**Differential regulation of oligodendrocyte development and myelination by protein tyrosine phosphatase alpha and Wnt signaling**

by

Yuda Shih

B.Sc., The University of British Columbia, 2008

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Pathology and Laboratory Medicine)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2015

**Abstract**

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS). The myelination process is preceded by molecular and morphological differentiation of oligodendrocyte precursor cells (OPCs) into mature myelinating OLs. Protein tyrosince phosphatase alpha (PTPα) is a brain-enriched tyrosine phosphatase that regulates many cellular processes, including OPC differentiation. Our laboratory has previously demonstrated that PTPα null OPCs have impaired differentiation and brains of PTPα KO mice are hypomyelinated. In this study, I observed defective myelination in OL/neuron co-cultures where wild type (WT) and PTPα knock-out (KO) OPCs were introduced to neurite beds formed by dorsal root ganglion neurons for 14 days and immunostained for myelin basic protein (MBP), a component of the myelin sheath, and neurofilament (NFH), an axonal protein. MBP/NFH co-localization was used as an indicator of potential myelination. Co-localization is significantly reduced by ~50% in co-cultures with KO OPCs as compared to WT OPCs. Additionally, co-cultures with KO OPCs exhibit a reduced ability to form elongated MBP/NFH immunopositive segments, suggestive that in co-cultures with KO OPCs the ability to form elongated axo-glial contacts, a prerequisite for myelination, is impaired. This coincides with a reduction in MBP immunopositivity from KO OPCs, indicating a differentiation defect in the absence of PTPα.

Pharmacological modulation of several signaling pathways has recently been shown to affect OL differentiation, myelination and remyelination. XAV939 is an inhibitor of canonical Wnt signaling and is known to promote OL differentiation and remyelination. Therefore, I investigated whether inhibition of Wnt signaling can remediate PTPα-dependent impairments in OL differentiation and myelination. I observed that inhibiting Wnt signaling can partially rescue

PTPα-dependent impairments in differentiation; however, inhibition of Wnt signaling could not remediate the defects in elongation of MBP/NFH immunopositive segments in co-cultures with KO OPCs. While these studies reveal no apparent common molecular candidates between PTPα and Wnt signaling that may regulate OL differentiation, the findings described suggest that PTPα has at least two distinct roles during oligodendrocyte development: promoting OL differentiation by regulating MBP expression, formation and elongation of axo-glial contacts, both of which are prerequisite for myelination.

**Preface**

The research project presented in this thesis was developed in Dr. Catherine J. Pallen's laboratory. I have participated in experimental design under Dr. Pallen's supervision and conducted all listed experiments with exception of the experiments described in Figures 4.1 and 4.2. Figure 4.1 was conducted in collaboration with Dr. Philip Ly. The experiments described in Figure 4.2 were carried out by Dr. P. Ly. Figures 1.1 and 4.2 were contributed by Dr. P. Ly. I was responsible for data collection and analysis. Mouse colony was maintained by Dr. Jing Wang. Electron microscopy was performed by Fanny Chu at the UBC Centre of Heart Lung Innovation at St. Paul's Hospital

Animal care and use followed the guidelines of the University of British Columbia and the Canadian Council on Animal Care, and were reviewed and approved by the University of British Columbia Animal Care Committee with Certificate Number A14-0292 (Neurobiology of PTP Alpha) and with Certificate Number A14-0020 (PTP Alpha Mouse Study).

**Table of Contents**

**List of Tables**

**List of Figures**

**List of Abbreviations**

| | |
|---|---|
| APC | adenomatous polyposis coli |
| ATP | adenosine triphosphate |
| BCAR3 | breast cancer anti-estrogen resistance 3 |
| bFGF | basic fibroblast growth factor |
| BSA | bovine serum albumin |
| Caspr | contactin associated protein |
| Cdc42 | cell division control protein 42 homolog |
| CK | casein kinase |
| CNPase | 2'-3'-cyclic nucleotide 3'-phosphodiesterase |
| CNS | central nervous system |
| CNTF | ciliary neurotrophic factor |
| CXCL1 | chemokine (C-X-C motif) ligand 1 |
| Dcc | deleted in colorectal carcinoma |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | dimethyl sulfoxide |
| DRG | dorsal root ganglion |
| Dvl | dishevelled |
| EAE | experimental autoimmune encphalomyelitis |
| ECM | extracellular matrix |
| EDTA | ethylenediaminetetraacetic acid |
| EGF | epidermal growth factor |
| eIF2B | translation initiation factor 2B |
| Erk | extracellular signal-regulated kinase |
| FAK | focal adhesion kinase |
| FBS | fetal bovine serum |
| FuDR | 5-Fluoro-2’-deoxyuridine |
| Fz | Frizzled |
| GalC | galactocerebroside |
| GFP | green fluorescent protein |

| | |
|---|---|
| GPI | glycosylphosphatidylinositol |
| GPR17 | G-protein coupled receptor 17 |
| GSK | glycogen synthase kinase |
| HBSS | Hank's Balanced Salt Solution |
| HDAC | histone deacetylase |
| hnRNP | heterogenous nuclear ribonuclear protein |
| Ig | immunoglobulin |
| KO | knockout |
| Kv2.1 | delayed rectifier voltage-gated potassium channel |
| LEF | lymphoid-enhancer binding factors |
| LINGO-1 | leucine-rich repeat and immunoglobulin-domain-containing, Nogo-receptor interacting protein-1 |
| LRP | low-density lipoprotein receptor protein |
| MAG | myelin associated glycoprotein |
| MAPK | mitogen-activated protein kinase |
| MBP | myelin basic protein |
| MS | multiple sclerosis |
| mTOR | mammalian target of rapamycin |
| NAC | N-acetyl-L-cysteine |
| NCAM | neural cell adhesion molecule |
| NCM | neural culture medium |
| NF155 | neurofascin 155 |
| NFH | neurofilament |
| NG2 | neural/glial antigen 2 |
| NRG | neuregulin |
| NT3 | neurotrophin-3 |
| OL | oligodendrocyte |
| OPC | oligodendrocyte progenitor cell |
| PBS | phosphate buffered saline |
| PDGF | platelet derived growth factor |

| | |
|---|---|
| PDGFR | platelet derived growth factor receptor |
| PDL | poly-D-lysine |
| PI3K | phosphatidylinositol 3-kinase |
| PIP3 | phosphatidylinositol-3,4,5-triphosphate |
| PKB | protein kinase B |
| PLP | proteolipid protein |
| PMSF | phenylmethylsulfonyl fluoride |
| PNS | peripheral nervous system |
| POA | proligodendroblast antigen |
| PSA | polysialylated |
| PTEN | phosphatase and tensin homolog |
| PTP | protein tyrosine phosphatase |
| PVDF | polyvinylidene fluoride |
| QKI | quaking homolog, KH domain RNA binding |
| RTK | receptor tyrosine kinase |
| SDS | Sodium dodecyl sulfate |
| SDS-PAGE | SDS polyacrylamide gel electrophoresis |
| SFK | Src family kinase |
| SH1 | Src homology domain 1 |
| SH2 | Src homology domain 2 |
| SH3 | Src homology domain 3 |
| SH4 | Src homology domain 4 |
| T3 | triiodothyronine |
| T4 | thyroxine |
| TAG | transient axonal glycoprotein-1 |
| Tcf | T-cell factor |
| TGF | transforming growth factor |
| VLCFA | very long unbranched fatty acid |
| WT | wild-type |

**Acknowledgements**

First and foremost, I wish to express my heartfelt gratitude to my research supervisor, Dr. Catherine J. Pallen for the incredible support she has provided during the course of my graduate studies and research experience. Her patience, immense knowledge, passion for science and encouragement has guided me through my research and thesis writing. I am very blessed to have had her as my mentor during my Masters study.

I would like to thank Dr. Jing Wang for managing the lab, generating and maintaining the mice used for my studies. In particular, I am extremely grateful for her tireless efforts in establishing and training me in the OPC/DRGN co-culture technique, a major component of my research project. In addition, I enjoyed all the delicious homemade treats.

I would also like to thank Dr. Philip Ly for all the support he's provided me at the bench. I am extremely grateful for his patience, experience, knowledge, ideas, and the never-ending discussions about our research. I am extremely fortunate to have had the opportunity to work with Dr. Ly.

I am grateful to my supervisory committee: Dr. Janet Chantler, Dr. Katerina Dorovini-Zis, and Dr. Wolfram Tetzlaff for their insightful suggestions, as well as their efforts in reading my thesis.

Additionally, I would like to thank Dr. C. James Lim for his patience and advice with regards to immunofluorescence microscopy.

I am also grateful to my graduate advisor, Dr. Haydn Pritchard. Without his help and generosity, I would not have had the opportunity to carry my graduate studies.

All the members of the Pallen Lab, I thank you for making it a fun place to do research. In particular, I wish to thank Dominik Sommerfeld for putting up with me while we shared a bench, and for the motivation and support.

I also wish to thank my incredible friends for the tremendous support they have provided over the years. I am particularly indebted to Jaime Yen for being my voice of reason. Without her encouragement and endless support, I would have never explored the prospect of pursuing graduate studies.

I also want to express my gratitude to the Multiple Sclerosis Society of Canada for funding an operating grant to Dr. C.J. Pallen that supported this research.

Last but definitely not least, I wish to thank my family: my parents and my brother Yulin Shih for their encouragement and moral support throughout my life; and my youngest brother Yuder Huang for providing comic relief and being my inspiration.

# Chapter 1: Introduction

## 1.1 An overview of oligodendrocyte development and central nervous system myelination

The vertebrate CNS consists of two main classes of cells: neurons and glial cells. Neurons primarily function to transmit electrical signals and information throughout the nervous system. Neuronal function is dependent on interactions with glial cells such as astrocytes and oligodendrocytes for support (Miller, 2002). Astrocyte functions are diverse and not well characterized (Molofsky, 2012), whereas oligodendrocytes are the myelin forming cells of the central nervous system (CNS). Oligodendrocytes (OLs) are derived from progenitors that pass through a series of developmental stages before reaching the mature myelinating form (Figure 1.1). Oligodendrocyte progenitor cells (OPCs) arise and proliferate in distinct locations of the developing nervous system and subsequently migrate throughout the CNS and continue to undergo proliferation upon arriving at their destinations in developing white matter (de Castro and Bribian, 2005). When sufficient numbers of OPCs have been generated, OPCs differentiate into immature pre-myelinating OLs. As differentiation continues, OLs increase expression of myelin-associated molecules and begin to contact and ensheath appropriate axons (Miller, 2002). The myelin sheath is composed of layers of modified plasma membrane and functions to protect axons from degeneration and insulate axons to promote saltatory conduction, where neuronal action potentials propagate between unmyelinated nodes of Ranvier. This increases both speed and energy efficiency of nerve signal conduction (de Castro and Bribian, 2005; Mitew *et al.,* 2014).

### 1.1.1. Diseases of oligodendrocytes and myelin

Oligodendrocytes are the sole source of myelin in the CNS; therefore, dysfunction or aberrations in oligodendrocyte development and function can give rise to myelin disorders. Myelin disorders are grouped into two main classes: inherited dysmyelinating disorders and acquired demyelinating disorders.

#### 1.1.1.1 Inherited dysmyelinating disorders

Inherited disorders of myelin, such as leukodystrophies, result from myelin failure or loss. Children with these disorders often present general neurological symptoms related to motor function, including changes in gait and muscle tone; and to cognitive functions such as speech difficulties and impaired intellectual skills (Franklin and ffrench-Constant, 2008). In general, leukodystrophies pertaining to lipid catabolism result in demyelination, whereas deficiencies in myelin-associated proteins give rise to hypomyelination (Schiffmann *et al.,* 1994). X-linked adrenoleukodystrophy is an inheritable disorder of lipid catabolism characterized by the accumulation of very long chain unbranched fatty acids (VLCFAs) in the brain, attributable to dysfunctional peroxisomal β-oxidation of VLCFAs (Moser *et al.,* 1999; Yamada, *et al.,* 1999). VLCFA accumulation initiates immune infiltration and inflammation, resulting in myelin destruction and the formation of demyelinated lesions (Kaye, 2001). Tay-Sachs and Krabbe's diseases are autosomal recessive disorders of myelin related to aberrant lysosome function and lipid storage (Kaye, 2001).

Pelizaeus-Merzbacher disease is a X-linked disorder affecting the gene encoding the myelin-associated protein proteolipid protein (PLP) and its isoform DM20, both of which are major components of the myelin sheath (Kaye 2001). DM20 is implicated in oligodendrocyte

maintenance and myelin formation, and mutations affecting the trafficking of DM20 and PLP are associated with a severe congenital variant of Pelizaeus-Merzbacher disease (Gow and Lazzarini, 1996).

Childhood ataxia with diffuse CNS hypomyelination (vanishing white matter disease) is a leukodystrophy characterized by a generalized reduction in myelin-specific protein and lipids (Schiffmann *et al.,* 1994). Abnormal control of protein translation resulting from mutations in initiation factor 2B (eIF2B) has recently been attributed to vanishing white matter disease; however, it remains unclear why mutations in eIF2B predominantly affect oligodendrocytes (Schiffmann and Elroy-Stein, 2006).

#### 1.1.1.2 Acquired demyelinating disorders

Multiple sclerosis (MS) is an intensively studied CNS demyelinating disease of unknown cause. MS involves the inflammation-mediated destruction of myelin and accumulating axonal loss (Compston and Coles, 2002). It is estimated that MS affects approximately 100-200 out of 100,000 people in North America (Hauser and Oksenberg, 2006). In approximately 80% of clinical cases, patients present with the relapsing-remitting form of disease, characterized by functional recovery and remyelination following an individual demyelination episode (Compston and Coles, 2002; 2008). As the disease progresses, recovery from individual demyelinating episodes is incomplete and remyelination becomes less efficient. Eventually 65% of patients enter a secondary progressive phase where increasing axonal loss results in the permanent loss of neurological function (Compston and Coles, 2008). Current treatments aim to suppress inflammation or modify the immune response. Some of the clinically approved treatments include beta-interferons, glatiramer acetate, teriflunomide, fingolimod, and natalizumab (Cross

and Naismith, 2014). While some of these therapies can be efficacious, they are often associated with severe complications and side effects; for example, natalizumab is associated with progressive multifocal leukoencephalopathy (Cross and Naismith, 2014).

Periventricular leukomalacia is an important disease of myelin and is the major cause of cerebral palsy (Franklin and ffrench-Constant, 2008). It is thought to result from oligodendrocyte loss or damage due to ischemia or infection during fetal development (Levison *et al.,* 2001; Robinson *et al.,* 2005; Haynes, *et al.,* 2005). Aberrations in developmental myelination are also observed with hypothyroidism, nutritional deficiencies, and fetal alcohol and cocaine syndromes (Noble *et al.,* 2005).

### 1.1.2 Structure and function of the myelinated axon

Myelination by oligodendrocytes reorganizes CNS axons into distinct domains by restricting various axonal molecules to specific regions of the axon (Figure 1.2). Myelination leads to the formation of myelinated internodes, intervened by unmyelinated nodes of Ranvier where sodium channels become clustered. Segregation of sodium channels to the nodes restricts action potentials to the nodes, thereby increasing the velocity of nerve conduction. Axonal myelination not only increases conduction velocity, but also reduces energy consumption by ion channels, especially by $Na^+/K^+$ ATPase during axonal repolarization (Naves, 2010). In demyelinated axons, ATP consumption increases such that re-establishment of ion gradients by $Na^+/K^+$ ATPase pumps cannot be achieved, leading to abnormal calcium entry causing proteolysis and axonal destruction (Trapp and Stys, 2009). The nodes of Ranvier are flanked by paranodes, which are specialized zones of contact between axons and glial cells. The paranodes, are characterized by loops of uncompacted oligodendroglial cytoplasm and function as a

membrane barrier for the separation of sodium channels, and potassium channels, located in the juxtaparanodes (Zoupi *et al.,* 2011). The juxtaparanodes also contain compact myelin characteristic of myelinated internodes (Peles and Salzer 2000).

#### 1.1.2.1 Axo-glial interaction in myelination

Myelination requires physical and functional interactions between axons and oligodendrocytes. Axo-glial communication is bidirectional and complex, acting through direct contact or through secreted factors. These interactions are essential for oligodendrocyte maturation and myelination during development.

##### 1.1.2.1.1 Contact-mediated axo-glial interactions

*Polysialylated-neural cell adhesion molecule (PSA-NCAM)*

Neural cell adhesion molecules (NCAMs) are expressed on both oligodendrocytes and neurons (Fields and Itoh, 1996; Decker *et al.,* 2000). Transient addition of polysialic acid (PSA) to NCAMs prevents homophilic NCAM-NCAM interactions and negatively regulates cell-cell interactions (Kiss and Rougon, 1997; Rutishauser and Landmesser, 1996). Down-regulation of axonal and oligodendroglial PSA-NCAM favors oligodendrocyte differentiation and myelination, and increased formation of myelinated internodes, but does not affect the timing of myelination onset (Charles *et al.* 2000; Fewou *et al.,* 2007).

*Leucine-rich repeat and immunoglobulin-domain-containing, Nogo receptor-interacting protein-1 (LINGO-1)*

LINGO-1 is a transmembrane protein expressed on both oligodendrocytes and axons, and interacts with the NOGO-66 receptor/p75 signaling complex to inhibit neurite outgrowth (Mi *et al,* 2004). Oligodendroglial expression of LINGO-1 negatively regulates OPC differentiation and myelination through reduced Fyn activity and increased RhoA activity, signaling molecules that have been implicated in oligodendrocyte differentiation and myelination (Mi *et al.,* 2005; Section 1.2). Additionally LINGO-1 knockout mice exhibit early-onset myelination (Mi *et al.* 2005). Oligodendroglial and axonal LINGO-1 can also interact in *trans* to inhibit oligodendrocyte differentiation and myelination (Jepson *et al.,* 2012).

*G-protein coupled receptor 17 (GPR17)*

GPR17 is an oligodendrocyte-specific receptor that is transiently expressed in the early stages of differentiation (Chen *et al.,* 2009; Fumagalli *et al.,* 2011). GPR17 overexpression in oligodendrocytes inhibits OPC differentiation, whereas GPR17 knockout mice exhibit precocious myelination, suggesting GPR17 functions as a negative regulator of oligodendrocyte differentiation and myelination (Chen *et al.,* 2009).

*Notch-1*

Oligodendrocytes express Notch-1, which interacts with the axonally expressed ligands Jagged-1 and Delta 1 to exert inhibitory effects on OPC differentiation (Wang *et al.,* 1998; Kondo and Raff, 2000). Conversely, oligodendroglial Notch-1 interactions with axonal

F3/contactin promote differentiation as indicated by expression of differentiation markers such as myelin-associated glycoprotein (MAG) (Hu *et al.,* 2003).

*Integrin signaling*

Pre-myelinated axons also express the α2 chain of laminin, which binds to the β1 integrin receptors on oligodendrocytes to promote OPC survival and differentiation (Colognato *et al.* 2002). Laminin and integrin interactions in oligodendrocyte development are described further in section 1.2.

#### 1.1.2.1.2 Axo-glial signaling through secreted factors

Recent studies have revealed that the secreted Wnt glycoprotein, which signals through the canonical Wnt signaling pathway, has inhibitory effects on OPC differentiation, myelination and remyelination (described in section 1.2.5). In addition, netrin 1, a laminin family extracellular matrix protein present in the local environment of immature OLs, is important for the transition of an OL from its immature premyelinating stage to the mature myelinating stage (Rajasekharan *et al.,* 2009). Mitogen withdrawal and addition of the thyroid hormone, triiodothyronine (T3), to OPCs in culture can stimulate OPCs to cease proliferation and promote differentiation *in vitro* (Barres *et al*., 1994).

