of functional activation of canonical Wnt signaling. Using Bat-lacZ reporter mice (Maretto et al., 2003), it has been reported that nuclear β-gal is expressed in PDGFRα$^+$ OPCs, but not in CC1$^+$ differentiated OLs in the early postnatal spinal cord and corpus callosum (Fancy et al., 2009). However, β-gal is expressed in CC1$^+$ differentiated OLs but not in PDGFRα$^+$ OPCs (Fancy et al., 2011) in another mouse Wnt reporter line, Axin2-lacZ (Lustig et al., 2002). The discrepancy between these observations likely reflects differences in kinetics of lacZ expression or in the reporting efficacy or dependency on the β-catenin/TCF complex in these reporter lines (Barolo, 2006; Al Alam et al., 2011).

The response of oligodendroglial lineage cells to canonical Wnt signaling after traumatic injury also remains controversial. In a contusion model of spinal cord injury (SCI), Axin2 immunostaining suggested that canonical Wnt/β-catenin signaling is substantially induced in PDGFRα$^+$ OPCs (Rodriguez et al., 2014), but the number of Bat-lacZ reporter$^+$ cells was not elevated in a hemisection spinal cord model (White et al., 2010). These disparate results suggest

that there are intrinsic and/or extrinsic differences in oligodendroglial lineage responses to canonical Wnt signaling in different contexts.

IV: Wnt/β-catenin signaling in OPC generation from NSCs

Studies suggested that Wnt/β-catenin signaling inhibits OPC generation from NSCs in both the brain and spinal cord during embryonic development (Shimizu et al., 2005; Ye et al., 2009; Langseth et al., 2010; Dai et al., 2014). However, other studies have complicated the original straightforward concept that Wnt/β-catenin signaling inhibits oligodendrogenesis and differentiation. The following sections review current knowledge about Wnt/β-catenin regulation of OPC generation from NSCs in the spinal cord and forebrain (See **Table 1** for a glossary of commonly used terms, and **Table 2** for major observations arising from genetic manipulations of Wnt/β-catenin signaling in mice).

1) The role of Wnt/β-catenin signaling in OPC generation in the spinal cord

OPCs are specified at embryonic day ~12.5 (E12.5) in the ventral Olig2$^+$ pMN domain of the mouse spinal cord (Richardson et al., 2006). Most Wnt ligands (Wnt1, Wnt3a Wnt3 and Wnt4) are restricted to the dorsal domains of the embryonic spinal cord from E9.5-E12.5 (Shimizu et al., 2005; Yu et al., 2008), though some (Wnt7a and Wnt7b) are distributed in a ventral (high)-to-dorsal (low) gradient (Megason and McMahon, 2002; Daneman et al., 2009). Wnt/β-catenin signaling is active throughout the spinal neural tube at E8.5, but then becomes confined to dorsal domains during E9.5-E12.5 (Yu et al., 2008; Bluske et al., 2009). There is little evidence thus far suggesting the activation of endogenous Wnt/β-catenin signaling in the ventral pMN domain when OPC specification occurs.

Some evidence suggests that Wnt/β-catenin signaling represses the fate of ventral progenitors (or NSCs) including Olig2$^+$ pMN domain progenitors prior to OPC specification (Yu et al., 2008). In brief, expression of constitutively active (CA)-β-catenin driven by *Olig1-Cre* (*Ctnnb1 floxed-exon3* allele, β-catenin gain-of-function, **CA-β-catenin**, hereafter) (Harada et al., 1999) decreases the numbers of ventral progenitors, including Olig2$^+$ NSCs by E10.5, 2 days prior to OPC specification (Yu et al., 2008). Conversely, β-catenin loss-of-function driven by *Olig1-Cre* (*Ctnnb1 floxed-exon2-6,* **β-catenin KO**, hereafter) (Brault et al., 2001) increases

numbers of ventral progenitors. Of note, numbers of Olig2$^+$ progenitors did not change in the β-catenin loss-of-function mutants (Table 2).

Subsequent studies employed *Olig1*-Cre or *Olig2-Cre* to drive expression of either CA-β-catenin or β-catenin KO, and analyzed spinal cord after the onset of OPC specification. Those studies have yielded diverse results (**Table 2**). In *Olig1*-Cre/CA-β-catenin mice, OPCs were virtually absent in the spinal cord from E13.5 through P15, and conversely in *Olig1*-Cre/β-catenin KO mice OPCs were precociously generated at E12.5, but returned to normal at later time-points (Ye et al., 2009; Dai et al., 2014). In sharp contrast, OPCs generation was not affected in either *Olig2*-Cre/CA-β-catenin or *Olig2*-Cre/β-catenin KO mutants (Fancy et al., 2009). Since these studies used *Olig1-Cre* or *Olig2-Cre* to manipulate CA-β-catenin or β-catenin KO, this discrepancy may reflect differences in the cellular specificity of Olig1 and Olig2 promoters in embryonic development. Olig1 expression begins at ~E8.5 in the dorsal portion of the Nkx2.2$^+$ p3 domain of the ventral spinal cord, and becomes restricted to the pMN domain by ~E10.5, whereas Olig2 expression begins before neural tube closure in the ventral part of the Nkx2.2$^+$ p3 domain, and then becomes confined to the pMN domain at ~ E9 (Zhou et al., 2000; Meijer et al., 2012). Alternatively, if *Olig1-Cre* and *Olig2-Cre* activate Wnt/β-catenin in the same precursor population, the discrepancy may result from the differential dosage of signaling, since Olig2 expression is much stronger than Olig1 in the OPC-generating pMN domain (Zhou et al., 2000), so that the Wnt/β-catenin activity in *Olig1-Cre*/CA-β-catenin mutants woud appear lower than that in *Olig2-Cre*/CA-β-catenin mutants. Although repressing the ventral NSC fate prior to OPC specification (Yu et al., 2008), Wnt/β-catenin signaling likely has a direct effect on OPC generation from NSCs in the spinal cord, since Olig2/Olig1$^+$ progenitors are unaltered in *Olig1-Cre*/CA-β-catenin mutants at the time of OPC specification (Ye et al., 2009). A cautionary note in interpreting the above CA-β-catenin studies is that this approach may modulate additional pathways that are not physiologically regulated by β–catenin. This is especially a concern considering the paucity of evidence for activation of endogenous Wnt/β-catenin signaling in the oligodendrogenic Olig2$^+$ pMN progenitor domain around the time that OPC specification begins.

2) The role of Wnt/β-catenin signaling in OPC generation in the forebrain during embryonic and early postnatal development

Aberrant activation of Wnt/β-catenin signaling prevented OPC generation from embryonic NSCs in *Olig1-Cre*/CA-β-catenin forebrains (Ye et al., 2009) whereas OPC generation was normal in *Olig2-Cre*/CA-β-catenin neonatal forebrains (Fancy et al., 2009). Conversely, *in utero* electroporation at E15.5 to induce expression of dominant negative (DN)-*Lef1* (a Tcf family member) or *Dkk1* (an inhibitor of Wnt LRP receptors) enhanced OPC production from cortical NSCs (Langseth et al., 2010), indicating that Wnt/β-catenin activity delays the timing of neocortical OPC generation. In contrast, β-catenin KO driven by *Olig2*-Cre did not alter corpus callosum OPC numbers in the early postnatal period (Fancy et al., 2009). These studies collectively suggest that Wnt/β-catenin signaling exerts a context-dependent effect on the timing of OPC generation in the forebrain. Alternatively, the different phenotypes of OPC genesis in studies by Ye et al., 2009, Fancy et al., 2009 and Langseth et al., 2010 may simply reflect intrinsic temporal differences in NSC responses to Wnt/β-catenin signaling. The disparate results from these important studies prompt us to hypothesize that the effects of Wnt/β-catenin signaling on OPC generation are also dosage-dependent. Compared to normal OPC generation under physiological conditions of tightly-controlled Wnt dosage, constitutively enforced Wnt activation (as achieved in *Olig1-Cre*/CA-β-catenin mutant mice) results in a block of OPC generation, transiently diminished Wnt signaling strength (as achieved by *in utero* electroporation of DN-lef1 or Dkk1) results in a temporal shift of OPC generation, and completely ablated Wnt signaling tone (as achieved in *Olig2-Cre*/β-catenin KO mutant mice) has only a minimal effect on OPC generation from NSCs in the forebrain. It is also possible that genetic manipulation of CA-β-catenin or β-catenin KO affects additional signaling pathways (Haq et al., 2003; Ji et al., 2009), and that the results of these manipulations reflect the integrated readout of canonical Wnt and these other β-catenin-mediated, as yet unspecified, pathways. The different results obtained with *Olig1-Cre*/CA-β-catenin (no OPC genesis) and *Olig2-Cre*/CA-β-catenin (normal OPC genesis) mutants may also be because, though Olig1 and Olig2 expressions overlap during oligodendrogenesis, *Olig1-Cre* drives CA-β-catenin expression in early NSCs (Yu et al., 2008) whereas *Olig2-Cre* drives CA-β-catenin expression predominantly in OPC-committed NSCs, thus minimally affecting the OPC population. A discrepancy in recombination efficiency - higher in *Olig1-Cre* (Ye et al., 2009) than that in *Olig2-Cre* mice (~85%) (Fancy et al., 2014) might also contribute to this discrepancy – OPCs generated from the 15% of non-

recombined NSCs may repopulate the CNS (Kessaris et al., 2006), thus leading to an eventually normal OPC population (Fancy et al., 2009).