### 1.1.3 Development and maturation of oligodendrocytes

#### 1.1.3.1 Origins of oligodendrocyte progenitors

In the spinal cord, oligodendrocyte progenitors (OPCs) arise in sequential waves with the initial wave commencing in the ventral neuroepithelium within the pMN domain, a progenitor

domain of the embryonic neural tube, that also gives rise to motor neurons around embryonic day 12 (E12.5) (Lu *et al.,* 2000). A second wave of dorsally derived of OPCs arises around E15.5 (Cai *et al.,* 2005). Ventrally derived OPCs account for approximately 85-90% of the adult oligodendrocyte population (Mitew *et al.,* 2014). In the forebrain, three sequential waves of OPCs are generated: the first arises in the medial ganglion eminence and anterior entopeduncular areas of the ventral forebrain; the second wave is derived from the lateral and caudal ganglion eminences (Kessaris *et al.,* 2006). A third wave of OPCs arises post-natally; however, their origins remain unclear (Rowitch and Kriegstein, 2010). These populations are likely functionally redundant, as when one population is destroyed using targeted expression of the diphtheria toxin, the remaining OPCs take over and mice survive with normal complements of oligodendrocytes and myelin (Kessaris *et al.,* 2006).

Populations of $NG2^+$ oligodendrocyte progenitors are present in the adult CNS, some of which are embryonically derived OPCs that remained undifferentiated (Dawson *et al.,* 2003; Crawford *et al.,* 2014). $NG2^+$ OPCs can also arise from TypeB cells of the adult subventricular zone, and cells of this origin can become activated following demyelination and contribute to remyelination of demyelinated axons, such as within the corpus callosum (Nait-Oumesmar *et al.,* 1999; Menn *et al.,* 2006).

#### 1.1.3.2 Proliferation of oligodendrocyte progenitors

Early expansion of OPCs occurs in the ventricular and subventricular zone following commitment to the OPC fate; however, the majority of OPC proliferation occurs following migration of OPCs to sites of developing white matter (Miller, 2002). OPCs are proliferative and respond to mitogens including neurotrophin-3 (NT3), neuregulins (NRGs), platelet derived

growth factor (PDGF), basic fibroblast growth factor (bFGF), and the chemokine CXCL1 (Miller, 2002). PDGF and bFGF are the best characterized mitogens regulating OPC development. PDGF induces OPC proliferation and prevents premature differentiation (Richardson *et al.,* 1988; Noble *et al.,* 1988). Expression of the PDGF receptor alpha (PDGFRα) is upregulated by bFGF, and acts in concert with PDGF to maintain OPCs in a proliferative state (Bogler *et al.,* 1990; McKinnon *et al.,* 1990; McKinnon *et al.,* 1991).

#### 1.1.3.3 Differentiation of oligodendrocytes

Maturation of oligodendrocytes can be divided into distinct stages: the progenitor stage, the pro-oligodendrocyte stage, pre-myelinating oligodendrocytes, and mature myelinating oligodendrocytes (Merrill, 2009) (Figure 1.1). Oligodendrocyte progenitors are bipolar, proliferative, and migratory and express PDGFRα, the NG2 proteoglycan, and several surface gangliosides that are recognized by the monoclonal A2B5 antibody (Pfeiffer *et al.,* 1993; Dawson *et al.,* 2000). As oligodendrocyte progenitors mature into pro-oligodendrocytes, they begin to elaborate processes. Pro-oligodendrocytes are post-migratory and can be recognized using the O4 antibody, which detects sulfated surface antigens, including proligodendroblast antigen (POA) (Bansal *et al.,* 1992). Pre-myelinating oligodendrocytes are post-mitotic cells that express galactocerebroside (GalC), which can be identified by the monoclonal O1 antibody, and 2'-3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), which is detectable by anti-CNPase antibodies (Merrill, 2009). Mature oligodendrocytes enter a terminally differentiated stage, extend membranous sheets which ensheath axons, and in addition to CNPase, express myelin specific proteins such as myelin-associated glycoproteins (MAG), myelin basic protein (MBP), and proteolipid protein (PLP) (Pfeiffer *et al.,* 1993; Merrill, 2009).

### 1.1.4 Experimental models to study myelination

#### 1.1.4.1 *In vitro* models

Purified populations of independently prepared neurons and oligodendrocyte progenitors can be co-cultured to study oligodendrocyte-mediated myelination *in vitro*. Dorsal root ganglion neurons (DRGNs) are commonly used in oligodendrocyte/neuron co-cultures due to their ease of extraction, minimal presence of contaminating cells, and ability to form dense neurite beds (Wood *et al.,* 1980; O'Meara *et al.,* 2011). Neuronal cultures are exposed to fluorodeoxyuridine (FuDR) to inhibit proliferation and division of non-neuronal cells and further cultured prior to the addition of oligodendrocyte progenitors to facilitate neurite bed formation (Stevens *et al.,* 2002; Ishibashi *et al.,* 2006; Huang *et al.,* 2011; O'Meara *et al.,* 2013). An advantage of oligodendrocyte/neuron co-cultures is the minimal presence, or complete absence, of contaminating glia cells that might affect axo-glial interactions or influence effects of exogenous agents added to the co-cultures, thereby providing controlled conditions to study the effects of pharmacological agents (Merrill, 2009).

Organotypic cerebellar slices are also commonly used to study myelination. Slices prepared from tissue of neonatal mice are cultured on a semi-porous membrane, and over 2-4 weeks, endogenous OPCs within the slice differentiate and myelinate axons (Gahwiler *et al.,* 1997; Jarjour *et al.,* 2008). Organotypic cerebellar slice cultures are viable in culture for at least 4 weeks, which enables the study of myelination in transgenic mice, or in mice with naturally occurring mutations (Jarjour *et al.,* 2012). Additionally, the microenvironment and architecture of organotypic cerebellar slice cultures more closely resembles the *in vivo* environment and can achieve robust myelination (Jarjour *et al.,* 2008; Merrill, 2009).

#### 1.1.4.2 *In vivo* models

Several experimental models exist to study remyelination in rodents following induction of demyelination, such as experimental autoimmune encephalomyelitis (EAE), and cuprizone and lysolecithin treatments.

*Experimental autoimmune encephalomyelitis* (EAE)

EAE is primarily used as an animal model of autoimmune inflammatory diseases, and it resembles MS in various ways (Gold *et al.,* 2006; Constantinescu *et al.,* 2011). EAE is induced by immunization with antigens such as CNS myelin, MBP, or PLP, leading to immune-mediated inflammation of the CNS. Clinical symptoms arise 9-12 days following immunization and are followed by variable clinical and pathological outcomes, likely stemming from inflammation-related injuries (Constantinescu *et al.,* 2011). While EAE and MS share clinical features such as T-cell mediated inflammation and demyelination, it has been proposed that EAE represents a model of acute CNS inflammation rather than the pathology of a progressive disorder, which is characteristic of MS (Sriram and Steiner, 2005).

*Cuprizone induced demyelination*

Cuprizone is a neurotoxicant that can be orally administered to induce non-inflammatory demyelination. It functions as a copper chelator and reduces cytochrome oxidase activity in oligodendroglial mitochondria, resulting in decreased oxidative phosphorylation (Matsushima and Morell, 2001). Demyelination is observed 3-6 weeks following administration of cuprizone; however upon removal of dietary cuprizone, complete remyelination is observed within 4 weeks (Matsushima and Morell, 2001; Merrill, 2009).

*Lysolecithin induced demyelination*

Lysolecithin is a detergent-like membrane-solubilizing agent that induces myelin breakdown and oligodendrocyte apoptosis (Shields *et al.,* 1999; Blakemore and Franklin, 2008). Injection of a 1% solution of the agent creates locally demyelinated lesions one week post-injection and is followed by rapid remyelination, with total remyelination complete by one month (Merrill, 2009).

#### 1.1.4.3 Genetic models

Several rodent mutants exhibit developmental defects in myelination and include the jimpy and shiverer mouse models. Premature oligodendrocyte death was observed in jimpy mice, and was later attributed to mutations in the *plp1* gene, which encodes proteolipid protein (Knapp *et al.,* 1986; Nave *et al.,* 1986; Hudson *et al.,* 1987). These mice develop tremors approximately 7-10 days following birth, seizures by postnatal days 18-20, and usually die by 25 days (Duncan *et al.,* 2011). The shiverer mouse is an autosomal recessive mutant, which carries a major deletion in the *mbp* gene, resulting in severe CNS myelin deficiencies (Duncan *et al.,* 2011). Homozygous shiverer mice develop notable tremors approximately 12 days after birth, seizures at approximately 30 days, and usually death by 20-22 weeks (Windrem *et al.,* 2004; Duncan *et al.,* 2011). Germline introduction of MBP into homozygous shiverer mice through microinjection into fertilized zygotes partially restores MBP expression, alleviates shivering and prevents premature death (Readhead *et al.,* 1987). Additionally, transplantation of human glial progenitor cells into forebrains of neonatal shiverer mice results in myelin production, compaction, and axonal myelination (Yandava *et al.,* 1999; Windrem *et al.,* 2004; Goldman *et al.,* 2008).

## 1.2 Molecular signaling mechanisms that regulate OL development and myelination

Myelination involves a series of complex events that drive changes in morphology and gene expression associated with OL maturation, as well as bi-directional axo-glial signaling events during myelination. Through manipulation of signaling systems, intracellular signaling mechanisms and pathways that regulate OL differentiation, myelination and remyelination have been identified. Some of these pathways, which are briefly described below, involve receptor-mediated transduction of extracellular signals, while other pathways are mediated through kinase activity.

### 1.2.1 Receptor-dependent signaling mechanisms in OL development

#### 1.2.1.1 Integrin signaling

Integrins are the major receptors for the extracellular matrix (ECM) components, and function in cell-cell adhesion processes, mediation of transmembrane connections to the cytoskeleton, and activation of various intracellular signaling pathways (Hynes, 2002). In oligodendrocytes, several integrin receptors have been identified: the $\alpha1\beta1$ receptor which binds collagen/laminin; the vitronectin/fibronectin receptors $\alpha_v\beta1$, $\alpha_v\beta3$, $\alpha_v\beta5$; the fibronectin receptor $\alpha5\beta1$; and the laminin receptor $\alpha6\beta1$ (Baron *et al.,* 2005). PDGF, a mitogen for OPCs, promotes OPC proliferation at physiological concentrations (0.1-1.0 ng/ml). This effect is enhanced by $\alpha_v\beta3$ integrin engagement and is dependent on the activity of the Src family kinase (SFK) Lyn (Baron *et al.,* 2002; Colognato *et al.,* 2004). Growth factors such as PDGF and neuregulins (NRGs) can also function as survival factors for oligodendrocytes (Barres *et al.,* 1992; Calver *et al.,* 1998; Fernandez *et al.,* 2000). Myelinating axonal tracts express laminins, such as the $\alpha2$

subunit of laminin, to potentiate the effects of growth factor-mediated survival (Colognato *et al.,* 2002). Engagement of α6β1 integrins by laminin-2 amplifies PDGF-mediated oligodendrocyte survival in a Fyn (a SFK)-dependent manner (Colognato *et al.,* 2004). The immunoglobulin superfamily member F3/contactin is expressed in oligodendrocytes and interacts with α6β1 integrin to amplify PDGF-mediated oligodendrocyte survival. F3/contactin stimulation by the axonal ligand L1 increases phosphorylation of both Fyn Y531 (in rats) and the Fyn activation tyrosine residue Y420 (in rats), resulting in enhanced Fyn activity. Additionally, integrin stimulation induces dephosphorylation of the inhibitory tyrosine Y531 residue of Fyn (Laursen *et al.,* 2009). Therefore, these findings indicate that Fyn may function in the collaborative effects of F3/contactin and integrins to amplify PDGF-dependent oligodendrocyte survival.

#### 1.2.1.2 TGFβ and activin signaling

TGFβ1 and activins belong to the same superfamily of TGFβ ligands, which bind to type II transmembrane serine/threonine kinase receptors (TGFβ RII and Act RII), forming a stable ligand/receptor complex (Weiss and Attisano, 2013; Marino *et al.,* 2013). Type II receptors are considered to be constitutively active, and within a stable ligand/receptor complex, phosphorylate type I receptors, enabling the recruitment of intracellular effectors termed receptor-regulated SMADs, which include SMAD2 and SMAD3 (Schmierer and Hill, 2007). Type I receptors phosphorylate receptor-regulated SMADs at the C-terminus, allowing the formation of complexes with the common mediator SMAD4. These translocate and accumulate in the nucleus to regulate transcription of target genes (Shi and Massague, 2003; Schmierer and Hill, 2007). TGFβ signaling is a positive regulator of oligodendrocyte development (McKinnon *et al*., 1993). TGFβ1 promotes cell cycle exit in OPCs to accelerate differentiation and

myelination (Palazuelos *et al.,* 2014), and may also act in concert with activin-B to promote OL maturation and myelination in the spinal cord (Dutta *et al.,* 2014). Additionally, activin-A has also been recently implicated as a positive regulator of OL differentiation as well as remyelination following lysolecithin-induced demyelination (Miron *et al.,* 2013). TGFβ ligands can signal independently of SMAD proteins via the MAP kinase and PI3K/Akt signaling cascades, both of which have been implicated in OPC differentiation (Weiss and Attisano, 2013; Massague, 2012), and are described in section 1.2.2.2.

#### 1.2.1.3 Wnt signaling

Wnt signaling pathways are highly evolutionarily conserved and control developmental processes such as proliferation, stem cell renewal, cell fate commitment, developmental patterning, and establishment of tissue polarity (van Amerongen and Nusse, 2009). Wnt signaling pathways are also required for maintenance of adult tissue, as aberrations in Wnt signaling have been implicated in degenerative diseases, as well as cancers (Logan and Nusse, 2004). For example, aberrant activation of Wnt signaling promotes cell proliferation and survival, and may play a role in carcinogenesis (Barker and Clevers, 2006). Conversely, down-regulation of Wnt signaling effectively inhibits proliferation of colon cancer cells *in vitro* and stimulates differentiation (Tetsu and McCormick, 1999; van de Wetering *et al.,* 2002). A recent study reported that OPCs express markers of high Wnt activity following neonatal hypoxic ischemic encephalopathy, similar to markers of high Wnt activity expressed in colon cancer (Fancy *et al.,* 2014).

Wnt signaling is initiated through the binding and interaction of Wnt ligands with Frizzleds, the primary Wnt receptors, which are seven-transmembrane receptors that contain

extracellular cysteine-rich domains that interact with Wnt ligands (Clevers and Nusse, 2012). Wnt signals are transduced through Frizzled (Fz) receptors and associated co-receptors, such as low-density lipoprotein receptor proteins (LRPs), to the canonical or non-canonical Wnt signaling cascades (Katoh and Katoh, 2007). Emphasis here is placed on the canonical Wnt signaling pathway, as several studies have recently implicated canonical Wnt signaling in oligodendrocyte development and myelination (described in 1.2.1.3.3).

#### 1.2.1.3.1 Wnt proteins

Wnts are secreted glycoproteins approximately 350 residues in length with a molecular mass of approximately 40kDa (Tanaka *et al.,* 2002; Logan and Nusse, 2004). Most mammalian genomes contain 19 Wnt genes, twelve of which fall into conserved Wnt subfamilies (Clevers and Nusse, 2012). Wnt proteins are hydrophobic, and are modified through palmitoylation of conserved residues, such as Ser209 in Wnt3a, a process that is essential for Wnt function, as prevention of palmitoylation results in the formation of an inactive Wnt protein (Willert *et al.,* 2003). The interaction between Wnt8 and Fz was revealed through crystallization of bound Wnt8 and the cysteine-rich domain of Fz and demonstrated that two domains on Wnt interact with the receptor. One of these Wnt domains contains the palmitoylated residue, which interacts with a hydrophobic pocket in the cysteine-rich domain of the Fz receptor (Janda *et al.,* 2012).

#### 1.2.1.3.2 Canonical Wnt signaling

The canonical Wnt pathway signals through a protein called β-catenin, whose stability plays a key role in the outcome of the canonical Wnt signaling cascade. Wnt-dependent transcriptional activation is mediated by β-catenin interactions with two major classes of

transcription factors: T-cell factors (Tcfs), and lymphoid enhancer-binding factors (LEFs) (Logan and Nusse, 2004). In the cytoplasm, the so-called destruction complex regulates cytoplasmic β-catenin stability. This complex consists of the tumor suppressor protein Axin, the scaffold of the destruction complex, which interacts with β-catenin, the tumor suppressor protein adenomatous polyposis coli (APC), and two serine/threonine kinases: casein kinase 1 (CK1) and glycogen synthase kinase α/β (GSK3α/β) (Clevers and Nusse, 2012) (Figure 1.3). When Fz/LRP receptors are not engaged by Wnt ligands, CK1 and GSK3 phosphorylate Axin-bound β-catenin at several N-terminal Ser/Thr residues. This triggers its recognition by the E3 ubiquitin ligase complex and as a consequence, phosphorylated β-catenin is ubiquitinated and targeted for proteasome degradation (Aberle *et al.,* 1997) (Figure 1.3A). In the absence of β-catenin, TCF interacts with Groucho transcriptional repressors, thereby preventing transcription of Wnt-targeted genes (Cavallo *et al.,* 1998).

Upon Fz/LRP5/6 activation by Wnt ligands, the cytoplasmic protein Dishevelled (Dvl) becomes phosphorylated and interacts with the C-terminus of the Fz receptor at the cell surface (Wong *et al.,* 2003). Additionally, Wnt activation recruits Axin to the cell surface where it associates with the phosphorylated tail of the LRP5/6 co-receptor, preventing subsequent ubiquitination of β-catenin and leads to stabilization of β-catenin in the cytoplasm (Li *et al.,* 2012). Stabilized β-catenin accumulates in the cytoplasm and translocates into the nucleus where it interacts with Tcf/LEF to activate transcription of Wnt targeted genes (Logan and Nusse, 2004; Clevers and Nusse, 2012).

#### 1.2.1.3.3 Wnt signaling in OL development, myelination, and remyelination

In recent years, the canonical Wnt signaling pathway has been implicated in OL development and myelination as several studies have demonstrated that down-regulation of canonical Wnt signaling promotes OPC differentiation and myelination (Fancy *et al.,* 2011; Ye *et al.,* 2009). In an *ex vivo* cerebellar slice culture model, pharmacological inhibition of canonical Wnt signaling using the small molecule inhibitor XAV939 increases myelination and remyelination following hypoxic insult (Fancy *et al.,* 2011). Ablation of the chromatin re-modeling enzymes histone deacetylase 1/2 (HDAC1/2) increases β-catenin accumulation and expression of *Id2/4,* Wnt targeted genes that repress differentiation. Active Wnt signaling also reduces expression of *Olig2* (an OPC lineage marker) and *Mbp* (Ye *et al.,* 2009). Additionally, expression of dominant-active β-catenin results in hypomyelination in mice, and significantly delays OPC differentiation (Fancy *et al.,* 2009). Wnt3a activation of canonical Wnt signaling in OPC cultures represses OPC differentiation, delays myelination *in vivo* (Feignson *et al.*, 2009), and prevents initial OPC differentiation in the spinal cord (Shimizu *et al.,* 2005). Despite these results, recent studies have reported that active Wnt signaling is required to promote OPC differentiation (Dai *et al.,* 2014; Azim and Butt, 2011). The paradoxical effects of manipulating Wnt/β-catenin suggest more complex roles of this signaling pathway in OL development and myelination.

### 1.2.2 Kinase dependent signaling cascades in OL development

#### 1.2.2.1 Fyn signaling

The non-receptor tyrosine kinase Fyn, a Src family kinase (SFK), is a central regulator of OPC differentiation whose expression and activity increases during differentiation (Wang *et al.,*

2009; Osterhaut, *et al.,* 1999). While other SFKs (Src, Yes, and Lyn) are expressed by oligodendrocytes, no defects in CNS myelination are observed in Src, Yes, or Lyn deficient mice; however Fyn knockout, or mutant mice exhibit severe CNS hypomyelination (Umemori *et al.,* 1994; Sperber *et al.,* 2001). Fyn activity, which is controlled through the integration of extracellular signals from the axonal surface and ECM, through integrins, regulates both OL survival and myelination (Laursen *et al.,* 2009; Rajasekharan *et al.,* 2009; Colognato *et al.,* 2004; Colognato *et al.,* 2002). Additionally, Fyn is known to signal through several downstream effectors implicated in morphological changes involving the cytoskeleton, and promotes myelin gene expression during oligodendrocyte differentiation.

#### 1.2.2.1.1 Fyn-dependent regulation of cytoskeletal changes during OL differentiation

Fyn targets molecules that function in regulating cytoskeletal dynamics, which include focal adhesion kinase (FAK), the Rho GTPases RhoA, Rac, Cdc42, and the Rho GTPase family regulator p190RhoGAP (Liang *et al.,* 2004; Wolf *et al.,* 2001). FAK, a tyrosine kinase, is a downstream effector of integrin signaling and extracellular signals that regulates cell adhesion and motility (Mitra *et al.,* 2005). FAK activation is Fyn-dependent during differentiation in the rat oligodendroglial CG4 cell line (Hoshina *et al.,* 2007). The shRNA-mediated knockdown of Fyn reduces FAK activity and decreases laminin-induced process outgrowth in CG4 cells (Hosina *et al.,* 2007). The activity of Rho GTPases is dependent on Fyn activation as inhibition of integrin engagement, or Fyn activation, blocks Cdc42 and Rac1 activation and differentiation (Liang *et al.,* 2004). In addition, phosphorylation of p190RhoGAP by Fyn decreases RhoA activity and increases oligodendroglial process extension (Liang *et al.,* 2004). Expression of a dominant-negative form of RhoA results in hyperextension of oligodendroglial processes (Wolf

*et al.,* 2001). Taken together, these results suggest that integrin-dependent Fyn signaling plays a crucial role in morphological differentiation of OLs by regulating the activity of Rho GTPases.

#### 1.2.2.1.2 Fyn-dependent regulation of myelin basic protein expression

Fyn activity controls the expression of myelin basic protein (MBP), a major component of the myelin sheath, by transcriptional and translational regulation. Umemori *et al.* (1999) demonstrated, using a CAT reporter assay, that Fyn activation transcriptionally activated MBP expression and furthermore identified the Fyn response element in the MBP gene, located 647-675 base pairs upstream of the MBP promoter sequence. Transcription factor binding to the Fyn response element is developmentally regulated, as interactions between transcription factors and the Fyn response element in the MBP gene is correlated with Fyn activity (Umemori *et al.*, 1994), indicating that Fyn stimulates transcription factor binding to the promoter region of the MBP gene during myelination (Umemori *et al.,* 1999).

Preferential reduction of exon2-containing MBP mRNA was observed in Fyn deficient mice, suggesting that Fyn may regulate MBP post-transcriptionally (Lu *et al*., 2005). QKI is a RNA binding protein that maintains MBP mRNA stability and can be phosphorylated by Fyn (Lu *et al.,* 2005). C-terminal phosphorylation of QKI by SFKs attenuates the binding ability of QKI to MBP mRNA as opposite expression patterns of MBP mRNA were observed between Fyn-deficient and QKI-deficient mice (Zhang *et al.,* 2003; Lu *et al.,* 2005), suggesting that Fyn may regulate MBP mRNA homeostasis during myelination through phosphorylation of QKI.

MBP mRNA is translated locally at points of axon-glia contact in oligodendroglial process extensions; therefore, repression of MBP mRNA is required until it arrives at its destination via transport in RNA granules (White *et al.,* 2008). The 3'UTR region of MBP

mRNA contains an 11-nucleotide long A2 response element (Ainger *et al.,* 1997), which binds heterogeneous nuclear ribonuclear protein A2 (hnRNP A2). hnRNP A2 can recruit and interact with hnRNP E1 in RNA granules to repress translation of mRNA sequences containing the A2 response element, such as MBP mRNA (Kosturko *et al.,* 2006). Tyrosine phosphorylation of hnRNP A2 by Fyn in response to neuronal L1 binding to oligodendroglial contactin releases hnRNP E1 and hnRNP A2 from RNA granules thereby alleviating translation repression (White *et al.,* 2008).

#### 1.2.2.2 PI3K/Akt/mTOR and MAP kinase signaling

Phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases that have been implicated in key regulatory roles in many cellular processes including survival, proliferation, and differentiation (Engelman *et al.,* 2006; Vivanco and Sawyers, 2002). PI3Ks are downstream effectors of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), transducing signals from cytokines and various growth factors into intracellular signals through the generation of phospholipids such as phosphatidylinositol-3,4,5-triphosphate ($PIP_3$). $PIP_3$ activates the serine/threonine kinase AKT (also known as protein kinase B) and other downstream effectors (Liu *et al.,* 2009).

While the PI3K/Akt signaling pathway mediates survival and proliferation of OPCs, and the survival of mature OLs through induction by various compounds (Flores *et al.,* 2000; Cui *et al.,* 2007; Ebner *et al.,* 2000; Baron *et al.,* 2003), it has been revealed in recent years that this signaling pathway is critical for OPC differentiation, as well as myelination. Expression of constitutively active Akt results in CNS hypermyelination *in vivo,* likely through increasing myelin formation by oligodendrocytes as no difference in the number of OLs was observed

(Flores *et al.,* 2008). This effect is dependent on a downstream effector of Akt, the serine/threonine kinase mammalian target of rapamycin (mTOR), as rapamycin inhibition of mTOR signaling *in vitro* reduced the expression of myelin-associated proteins such as MBP and PLP (Tyler *et al.,* 2009). Rapamycin inhibition of mTOR *in vivo* also reduced Akt-dependent hypermyelination reported by Flores *et al.* (2008). Rapamycin inhibition of mTOR signaling *in vivo* during postnatal days 21-42, a period of active myelination, reduces expression of myelin-associated proteins in mice (Narayanan *et al.*, 2009). Erk1/2 signaling, a component of the MAP kinase cascade, has been implicated in OPC differentiation, and it has been demonstrated that crosstalk exists between Erk1/2 and mTOR signaling in OPCs, as inhibition of mTOR increased Erk1/2 activity, but not *vice versa* (Dai *et al.,* 2014). These pathways appear to be important at distinct stages in OPC differentiation, as pharmacological inhibition of Erk1/2 prevented transition from the progenitor stage into an immature differentiated pre-myelinating OL, and rapamycin inhibition of mTOR signaling prevented the transition from a differentiated pre-myelinating OL to a mature OL as MBP expression was reduced (Guardiola-Diaz *et al.,* 2012). Additionally, loss of phosphatase and tensin homolog (PTEN), a major lipid phosphatase opposing Akt signaling, in the OL lineage accelerates differentiation and results in hypermyelination in mice (Goebbels *et al.,* 2010).

## 1.3 Protein tyrosine phosphatase alpha (PTPα)

### 1.3.1 Structure of PTPα

PTPα is a ubiquitously expressed receptor-like transmembrane PTP that is particularly enriched in the brain (Kaplan *et al.,* 1990; Shock *et al.,* 1995). Unlike other PTPs, the extracellular domain of PTPα is short, heavily glycosylated and lacks adhesion domains (Pallen,

2003). Like other PTPs, PTPα contains two tandem catalytic domains: the D1 domain, which is responsible for the majority of the catalytic activity (amino acids 241-500); and the D2 domain (amino acids 501-802), which exhibits minimal catalytic activity (Kaplan *et al.,* 1990; Wang and Pallen, 1991) (Figure 1.4). Because the D2 domain has intrinsically low catalytic activity and no known physiological substrate, it has been proposed that the D2 domain of PTPα plays a regulatory role (Pallen, 2003).

### 1.3.2 Src family kinases (SFKs) are substrates of PTPα

SFKs are non-receptor tyrosine kinases approximately 52-62kDa in size that share a common structure consisting of several functional domains (Thomas and Brugge, 1997; Kim *et al.,* 2009): (1) The N-terminal SH4 domain contains a myristoylation sequence, important for membrane localization at the cell surface (Xu *et al.,* 1997); (2) The unique sequence, which is important for mediation of interactions with receptors, or proteins specific for each member of the SFKs; (3) The SH3 domain, which binds proline-rich regions; (4) The SH2 domain, which interacts with phosphotyrosine residues; (5) The SH1 domain, which contains the catalytic kinase domain, within which Tyr416 is residue is located that is autophosphorylated to maximize Src activity (Kim *et al.,* 2009). Finally, the C-terminal region contains the inhibitory regulatory Tyr527 residue (Cartwright *et al.,* 1987; Thomas and Brugge, 1997; Kim *et al.,* 2009). In its inactive state, the phosphorylated C-terminal regulatory tyrosine residue of Src is recognized and bound by its own SH2 domain to produce a closed inactive conformation that prevents interaction of Src substrates with the catalytic kinase domain (Yaffe, 2002). Dephosphorylation of the inhibitory tyrosine residue induces dissociation of the C-terminal region from the SH2

domain, resulting in an open state that permits substrate proteins to access the catalytic kinase domain in the SH1 domain (Cowan-Jacob *et al.,* 2005).

PTPα is known to interact with Src family kinases (SFKs) by dephosphorylating the inhibitory regulatory C-terminal Tyr527 site in Src, thereby activating Src activity (Zheng *et al.,* 1992; den Hertog *et al.,* 1993; Bhandari *et al.,* 1998). In cells expressing both SFKs and PTPα, PTPα associates with the SFKs Src, Fyn, and Yes (Bhandari *et al.,* 1998; Harder *et al.,* 1998). The brains and embryonic fibroblasts of PTPα-null mice exhibit reduced Src and Fyn activities, approximately 30-50% of the SFK activity in wild type cells and tissues, accompanied by increased inhibitory tyrosine phosphorylation (Ponniah *et al.,* 1999; Su *et al.,* 1999). This indicates that PTPα is a physiological regulator of SFK activation.

### 1.3.3 PTPα is involved in CNS myelination

PTPα null mice are viable and have normal appearance suggesting that compensatory or redundant mechanisms exist to regulate SFK activity; however, various studies have revealed a variety of developmental and functional impairments in PTPα null mice. These include hippocampal and neocortex development, myelination, learning, and memory (Petrone *et al.,* 2003; Skeleton *et al.,* 2003; Ye *et al.,* 2008). Previous results from our laboratory have demonstrated that PTPα plays at least two roles in oligodendrocyte development: it acts as an upstream activator of the SFK Fyn activity to coordinate downstream signaling events to promote morphological changes by activating FAK, Rac1, and Cdc42, and inhibiting Rho (Wang *et al.,* 2009); and it promotes cell cycle exit by mediating Fyn-dependent suppression of Ras and Rho. Suppression of the latter upregulates p27Kip1 expression (Wang *et al.,* 2012), which is

critical for reducing OPC proliferation (Casaccia-Bonnefil *et al.,* 1997; Durand *et al.,* 1997; Casaccia-Bonnefil *et al.,* 1999). OPCs derived from PTPα null mice exhibit impairments in differentiation as well as hypomyelination in the forebrain (Wang *et al.,* 2009). Additionally, PTPα has also been implicated down-regulating the activity of a delayed rectifier voltage-gated potassium channel (Kv2.1) in Schwann cells, a process required for Schwann cell maturation, since PTPα null mice exhibit increased Kv2.1 activity and defective peripheral nervous system (PNS) myelination (Tiran *et al.,* 2006).

### 1.4 Rationale and hypothesis

Myelin, the multilamellar membranous structure that ensheathes and insulates axons of the vertebrate nervous system is synthesized by OLs in the CNS, and Schwann cells in the PNS. Myelination of the CNS is crucial for the molecular organization, protection, and maintenance of axons and normal function in the brain and spinal cord. As OLs are the only source of CNS myelin, absence or dysfunction of OLs give rise to several myelin disorders that can be separated into two main classes: inherited dysmyelinating disorders, such as leukodystrophies; and acquired demyelinating disorders such as multiple sclerosis (Goldman, 2005). Traumatic events such as spinal cord injuries can also contribute to demyelination (Mueller *et al.,* 2005). In the absence of myelin, transmission of neuronal impulses is impaired, resulting in various symptoms that may consequently lead to impaired cognitive, autonomic, sensory and motor functions.

Currently, there are no available therapies that promote myelin formation or repair; therefore, an opportunity exists to remediate the effects of dysmyelination or demyelination through therapeutic enhancement of myelin repair. However, this requires a more comprehensive understanding of the intricate cellular and molecular events that orchestrate myelin formation

and repair. The "recapitulation hypothesis of remyelination" postulates that mechanisms of myelination and remyelination are conserved (Franklin and Hinks, 1999; Fancy *et al.,* 2011); therefore, increased understanding of developmental myelination may provide essential insight into the remyelination, as the inability of oligodendrocytes to properly differentiate is believed to be a major barrier in achieving remyelination.

Myelination involves bi-directional signaling events between axons and oligodendrocytes (described in section 1.1.2.1). A major focus of our research is the brain enriched receptor-like transmembrane protein tyrosine phosphatase α (PTPα). Previous results from our laboratory identified multiple roles for PTPα in oligodendrocyte development (described in section 1.3). Using an *in vitro* OPC differentiation cell culture model, a mechanism was identified demonstrating that PTPα signaling promoted morphological changes associated with OL differentiation, and that forebrains of PTPα null mice exhibited hypomyelination (Wang *et al.,* 2009). While studying oligodendrocyte development provides valuable insight into mechanisms that regulate the complex events associated with OL differentiation, myelination cannot be modeled on the basis of OL differentiation alone, as modeling myelination also requires addressing the bi-directional signaling events between axons and oligodendrocytes. It has been reported that PTPα forms a receptor complex with the neuronal adhesion molecule F3/contactin (Zeng *et al.,* 1999), and F3/contactin functions as an activator of Notch signaling that upregulates OL maturation (Hu *et al.,* 2003), therefore PTPα may also play a role in the axo-glial signaling events during myelination.

The canonical Wnt signaling pathway has recently been implicated in OL development, myelination, and remyelination (described in section 1.2.1.3). Inhibition of canonical Wnt

signaling promotes differentiation of oligodendrocytes, and represses proliferation of colon cancer cells, (Fancy *et al.,* 2011; Tetsu and McCormick, 1999; van de Wetering *et al.,* 2002). Since PTPα signaling and down-regulation of canonical Wnt signaling are both required for OPC differentiation and myelination, **I hypothesize that PTPα functions as coordinator of bi-directional axo-glial events that regulate myelination, and that PTPα–dependent effects on OL differentiation and myelination can be altered through pharmacological manipulation of the canonical Wnt signaling pathway**. This will be investigated through the following specific aims:

**Aim 1:** Establish an *in vitro* neuron-glia myelinating co-culture system to study the roles of both oligodendroglial and neuronal PTPα in myelination.

**Aim 2:** Determine whether PTPα-dependent effects on OL differentiation and myelination can be altered through pharmacological manipulation of the canonical Wnt signaling pathway in the *in vitro* neuron-glia myelinating co-culture system.

The results of these studies will reveal the roles of PTPα and its potential interactions with the canonical Wnt signaling pathway in coordinating axo-glial signaling events that regulate myelination. This will enhance our understanding of the intricate processes that regulate the physiological process of myelination, and may also provide insight into mechanisms that may orchestrate remyelination. A comprehensive understanding of the cellular and molecular events that regulate myelination provides opportunity for the development of targeted pharmacological interventions that may translate into effective therapies for myelin formation and repair.

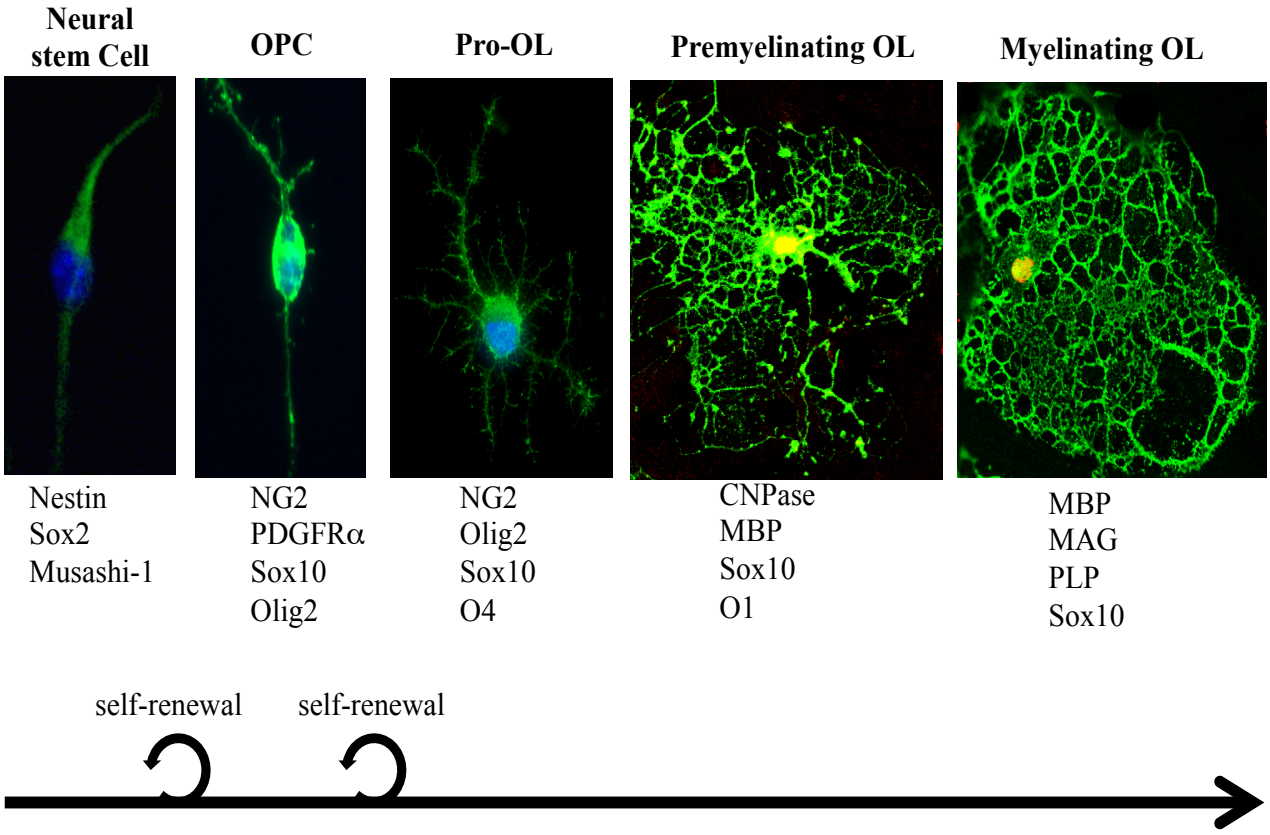


**Figure 1.1 Schematic representation of major stages of OL development.** Neural stem cells express Nestin, Sox2 and Musashi-1. Upon commitment to OPC fate, OPCs express the chondroitin sulfate proteoglycan NG2, PDGFRa, Sox10, and Olig2. As OPCs begin to mature into pro-OLs, they begin to extend processes and continue to express NG2, Sox10 and Olig2. Pro-OLs also express antigens recognized by the monoclonal O4 antibody. Pre-myelinating OLs extend complex secondary and tertiary processes. Pre-myelinating OLs no longer express progenitor markers but express 2'3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) and surface antigens recognized by the monoclonal O1 antibody. Mature OLs extend membrane-like sheets and express myelin components including myelin basic protein (MBP), myelin associated glycoproteins (MAGs), and proteolipid protein (PLP).