Two recent studies add still more complexity to the role of Wnt/β-catenin signaling in OPC genesis. In the first, oligodendrogenesis (*Mbp mRNA*$^+$ cells) was diminished in the *hGFAP-Cre*-mediated genetic β-catenin-KO forebrain (Gan et al., 2014), suggesting that endogenous β-catenin-mediated signaling is a positive regulator for OPC genesis in subcortical white matter. However, this study did not definitively determine whether decreased *Mbp*$^+$ cells was due to a diminished NSC pool, to decreased OPC generation from NSCs or attenuated OPC differentiation, or to a combination of these effects. In the second study, when a Cre-expressing plasmid was electroporated into the dorsal SVZ of CA-β-catenin mutant (Wnt activation) at P2, OPC generation from dorsal SVZ was increased, indicating that β-catenin-mediated signaling is important for OPC generation in the dorsal SVZ region. Interestingly, electroporation into the lateral SVZ had no effect (Azim et al., 2014b). These contrasting observations suggest that the local environment, an intrinsic difference between NSC populations in responses to Wnt/β-catenin signaling, or both influence the effects of dominant activation of β-catenin, and that Wnt/β-catenin exerts its effect on OPC generation in a context-dependent manner. Another interesting observation was that, though increased tone of Wnt/β-catenin signaling (achieved by CA-β-catenin) promoted OPC generation from dorsal SVZ NSCs, decreased tone (achieved by β-catenin KO) had no effect (Azim et al., 2014b). In this context, it is also possible that other factors or pathways affected by CA-β-catenin mutation may activate genes independent of (or downstream from) the canonical Wnt pathway to induce OPC genesis.

Recent studies have suggested both cell intrinsic and cell extrinsic roles for Wnt/β-catenin signaling in Olig2$^+$ and Olig2$^-$ NSCs in the embryonic brain. The distinct roles of Wnt/β-catenin signaling in Olig2$^+$ and Olig2$^-$ NSCs are apparent from β-catenin KO studies. Results of *hGFAP*-Cre/β-catenin KO provide strong evidence that endogenous Wnt/β-catenin plays a critical role in generation of OLs from NSCs (both Olig2$^+$ and Olig2$^-$) in the late embryonic and postnatal brain (Gan et al., 2014). In contrast, the normal OPC generation in *Olig2*-Cre/β-catenin KO mutants (Fancy et al., 2009) suggests that endogenous Wnt/β-catenin does not regulate oligodendrogenesis from Olig2$^+$ NSCs. Thus, Wnt/β-catenin signaling appears to promote oligodendrogenesis from Olig2$^-$ NSCs. It remains to be determined whether the deficiency in oligodendroglia in *hGFAP*-Cre/β-catenin KO mice is due to reduced Wnt/β-catenin activity or to

inhibition of β-catenin-mediated adhesion function. Future studies employing a β-catenin floxed allele (**Table 1**) that disrupts Wnt/β-catenin signaling while preserving the cell adhesion function of β-catenin (Valenta et al., 2011) should help resolve this issue.

It is clear, however, that the effects of forced Wnt/β-catenin activation and of inhibiting endogenous Wnt/β-catenin signaling vary with CNS developmental stage. Forced Wnt/β-catenin activation in embryonic NSCs diminishes the generation of OPCs in the *Olig1*-Cre/CA-β-catenin forebrain (Ye et al., 2009; Dai et al., 2014). Consistently, inhibiting endogenous Wnt/β-catenin activity promotes OPC generation from the embryonic VZ (Langseth et al., 2010; Dai et al., 2014) but has no effect by late embryonic stages (Fancy et al., 2009; Dai et al., 2014). In contrast, forced Wnt/β-catenin activation in postnatal dorsal SVZ NSCs enhanced OPC generation (Azim et al., 2014b; Azim et al., 2014a) and inhibiting endogenous Wnt/β-catenin activity has no effect (Azim et al., 2014b). Collectively, endogenous Wnt/β-catenin signaling (rather than forced Wnt/β-catenin activation) regulates the timing of OPC generation from embryonic NSCs, and this regulatory role is dispensable at later developmental stages.

Of note, GSK3β inhibition-elicited Wnt/β-catenin activation overrides other GSK3β inhibition-elicited signaling pathways to promote OPC generation from dorsal SVZ NSCs (Azim et al., 2014a), whereas GSK3β inhibition-elicited other signaling pathways override the Wnt/β-catenin signaling pathway to promote subsequent OPC differentiation to OLs during postnatal development (Azim and Butt, 2011). These results suggest that the effects of Wnt/β-catenin signaling on oligodendroglial development are both temporally regulated and modulated (synergistically or antagonistically) by other signaling pathways.

3) Wnt/β-catenin regulation of OPC generation from adult NSCs

A recent publication has provided new evidence that Wnt/β-catenin signaling promotes oligodendrogenesis from the adult SVZ by lentivirus-mediated local Wnt3 expression and DN-TCF7l2 (Ortega et al., 2013). Furthermore, FACS analysis revealed that Wnt3, the most abundant Wnt ligand in the adult SVZ, is predominantly expressed by OPCs, suggesting a positive feedback loop driving OPC generation from adult SVZ NSCs (Ortega et al., 2013). The enhanced OPC generation is likely to be due, at least in part, to the increased pool of adult NSCs and/or progenitor cells, since Wnt/β-catenin has been reported to promote the proliferation of progenitor cells in the adult SVZ (Adachi et al., 2007). It is not clear, however, whether the local

Wnt3 expression specifically affects Wnt/β-catenin signaling or, instead, a non-canonical, β-catenin-independent Wnt pathway (Kobune et al., 2007; Davis et al., 2008), and whether DN-TCF7l2 expression has a gain-of-function effect (Labbe et al., 2000) that modulates OPC genesis independent of Wnt/β-catenin signaling. In order to pinpoint the role of canonical Wnt/β-catenin, it would be useful to simultaneously express Wnt3 and DN-TCF7l2 and assess whether OPC generation is rescued to the level of wild type SVZ.

Interestingly, *in vivo* Wnt/β-catenin activation did not stimulate OPC generation from early postnatal lateral SVZ NSCs (Azim et al., 2014b), but did promote OPC production from adult lateral SVZ cells (Ortega et al., 2013). Notably, as well, Wnt3 did not induce oligodendrogenesis in adult hippocampal progenitors (Lie et al., 2005). These data again support the hypothesis that Wnt/β-catenin signaling exerts distinct, context-dependent effects during normal development in different CNS regions.

4) Role of Wnt/β-catenin in OPC proliferation

Previous *in vitro* culture studies suggested that Wnt/β-catenin signaling plays an important role in OPC proliferation (Ye et al., 2010; Chew et al., 2011; Hill et al., 2013). However, Wnt 3a treatment does not affect the proliferation of cultured rat OPCs (Feigenson et al., 2009), indicating that *in vitro* results was likely due to differences in the culture conditions and/or intrinsic difference of the cultured cells. In sharp contrast to the *in vitro* analyses, *in vivo* studies (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009) have consistently demonstrated that Wnt/β-catenin signaling does not affect OPC proliferation in either white matter or gray matter. Furthermore, inhibition of Wnt/β-catenin signaling by XAV939 treatment (Hill et al., 2013) promotes oligodendrocyte differentiation without affecting OPC proliferation in an *in vivo* demyelination model (Fancy et al., 2011). In conclusion, it is unlikely that Wnt/β-catenin signaling plays a critical role in OPC proliferation during *in vivo* CNS developmental myelination and remyelination.

V: Wnt/β-catenin regulation of oligodendroglial differentiation and myelination

The current literature has reached a strong consensus that forced Wnt/β-catenin activation inhibits oligodendrocyte differentiation. However, the role of endogenous Wnt/β-catenin signaling in oligodendrocyte differentiation and remyelination is still controversial. This section

briefly reviews findings on this topic, discusses possible interpretations for disparate observations, and proposes future studies.

1) Dysregulated Wnt/β-catenin activation: insights from *in vivo* genetic manipulation and questions

Four years after Shimizu et al demonstrated that Wnt/β-catenin inhibited oligodendrocyte differentiation in organotypic cultures derived from embryonic spinal cord (Shimizu et al., 2005), several laboratories, using Cre-loxP methodology, provided compelling evidence that forced Wnt/β-catenin activation delays the timing of oligodendrocyte differentiation (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009) (see Table 2 for details).

Both the Franklin/Rowitch and the Grinspan laboratories (Fancy et al., 2009; Feigenson et al., 2009) reported a temporal delay in oligodendrocyte differentiation in CA-β-catenin mutants driven by *Olig2-Cre* or *Cnp-Cre*, respectively, and found that oligodendrocyte differentiation returned to normal in the adult. The Franklin/Rowitch groups attributed the adult normal oligodendrocyte differentiation to the near absence of TCF7l2 expression in adult oligodendroglia, since β-catenin requires a TCF/LEF1 family member to activate transcription. According to this TCF7l2-dependent Wnt model, oligodendroglial Wnt effector TCF7l2 plays a key role of Wnt/β-catenin signaling in the temporal inhibition of oligodendrocyte differentiation during both developmental myelination and remyelination. Consistent with that proposal, TCF7l2 was reported to be expressed in OPCs but not in mature OLs, and constitutive activation of β-catenin in OPCs inhibited their differentiation (Fancy et al., 2009). But inconsistent with that model, constitutive deletion of TCF7l2 inhibited perinatal oligodendrocyte differentiation in the spinal cord of Tcf7l2-null mutants (Fu et al., 2009; Ye et al., 2009). To reconcile these disparate results, Ye et al. proposed a two-modal, "switch" model of TCF7l2 in which when TCF7l2 is switched "on" by binding β-catenin, oligodendrocyte differentiation is inhibited, but, when switched "off" by binding HDAC1/2, TCF7l2 promotes oligodendrocyte differentiation (Ye et al., 2009). This "switch" model is consistent with the established role of TCF7l2 outside the CNS – in the presence of Wnt, TCF7l2 activates gene expression by binding β-catenin, whereas in the absence of Wnt, it acts a gene repressor (Lien and Fuchs, 2014).