Courtesy of P. Ly

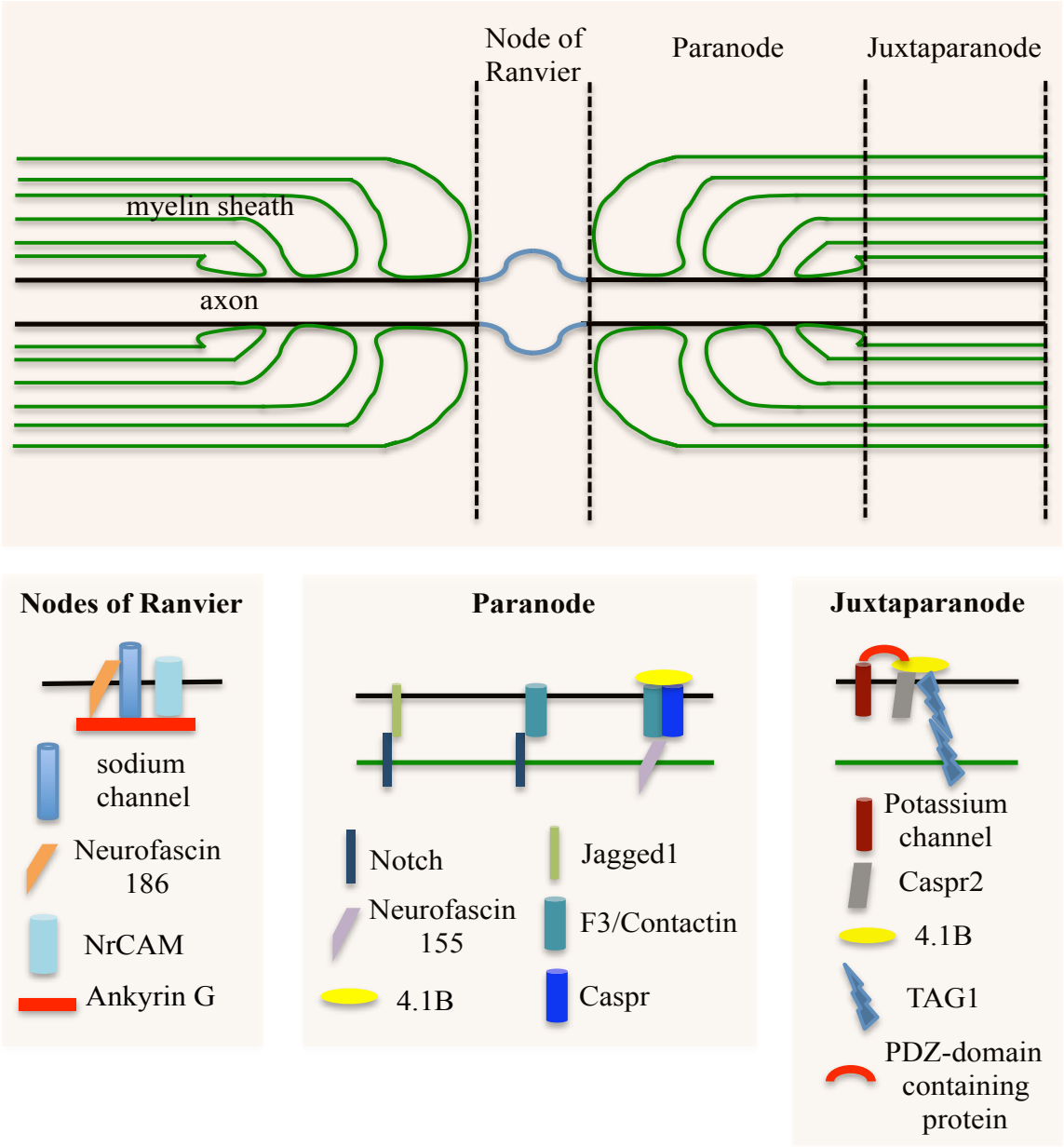


**Figure 1.2 Schematic of myelinated axon.** Molecular composition of the domains of a myelinated axon. The nodes of Ranvier express voltage gated sodium channels, Neurofascin 186, and NrCAM, which are tethered to Ankyrin G. The paranodes express complexes including: Notch interactions with Jagged1; Notch interactions with F3/Contactin; and Neurofascin 155 interactions with an axonal complex consisting of F3/Contactin and Caspr, which is anchored to protein 4.1B. The juxtaparanodes consist of an axonal complex containing voltage gated potassium channels linked by PDZ-domain containing protein to a complex containing Caspr2/TAG1, which is anchored to Protein 4.1B. This axonal complex interacts with glial TAG1.

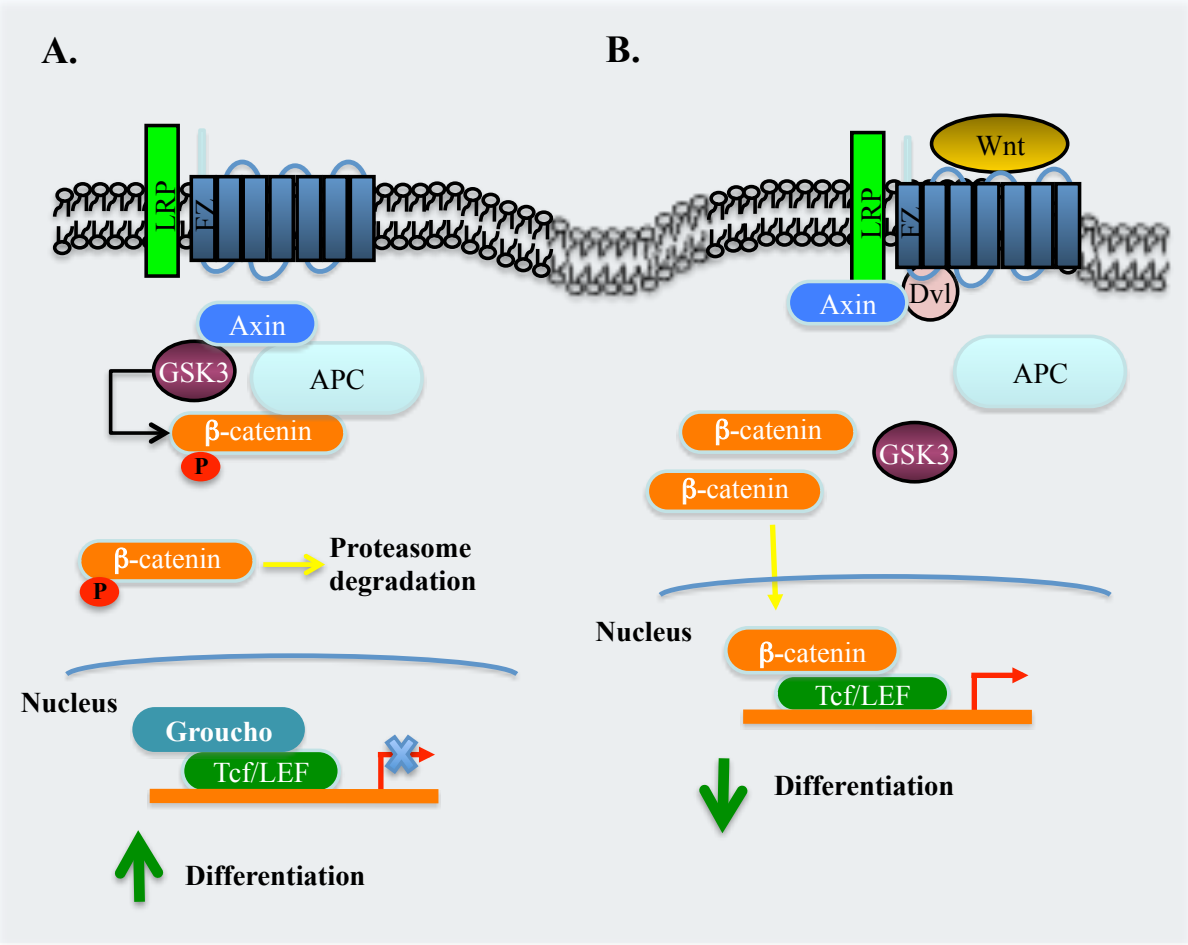


**Figure 1.3 Schematic of the canonical Wnt signaling cascade.** (A) In the absence of Wnt engagement of the Frizzled receptor, the destruction complex consisting of Axin/APC/GSK3β phosphorylates β-catenin. Phosphorylated β-catenin is ubiquinated by E3 ubiquitin ligase and targeted for proteasome degradation. In the nucleus, the Tcf/LEF transcription interacts with Groucho to repress transcription of Wnt target genes. (B) Upon Wnt engagement, dishevelled becomes activated and binds to Frizzled. Axin is also recruited to the cell surface where it interacts with the phosphorylated LRP5/6 co-receptor thus preventing the assembly of the degradation complex. β-catenin cannot be phosphorylated and accumulates in the cytoplasm before translocating into the nucleus where it displaces Groucho and interacts with Tcf/LEF to activate transcription of Wnt target genes.

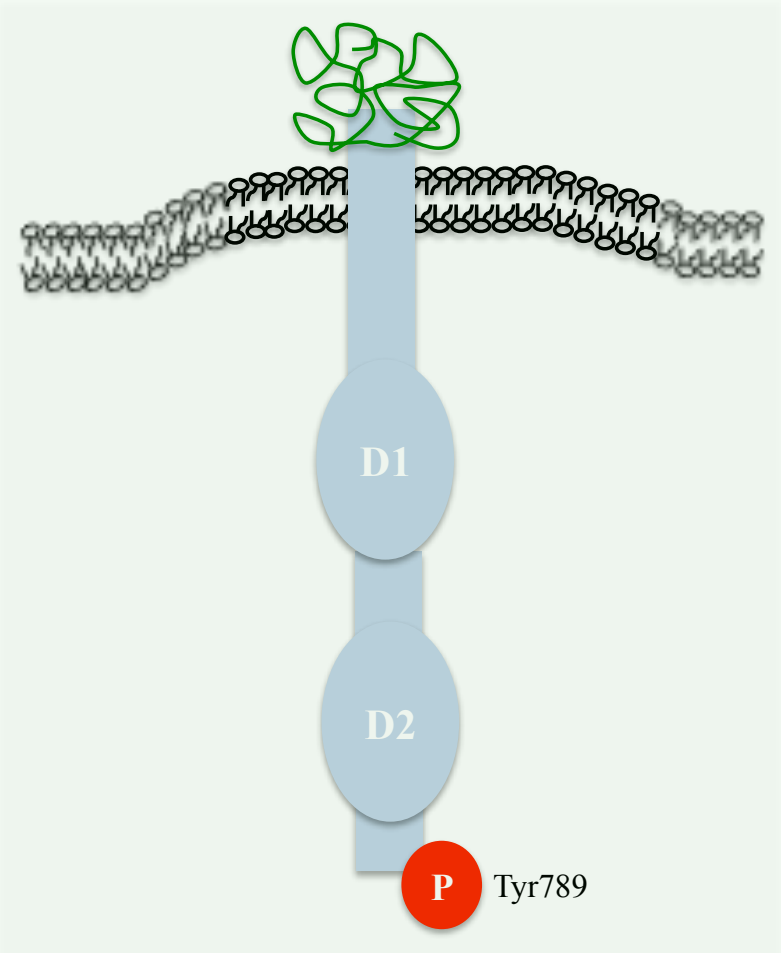


**Figure 1.4 Structure of PTPα.** PTPα is a 793 amino-acid transmembrane receptor-like protein tyrosine phosphatase. The extracellular domain of PTPα is short and heavily glycosylated. The extracellular region is also able to interact with contactin in *cis*. PTPα contains two catalytic domains (D1 and D2), with the D1 domain responsible for the majority of the catalytic activity. PTPα can be phosphorylated at its C-terminal Tyr789 residue, which facilitates interactions with Src, Grb2, and BCAR3.

# Chapter 2: Materials and methods

## 2.1 Materials

### 2.1.1 Animals

The 129*SvEv* PTPα$^{-/-}$ mice (Ponniah et al., 1999) were backcrossed with C57BL/6 mice for 10 generations. PTPα$^{-/-}$ wild type (WT) C57BL/6 mice were housed under specific pathogen-free conditions. Animal care and use followed the guidelines of the University of British Columbia and the Canadian Council on Animal Care, and were reviewed and approved by the University of British Columbia.

### 2.1.2 Reagents

Reagents were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada) unless otherwise stated. Murine recombinant Wnt3a was purchased from R&D Systems (Minneapolis, MN, USA) and PeproTech (Rocky Hill, NJ, USA). XAV939 was purchased from Tocris Bioscience (Bristol, United Kingdom).

### 2.1.3 Growth factors

Human recombinant PDGF-AA, bFGF, and EGF were purchased from PeproTech (Rocky Hill, NJ, USA).

### 2.1.4 Antibodies

Anti-PTPα has been previously described (Chen *et al.,* 2006). Antibodies to NFH were purchased from Aves Labs (Tigard, OR, USA). Antibodies to Fyn were purchased from BD Transduction Laboratories (San Jose, CA, USA). Antibodies to phosphoTyr527-Src were purchased from Biosource (Camarillo, CA, USA). Antibodies to MBP and Sox10 were

purchased from Millipore (Billerica, MA, USA). Antibodies to actin and NFH were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Secondary antibodies conjugated with FITC were purchased from Aves Labs. Secondary antibodies conjugated with Alexa Fluor 488, 594, or 694 (Molecular Probes) were purchased from Invitrogen Canada (Burlington, ON, Canada).

## 2.2 Cell culture

### 2.2.1 CG4 cells

CG4 cells were a gift provided by Dr. Y. Feng (Emory School of Medicine, USA). CG4 cells were maintained in CG4 proliferation medium (DMEM [High Glucose, Cat. # SH30243.01, HyClone], 1% FBS, 1x N1 medium supplement (Sigma-Aldrich, Cat. #N6530), 10ng/mL PDGF, and 10ng/mL bFGF.

#### 2.2.1.1 Reporter assay for Wnt activity

##### 2.2.1.1.1 Transfection

CG4 cells were plated onto 24-well tissue culture plates at a density of $2x10^4$ cells per well. Following overnight attachment, cells were incubated with Lipofectamine LTX (Invitrogen Canada) in OPTI-MEM (Gibco, Cat#. 31985), and 500ng of either the TOPFlash or the FOPFlash plasmid (Both plasmids were a kind gift from Dr. P. Leung). The TOPFlash plasmid contains three tandem repeats of the Tcf transcription factor binding site that drives the expression of a firefly luciferase gene. The FOPFlash plasmid also contains three tandem repeats of the Tcf transcription binding site; however, the sequences of the binding site are mutated

therefore, Wnt-dependent expression of the luciferase gene is impaired. Thus, the FOPFlash plasmid can be used as a negative control.

CG4 cells were also co-transfected with 1ng of a plasmid carrying the CMV promoter driving the expression of the *Renilla* luciferase gene, which is used as an internal control.

#### 2.2.1.1.2 Dual-luciferase reporter assay

24h following transfection cells were treated with XAV939, the Wnt3a ligand, or a combination of Wnt3a and XAV939. Luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega, Cat#. E1910). Luciferase activity was measured in a luminometer and TCF/β-catenin promoter-dependent luciferase activity of the TOPFlash and FOPFlash plamsids was measured as a ratio of firefly luciferase activity to *Renilla* luciferase activity.

### 2.2.2 Isolation and culture of primary neural stem cells and OPCs from murine embryos

Primary murine neural stem cells and OPCs were generated from neurospheres as previously described (Chen *et al.*, 2007). Following the removal of meninges and cerebellum in ice-cold Hank's balanced salt solution (HBSS) (Gibco, Cat. #24020), cerebral cortex tissue from E14-E18 mouse embryos was placed in a sterile dish containing HBSS, cut into small pieces and transferred into ice-cold neuro culture medium (NCM) (Table 1) supplemented with 20 ng/ml bFGF and 20 ng/ml EGF. Tissues were mechanically triturated with a 1ml Gilson pipette until the cell suspension contained very few small clumps. The suspension was then filtered through a 40μM cell strainer and plated at $1x10^6$ cells/ml in a $25cm^2$ rectangular tissue culture flask (10 ml per well of NCM supplemented with 20 ng/ml bFGF and 20 ng/ml EGF) (Corning, Cat#.

431463). Following 3-4 days, floating neurospheres were passaged at a 1:3 ratio in the same medium every 3-4 days. Passage 2-5 (P2-5) neurospheres were used for experiments, cryopreserved in media containing 10% DMSO, or dissociated into single cell suspensions using the StemPro Accutase Cell Dissociation Reagent (Life Technologies, Cat#A11105-01). To induce oligosphere formation, neurospheres were resuspended in NCM supplemented with 20ng/ml PDGF-AA and 20ng/ml bFGF (oligosphere medium). Aggregates of oligospheres were passaged at a 1:2 ratio every 4-6 days. Oligospheres (P2-5) were dissociated using the StemPro Accutase Cell Dissociation Reagent and plated on poly-D-lysine (PDL, 10μg/ml)-coated chamber slides at density of 3-4x$10^4$/$cm^2$ in oligosphere medium for 2 days. To induce differentiation, the medium was changed to NCM supplemented with 5μg/ml N-acetyl-L-cysteine, 10ng/mL of ciliary neurotrophic factor (CNTF), and 50nM 3,3',5-Triiodo-L-thyronine (T3) for 5 days.

### 2.2.3 Dorsal root ganglion neurons

#### 2.2.3.1 Isolation of DRGNs

Isolation and culture of primary post-natal murine dorsal root ganglions has been previously described (O'Meara *et al.*, 2011). Spinal columns were extracted from P5-P8 post-natal mice. Muscles and bones were trimmed away from the spinal column and transferred to a clean petri dish with the ventral side facing up (Figure 2.2a). To open the spinal column, a longitudinal cut was made caudal to rostral along the ventral midline of the spinal column. Using fine-tipped forceps, the spinal column was gently pried open to expose the spinal cord. The spinal cord was gently peeled aside to expose DRGs, which are located underneath and lateral to the spinal cord (Figure 2.2b). Using fine tipped forceps, DRGs were gently removed and

transferred into a clean petri dish containing ice-cold HBSS. Once DRGs were removed from the spinal column, excessively long roots were trimmed away such that only cell bodies remain (Figure 2.2c).

#### 2.2.3.2 Dissociation of DRGNs

Using a pipette tip coated pre-coated with 0.3% BSA, DRGs were transferred into a 1.5mL microtube containing ice cold HBSS and centrifuged at 1800rpm for 5 minutes at 4$^{o}$C. The HBSS was gently removed and replaced with a pre-warmed papain solution (37$^{o}$C) containing 20U/ml papain, 1mM L-cysteine with 0.5mM EDTA, and 200U/ml deoxyribonuclease (Papain Dissociation Kit, Worthington Biochemical Corporation, Cat. #LK003150). DRGs were incubated in papain solution for 12mins at 37$^{o}$C and inverted every 2mins to prevent tissue aggregation. DRGs were pelletized by centrifugation at 1800rpm for 5mins at 4$^{o}$C. DRGs were then incubated with a pre-warmed solution (37$^{o}$C) containing 4mg/mL collagenase A (Collagenase A, Roche, Cat. #10103578001) for 10mins at 37$^{o}$C and inverted every 2mins to prevent tissue aggregation. DRGs were pelletized at 1800rpm for 5mins at 4$^{o}$C and subsequently washed with 1mL of DRGN media (NCM (Table 1) supplemented with 10% FBS, and 100U/ml penicillin/streptomycin (Gibco, Cat #. 15140-122). DRGs were centrifuged for 1800rpm for 5mins at 4$^{o}$C and re-suspended in DRGN media and passed through a 40μm cell strainer onto a petri dish containing DRGN media. DRGNs were incubated at 8.5% $CO_2$ at 37$^{o}$C for 1.25-1.5hrs to allow contaminating cells to attach. The cell suspension was transferred to a 15ml conical tube. The petri dish was rinsed gently with 4mL of DRGN media to collect any residual DRGs. The additional 4mL was also transferred into the conical tube. DRGs were pelletized by centrifugation at 1200rpm for 4mins at room temperature. The supernatant was

aspirated and DRGs were re-suspended in DRGN media. The yield was calculated by using a hemocytometer (note: DRGs can be distinguished by their large spherical bodies). 4-5x$10^4$ DRGs were seeded onto 8-welled chamber slides (Thermo/Fisher Scientific, Cat. #177445) pre-coated with10μg/mL of laminin-2 (human merosin) (Millipore, Cat. #CC085).

#### 2.2.3.3 Culture of DRGNs

A full media change was performed the following day by replacing DRGN media with OPC/DRGN co-culture media containing 10μM of 5-Fluoro-2'-deoxyuridine (FuDR) (Sigma-Aldrich, Cat. #F0503), and 100U/ml penicillin/streptomycin. 3/4 media changes were performed on days 3 and 5. On day 7, a full media change was performed with OPC/DRGN co-culture media without FuDR and penicillin/streptomycin. 3/4 media changes were performed with OPC/DRGN co-culture media ever other day until the co-cultures were processed for immunostaining.

#### 2.2.3.4 OPC/DRGN co-culture

Following 14 days of neuronal culture, P2-P5 oligospheres derived from either WT or PTPα KO murine embryos were dissociated into single cells using the StemPro Accutase Dissociation Reagent and resuspended in OPC/DRGN co-culture media. 4-5x$10^4$ WT or PTPα KO OPCs were plated onto laminin-2 coated chamber slides containing DRGNs. A 3/4 media change was performed every other day for either 14 or 21 days of OPC/DRGN co-culture.

For XAV939 studies, cultures were treated with 0.05, 0.5, or 2.5μM of XAV939 dissolved in 0.05% DMSO. Control cultures were treated with a vehicle solution (0.05%

DMSO). For Wnt studies, cultures were treated with 100ng/ml of Wnt3a, or with a vehicle solution (0.1% BSA dissolved in PBS).

### 2.3 Immunoblotting

Cells were harvested by washing twice with ice-cold PBS on ice. For preparation of lysates, cells were lysed on ice by adding RIPA lysis buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS, 1mM EDTA, 2 mM sodium orthovanadate, 50 mM sodium fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF) directly onto the cells. Cell lysates were then transferred to microtubes and incubated for 30 min on ice and centrifuged at 13000 rpm for 15 min at 4°C. The supernatants collected to obtain protein extracts. Protein concentration was determined with the BioRad Protein Assay Dye Reagent Concentrate (BioRad, Cat #. 500-0006) (Mississauga, ON, Canada). Protein extracts were resolved by SDS-PAGE and transferred to a PVDF membrane, which was then blocked with 3% bovine serum albumin in PBS with 0.1% Tween 20 (PBST) for 1 h at room temperature. The membranes were probed overnight at 4°C with primary antibodies, washed with PBST, and probed with species-specific secondary antibodies conjugated to horseradish peroxidase. The addition of chemiluminescent reagents was used for signal detection.

### 2.4 Immunofluorescence labeling of cells and cultures

Cells cultured on 8-welled chamber slides were fixed with 4% paraformaldehyde for 15min at room temperature and subsequently washed three times with PBS. Cultures were permeabilized with 0.5% Triton-X-100 in PBS for 10min at room temperature. Following permeabilization, cultures were incubated with blocking buffer (0.1M phosphate buffer, 10%

goat serum and 0.5% Triton-X-100) for 30min, followed by incubation with primary antibodies overnight at 4°C. After washing three times with PBS, cells and tissues were incubated with secondary antibodies for 2h at room temperature. The slides were washed three times with PBS followed by mounting in Prolong Gold Antifade Reagent (Invitrogen Canada) with DAPI and viewed using the Olympus IX81 fluorescence microscope. Images were captured using the CoolSnap HQ2 camera (Photometrics). The chamber slide was navigated using the H-117 linear-encoded stage and controlled using the MetaMorph software (Molecular Devices). Ten z-stacks at 0.5μm intervals were obtained for each image.

### 2.5 Electron microscopy

Electron microscopy was performed at the UBC Centre of Heart Lung Innovation at St. Paul's Hospital. A sample of oligodendrocyte/dorsal root ganglia was co cultured on a Permanox dish. Sample was fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for 30 minutes, washed in 0.1M sodium cacodylate buffer, post fixed in 1% osmium tetroxide, washed in 0.1M acetate buffer and then in 2% aqueous uranyl acetate. The following steps follow: dehydration in graded series of alcohol and then infiltration and embedding in Epon. Cured blocks of samples were sawed out and ultra thin sections were cut either en face or at cross section of the cells. Sections were contrast stained with 2% uranyl acetate and Sato's Lead and then viewed and imaged on a Tecnai 12 Transmission Electron Microscope.

### 2.6 Quantification of OPC/DRGN co-cultures

Original images were stored in TIFF format. For each visual field, ten z-stacks at 0.5μm intervals were imaged. To avoid bias, images were obtained using only the channel for NFH

immunopositivity. For each experiment, ten random visual fields of equivalent neuronal bed density taken at 20x objective were used for quantification. For each visual field analyzed, a binary mask for neurofilament (NFH), which labels the neurite bed, was created and the percentage of the visual field immunopositive for NFH was measured to determine the density of the neuronal bed. Similarly, a binary mask was also created for myelin basic protein (MBP), which labels mature oligodendrocytes. The percentage of MBP immunopositivity per visual was used as an indicator of differentiation. As an indicator of potential myelination, a binary mask representing MBP/NFH co-localization was extracted using the co-localization plugin in ImageJ. The total $MBP^+$/NFH area was then divided by the NFH-immunopositive area measured in that field and multiplied by 100 to yield the percentage of the neuronal bed that co-localized with MBP.

To measure the length of $MBP^+$/NFH segments, binary masks representing MBP/NFH co-localization were extracted. $MBP^+$/NFH segments were traced using the NeuronJ plugin in ImageJ, and the length per co-localized segment was calculated using the software. Three representative binary masks per condition were analyzed per experiment.

### 2.7 Statistical analysis

Student's unpaired t-test, one-way ANOVA, and two-way ANOVA were used for statistical analyses in this report. Where appropriate, Tukey's post-hoc, and Bonferroni post-tests were performed.

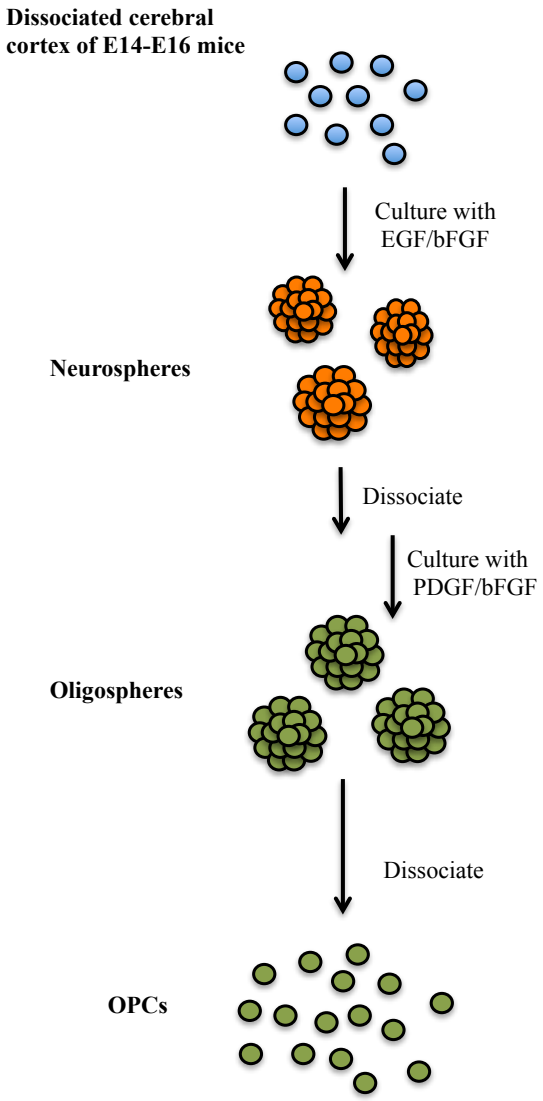


**Figure 2.1 Schematic of isolation and culture of neural progenitor/stem cells and OPCs.** The procedure is detailed in section 2.2.2

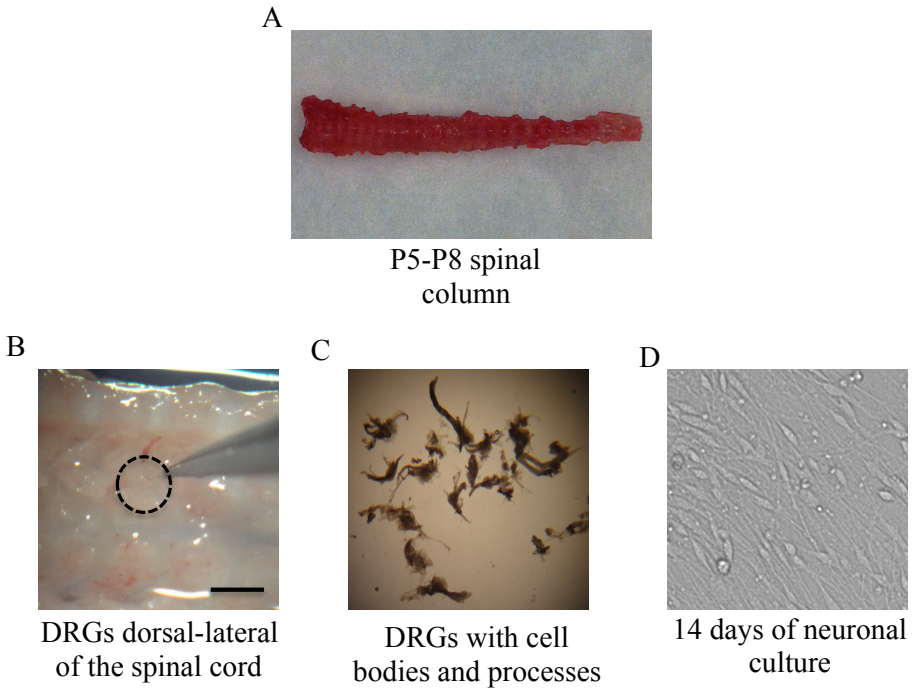


**Figure 2.2 Schematic representing isolation of DRGs.** Details for isolating DRGs are described in section 2.2.3. **(B)** Scale bar 1mm.

**Table 2.1. Neuro culture media**

| Component | Company | Catalogue Number | Amount /concentration |
|---|---|---|---|
| DME/F12 | Thermo Scientific | SH30023.01 | 25ml |
| B27 supplement | GIBCO, by Life Technologies | 17504-44 | 1x |
| Glutamax | GIBCO, by Life Technologies | 35050-61 | 1mM |
| Sodium pyruvate | GIBCO, by Life Technologies | 11360-070 | 1mM |

**Table 2.2. OPC/DRGN co-culture media**

| Component | Company | Catalogue Number | Amount /concentration |
|---|---|---|---|
| Neuro culture media | N/A | N/A | 24.3ml |
| FBS | GIBCO, by Life Technologies | 12483-020 | 125µl (0.5%) |
| Holo-transferrin | Sigma-Aldrich | T0665 | 49.5µg/ml |
| Bovine insulin | Sigma-Aldrich | I0516 | 5µg/ml |
| L-Thyroxine | Sigma-Aldrich | T1775 | 400ng/ml |
| 100x OL supplement | N/A | N/A | 250µl |

**Table 2.3. 100x OL supplement (Stored at -80$^{o}$C in 250µl aliquots)**

| Component | Company | Catalogue Number | Amount |
|---|---|---|---|
| DME/F12 | Thermo Scientific | SH30023.01 | 100ml |
| BSA | Sigma-Aldrich | A3912 | 1.02g |
| Progesterone | Sigma-Aldrich | P0130 | 0.6mg |
| Putrescine | Sigma-Aldrich | P7505 | 161mg |
| Sodium Selenite | Sigma-Aldrich | 214485 | 50µg |
| 3,3',5-Triiodo-L-thyronine | Sigma-Aldrich | T2877 | 4mg |

## Chapter 3: PTPα is involved in oligodendrocyte differentation and myelination in a neuronal-glial co-culture system

### 3.1 Introduction and rationale

Our lab has previously identified a role for PTPα in OPC differentiation in an *in vitro* cell culture system, and that myelination is impaired in the CNS of PTPα knockout mice (Wang *et al.*, 2009). However, without neuronal involvement, myelination cannot be modeled in an *in vitro* cell culture system comprised of only OPCs/OLs. Since myelination is directed by axon-glial interactions and bi-directional signaling events (Charles *et al.*, 2000; Fancy *et al.*, 2009; Mi *et al.*, 2005; and Chen *et al.*, 2009) (and described in Section 1.1.2.1), I established an *in vitro* neuronal-glial myelinating co-culture system to investigate the involvement of oligodendroglial PTPα in myelination. Dorsal root ganglion neurons (DRGNs) are standard sources of neurons that have been used for neuron-glial co-cultures to study myelination (Bernard *et al.,* 2012; O'Meara, *et al.,* 2013; Wong, *et al.*, 2013; Wood *et al.,* 1980). Therefore, to investigate the role of oligodendroglial PTPα in myelination, OPCs derived from neural stem cells of either WT or PTPα KO E14-18 murine embryos (Materials and Methods 2.2.2**)**, were co-cultured with purified DRGNs isolated from WT mice.

### 3.2 Results

#### 3.2.1 PTPα promotes OPC differentiation in cultures grown on PDL/laminin-2

It has been reported that mice lacking laminin-2 display defective central nervous system myelination in a region-specific manner (Chun *et al.,* 2003) and that laminin-2 is an OL differentiation promoting extracellular matrix substrate (Colognato *et al.,* 2004). Therefore, using

an improved *in vitro* differentiation culture model (developed in our lab by Dr. Philip Ly, see section 2.2.2), I investigated whether PTPα is promotes laminin-2 induced OPC differentiation. Oligosphere-derived WT or KO OPCs were plated onto PDL and laminin-2 coated 8-welled chambers slides and maintained in proliferation medium for 5 days followed by differentiation for 5 days. Following differentiation, the cells were immunostained for Sox10, a transcription factor expressed by cells of oligodendroglial lineage, and MBP, to mark mature oligodendrocytes (Fig 3.1A). The percentage of mature oligodendrocytes was determined by counting the number of MBP/Sox10$^+$ as a percentage of Sox10$^+$ cells per visual field. There was no difference in the number of Sox10$^+$ cells per chamber when comparing WT vs. KO OPCs (169.2±36.06 vs. 166.67±12.90 respectively, n=1) (Fig 3.1B). There was a 1.7-fold increase in the percentage of MBP/Sox10$^+$ cells in chambers containing WT OPCs versus chambers containing KO OPCs (9.56±1.69% vs. 5.54±0.98% respectively) (Fig 3.1C). These results are consistent with previous observations in our laboratory (Dr. Philip Ly, personal communication). In conjunction with the previous observations of our laboratory (Wang *et al.,* 2009), this confirms that PTPα promotes laminin-2 dependent OPC differentiation.

### 3.2.2 Oligodendroglial PTPα is promotes MBP expression and MBP/NFH co-localization in neuronal/glial co-cultures grown on laminin-2

O'Meara *et al.* (2011) have shown that laminin-2 promotes OL differentiation and myelination in a murine DRGN/OPC co-culture system. However, the myelinating ability of neural stem cell/oligosphere derived OPCs in DRGN/OPC co-cultures has not been determined. Others who have used a myelinating co-culture system obtain OPCs through other methods such

as immunopanning, or shake off from mixed glial cultures (Bin *et al.*, 2012; Cui *et al.,* 2014; Watkins, *et al.*, 2008).

Therefore, to determine if PTPα promotes OPC differentiation and myelination, I co-cultured oligosphere-derived OPCs in differentiation-promoting conditions in the presence of DRGNs derived from WT mice grown on laminin-2 coated chamber slides. DRGNs were isolated from P5-P8 mice and cultured for 14 days to allow neurites to extend axonal processes, forming a dense neuronal bed. Following 14 days of neuronal culture, WT or PTPα KO oligosphere-derived OPCs were plated onto DRGNs and the co-cultures were maintained for an additional 14 days (See materials and methods 2.2.3.4). DRGN/OPC co-cultures were fixed and immunostained following 14 days of co-culture for neurofilament (NFH) to mark the neuronal bed (Fig. 3.2A, 3.2E), and myelin basic protein (MBP) to mark mature oligodendrocytes (Fig. 3.2B, 3.2F). For each of three independent experiments, ten random visual fields of equivalent neuronal bed densities were used for quantification. I first determined the neuronal bed density by measuring the percentage of NFH signal per visual field to confirm that WT and KO OPCs were co-cultured on neuronal beds of equivalent densities. I observed that there was no significant difference in neuronal bed density following co-culture with WT OPCs or PTPα KO OPCs (21.38±5.09% vs. 21.31±4.75% respectively, n =3) (Fig. 3.3A). The percentage of MBP signal per visual field was determined as a measure of OPC differentiation. When the MBP signal per visual field was quantified following 14 days of DRGN/OPC co-culture, I observed a significant difference in MBP signal per visual field between WT OPCs and PTPα KO OPCs (4.00±0.86% vs. 2.64±0.48% respectively, $P<0.05$, n=3) (Fig. 3.3B).

Myelination requires axo-glial contact; therefore areas co-immunopositive for MBP and NFH represent areas where myelination may potentially occur in the co-cultures. The percentage

of MBP/NFH co-localization per NFH signal was measured as an indicator of potential myelination. Quantitative analysis revealed a significant difference in MBP/NFH co-localization per NFH signal in WT OPC/DRGN co-cultures in comparison to PTPα KO OPC/DRGN co-cultures (16.83±3.48% vs. 8.42±1.57% respectively, $P<0.01$, n=3) (Fig. 3.3C). In WT OPC/DRGN co-cultures, I observed longer MBP/NFH co-localized segments in comparison to DRGNs co-cultured with PTPα KO OPCs **(**discussed in section 3.2.3**)**, suggesting that myelination was occurring (Figs. 3.2D and 3.2H, respectively).