Here, we hypothesize that the "off" switch role of TCF7l2 (Wnt-independent role) overrides the "on" switch role (Wnt-dependent role) during oligodendroglial development *in vivo* and that TCF7l2 expression plays a minor, if any, role in Wnt/β-catenin signaling in

oligodendroglial lineage cells. In this context, we would expect that conditional ablation of TCF7l2 would not alter the activity of endogenous Wnt/β-catenin signaling during either perinatal or postnatal development. Interestingly, genetic ablation of the Wnt negative regulators APC and Axin2 inhibited oligodendrocyte differentiation (Fancy et al., 2011; Lang et al., 2013; Dai et al., 2014; Fancy et al., 2014), but surprisingly, TCF7l2 was downregulated in these APC- and Axin2-KO mutants (Lang et al., 2013; Dai et al., 2014). These data indicate that TCF7l2 expression is uncoupled from activation of Wnt/β-catenin signaling and that the downregulation of TCF7l2 simply reflects the inhibition of oligodendrocyte differentiation.

2) Dysregulated Wnt/β-catenin activation: insights from *in vivo* pharmacological manipulation and questions

Though there is a strong consensus from *in vivo* genetic data that dysregulated Wnt/β-catenin activation inhibits oligodendroglial differentiation (discussed above), a panel of GSK3β inhibitors (resulting in Wnt/β-catenin signaling activation and alterations of other signaling pathways) have been reported to stimulate myelin gene expression *in vitro* and *in vivo* (Azim and Butt, 2011; Meffre et al., 2015) and to accelerate postnatal oligodendroglial differentiation despite the activation of Wnt/β-catenin signaling (Azim and Butt, 2011). This argues that other signaling pathways or factors modulated by GSK3β inhibition can override the effect of Wnt/β-catenin activation and thereby promote oligodendroglial maturation (Doble and Woodgett, 2003; Azim and Butt, 2011). However, inhibition of GSK3β was also recently reported to diminish oligodendroglial differentiation *in vitro* (Zhou et al., 2014), presumably reflecting an intrinsic difference between *in vivo* and *in vitro* contextual cues and differences amongst *in vitro* culture systems. Fancy et al., 2011 (Fancy et al., 2011) used the small molecule tankyrase inhibitor XAV939 to stabilize Axin (both constitutive Axin1 and inducible Axin2) protein, thereby enhancing proteasomal degradation of β-catenin and antagonizing Wnt/β-catenin activity (Huang et al., 2009). Treatment of OPC-enriched cultures derived from cerebral cortex with XAV939 increased the percentage of $Olig2^+$ and MBP^+ cells and total MBP protein levels, and promoted myelination and remyelination in CNS slice cultures and in demyelination model (Fancy et al., 2011). However, in addition to down-regulating canonical Wnt/β-catenin signaling, Axin, a scaffold protein, may modulate a panel of other signaling pathways, including TGFβ (Furuhashi et al., 2001; Guo et al., 2008), JNK (Rui et al., 2007) and p53(Rui et al., 2004). Notably, TGFβ

signaling has been reported to positively regulate the timing of oligodendrocyte differentiation (McKinnon et al., 1993; Palazuelos et al., 2014). Hence, it remains unclear whether XAV939-mediated enhancement of oligodendrocyte differentiation is a direct consequence of modulation of Wnt/β-catenin alone or, instead, is due to other altered signaling pathways (Casaccia, 2011), as in the GSK3β inhibitor study (Azim and Butt, 2011). Nevertheless, XAV939 may still prove to be therapeutically valuable in periventricular leukomalacia and multiple sclerosis since it enhances remyelination in the animal demyelination model.

3) Disruption of endogenous Wnt/β-catenin activity – insights from inactivation of Wnt/β-catenin signaling

As discussed above, genetic and pharmacological activation of Wnt/β-catenin inhibits oligodendrocyte differentiation; however, inactivation of endogenous Wnt/β-catenin signaling yields conflicting results. A recent study reported that the number of *Plp* mRNA^{+} differentiated OLs was significantly decreased from E18.5 through P15 in *Olig1*-Cre/β-catenin KO spinal cord (inactivating endogenous Wnt/β-catenin signaling) (Dai et al., 2014). However, a previous study found that the numbers of both Olig2^{+} oligodendroglial lineage cells and *Plp* mRNA^{+} differentiated oligodendrocytes were normal in *Olig2*-Cre/β-catenin KO spinal cord at P1 (Fancy et al., 2009). Like the contrasting OPC generation in the *Olig1*-Cre/CA-β-catenin and *Olig2*-Cre/CA-β-catenin mutants discussed in section IV, the reason for the disparate results in these two β-catenin-KO systems is elusive. However, the genetic manipulation of β-catenin in these two systems is likely different from each other in cellular specificity and deletion efficiency (one-allele vs two-allele deletion even in the same β-catenin$^{fl/fl}$). A systematic side-by-side comparison of deletion efficiency and cellular specificity between constitutive *Olig1-Cre* and *Olig2-Cre* would help to resolve this issue. Moreover, a comparison of β-catenin$^{+/+}$ (intact Wnt tone), β-catenin$^{fl/+}$ (decreased Wnt tone), and β-catenin$^{fl/fl}$ (extinguished Wnt tone) mutations driven by the same constitutive *Olig1-Cre* or *Olig2-Cre* would help to test the hypothesis that endogenous Wnt/β-catenin signaling plays a dose-dependent role in oligodendrocyte development.

Our laboratory employed three independent Cre drivers, *Cnp*-Cre, *NG2*(*Cspg4*)-Cre, and *Olig2*-CreER^{T2}, to delete β-catenin and found that β-catenin gene expression was significantly decreased but oligodendrocyte differentiation was normal in those three Cre-loxP KO systems

(Lang et al., 2013). The densities and proportions of OPCs and differentiated OLs and the levels of expression of Plp and Mbp mRNAs were normal in these three β-catenin KO systems. There was a 50% lower *Cnp* mRNA level in *Cnp*-Cre/β-catenin KO than in no Cre controls, which was to be expected because the *Cnp-Cre* knock-in transgene replaces one allele of the endogenous *Cnp* gene in the *Cnp-Cre* transgenic mice (Lappe-Siefke et al., 2003). Our data are consistent with those derived from the *Olig2-Cre*/β-catenin KO system (Fancy et al., 2009) but different from the *Olig1-Cre*/β-catenin KO system (Dai et al., 2014).

There are several cautionary considerations when interpreting data collected from β-catenin KO experiments. All published data assume that oligodendroglial lineage cells respond to Wnt *in vivo* through canonical β-catenin signaling and that β-catenin KO blocks this signaling activation. But does the disruption of β-catenin affect endogenous Wnt/β-catenin activity in oligodendroglial lineage cells *in vivo*? Unfortunately few studies have assessed this important question. Also, another catenin, γ-catenin (plakoglobin) can compensate for the loss of β-catenin in cardiomyocytes (Zhou et al., 2007), hepatocytes (Wickline et al., 2011) and in a β-catenin-null malignant cell line (Maeda et al., 2004). Before drawing conclusions regarding the role of Wnt/β-catenin signaling in oligodendroglial development, it will be critically important to prove that canonical Wnt signaling activity is indeed reduced or extinguished in the *in vivo* β-catenin KO system being studied. We approached this issue by RT-qPCR quantifying gene expression of Wnt target gene Axin2 and Sp5, readouts of Wnt/β-catenin activity, in different β-catenin KO system systems at different developmental stages. In the P26 *NG2-Cre*/β-catenin KO spinal cord, both γ-catenin and Axin2 mRNAs were normal compared to age-matched controls, though β-catenin remained downregulated (**Fig. 3A**). In the P14 *Olig2-CreER*^{T2}/β-catenin KO spinal cord (KO at P6 and P7), β-catenin mRNA was significantly reduced (**Fig. 3B**), but Axin2 mRNA was at control levels (**Fig. 3C**). In the neonatal *Cnp-Cre*/β-catenin KO spinal cord, the Wnt effector TCF7l2, which we have shown to upregulate in newly differentiated myelin gene-expressing OLs (Hammond et al., unpublished data), was unaffected, nor were the expressions of Axin2 and Sp5 (**Fig. 3D**). These data suggest that it is unlikely that γ-catenin compensates for the loss of β-catenin in oligodendroglial lineage cells, but further biochemical and molecular assays are needed to definitively exclude this possibility. The normal Axin2 mRNA expression concomitant with the reduced level of β-catenin indicates that postnatal oligodendroglial lineage cells are unlikely to respond to *in vivo* Wnt via canonical β-catenin-dependent Wnt signaling under

physiological conditions. Therefore, it will also be important to assess whether *in vivo* Axin2 expression (i.e. Wnt tone) is indeed reduced in the oligodendroglial lineage cells of *Olig1-Cre*/β-catenin KO mutants in which β-catenin KO has an effect on postnatal oligodendrocyte differentiation (Dai et al., 2014).

The Wnt negative regulator APC, in contrast to clone CC1 (Bhat et al., 1994; Brakeman et al., 1999), has been reported to be transiently upregulated in newly differentiated OLs and to co-label with Wnt effector TCF7l2 in the same population (Lang et al., 2013). The co-expression of APC and TCF7l2 suggests that APC tightly controls the transcriptional pool of β-catenin (in response to Wnt) (Rosin-Arbesfeld et al., 2003; Choi et al., 2013) to prevent it from binding nuclear TCF7l2 during active myelination, thus explaining why conditional KO of TCF7l2 in the oligodendroglial lineage cells does not alter the expression of canonical Wnt target genes (Hammond et al., unpublished data).

The second cautionary consideration when interpreting β-catenin KO data is that β-catenin is involved not only in canonical Wnt signaling but also cadherin-mediated cell adhesion and in the actin cytoskeleton (Valenta et al., 2012) and IGF-1-mediated cell response (Ye et al., 2010). The inhibition of oligodendrocyte differentiation caused by β-catenin KO may result from a loss in its adhesive function (Nielsen et al., 2006; Laursen and Ffrench-Constant, 2007; Lewallen et al., 2011) and the normal oligodendrocyte differentiation upon β-catenin KO may also stem from its altered adhesive function, which might negate the inhibitory effect of its signaling activity (Kakinuma et al., 2004; Czopka et al., 2009). Therefore, genetic disruption of the signaling function while retaining the adhesive function of β-catenin (e.g., using $Ctnnb1^{dm/lox}$ mice, see **Table 1**) would be useful in determining whether endogenous β-catenin-mediated Wnt signaling promotes, inhibits, or is dispensable for postnatal oligodendrocyte differentiation.

A recent study used dominant negative expression of LRP6 (DN-LRP6) and DN-TCF7l2 at 2 days post-fertilization when OPCs have already specified in zebrafish, and reported that Wnt/β-catenin signaling is a positive regulator for myelin gene expression and myelination (Tawk et al., 2011). However, a prior study, using the GSK3β inhibitors 6-bromoindirubin-3-oxime (Bio) and LiCl at 2 days post-fertilization, reported that Wnt/β-catenin activity has to be downregulated in order for OPC to differentiate and express myelin genes (Kim et al., 2008). Those disparate results again suggest that the experimental manipulations employed in these two studies additionally modulated other signaling pathways, thus yielding different endpoint

readouts of myelin gene expression and oligodendrocyte differentiation. In particular, GSK3β is a master regulator determining the output of numerous signaling pathways (Doble and Woodgett, 2003) and LRP6 can also mediate β-catenin-independent non-canonical signaling (Gray et al., 2013). Considering the observations that conditional KO of β-catenin by *Olig2-Cre, NG2-Cre, Cnp-Cre* and *Olig2-Cre-ER^{T2}*, respectively, did not alter myelin gene expression and kinetics of postnatal oligodendrocyte differentiation (Fancy et al., 2009; Lang et al., 2013), it is still too soon to conclude that Wnt/β-catenin signaling drives myelin gene expression and myelination (Tawk et al., 2011). Our own laboratory, using inducible *Plp-CreER^{T2}*/Tcf7l2$^{fl/fl}$ to conditionally delete TCF7l2 in differentiated murine OLs, found that TCF7l2 disruption did not affect the mRNA levels of *Plp, Mbp* and *Cnp* in oligodendrocytes (Hammond et al., unpublished data), therefore weakening the hypothesis that TCF7l2-mediated Wnt signaling in differentiated OLs is required for myelin gene expression.

In summary, some *in vivo* genetic studies employing aberrant Wnt/β-catenin activation have supported the concept that dysregulated Wnt/β-catenin activation inhibits the timing of transition of OPCs to OLs, and have suggested that inhibiting this signaling would promote oligodendrocyte differentiation in demyelinating and dysmyelinating disorders. However, genetic studies employing β-catenin KO and dominant negative TCF/LEF1 have yielded conflicting results, and it still remains unclear whether endogenous Wnt/β-catenin activity plays a critical role in oligodendrocyte differentiation.

VI: Wnt/β-catenin signaling in oligodendroglial regeneration and remyelination

Current published data from demyelination animal models support the hypothesis that aberrant activation of Wnt/β-catenin signaling inhibits oligodendrocyte differentiation during remyelination. Wnt/β-catenin signaling was reported to be aberrantly activated in OPCs in human hypoxic/ischemic WM injury and multiple sclerosis (MS) and in animal models of these disorders and of spinal cord injury (Fancy et al., 2009; Fancy et al., 2011). In human MS tissues, the Wnt effector TCF7l2 is upregulated in the active lesions (Fancy et al., 2009; Pedre et al., 2011; Lurbke et al., 2013), suggesting that TCF7l2 and/or possibly TCF7l2-mediated Wnt/β-catenin activation inhibits the timing of oligodendrocyte differentiation, a major culprit for remyelination failure (Franklin, 2002; Franklin and Gallo, 2014). However, the observation that spontaneous remyelination is robust in those active lesions also suggests that TCF7l2 and/or

TCF7l2-mediated Wnt signaling, rather than inhibiting, instead promotes oligodendrocyte differentiation. Future genetic studies using TCF7l2 conditional KO in demyelination animal models will help determine the function of TCF7l2 in these demyelinating disorders.

Studies of remyelination after lysolecithin-induced demyelination in spinal cords of *Olig2*-Cre/CA-β-catenin mice, in which adult myelination is normal, revealed that constitutive activation of β-catenin delays remyelination in an oligodendroglial lineage autonomous manner (Fancy et al., 2009). OPC proliferation and recruitment to the lesion were not affected in these mice, but there was a delay in oligodendrocyte differentiation. Consistent with those results, mice deficient in *Axin2* or carrying a truncated allele of Apc (Apc^{min} mice) also had delayed oligodendroglial differentiation and remyelination after lysolecithin lesioning (Fancy et al., 2011). However, subsequent studies reported that one-allele ablation or truncation of APC does not result in dysregulation of Wnt/β-catenin activity in oligodendroglial lineage cells (Lang et al., 2013; Fancy et al., 2014). This suggests that the delayed remyelination in Apc^{min} mice may be a consequence of a Wnt-independent effect of APC haploinsufficiency or an injury-specific role of Apc^{min}. Stabilizing Axin by administration of XAV939 improved spinal cord remyelination (Fancy et al., 2011), though it remains possible that this effect was also mediated, at least in part, via non-Wnt/β-catenin pathways.

VII: Interplay between Wnt/β-catenin and TGF-β/BMP signaling pathways

BMP signaling via the BMPRI/II-SMAD1/5/8-SMAD4 cascade inhibits oligodendrogenesis and oligodendroglial differentiation by induction of Id and Hes genes and repression of Olig1 and Olig2 in the oligodendroglial lineage (Grinspan et al., 2000; Mekki-Dauriac et al., 2002; Miller et al., 2004; Samanta and Kessler, 2004; See et al., 2004; Cheng et al., 2007). Both BMP signaling and Wnt/β-catenin signaling have been reported to inhibit oligodendrocyte differentiation. These two pathways interact with each other at multiple levels (**Fig. 4**). Recent reports have shed light on crosstalk between these signaling pathways in the context of oligodendroglial lineage cells. Using primary OPC cultures, Feigenson et al demonstrated that both Wnt3a and BMP4 inhibited OL differentiation; however, Wnt3a did not inhibit OL differentiation when BMP signaling was blocked at the receptor level. Conversely, BMP4 retained its inhibitory effect on OL differentiation even when Wnt/β-catenin signaling was blocked by β-catenin KO (Feigenson et al., 2011). These *in vitro* results support the novel

hypothesis that BMP signaling acts downstream of Wnt/β-catenin signaling to inhibit OL differentiation (Feigenson et al., 2011). Recently, Weng et al. reported that SMAD7, a direct target and also a negative regulator of BMP signaling, negatively regulates BMP signaling by targeting BMP receptor for degradation in oligodendroglial lineage cells. The SMAD-interacting protein 1 (Sip1) directly activates SMAD7 and also inhibits p-SMAD/p300-mediated Id2/Id4 gene expression (Weng et al., 2012). Interestingly, SMAD7/Smurf1 complex also decreases the level of β-catenin (Weng et al., 2012), therefore presumably down-regulating Wnt/β-catenin signaling. It appears that the BMP signaling target SMAD7 links BMP and Wnt/β-catenin signaling in the oligodendroglial lineage (**Fig. 4**). Based on the working model proposed by Weng et al., we propose that BMP4 does not inhibit OL differentiation when BMP receptors are deleted, however BMP-independent transcription factor Sip1 still directly activates SMAD7 in the absence of BMP signaling activation, and that SMAD7 upregulation reduces or extinguishes the transcriptional pool of β-catenin (Han et al., 2006; Tang et al., 2008), thus making OLs resistant to Wnt3a activation. This interpretation is compatible with the report by Feigenson et al., that Wnt3a did not exert inhibit oligodendrocyte differentiation if BMP signaling was blocked (Feigenson et al., 2011). Future studies are needed to validate this hypothesis. To further explore the proposed role of Sip1 target SMAD7 in repressing Wnt/β-catenin signaling, it will be important to determine whether SMAD7 cKO increases Wnt/β-catenin activity in OLs and restores the response of BMP signaling-blocked OPCs to Wnt3a.

A recent study showed that TGFβ signaling through the TGFβ-TGFβRI/II-SMAD2/3-SMAD4 cascade promotes OL differentiation by accelerating OPC cell cycle exit via p21 activation (**Fig. 4**) (Palazuelos et al., 2014). This suggests that enhanced OL differentiation and myelination achieved by Axin stabilization (Fancy et al., 2011) is mediated, at least in part, by Axin-facilitated upregulation of TGFβ signaling (Furuhashi et al., 2001).

Another possible convergent point linking TGFβ/BMP/SMAD and Wnt signaling is the SMAD/TCF/LEF1 complex (**Fig. 4**). TGFβ-related SMAD3 interacts with TCF/LEF1 to synergistically activate the Wnt target gene Xenopus homeobox twin (Xtwn). Interestingly, this synergistic interaction occurs in the absence of β-catenin, and requires both SMAD and TCF/LEF1 DNA binding sites in the target genes (Labbe et al., 2000). BMP-dependent activation of Msx2, a key regulator of cell fate of embryonic stem cells, was synergized via the binding of both SMAD4 and TCF/LEF1 with Msx2 promoter, and Wnt/β-catenin-dependent

activation of Msx2 was defective in Smad4-deficient embryonic stem cells (Hussein et al., 2003) which suggest a cooperative interaction of SMAD4 and TCF/LEF1 in activating certain genes. Similarly, both SMADs and TCF/LEF1 could synergistically regulate Emx2 activation via binding to its enhancer region (Theil et al., 2002). Since activation of Wnt/β-catenin signaling and TGFβ/SMAD signaling were reported to inhibit and promote oligodendrocyte differentiation, respectively, how these two pathways are antagonized and balanced to ensure the timing of oligodendrocyte differentiation is likely to prove to be an interesting direction for future research.