To determine if longer co-culture time altered MBP/NFH co-localization or MBP expression, DRGN/OPC co-cultures grown on laminin-2 coated chamber slides were fixed following an additional week (21 days) of co-culture and immunostained for NFH and MBP to identify neurons and OLs respectively. For each of the three independent cultures, ten random visual fields matched for equivalent neuronal bed density were used for quantification. There was no significant difference observed in neuronal bed densities when DRGNs were co-cultured with either WT OPCs or PTPα KO OPCs (18.83±4.13% vs. 19.62±3.94% respectively, n=3) (Fig. 3.3A). Analysis of MBP signal per visual field indicated a significant difference in OPC differentiation between WT OPCs and PTPα KO OPCs (4.65±1.20% vs. 2.49±0.44% respectively, $P<0.05$, n=3) (Fig. 3.3B) suggesting that loss of oligodendroglial PTPα results in impaired OPC differentiation. MBP/NFH co-localization per NFH signal was also measured as an indicator of potential myelination. Quantitative analysis revealed a significant difference in MBP/NFH co-localization per NFH signal in DRGNs co-cultured with WT OPCs versus DRGNs co-cultured with PTPα KO OPCs (19.14±2.31% vs. 9.00±1.48% respectively, $P<0.01$, n=3) (Fig. 3.3C). Collectively, these results indicate that oligodendroglial expression of PTPα promotes

MBP/NFH co-localization, suggestive of myelination, in our primary DRGN/OPC co-culture system.

### 3.2.3 Myelination occurs in DRGN/OPC co-cultures and PTPα promotes formation of elongated $MBP^+$/NFH co-localized segments

Watkins *et al.* (2008) describe myelination by referring to stages of oligodendrocyte development. A mature OL expresses myelin-associated proteins (such as MBP) and extends multiple branched processes. These processes contact and ensheath axons, depositing smooth layers of $MBP^+$ membrane along axons. The final stage involves the wrapping of multiple layers of membranes around the axon to form compact myelin. While MBP/NFH co-localization is suggestive of myelination, it is not necessarily representative of a myelinated axon ensheathed by multiple layers of myelin since oligodendrocytes expressing MBP can contact axons without forming a myelin sheath. To confirm myelination in neuron-glial co-cultures, other groups have immunostained co-cultures with antibodies towards molecules associated with the organized domains of a myelinated axon (Fig. 1.1) such as the potassium channels of the juxtaparanode, sodium channels of the nodes of Ranvier, and Caspr in the paranodes (Bin *et al.,* 2012; Stettner *et al.,* 2013; Heller *et al.,* 2014). Alternative methods for confirming myelination include counting the number and length of myelinated internodes, staining with lipophilic stains such as Sudan Black, and electron microscopy to visualize myelinated axons (Lehmann *et al.,* 2009; Stettner *et al.,* 2013; Pang *et al.,* 2012, Heller *et al.,* 2014).

Therefore, to determine whether myelination might be occurring in my DRGN/OPC co-cultures, I counted the number of $MBP^+$/NFH segments and measured the length of each segment using Neuron J. Three representative visual fields per independent experiment were

selected for quantification for each co-culture condition (WT or KO OPCs). $MBP^+$/NFH segments less than 1μm were excluded from quantifications. There was no significant difference in the number of $MBP^+$/NFH segments in DRGNs co-cultured with WT or PTPα KO OPCs (68.00±9.17 vs. 53.00±18.33 respectively, n = 3) (Fig 3.4B). However, $MBP^+$/NFH segments in co-cultures with WT OPCs were 1.35-fold longer than in co-cultures with PTPα KO OPCs (4.72±0.24μm vs. 3.50±0.30μm respectively, $P<0.01$, n=3) (Fig. 3.4C), representing a significant difference. The longest segment observed in WT OPC/DRGN co-cultures was 21.14μm. In mice, the average length of CNS internodes formed by OLs range from 20-200μm (Chong, *et al.,* 2012).

To determine whether structures reminiscent of compact myelin ensheathing axons are formed in our *in vitro* DRGN/OPC myelinating co-culture model, I conducted a pilot study where I co-cultured WT OPCs with DRGNs derived from WT mice for 28 days and processed these for electron microscopy (EM). EM visualization revealed the presence of myelinated axons (Fig. 3.4D). Taken together, these results validate the occurrence of myelination in our DRGN/OPC co-culture system and that oligodendroglial expression of PTPα promotes the formation of longer myelinated segments.

### 3.2.4 Neuronal expression of PTPα promotes *in vitro* myelination in DRGN/OPC co-cultures

So far, I have shown that oligodendroglial expression of PTPα promotes MBP expression and MBP/NFH co-localization in an *in vitro* DRGN/OPC co-culture system. In addition to being expressed in glial cells, PTPα is also expressed in neuronal cells (Ye *et al.*, 2011) and has been

shown to form a receptor complex with the neuronal adhesion molecule contactin (Zeng *et al.,* 1999). Myelination requires interactions between axons and glial cells. In addition, several neuronally expressed molecules are known to be involved in OPC differentiation and myelination (described in section 1.1.2.1); therefore, I investigated whether neuronally expressed PTPα promotes axo-glial signaling during myelination. For this purpose, I co-cultured DRGNs isolated from PTPα KO mice with OPCs derived from WT or PTPα KO mouse embryos.

#### 3.2.4.1 PTPα is expressed in DRGNs

It has been reported that PTPα is expressed in murine neuronal cells. I first confirmed that PTPα is expressed in DRGNs prior to further investigating the potential role of neuronal PTPα in myelination. DRGNs isolated from WT and PTPα KO pups were cultured for 14 days and PTPα expression was determined by immunoblot analysis (Fig. 3.5A). I confirmed that PTPα is indeed expressed in DRGNs derived from WT but not PTPα KO mice. Since PTPα is an upstream activator of the Src family kinase Fyn (Ponniah *et al*., 1999; Su *et al.,* 1999), I also determined the expression of Fyn and its activity based on its phosphorylation status at its negative regulatory C-terminal tail residue Tyr528. I observed that total Fyn expression was approximately 1.5-fold higher (Fig. 3.5A and B) in DRGNs derived from PTPα KO mice and that the ratio of P-Fyn Tyr528/total Fyn was approximately 1.25-fold higher (Fig. 3.5A and C) in DRGNs isolated from PTPα KO mice in comparison to DRGNs isolated from WT mice. These results are consistent with our laboratory's previous findings, which demonstrate that both P-Fyn Tyr528 and total Fyn expression are increased in fibroblasts derived from PTPα KO mice.

Furthermore, these results confirm PTPα expression in DRGNs; therefore the potential role of neuronal PTPα in myelination can now be investigated using the DRGN/OPC co-culture system.

#### 3.2.4.2 Neuronal expression of PTPα promotes MBP/NFH co-localization but not MBP expression in DRGN/OPC co-cultures

Primary OPCs derived from WT and PTPα KO mice were cultured under differentiation promoting conditions with DRGNs isolated from PTPα KO mice for 14 days on laminin-2 coated chamber slides. Co-cultures were immunostained for NFH and MBP to mark the neurite bed and mature OLs respectively. For each independent co-culture, ten random visual fields of equivalent neuronal bed densities were used for quantification. There was no significant difference observed in neuronal bed density (Fig. 3.6A) in DRGNs co-cultured with WT OPCs or PTPα KO OPCs (25.37±3.65% vs. 25.29±3.74% respectively, n=3) (Fig. 3.7A). MBP expression based on $\%MBP^{+}$ signal per visual field was used as an indicator of OPC differentiation (Fig. 3.6B, F). Quantitative analysis of %MBP signal per visual field showed a significant difference in MBP expression between WT OPCs and PTPα KO OPCs (4.81±0.14% vs. 3.14±0.40% respectively, $P<0.01$, n=3) (Fig. 3.7B). Analysis of MBP/NFH co-localization revealed a significant difference between DRGNs co-cultured with WT OPCs and PTPα KO OPCs (9.81±0.85% vs. 7.15±0.54%, $P<0.05$, n=3) (Fig. 3.7C).

To determine whether neuronal PTPα expression affected MBP expression or MBP/NFH co-localization, I compared neurite bed densities, MBP expression, MBP/NFH co-localization for WT/KO OPCs co-cultured with either WT or KO DRGNs. There was no significant difference in neurite bed densities between cultures of WT or PTPα KO DRGNs (Fig. 3.8A).

MBP expression by WT OPCs was not affected when co-cultured with WT or PTPα KO DRGNs (4.00±8.62% vs. 4.81±0.14% respectively, n=3, Fig. 3.8B). No significant difference in MBP expression by PTPα KO OPCs was observed when co-cultured with WT or PTPα KO DRGNs (2.66±0.43% vs. 3.14±0.40% respectively, n=3) (Fig. 3.8B). MBP/NFH co-localization was reduced when WT OPCs were co-cultured with PTPα KO DRGNs. Indeed, a 1.5-fold decrease in MBP/NFH co-localization was observed in WT OPCs co-cultured with WT DRGNs compared to WT OPCs co-cultured with PTPα KO DRGNs (16.83±3.49% vs. 9.81±0.85%, $P<0.05$, n=3) (Fig. 3.8C). No significant difference was seen in co-cultures where PTPα KO OPCs were co-cultured with either WT or PTPα KO DRGNs (8.42±1.57% vs. 7.15±0.54% respectively, n=3) (Fig. 3.8C). Taken together, these results indicate neuronal expression of PTPα promotes OL and neuronal contact and segment formation indicative of myelination, but not OPC differentiation.

### 3.3 Discussion

These results suggest PTPα plays two roles during myelination. Using an *in vitro* differentiation cell culture model, I have shown that PTPα promotes oligodendrocyte differentiation and that MBP expression is impaired in PTPα-deficient OPCs, consistent with previous results in our laboratory (Dr. Philip Ly, personal communication) (Wang *et al.,* 2009). I have also established an *in vitro* neuron-glia co-culture system to study the role of PTPα in myelination. Studies using the co-culture system demonstrated that oligodendroglial expression of PTPα promotes not only MBP/NFH co-localization, but also the formation of significantly longer $MBP^{+}$/NFH segments. In addition, EM visualization revealed the presence of myelinated

axons, indicating that myelination occurred in the co-culture system and not only the axo-glial contact that occurs prior to myelination.

I have also shown that neuronal expression of PTPα promotes MBP/NFH co-localization. Loss of neuronal PTPα reduced MBP/NFH co-localization by WT oligosphere-derived OPCs. However, MBP expression by WT and PTPα KO OPCs was unaffected suggesting that OPC differentiation in neuron-glial co-cultures is dependent on glial, rather than neuronal, expression of PTPα.

PTPα has been shown to form a neuronal receptor complex with the glycosyl phosphatidylinositol (GPI) linked receptor neural cell adhesion molecule F3/F11 contactin (Zeng *et al.*, 1999). In addition, neuronal contactins interact and form a complex with Caspr *in cis* that is targeted to the paranodal domain during myelination and form transverse bands, a defining feature of a mature paranode (Rios *et al.*, 2000; Dupree *et al.*, 1999). The paranodal junctions are sites of axo-glial contact that regulate myelination (Pedraza, *et al.*, 2001; Ma *et al.*, 2013). Contactin is also expressed on the oligodendroglial surface independent of Caspr and may mediate homophilic neuron-oligodendrocyte interactions (Faivre-Sarrailh and Rougon, 1997; Salzer, 2003). The neuronal Caspr/contactin complex is also known to bind oligodendroglial ligands such neurofascin 155 (NF155), and can also interact with Notch to promote oligodendrocyte differentiation (Charles *et al.*, 2002; Hu *et al.*, 2003). It is unknown whether the contactin/PTPα complex is expressed in oligodendrocytes, and whether oligodendroglial PTPα functions as a signal transducer of contactin mediated signals to stabilize paranodal junctions remains to be determined. As PTPα is known to form a neuronal complex with contactin, and the Caspr/contactin complex becomes localized at paranodal junctions, it would be interesting to

examine whether neuronal PTPα may function to transduce axo-glial signals through the Caspr/contactin complex to stabilize paranodal junctions. It would also be interesting to investigate whether the loss of neuronal PTPα may contribute to abnormal myelination by disrupting the organization of axonal domains during myelination.

### 3.4 Summary

In these studies, I established an *in vitro* model to study myelination. I have demonstrated that oligodendroglial expression of PTPα promotes OPC differentiation and myelination of DRGNs. I have shown that neuronally expressed PTPα is an important positive regulator of MBP/NFH co-localization and segment elongation, suggesting that it also promotes myelination. I also showed that OPC differentiation is dependent on oligodendroglial, and not neuronal expression of PTPα.

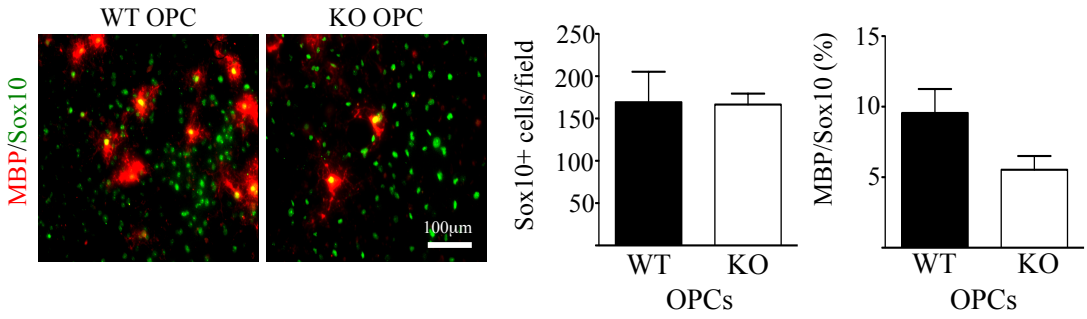


**Figure 3.1. Loss of PTPα impairs *in vitro* OPC differentiation.** Oligosphere-derived OPCs isolated from neural stem cells of WT or KO E14-E18 murine embryos were differentiated for 5 days on chamber slides coated with PDL and laminin-2. Chamber slides were fixed and immunostained for Sox10 to mark cells of oligodendroglial lineage, and for MBP to mark mature OLs. Ten visual fields per chamber were used for quantitation. Error bars represent mean ± S.D. from three chambers cultured in a single experiment.

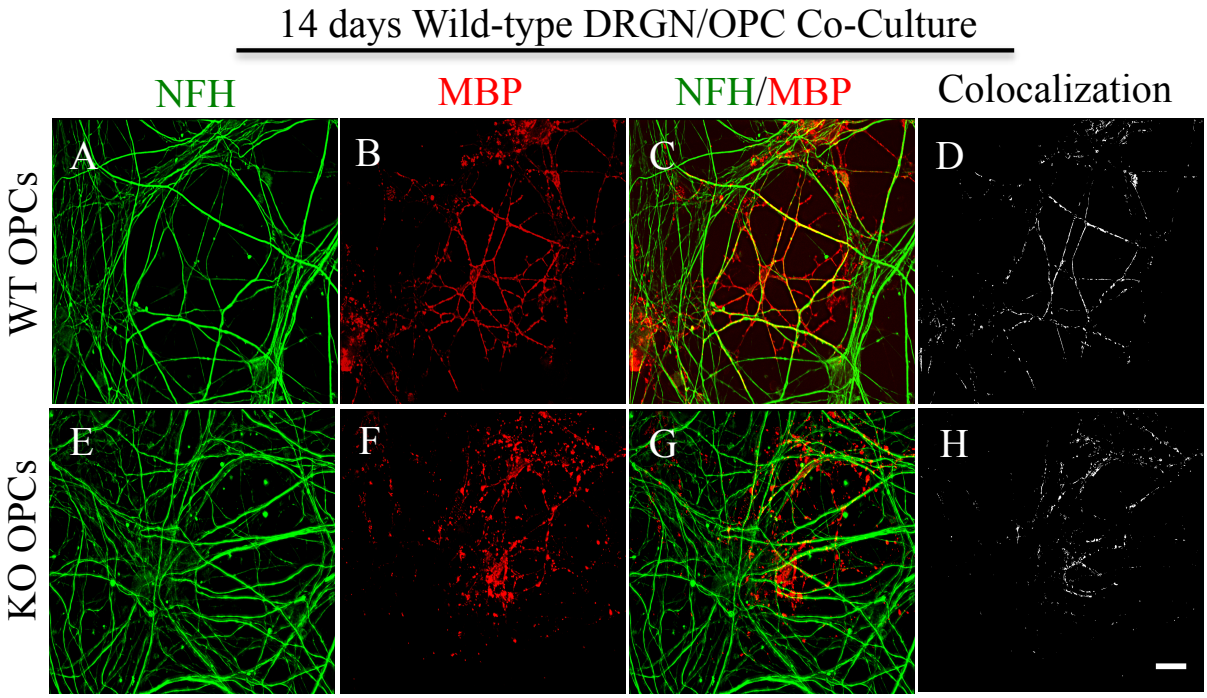


**Figure 3.2 Oligodendroglial PTPα in primary murine OPCs promotes the expression and co-localization of MBP along neurites during OPC/DRGN co-culture on laminin-2.** Dorsal root ganglion neurons (DRGNs) isolated from wild type P5-P8 mouse pups were cultured on laminin-2 coated chamber slides. Neuronal cultures were maintained for 7 days in medium containing 10μM fluorodeoxyuridine (FuDR) as detailed in Material and Methods (section 2.2.3.4). Neuronal cultures were maintained for an additional 7 days in medium without FuDR. OPCs from cultures of oligospheres, derived from (A-D) WT, and (E-H) PTPα null (KO) mice were plated onto the DRGNs at day 14 of the neuronal culture. DRGNs and OPCs were co-cultured for 14 or 21 days (14 day co-cultures shown) and immunostained for (A, D) neurofilament (NFH) to mark the neurite bed, (B, E) myelin basic protein (MBP) to mark mature myelinating OLs. (C, F) NFH and MBP channels were merged and (D, H) co-localized regions of NFH and MBP signals were identified using ImageJ. Scale bar 25μm.

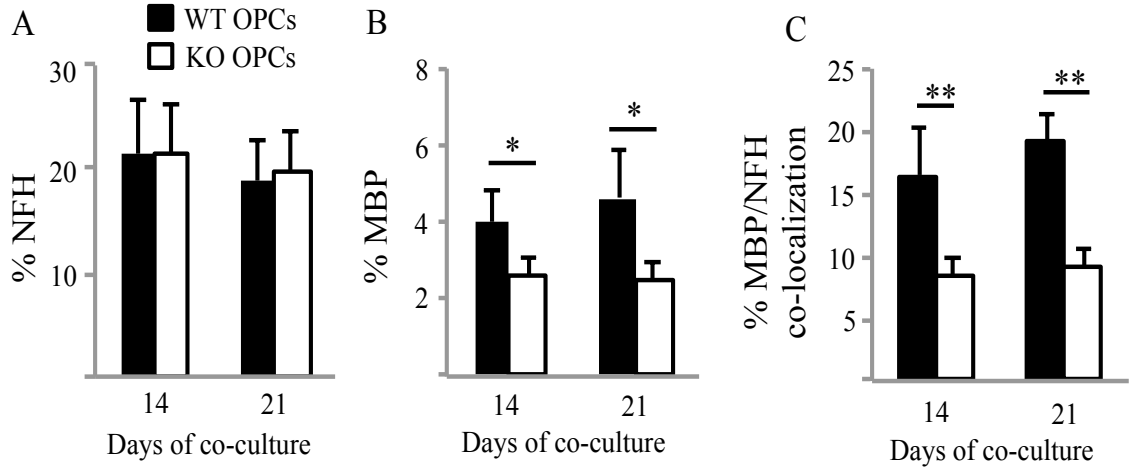


**Figure 3.3 PTPα promotes OPC differentiation and MBP/NFH co-localization in OPC/DRGNs co-cultures grown on laminin-2.** OPCs derived from WT or PTPα KO mice were co-cultured with DRGNs on laminin-2 coated chamber slides for 14 or 21 days. Co-cultures were immunostained for NFH to mark the neurite bed and MBP to mark mature OLs. (A) Neurite bed density was determined by forming a binary mask using Image J and calculating the percentage of a visual field positive for NFH Signal. (B) OPC differentiation was assessed by measuring the percentage of MBP signal per visual field. (C) As an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using unpaired Students t-test. (*P<0.05, **P<0.01) Data are from three independent experiments, and 10 random visual fields from each of the WT and KO OPC co-cultures that had equivalent neurite bed densities were quantified for each independent experiment.