VIII: Interplay between Wnt/β-catenin and HIF signaling pathways

Hypoxia inducible factor (HIF) signaling adapts cells to hypoxic conditions. HIF1α/β and HIF2α/β signaling have some common features, but redundancy between HIF1α and HIF2α is limited during development and in tumorigenesis (Maxwell, 2005). Under normal conditions, HIFα is constitutively translated, but is subject to rapid proteasome-mediated degradation mediated by O2- and $Fe2^{+}$-dependent prolyl-hydroxylases (PHD) and von Hippel Lindau protein (VHL). When this degradation is blocked, HIFα accumulates in the cytoplasm, translocates to the nucleus, and binds the constitutively expressed HIFβ to initiate the expression of target genes (Arany et al., 1996) that are involved in glycolysis, erythropoiesis, angiogenesis and cell survival, among others (Sharp and Bernaudin, 2004; Greer et al., 2012; Semenza, 2012).

In tumor cells, HIF1α competes with TCF7l2 for binding to β-catenin. Binding of HIF1α and β-catenin to the hypoxia response element (HRE) activates HIF1α-mediated target gene transcription and shuts off TCF7l2-medated transcription, thus decreasing Wnt/β-catenin signaling and enhancing tolerance to hypoxia (Kaidi et al., 2007). However, HIF1α can activate Wnt/β-catenin signaling in stem cells by inducing expression of Tcf1 and Lef1 (Mazumdar et al., 2010). Consistent with that study, conditional KO of HIF1α from hippocampal subgranular zone (SGZ) neural stem cells suppresses hippocampal neurogenesis (Mazumdar et al., 2010), which is normally driven by Wnt/β-catenin signaling (Lie et al., 2005). The interactions between Wnt/β-catenin and HIF1α signaling pathways seem to be cell-type-dependent: HIF1α signaling inhibits Wnt/β-catenin signaling in osteoblasts (Chen et al., 2012; Chen et al., 2013), human lung (A549) and colon cancer (HCT116) cell lines and cell lines derived from human lung and colon cancer

and embryonic kidney (293T) (Lim et al., 2008), but activates Wnt/β-catenin signaling in adipogenic cell lines (Park et al., 2013) and NSCs (Varela-Nallar et al., 2014).

Chronic hypoxia rearing and HIF1/2α signaling stabilization by disrupting *Vhl* were found to inhibit oligodendroglial differentiation without altering OPC numbers (Yuen et al., 2014). Mechanistically, HIF1/2α signaling activated oligodendroglial Wnt7a/7b expression, which, in turn, prevented OPC differentiation in an autocrine manner and increased angiogenesis in a paracrine manner. Consistent with that result, corpus callosum and cortex from *Sox10* (or *Olig1*)-Cre, $Vhl^{fl/fl}$ mutants showed increased angiogenesis, diminished oligodendroglial differentiation, and elevated Wnt/β-catenin activity (Yuen et al., 2014). Our own laboratory, using the *Cnp*-Cre driver (Lappe-Siefke et al., 2003), also observed increased angiogenesis in the spinal cord upon HIF stabilization in the oligodendroglial lineage of *Cnp*-Cre, $Vhl^{fl/fl}$ mutants. The blood-brain barrier appeared to be normal in these mice, as judged by expression of the tight junction molecule claudin 5 (Argaw et al., 2012) (**Fig. 5**). These observations suggest that postnatal OPC differentiation and angiogenesis are reciprocally regulated by oligodendroglial HIF signaling. However, the well-established HIF target VEGF (Forsythe et al., 1996; Igarashi et al., 2002) was unaltered by HIF stabilization in oligodendroglial lineage cells (Yuen et al., 2014), suggesting that the induction of VEGF is context-dependent and that OLs regulate postnatal angiogenesis in a VEGF-independent fashion. It has been reported that both HIF1α and TCF7l2 are upregulated in normal appearing white matter and active lesions, respectively, in multiple sclerosis brains (Graumann et al., 2003; Zeis et al., 2008; Fancy et al., 2009; Fancy et al., 2011). Future studies will be needed to determine whether the cross-talk between HIF and Wnt observed in early postnatal CNS also applies to injury conditions and, if so, how these two pathways interact to either promote or inhibit timing of oligodendroglial differentiation/maturation.

IX: Proposed working model, conclusions, and perspective

The role of the canonical Wnt/β-catenin signaling pathway during normal oligodendroglial development is much more complicated than was previously thought, varying with the stage of oligodendroglial development, age of the animal, and between spinal cord and brain. Strong consensus has been reached that dysregulated Wnt/β-catenin activation exerts an

inhibitory effect on postnatal differentiation of OPCs into mature OLs in both spinal cord and brain. However, the role of dysregulated Wnt/β-catenin activation in OPC generation from embryonic NSC is still controversial, with some laboratories observing an inhibitory effect and others no effect. Furthermore, dysregulated Wnt/β-catenin activation was reported to promote OPC generation from NSCs in the early postnatal and adult SVZ, but the role of Wnt/β-catenin signaling in the differentiation of SVZ-derived OPCs to mature OLs remains uncertain.

Many publications have utilized constitutively active β-catenin models, but it is now clear that the effects of this manipulation are often not reflective of those engendered by physiological signaling by the canonical Wnt/β-catenin signaling pathway, nor entirely confined to that signaling pathway (Haq et al., 2003; Song et al., 2003; Lu and Hunter, 2004; Valenta et al., 2012). Enforced activation of β-catenin may modulate other signaling pathways that are not normally regulated by β-catenin–mediated Wnt signaling under physiological conditions. It is still unclear whether endogenous Wnt/β-catenin signaling is required to ensure the timing of oligodendrocyte differentiation.

Based on data generated from β-catenin loss-of-function mutants and *Tcf7l2*-null embryos, and the expression patterns of Wnt effector TCF7l2 and negative regulator APC, we here propose a multistage model of the regulation of oligodendroglial development by endogenous Wnt/β-catenin signaling (**Fig. 6**).

1) **From embryonic NSCs to OPCs**. In the embryonic spinal cord, endogenous Wnt/β-catenin inhibits precocious OPC generation from embryonic Olig1/2$^+$ NSCs until the appropriate micro-environment is established (at ~E12.5 in mouse spinal cord) after which Wnt/β-catenin is dispensable for OPC genesis. This would explain the precocious OPC generation in the early but not late embryonic/postnatal spinal cord when β-catenin is ablated from Olig1/2$^+$ NSCs (Fancy, et al., 2009; Dai et al., 2014). Similarly, CA-β-catenin activation or Axin2-null decreases OPC number in early embryos, but not later in development (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009; Dai et al., 2014). However, distinct microenvironmental cues in the brain vs spinal cord NSC niches and/or in the embryonic vs postnatal brain NSC niches may interact with Wnt/β-catenin signaling to determine a different functional readout in the NSCs of postnatal brain (Azim and Butt, 2011; Ortega et al., 2013; Azim et al., 2014b; Azim et al., 2014a). Future studies on the interplay between Wnt/β-catenin and other pathways should shed light on the mechanism of this context-dependent role.

2) **From OPCs to immature OLs**. Low level Wnt/β-catenin signaling, mediated by TCF7l2 and/or other potential transcription factors (for example Bcl9 among others), promotes the transition of OPCs to immature OLs, in which myelin genes are barely expressed. This is supported by the normal numbers of OPCs and decreased numbers of immature OL in the late embryonic/perinatal spinal cord of both TCF7l2-null mutants and β-catenin loss-of-function mutants (Fu et al., 2009; Ye et al., 2009; Dai et al., 2014). This proposal is also compatible with the *in vivo* accumulation of immature OLs with low NG2/PDGFRa and low myelin gene expression in the Yin Yang 1 KO mutants in which TCF7l2 is upregulated (He et al., 2007). Constitutively active β-catenin or Axin2 mutations yield "pathologically" high Wnt/β-catenin activity that inhibits the transition of OPC to TCF7l2-low immature OLs, though oligodendrocyte differentiation eventually recovers presumably due to the dispensability of Wnt/β-catenin signaling (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009; Fancy et al., 2011; Lang et al., 2013; Dai et al., 2014; Fancy et al., 2014).

3) **From immature OLs to premyelinating mature OLs**. We propose that Wnt/β-catenin signaling is dispensable for this step. Upregulation of the Wnt negative regulator APC (Lang et al., 2013) and/or of Sip1/SMAD7 (Weng et al., 2012) down-regulates the transcriptional pool of β-catenin to a minimal level that prevents it from binding TCF7l2. However, TCF7l2 may collaborate with other factors (for example SMADs in BMP/TGFβ signaling) to promote the transition of immature OLs into premyelinating, mature OLs, presumably by upregulating myelin gene expression and/or myelin lipid production (Boj et al., 2012). Under this working model, β-catenin loss-of-function affects neither the number of postnatal mature OLs, nor myelin gene expression (Fancy et al., 2009; Lang et al., 2013). However, APC ablation prevents β-catenin degradation, thus allowing β-catenin to bind "ectopically" to TCF7l2 or other TCF/LEF family members or factors to activate Wnt/β-catenin signaling and block the generation of premyelinating mature OLs (Lang et al., 2013; Fancy et al., 2014). Consistently, LEF1, undetectable in OLs in APC WT mice, is upregulated in the post-mitotic, $NG2^-$/$PDGFRa^+$ immature OLs in APC KO mutants (Fancy et al., 2014) (Hammond et al., unpublished data).

4) **From premyelinating mature OLs to myelinated mature OLs**. It is still controversial whether canonical Wnt/β-catenin plays a role in this step. Dominant negative TCF7l2 expression, presumably blocking Wnt/β-catenin activity, was reported to decrease myelin gene expression and myelination (Tawk et al., 2011). However, dominant negative

TCF7l2 lacking β-catenin binding ability may retain the transcriptional repression function and its ability to participate in other signaling pathways. In this scenario, gain-of-function of dominant negative TCF7l2 may account for decreased myelin gene expression and myelination. In contrast, conditional KO of β-catenin during active myelination was reported not to affect either *in vivo* myelin gene expression or myelination (Fancy et al., 2009; Lang et al., 2013). Future studies employing independent approaches to manipulate Wnt/β-catenin activity are needed to draw conclusions concerning the role of TCF7l2 in driving myelin gene expression and myelination, and to prove or disprove our alternative interpretation.