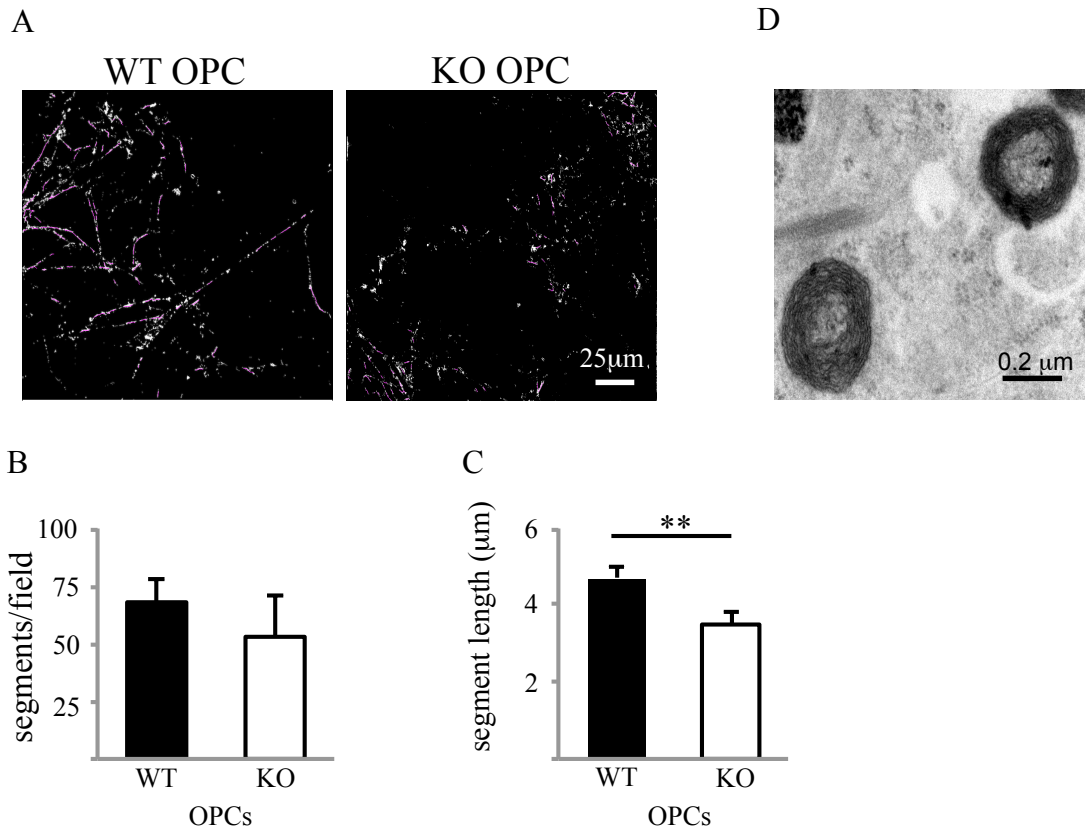


**Figure 3.4. Oligodendroglial PTPα promotes formation of longer MBP$^+$/NFH segments in co-cultures that can achieve myelination.** (A) The average length and total number of co-localized segments was quantified by tracing (shown in purple overlay) each co-localized segment per visual field using Neuron J. Three representative binary images of MBP/NFH co-localization per condition (WT or KO OPCs) were used per independent experiment for quantitation. (B) Graph representing the number of MBP$^+$/NFH segments per visual field and (C) a graph representing the average length per MBP$^+$/NFH segment. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using unpaired students t-test. (*P<0.05, **P<0.01) Data are from three independent experiments. (D) A 28 day DRGN/WT OPC co-culture was visualized using electron microscopy. The electron dense concentric rings are typical of compacted layers of myelin surrounding axons.

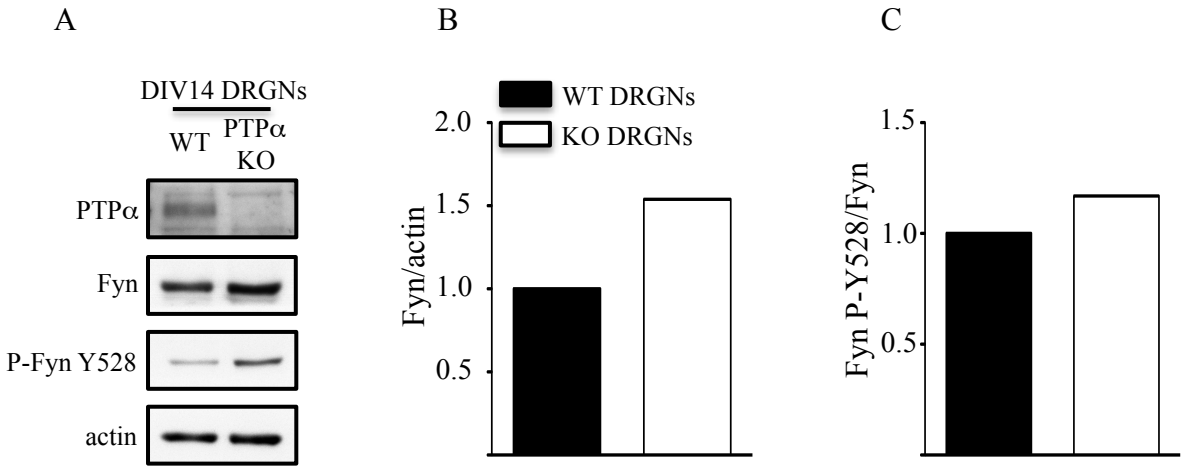


**Figure 3.5. DRGNs express PTPα and PTPα-null neurons exhibit decreased Fyn activity.** (A) Lysates prepared from PTPα KO DRGNs cultured for 14 days *in vitro* (DIV) were probed with antibodies against PTPα, Fyn, phosopho $Src^{527}$ to detect Fyn Y528 phosphorylation, and actin. (B) Total Fyn expression was normalized as a ratio of the band intensity of Fyn to the band intensity of actin. Fyn expression by PTPα KO DRGNs was ~1.5-fold greater than by WT DRGNs. (C) Fyn activity was determined by the phosphorylation status of the inhibitory phosphorylation site at the 528 tyrosine residue of Fyn. The band intensity of Fyn P-Y528 was normalized to the intensity of total Fyn expression. PTPα KO DRGNs expressed ~1.25-fold more Fyn P-Y528.

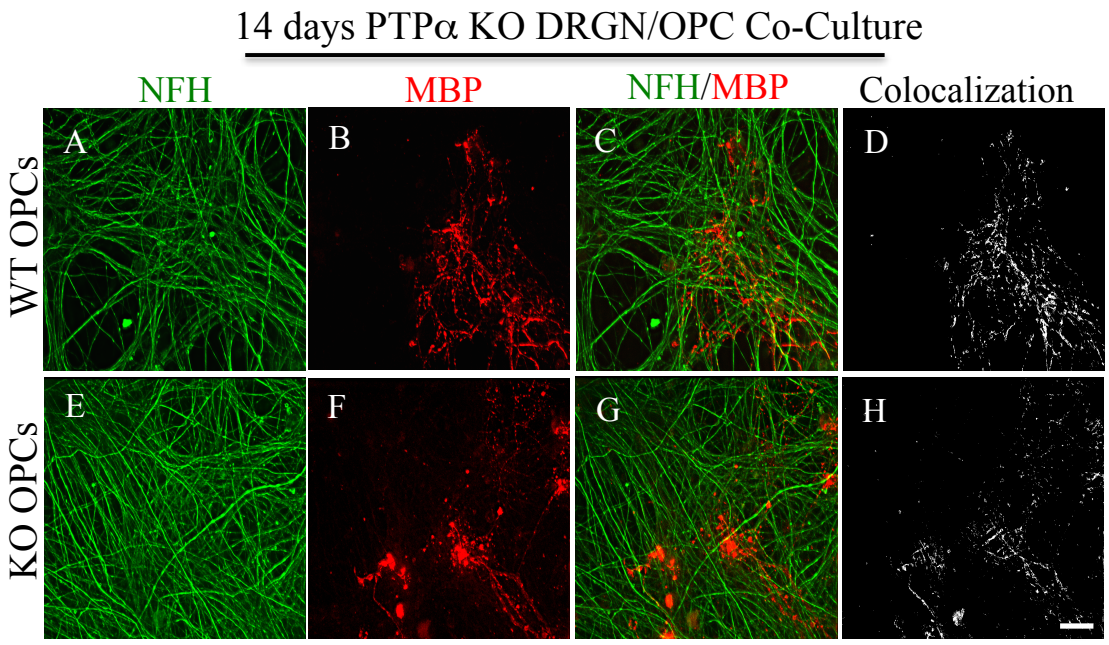


**Figure 3.6. Neuronal PTPα in primary murine OPCs promotes the expression and co-localization of MBP along neurites during OPC/DRGN co-culture on laminin-2.** Dorsal root ganglion neurons (DRGNs) isolated from wild type PTPα KO P5-P8 mouse pups were cultured on laminin-2 coated chamber slides. Neuronal cultures were maintained for 7 days in medium containing 10μM fluorodeoxyuridine (FdUR) as detailed in Material and Methods (section 2.3). Neuronal cultures were maintained for an additional 7 days in medium without FdUR. OPCs from cultures of oligospheres, derived from (A-D) WT, and (E-H) PTPα null (KO) mice were plated onto the DRGNs at day 14 of the neuronal culture. DRGNs and OPCs were co-cultured for 14 or 21 days (14 day co-cultures shown) and immunostained for (A, D) neurofilament (NFH) to mark the neurite bed, (B, E) myelin basic protein (MBP) to mark mature myelinating OLs. (C, F) NFH and MBP channels were merged and (D, H) co-localized regions of NFH and MBP signals were identified using ImageJ. Scale bar 25μm.

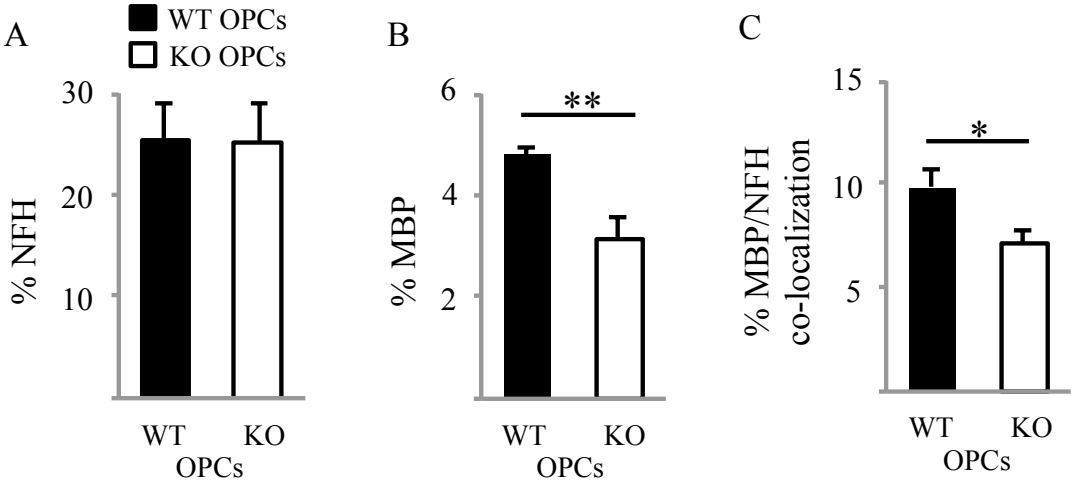


**Figure 3.7. Neuronal PTPα in primary murine DRGNs promotes MBP/NFH co-localization along neurites, but not MBP expression during OPC/DRGN co-culture on laminin 2.** OPCs derived from WT or PTPα KO mice were co-cultured with PTPα KO DRGNs on laminin 2 coated chamber slides for 14 days. Chamber slides were immunostained for NFH to mark the neurite bed and MBP to mark mature OLs. (A) Neurite bed density was calculated by forming a binary mask using Image J and calculating the percentage of a visual field positive for NFH Signal. (B) OPC differentiation was assessed by measuring the percentage of MBP signal per visual field. (C) As an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using unpaired students t-test. (*P<0.05, **P<0.01) Data are from three independent experiments and 10 random visual fields from each of the WT and KO OPC co-cultures that had equivalent neurite bed densities were quantified for each independent experiment.

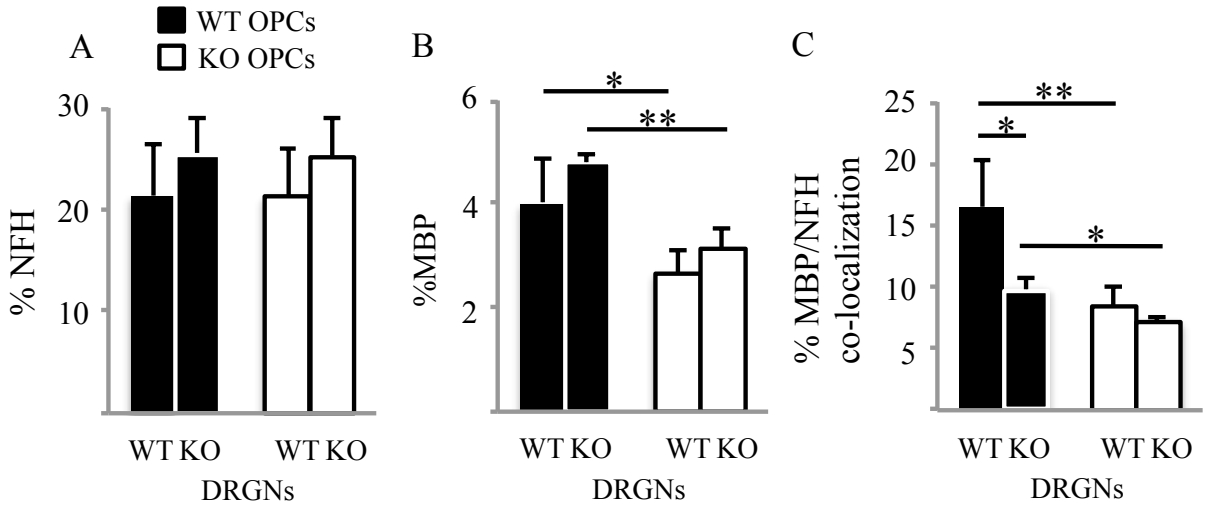


**Figure 3.8. Neuronal PTPα in primary murine DRGNs promotes MBP/NFH co-localization along neurites during OPC/DRGN co-culture on laminin 2.** (A-C) Neurite bed densities, MBP expression and MBP/NFH co-localization were compared between WT and KO DRGNs that were co-cultured with either WT or KO OPCs. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using unpaired students t-test. (*P<0.05, **P<0.01) Data are from three independent experiments and 10 random visual fields from each of the WT and KO OPC co-cultures that had equivalent neurite bed densities were quantified for each independent experiment.

## Chapter 4: Altering canonical Wnt signaling can affect oligodendroglial PTPα-dependent MBP/NFH co-localization in an *in vitro* DRGN/OPC co-culture system

### 4.1 Introduction and rationale

In accordance with our laboratory's previous findings (Wang *et al.*, 2009), I have shown that oligodendroglial expression of PTPα promotes OPC differentiation, process extension and elongated axo-glial contacts. In addition, I also showed that neuronal expression of PTPα promotes MBP/NFH co-localization. Our laboratory has also found that ablated PTPα signaling delays OPC differentiation and increases OPC proliferation (Wang *et al.,* 2012). However, additional signaling pathways are also involved in promoting or inhibiting OPC differentiation and myelination. The canonical Wnt signaling pathway (described in section 1.2.1.3) is of particular interest as pharmacological manipulation of Wnt signaling has recently been implicated in OPC differentiation and myelination.

During active Wnt signaling, β-catenin is not targeted for degradation, and therefore accumulates in the cytoplasm and translocates to the nucleus where it complexes with the TCFL2 (Tcf4) transcription factor to activate target gene expression. Active canonical Wnt signaling represses OPC differentiation, and suppresses myelination (Fancy *et al*., 2009; Ye *et al*., 2009). Experimental manipulation of β-catenin level has also revealed that down-regulation of Wnt signaling through promoting β-catenin degradation can promote OPC differentiation, myelination and remyelination (Fancy *et al.*, 2011). Conversely, stabilization of β-catenin through stimulation of Wnt signaling represses OPC differentiation (Feigenson *et al.*, 2009). Since PTPα signaling and down-regulation of canonical Wnt signaling both promote OPC differentiation and myelination, I investigated whether experimentally inhibiting or stimulating

the canonical Wnt signaling pathway can rescue PTPa-dependent impairments in differentiation and myelination. To address this, I used the DRGN/OPC *in vitro* myelinating co-culture system I established (Chapter 3). Co-cultures were treated with an inhibitor of Wnt signaling, or a Wnt ligand, and the effects on differentiation and myelination were examined.

## 4.2 Results

### 4.2.1 XAV939 inhibits Wnt reporter gene expression by CG4 cells in a dual-luciferase reporter assay system

XAV939 is a small molecule inhibitor known to selectively repress β-catenin mediated Wnt signaling. XAV939 prevents the degradation of axin, a component of the destruction complex, by inhibiting tankyrase mediated poly-ADP-ribosylation thereby stabilizing axin (Huang *et al.,* 2009). Fancy *et al.* (2011) also demonstrated that culturing OPCs and cerebellar slice cultures in the presence of XAV939 following lysolecithin induced demyelination also promotes OPC differentiation and remyelination. I first wanted to confirm that XAV939 targets the Wnt signaling pathway. To characterize the effects of XAV939 on Wnt signaling, I collaborated with a postdoctoral fellow in our lab, Dr. Philip Ly, and carried out a study using a TOPFlash dual-luciferase reporter assay system (described in section 2.2.1.1). The assay utilizes two plasmids: the TOPFlash plasmid, which contains three tandem repeats of the Tcf transcription factor binding site upstream of a firefly luciferase gene; and the FOPFlash plasmid, which is structurally similar to the TOPFlash plasmid except the sequences of the Tcf transcription factor binding site are mutated, therefore Wnt-dependent expression of the luciferase gene is impaired (Park *et al.,* 2011). Thus, the FOPflash plasmid can be used as a negative control. CG4 rat OPCs (a cell line) were transfected with either the TOPFlash plasmid

or FOPFlash plasmid. Cells were also co-transfected with a plasmid carrying the CMV promoter driving the expression of *Renilla* luciferase, which was used as an internal control. Following transfection cells were treated with XAV939, the Wnt3a ligand, or a combination of Wnt3a and XAV939. Luciferase activity was measured in a luminometer and TCF/β-catenin promoter-dependent luciferase activity of the TOPFlash and FOPFlash plasmids was measured as a ratio of firefly luciferase activity to *Renilla* luciferase activity (Figure 4.1).