In summary, canonical Wnt/β-catenin signaling pathway plays distinct roles in oligodendrogenesis, oligodendrocyte differentiation and myelination in a context-dependent manner: CNS developmental stage, oligodendrocyte developmental stage and CNS microenvironment together affect the strength of Wnt/β-catenin activity and determine the interplay of this activity with other signaling pathways. Physiologically regulated Wnt tone and its cross-talk with other pathways govern the effects of endogenous Wnt/β-catenin signaling during oligodendrocyte development and regeneration. In this context, successful therapeutics based on modulating canonical Wnt signaling may be possible to devise, but would require a greater understanding of the complexities of the canonical Wnt/β-catenin signaling pathway in oligodendroglial development.

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Figure legend

Figure 1 - Oligodendroglial development and regeneration. OPCs, which express high levels of NG2 and platelet-derived growth factor receptor α (PDGFRα), are initially derived from multipotent NSCs (i.e. radial glia (RG). There are two waves of OPC generation in spinal cord (Cai et al., 2005; Fogarty et al., 2005; Vallstedt et al., 2005) and three waves of OPC generation in brain (Kessaris et al., 2006; Richardson et al., 2006). OPCs migrate to their destination while proliferating to expand their population. Upon receiving appropriate cues, OPCs either die via programmed cell death or exit the cell cycle and differentiate. OPCs that remain in the adult CNS continue to generate myelinating OLs for

normal myelin turnover (Young et al., 2013). Proliferation by adult OPCs increases after demyelination, and generates new oligodendroglia for remyelination. NSCs resident in the adult forebrain subventricular zone (SVZ) also generate increased numbers of OPCs post-forebrain demyelination (Menn et al., 2006).

Figure 2 - Canonical Wnt signaling and its regulation. Please refer to the "Canonical Wnt/β-catenin signaling pathway and its regulation" section for details.

Figure 3 - RT-qPCR quantification of β-catenin and Wnt target Axin2 and Sp5 expression in β-catenin or APC KO spinal cords. Statistical analysis in **A** and **D**: two-tailed Student's t test; in **B** and **C**, one-way ANOVA with Bonferroni post-test. * $p<0.05$, ** $p<0.01$, ns, $p>0.05$.

Figure 4 - Interactions between TGFβ/BMP and Wnt/β-catenin signaling pathways. The canonical TGFβ/BMP signaling cascade starts with binding of a member of the TGFβ, BMP, Activin, Nodal superfamily to its receptors. This induces phosphorylation and activation of receptor-regulated SMADs (R-SMADs), including R-SAMD1/5/8 for BMP signaling and R-SMAD2/3 for TGFβ signaling. The activated R-SMADs form complexes with the essential SMAD4 which then translocate to the nucleus, where R-SMAD/SMAD4 binds defined DNA sequences, in conjunction with other factors, to induce target gene expression (Shi and Massague, 2003). SMAD7 and SMAD6 are among these target genes, and provide a negative TGFβ/BMP signaling feedback loop (Miyazono et al., 2005; Yan et al., 2009), just as Axin2 provides negative feedback to Wnt/β-catenin signaling. Cytoplasmic Axin, a key component of the β-catenin destruction complex, positively regulates TGFβ/BMP signaling by either promoting the degradation of inhibitory SMAD7 (Liu et al., 2006) or by activating SMAD3 (Furuhashi et al., 2001). Axin can also negatively regulate TGFβ/BMP signaling by promoting SMAD3 degradation (Guo et al., 2008). SMAD7 antagonizes Wnt/β-catenin signaling by promoting the degradation of β-catenin (Han et al., 2006) and by diminishing the transcriptional pool of β-catenin through stabilizing β-catenin to the E-cadherin complex (Tang et al., 2008). In the nucleus, SMAD4 can form a complex with β-catenin/TCF/LEF and affects expression of target genes under the control of both pathways (Nishita et al., 2000).

Figure 5 – Enhanced angiogenesis in the postnatal spinal cord upon HIF signaling stabilization in the Cnp-Cre, Vhlfl/fl mutants. A, Double immunohistochemistry for laminin, which labels blood vessel basal lamina and the thymidine analog EdU (2 hour pulse labeling) in P9 spinal cord of Vhlfl/fl control and a Cnp-Cre, Vhlfl/fl mutant mice. Note the increased number of EdU+ blood vessel endothelial cells (arrowheads). B, Quantification of laminin+ area by Image J (n=4 each group), *** $p<0.001$, two-tailed

Student's t test. C, Double immunostaining for laminin and the tight junction protein claudin 5, suggesting that the blood-brain barrier remains intact (Argaw et al., 2012). Scale bars: 50 μm.

Figure 6 – Multi-modal role of canonical Wnt/β-catenin signaling in oligodendroglial development. Please see "Proposed working model, conclusion and perspective" section for a detailed discussion.

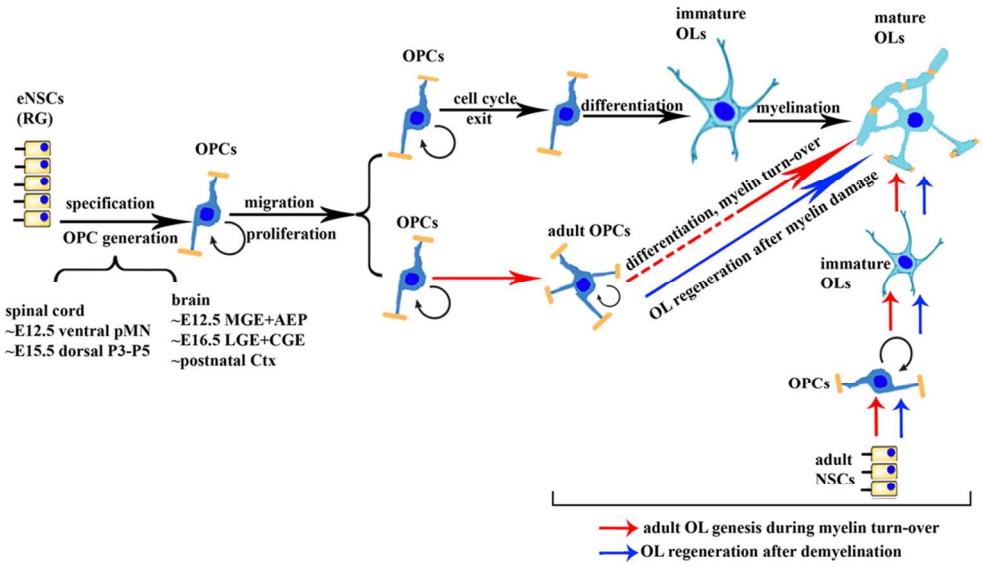
Table 1 – Glossary of abbreviations frequently used in this review.

Glossary	Explanation
Ctnnb1	Ctnnb1 is the official of the gene encoding β-catenin. β-catenin is a dual-role molecule, binding E-cadherins at cell adherens junctions and serving as a transcriptional activator in the nucleus.
Ctnnb1 exon3flox	Two loxP sites flank exon3 of Ctnnb1. Exon3 contains a phosphorylation site that targets β-catenin for degradation. Upon Cre-mediated exon3 deletion, the resulting "truncated" β-catenin escapes proteasome-mediated degradation, thus constitutively activating canonical Wnt signaling (termed CA-β-catenin in this review)
Ctnnb1 exon2-6flox	loxP sites are inserted in intron 1 and intron 6, so Cre-mediated recombination induces deletion of exons 2–6, resulting in ablation of β-catenin, thus blocking canonical Wnt signaling cascade. (termed β-catenin KO in this review)
Ctnnb1 dm/lox	Endogenous β-catenin protein is replaced by a mutant form that maintains the cell adhesion functions of β-catenin, but upon Cre-mediated recombination, its TCF/LEF-binding domains are disrupted, thus preventing activation of canonical Wnt/β-catenin nuclear targets
Bat-lacZ	The β-catenin activated transgene (BAT) driving expression of nuclear β-galactosidase was designed by fusing seven TCF/LEF binding sites upstream of a 0.13 kb fragment containing the minimal promoter-TATA box of the Siamois gene. This Wnt reporter transgenic strain is helpful in identifying Wnt responsive cells in different contexts in vivo. (also termed as BAT-Gal in the literature)
Axin2-lacZ	An in-frame insertion of a nuclear-localized β-galactosidase gene followed by a polyA signal and neo cassette is knocked into the endogenous Axin2 locus by homologous recombination. Homozygosity of Axin2-lacZ ablates the function of endogenous Axin2, resulting in decreased negative regulation of Wnt/β-catenin activity.
Apc^{min}	Apc multiple intestinal neoplasia (Apc^{min}), 1-allele truncated mutation of Apc gene, lacking DNA sequence for coding β-catenin-binding domain.
TCF/LEF1	T cell factor/lymphoid enhancing factor 1. TCF/LEF1 is a group of transcription factors which have a β-catenin binding domain and a high mobility group (HMG) DNA binding domain. They are required for canonical Wnt/β-catenin-mediated transcription. In human and mouse, there are four members of this family: TCF7, TCF7L1, TCF7L2 and LEF1
Dominant negative (DN) mutation	A dominant-negative mutation means that the resulting protein has lost a certain part of its function (negative), but it can out-compete the endogenous protein in some way (dominant). For example, DN-TCF/LEF1 is a mutated form of proteins that usually lack β-catenin binding domain or HMG DNA binding domain, thus inhibiting the normal function of endogenous TCF/LEF1.

Table 2 – Effects of *in vivo* genetic manipulations of canonical Wnt signaling on OPC generation and oligodendrocyte differentiation.

Method	Signaling activity	Major observations/conclusions	Reference
Olig1-Cre, Ctnnb1 exon3flox	Dysregulated activation	Decreased # of Olig2$^+$ progenitors at E10.5 (~2 days before OPC specification at E12.5) in the ventral spinal cord	Yu et al., 2008
Olig1-Cre, Ctnnb1 exon3flox	Dysregulated activation	Unaltered # of Olig2$^+$ progenitors at E12.5, but absence of OPCs and OLs thereafter in the spinal cord and brain	Ye et al., 2009 Dai et al., 2014
Olig2-Cre, Ctnnb1 exon3flox	Dysregulated activation	Unaltered # of OPCs, but decreased # of OLs at perinatal and early postnatal age; No difference of both OPCs and OLs in adulthood; delayed oligodendrocyte differentiation in lysolecithin-induced demyelination model	Fancy et al., 2009
Axin2-null	Increased activity	Decreased # of Pdgfrα and Sox10 mRNA$^+$ OPCs at E13.5; no difference in OPCs but decreased # of OLs at P0	Dai et al., 2014
Axin2-null	Increased activity	No difference in PDGFRα$^+$ OPCs, but less OLs postnatally; both OPCs and OLs return to normal in adulthood. Delayed oligodendrocyte differentiation in lysolecithin-induced demyelination model	Fancy et al., 2011
Olig1-Cre, Ctnnb1 exon2-6flox	Decreased activity	Increased # of ventral progenitors, but unaltered # of Olig2$^+$ progenitors at E10.5 in the spinal cord, 2 days prior OPC specification	Yu et al., 2008
Olig1-Cre, Ctnnb1 exon2-6flox	Decreased activity	Increased # of Pdgfrα mRNA$^+$ OPCs at E12.75 in the spinal cord	Ye et al., 2009
Olig1-Cre, Ctnnb1 exon2-6flox	Decreased activity	Increased # of Pdgfrα mRNA$^+$ OPCs at E12.5, but return to normal by late embryonic stage (E18) in the spinal cord	Dai et al., 2009
Olig2-Cre, Ctnnb1 exon2-6flox	Decreased activity	Unaltered # of PDGFRα$^+$ OPCs or Plp mRNA$^+$ OLs at perinatal age (P1) in both spinal cord and forebrain	Fancy et al., 2009
Tcf7l2-null	Decreased activity	Unaltered # of Pdgfrα mRNA$^+$ OPCs but decreased # of Plp mRNA$^+$ OLs in perinatal period (lethality in perinatal period) in the spinal cord	Ye et al., 2009 Fu et al., 2009
Cnp-Cre, Ctnnb1 exon3flox	Dysregulated activation	Unaltered # of Pdgfrα mRNA$^+$ OPCs, but absence of OLs till P7 in both spinal cord and brain	Ye et al., 209
Cnp-Cre, Ctnnb1 exon3flox	Dysregulated activation	No effects on OPC density, proliferation and apoptosis; decreased # of OLs during postnatal myelination in spinal cord, but return to normal in adulthood	Feigenson et a., 2009
Cnp-Cre, Ctnnb1 exon2-6flox	Decreased activity	No effects on OPCs and myelin gene expression at P1 in the spinal cord	Lang et al., 2013
NG2-Cre, Ctnnb1 exon2-6flox	Decreased activity	Unaltered # of OPCs and OLs; unaltered levels of myelin gene expression at both P7 and P26 in the spinal cord	Lang et al., 2013
Olig2-CreERT2 Ctnnb1 exon2-6flox	Decreased activity	Unaltered # of OPCs and OLs and unaltered levels of myelin gene expression at P14 in the spinal cord when Ctnnb1 is disrupted at P6 and P7	Lang et al., 2013
NG2 (Cnp)-Cre, Apcfl/fl; Olig2-CreERT2 Apcfl/fl	Dysregulated activation	Inhibition of oligodendrocyte differentiation: decreased # of OLs and levels of myelin gene expression; this inhibition persists into adulthood; Wnt-independent role of Apc also plays a part in oligodendrocyte differentiation.	Lang et al., 2013
Olig2-Cre, Apcfl/fl	Dysregulated activation	Inhibition of oligodendrocyte differentiation; this inhibition persists into adulthood	Fancy et al., 2014
hGFAP-Cre, Ctnnb1 exon2-6flox	Decreased activity	Decreased # of Mbp mRNA+ OLs in the corpus callosum at P14; reduced # of radial glia and intermediate progenitors in neocortex in late embryonic period	Gan et al. 2014
Pdgfrα-CreERT2, Ctnnb1 exon2-6flox	Decreased activity	No effects on adult OPC differentiation in the spinal cord; decreased proliferation of adult OPCs after spinal cord injury.	Rodriguez et al., 2014
In utero electroporations of DN-LEF1 and Dkk into E15.5 telencephalon	Decreased activity	Increased # of OPCs in the cortex at P0.5 or P2; no effects on OPC proliferation and apoptosis.	Langseth et al., 2010
In utero electroporations of Cre-expressing plasmid, Ctnnb exon3flox	Dysregulated activation	Increased # of OPCs when in utero electroporations of Cre into dorsal SVZ at P2; unaltered # of OPCs when EPs into lateral SVZ; unaltered # of OPCs when in utero electroporations of Cre into dorsal SVZ of Ctnnb1 exon2-6flox mice	Azim et al., 2014
Lentiviral expression of Wnt3 and DN-TCF7l2	Increased (Wnt3a) and decreased (DN-TCF7l2)	Increased # of Olig2$^+$ and PDGFRα$^+$ OPCs in adult SVZ after Wnt3 expression but decreased # of OPCs after DN-TCF7l2 expression	Ortega et al., 2013

	activity		
Expression of DN-TCF7l2 and DN-LRP6	Decreased activity	Decreased expression of myelin genes and extent of myelination in zebrafish CNS, oligodendrocyte differentiation unaltered	Tawk et al., 2011



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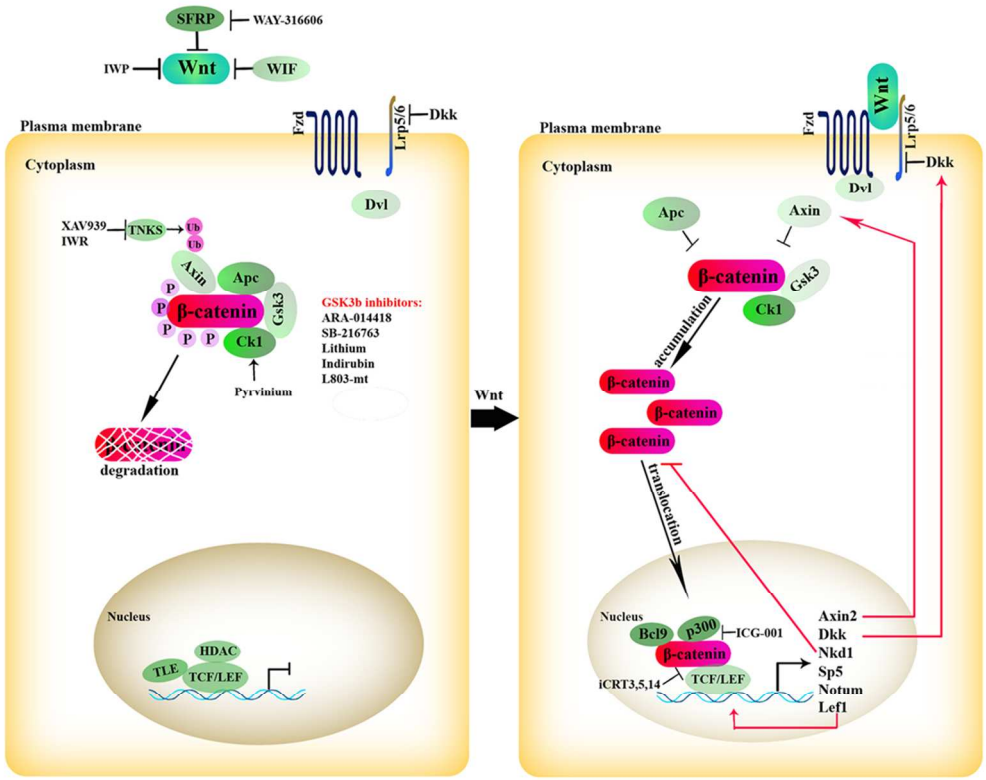

Figure 2 - Canonical Wnt signaling and its regulation. Please refer to the "Canonical Wnt/β-catenin signaling pathway and its regulation" section for details.
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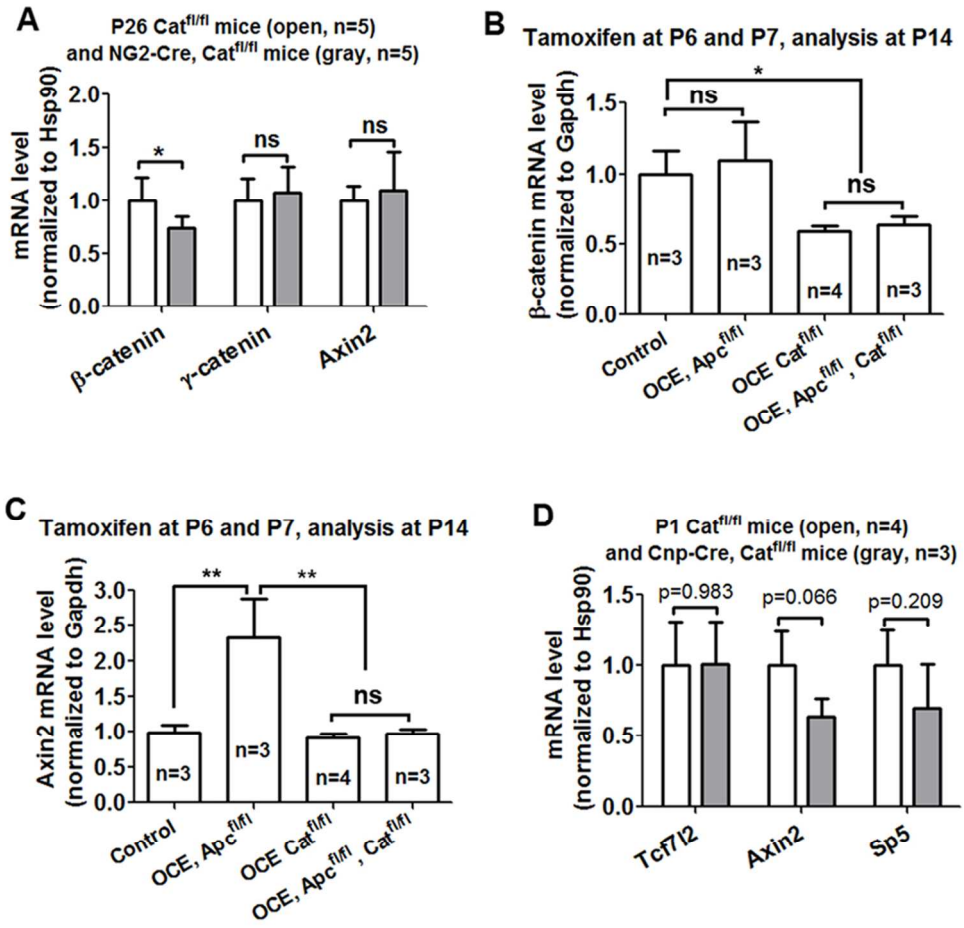

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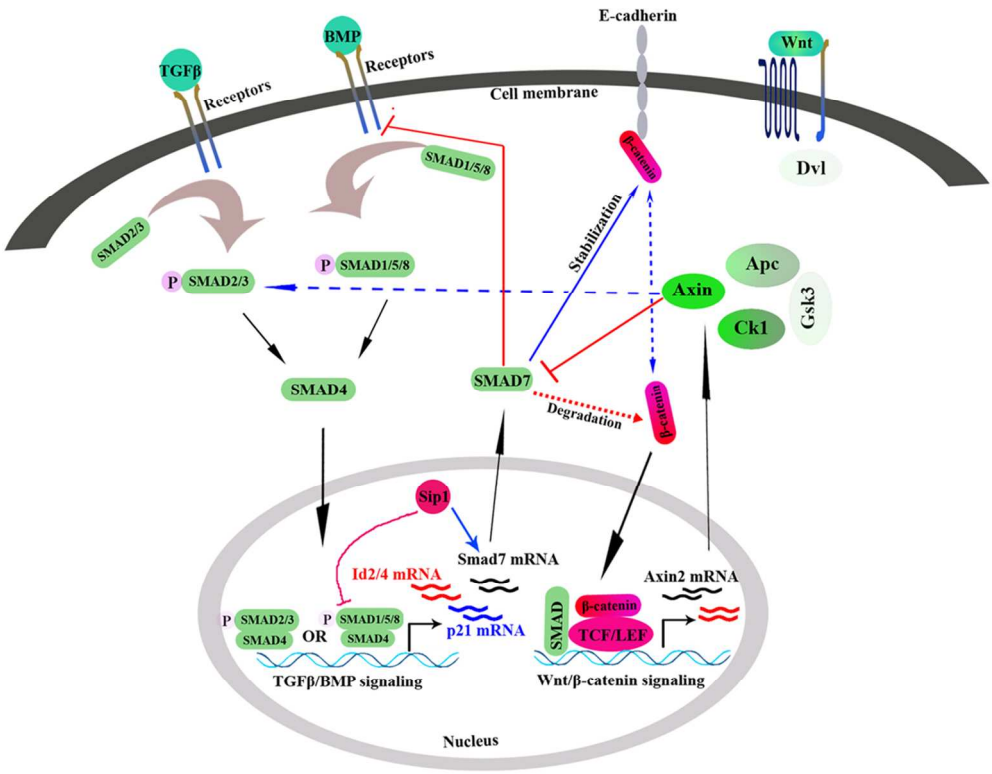

Figure 4 - Interactions between TGFβ/BMP and Wnt/β-catenin signaling pathways. The canonical TGFβ/BMP signaling cascade starts with binding of a member of the TGFβ, BMP, Activin, Nodal superfamily to its receptors. This induces phosphorylation and activation of receptor-regulated SMADs (R-SMADs), including R-SAMD1/5/8 for BMP signaling and R-SMAD2/3 for TGFβ signaling. The activated R-SMADs form complexes with the essential SMAD4 which then translocate to the nucleus, where R-SMAD/SMAD4 binds defined DNA sequences, in conjunction with other factors, to induce target gene expression (Shi and Massague, 2003). SMAD7 and SMAD6 are among these target genes, and provide a negative TGFβ/BMP signaling feedback loop (Miyazono et al., 2005; Yan et al., 2009), just as Axin2 provides negative feedback to Wnt/β-catenin signaling. Cytoplasmic Axin, a key component of the β-catenin destruction complex, positively regulates TGFβ/BMP signaling by either promoting the degradation of inhibitory SMAD7 (Liu et al., 2006) or by activating SMAD3 (Furuhashi et al., 2001). Axin can also negatively regulate TGFβ/BMP signaling by promoting SMAD3 degradation (Guo et al., 2008). SMAD7 antagonizes Wnt/β-catenin signaling by promoting the degradation of β-catenin (Han et al., 2006) and by diminishing the transcriptional pool of β-catenin through stabilizing β-catenin to the E-cadherin complex (Tang et al., 2008). In the nucleus, SMAD4 can form a complex with β-catenin/TCF/LEF and affects expression of target genes under the control of both pathways (Nishita et al., 2000).
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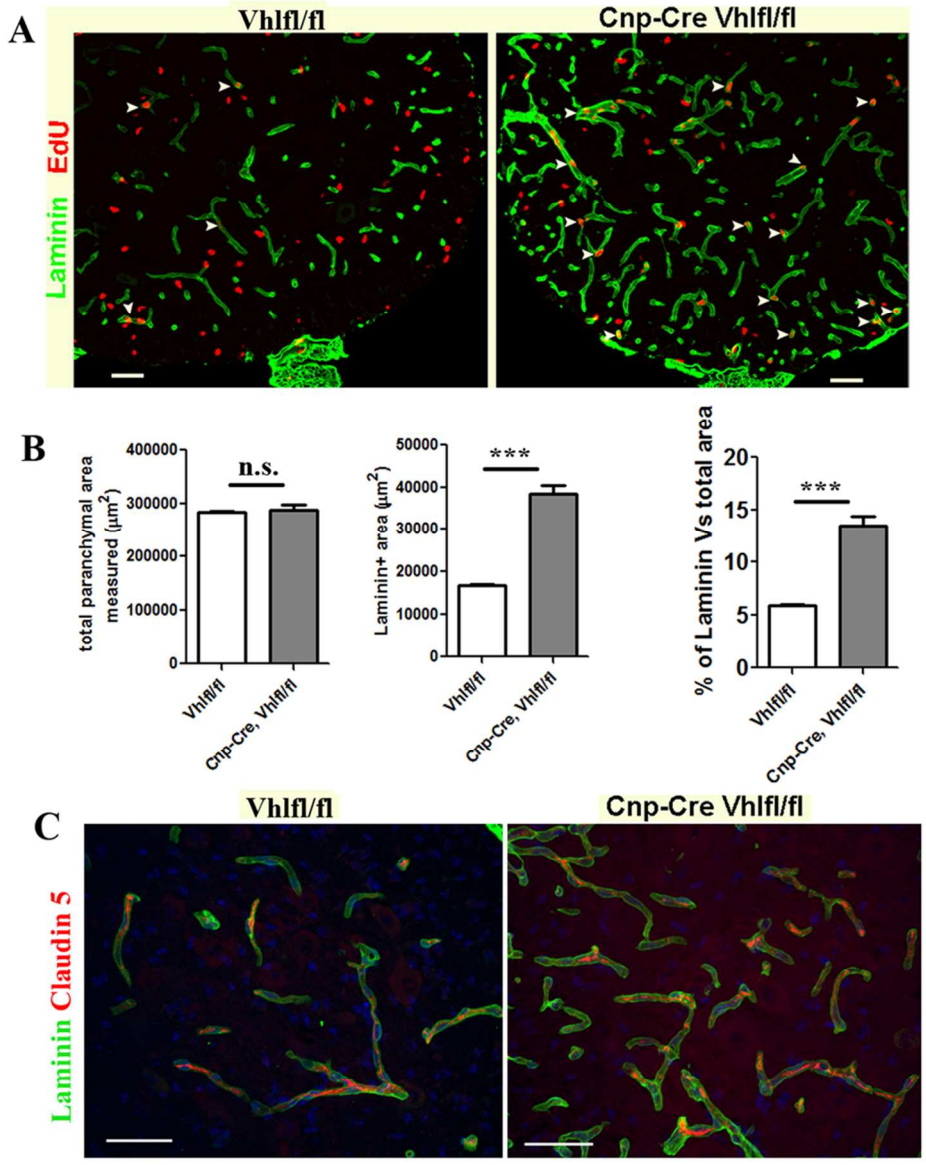

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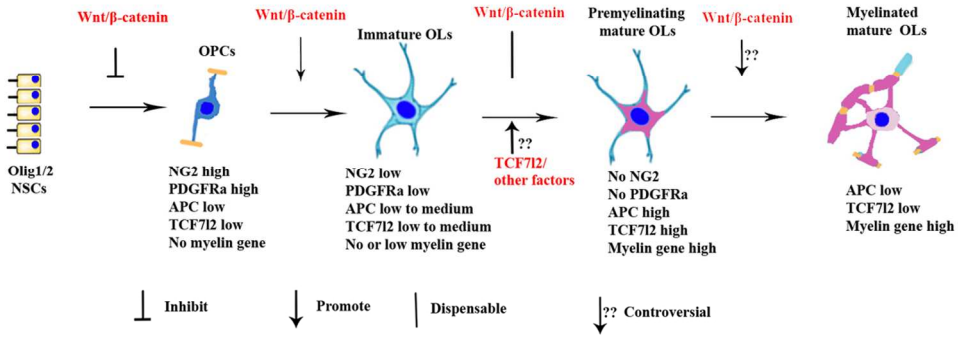
84x105mm (300 x 300 DPI)



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