As expected, no significant effect on luciferase activity was observed in TOPFlash/FOPFlash transfected CG4 cells treated with XAV939 since Wnt signaling was not stimulated (TOPFlash: 3.1±1.4 vs. 2.7±1.3 respectively). Upon treatment with Wnt3a, TOPFlash promoter activity increased by approximately 4-fold in comparison to untreated cells (12.3±4.5 vs. 3.1±1.4 respectively, $P<0.001$, n=3) (Figure 4.1), and had no effect on FOPFlash promoter activity. XAV939 blocked Wnt3a stimulated TOPFlash promoter activity (12.3±4.5 vs. 2.7±2.3, $P<0.001$, n=3) (Figure 4.1). Primary murine oligospheres derived from WT mice were transfected with either the TOPFlash or FOPFlash plasmid. Following transfection, oligospheres were treated with XAV939, Wnt3a, or a combination of Wnt3a and XAV939 to determine: the amount of Wnt3a to elicit Wnt reporter activity; and the amount of XAV939 needed to inhibit Wnt3a-dependent reporter gene expression. Our laboratory found that 100ng/ml of Wnt3a elicited optimal Wnt reporter activity, and 0.05μM of XAV939 was sufficient to suppress Wnt3a-dependent reporter gene expression (Dr. Philip Ly, personal communication). Taken together, these data indicate that XAV939 does indeed inhibit the Wnt signaling pathway.

### 4.2.2 XAV939 promotes OPC differentiation *in vitro* by both WT and PTPα KO OPCs

I next investigated the effect of XAV939 on OPC differentiation. Primary WT and PTPα KO murine OPCs were plated onto PDL and laminin-2 coated chamber slides and maintained under proliferative conditions for five days prior to inducing differentiation. Differentiating OPCs were cultured with or without XAV939, and at day 5 of differentiation, the cultures were immunostained for Sox10, a marker of OL lineage cells, and MBP. OPCs were immunostained for Sox10 and MBP (Figure 4.2A). The extent of differentiation was determined by counting the number of $MBP^+$/Sox10 cells.

In untreated cultures, WT OPCs expressed more $MBP^+$/Sox10 cells in comparison to PTPα KO OPCs, which is consistent with earlier observations. XAV939 increased MBP expression by $Sox10^+$ PTPα KO OPCs to levels comparable to those of untreated WT OPCs (4.7±0.58% untreated WT OPCs control vs. 3.8±0.56 KO OPCs with 50nM XAV939) (Figure 4.2B). XAV939 also significantly increased the number of $MBP^+$/Sox10 cells in WT cultures. The number of $Sox10^+$ cells expressing MBP increased by ~1.5 fold when treated with 50nM of XAV939, and increased by ~2 fold when treated with 100nM XAV939. Even though XAV939 treatment improved differentiation by PTPα KO OPCs, the extent of differentiation by PTPα KO OPCs was much lower in comparison to XAV939 treated WT OPCs as XAV939 also significantly improved differentiation by WT OPCs. As XAV939 improves differentiation by both WT and PTPα KO OPCs, it is possible that PTPα and Wnt signaling may affect OPC differentiation through different mechanisms.

### 4.2.3 XAV939 increases MBP expression and MBP/NFH co-localization by PTPα KO OPCs in OPC/DRGN co-cultures

To investigate the effects of down-regulating Wnt signaling on OPC differentiation and myelination, I treated co-cultures with varying doses of XAV939. Oligosphere derived OPCs from WT or PTPα KO mice embryos were co-cultured with DRGNs derived from WT mice for 14 days during which they were treated with varying doses of XAV939 (0, 0.05, 0.5, and 2.5μM) (Figure 4.3). Co-cultures were fixed and immunostained for MBP and NFH. No significant difference in neurite bed density was observed in DRGNs co-cultured with WT and PTPα KO OPCs (Figure 4.4A). In untreated co-cultures, there was a significant difference observed in MBP expression between WT and PTPα KO OPCs (5.59±1.44% vs. 3.31±0.82% respectively, $P<0.05$, n=3) (Figure 4.4B). XAV939 treatment did not increase MBP expression by WT OPCs; however, MBP expression by PTPα KO OPCs increased ~1.6 fold following treatment with 0.05μM XAV939 when compared to the control DMSO-treated co-cultures (3.31±0.82% Control vs. 5.35±0.72% 0.05μM XAV939, $P<0.05$, n=3) (Figure 4.4B). Increasing the XAV939 dosage further did not significantly enhance MBP expression by PTPα KO OPCs (5.44±0.95% 0.5μM vs. 5.50±1.35% 2.5μM) (Figure 4.4B). Taken together XAV939 addition to OPC/DRGN co-cultures can remediate deficiencies in MBP expression associated with the loss of oligodendroglial PTPα expression.

As an indicator of potential myelination, the percentage of MBP/NFH co-localization per NFH signal was measured. In untreated co-cultures, there was a significant difference observed in MBP/NFH co-localization between WT and PTPα KO OPCs (14.59±2.34% vs. 8.52±0.91% respectively, $P<0.01$, n=3) (Figure 4.4C). MBP/NFH co-localization in PTPα KO OPC/DRGN

co-cultures increased by 1.65 fold upon treatment with 0.05μM XAV939 (8.52±0.91% Control vs. 14.13±2.42% 0.05μM XAV939, P<0.01, n=3) (Figure 4.4C). Treatment of PTPα KO OPC/DRGN co-cultures with higher doses of XAV939 did not further increase MBP/NFH co-localization (13.46±1.77% 0.5μM vs. 15.28±2.24% 2.5μM) (Figure 4.3C). XAV939 increased MBP/NFH co-localization by PTPα KO OPC/DRGN co-cultures to levels comparable to MBP/NFH co-localization by WT OPC/DRGN co-cultures. Taken together, my data suggest that at 0.05μM, XAV939 treatment can remediate deficiencies MBP/NFH co-localization associated with the loss of oligodendroglial PTPα expression.

### 4.2.4 XAV939 increases the number of $MBP^+$/NFH segments, but does not increase the average length of $MBP^+$/NFH co-localized segment in PTPα null OPCs

I have shown that the addition of XAV939 to DRGN/OPC co-cultures improved both MBP expression and MBP/NFH co-localization (indicative of axo-glial contact and pro-myelination) in PTPα KO OPC/DRGN co-cultures. However, improved MBP/NFH co-localization does not necessary represent a rescue in myelination; therefore, the number of $MBP^+$/NFH segments was counted and the average length of the co-localized segments was measured to investigate whether XAV939 can rescue PTPα dependent impairments in forming axo-glial contacts.

Untreated OPC/DRGN co-cultures and co-cultures treated with 0.05μM XAV939 were quantified for comparison as increasing the concentration of XAV939 did not further affect MBP/NFH co-localization. Quantitative analysis revealed that the number of $MBP^+$/NFH segments in WT OPC/DRGN co-cultures were unaffected by XAV939 treatment (68.22±6.47

Control vs. 68.77±4.22 0.05μM XAV939, n=3, Figure 4.5B). The average length per $MBP^+$/NFH segment was unaffected in WT OPC/DRGN co-cultures (5.36±0.56μm Control vs. 5.79±0.38μm 0.05μM XAV939, n=3) (Figure 4.5C). In PTPα KO OPC/DRGN co-cultures, XAV939 treatment increased the number of $MBP^+$/NFH segments by ~1.7 fold (56.44±4.55 Control vs. 94.55±8.59 0.05μM XAV939, $P<0.01$, n=3) (Figure 4.5B). However, the average length of $MBP^+$/NFH segment did not increase with XAV939 treatment (Figure 4.5C). The majority of $MBP^+$/NFH segments were between 1-3μm in length in KO OPC/DRGN co-cultures, whereas in WT OPC/DRGN co-cultures, there were significantly more $MBP^+$/NFH segments longer than 8μm (14.63±5.03 Control, and 18.55±2.61, Figure 4.5D). Segments longer than 8μm were further analyzed to determine whether XAV939 promoted elongation of co-localized segments in WT OPC/DRGN co-cultures. There was no significant difference in the percentage of co-localized segments longer than 15μm (Figure 4.5E). The longest segment observed in control cultures was 23.48μm, and 22.94μm in XAV939 treated cultures. Collectively, these results indicate that XAV939 increases the number of axo-glial contacts established in PTPα KO OPC/DRGN co-cultures; however, elongation of axo-glial contact does not appear to be rescued by XAV939 suggesting that elongation of axo-glial contacts requires PTPα signaling.

### 4.2.5 Wnt3a decreases MBP expression and MBP/NFH co-localization by WT OPCs but not PTPα KO OPCs in DRGN/OPC co-cultures.

I have shown that treatment of DRGN/OPC co-cultures with the Wnt signaling inhibitor XAV939 increases MBP/NFH co-localization by PTPα KO OPCs. Stimulation of the Wnt signaling pathway using the Wnt3a ligand represses OPC differentiation and delays myelination

*in vivo* (Feignson *et al.*, 2009); therefore, I investigated whether activating Wnt signaling with the Wnt3a ligand affected MBP expression and MBP/NFH co-localization in the DRGN/OPC co-culture model. WT or PTPα KO OPC/DRGN co-cultures were treated with either vehicle solution (0.1% BSA in PBS), or 100ng/mL of the Wnt3a for 14 days. Co-cultures were fixed and immunostained for MBP and NFH (Figure 4.6).

There was no significant difference in neurite bed density in DRGNs co-cultured with WT and PTPα KO OPCs (Figure 4.7A). A significant difference in MBP expression was observed between WT and PTPα KO OPCs in untreated co-cultures, which is consistent with my earlier results (6.07±0.46% vs. 3.96±0.75% respectively, $P<0.01$, n=3) (Figure 4.7B). Wnt3a stimulation reduced MBP expression by WT OPCs by ~1.25 fold (6.07±0.46% Control vs. 4.86±0.17% 100ng/mL Wnt3a, $P<0.05$, n=3, Figure 4.7B). MBP expression by PTPα KO OPCs did not differ significantly (3.96±0.75% Control vs. 3.85±0.87% 100ng/mL Wnt3a, n=3) (Figure 4.7B). In Wnt3a treated co-cultures, MBP expression by WT OPCs was reduced to levels comparable with MBP expression levels of PTPα KO OPCs in both untreated and Wnt3a treated cultures. These results indicate that stimulation of the Wnt signaling pathway is sufficient to repress MBP expression.

In untreated co-cultures, MBP/NFH co-localization by WT OPCs was ~1.75 fold greater than PTPα KO OPCs (16.49±0.97% vs. 9.43±1.08% respectively, $P<0.001$, n=3, Figure 4.7C), consistent with previous experiments (Figures 3.3C and 4.4C). When co-cultures were treated with Wnt3a, MBP/NFH co-localization by WT OPCs was reduced by 1.6 fold when compared to WT OPCs in untreated cultures (16.49±0.97% vs. 10.24±1.00% respectively, $P<0.001$, n=3, Figure 4.7C). Wnt3a treatment did not have an observable effect on MBP/NFH co-localization

PTPα KO OPCs in both untreated and Wnt3a treated co-cultures (9.43±1.08% vs. 8.69±0.13% respectively, n=3, Figure 4.7C). Wnt3a treatment reduced MBP/NFH co-localization by WT OPCs to similar levels observed in PTPα KO OPC/DRGN co-cultures. Taken together, these data suggest that Wnt3a stimulation of the Wnt signaling pathway has a negative effect on promoting MBP/NFH co-localization.

## 4.3 Discussion

These studies indicate that the Wnt signaling pathway can be experimentally manipulated to regulate OPC differentiation and myelination. I have shown that the small molecule anti-tankyrase inhibitor XAV939 has a specific inhibitory effect on Wnt3a mediated of the Wnt induced reporter gene expression. Dr. Philip Ly (personal communication) has shown, using our *in vitro* cell culture model for differentiation, that XAV939 improved differentiation in both WT and PTPα null OPCs. XAV939 increased MBP expression by $Sox10^+$ PTPα KO OPCs to levels comparable to those of untreated WT OPCs. When comparing XAV939 treated WT OPCs with XAV939 treated PTPα KO OPCs, the number of $MBP^+$/Sox10 cells were not comparable as the extent of differentiation by PTPα KO OPCs was much lower in comparison to XAV939 treated WT OPCs. These data suggest that Wnt signaling may be functioning independently of PTPα signaling as XAV939 also further enhanced the number of $MBP^+$/Sox 10 cells in WT OPC cultures. Active Wnt signaling prevented initial differentiation of OPCs in the spinal cord, and it has also been proposed that Wnt signaling may act as a regulator of timing for oligodendrocyte development (Shimizu *et al.,* 2005). It is possible that down-regulating Wnt signaling may be

required to initiate OPC differentiation and PTPα may promote additional differentiation mechanisms.

I have shown, using the *in vitro* myelinating co-culture system, that XAV939 can rescue PTPα dependent impairments in differentiation, consistent with another report, which demonstrated that inhibition of Wnt signaling using XAV939 can improve OPC differentiation and remyelination following hypoxic white matter injury (Fancy *et al*., 2011). However, my results suggest that the reduced ability to from elongated axo-glial contacts cannot be rescued in PTPα null OPCs following XAV939 treatment, as the average length of $MBP^{+}$/NFH co-localized segments did not increase. This suggests that XAV939 can promote differentiation in PTPα KO OPCs and the formation of axo-glial contacts that enable myelination; however subsequent phases of myelination (described in section 3.2.3) may be dependent on PTPα signaling. As described in section 1.1.2, myelination segregates axonal molecules into specific domains (Figure 1.2). Caspr is a molecule that becomes restricted to the paranodes following myelination. Dr. Philip Ly (personal communication) has shown, using an *ex vivo* cerebellar slice culture model (described in section 1.1.4), that XAV939 treatment of cerebellar slices obtained from PTPα KO mice increased MBP/NFH co-localization. However, immunostaining for Caspr to identify paranodes of myelinated axons revealed that there was no increase in the formation of paranodal structures in comparison to untreated KO slices. These data support that down-regulating Wnt signaling increases OPC differentiation and formation of axo-glial contacts; however, formation of structures resembling the architecture of a myelinated axon may depend on PTPα activity. I have also shown that Wnt3a addition to WT OPC/DRGN co-cultures reduces differentiation and MBP/NFH co-localization; however differentiation and MBP/NFH co-

localization was not further reduced when Wnt3a was added to KO OPC/DRGN co-cultures. This indicates that OPC differentiation is already strongly impaired in the absence of PTPα, and that activation of Wnt signaling cannot further inhibit this process and therefore does not have an additive effect on repressing OPC differentiation.

## 4.4 Summary

These results indicate that inhibition of Wnt signaling can rescue PTPα-associated deficiencies in OPC differentiation but not myelination. These observations were supported as I also demonstrated that Wnt3a stimulation of the Wnt signaling pathway inhibited WT OPC differentiation, process extension and establishment of axo-glial contacts, processes that are prerequisite for myelination.

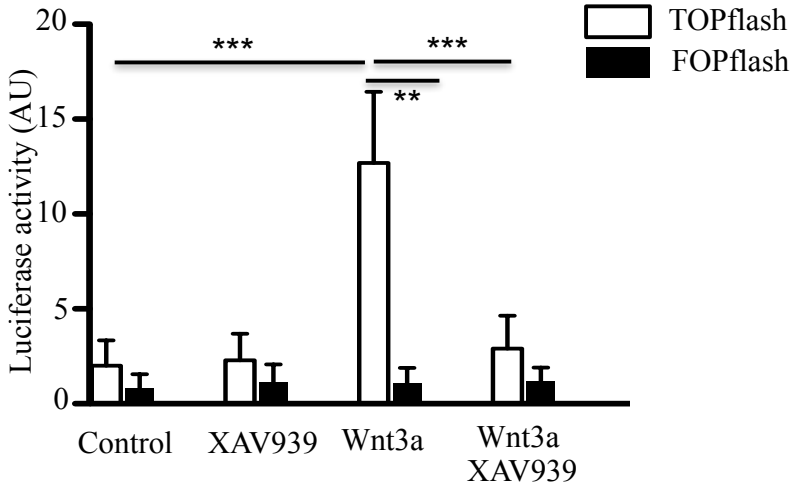


**Figure 4.1. XAV939 treatment inhibits Wnt3a stimulation in CG4 cells.** Luciferase assay readings of XAV939 and Wnt3a treatment in TOP/FOPflash-transfected CG4 cells. XAV939 treatment alone has no effect on TOP/FOPflash promoter activity. Wnt3a stimulation increase TOPflash promoter activity by ~3 fold, but has no effect on the FOPflash promoter. The tankyrase inhibitor, XAV939 stabilizes the Axin protein and blocked Wnt3a-stimulation of the TOPflash promoter, indicating a specific inhibitory effect of XAV939 on the Wnt pathway. Bars represent mean+/-SD. (N=3) One way ANOVA followed by *Tukey*'s *post hoc*. (*P<0.05, **P<0.01, ***P<0.001).

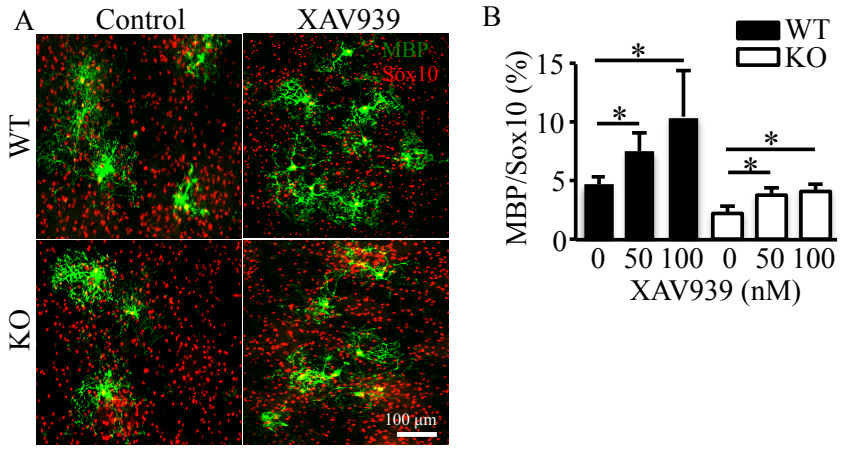


**Figure 4.2. PTPα functions independently of Wnt signaling to promote OL differentiation.** WT and PTPα null OPCs were seeded onto PDL and Laminin 2-coated chamber slides and maintained in proliferative conditions for 5 days prior to differentiation for another 5 days. XAV939 (50 and 100 nM) was added to differentiating cultures and the extent of differentiation was determined by immunostaining for MBP expression in $Sox10^+$ (oligodendrocyte lineage) cells. (A) Representative images of differentiated WT and KO OPC cultures treated with/without XAV939 for 5 days. (B) Quantification of $Sox10^+$ cells expressing MBP. 6-8 random areas were imaged and the number of MBP positive and Sox10 positive cells were counted. XAV939 treatment significantly improves WT OPC differentiation in a dose-dependent manner. KO OPCs treated with XAV939 also undergo differentiation, but to a much lower level as compared to WT OPCs. Bars represent mean ±SD, N=3 independent experiments. Two-way ANOVA followed by Bonferroni's *post hoc* test, *P<0.05.

Contributed by Dr. P. Ly (Unpublished data, 2014)

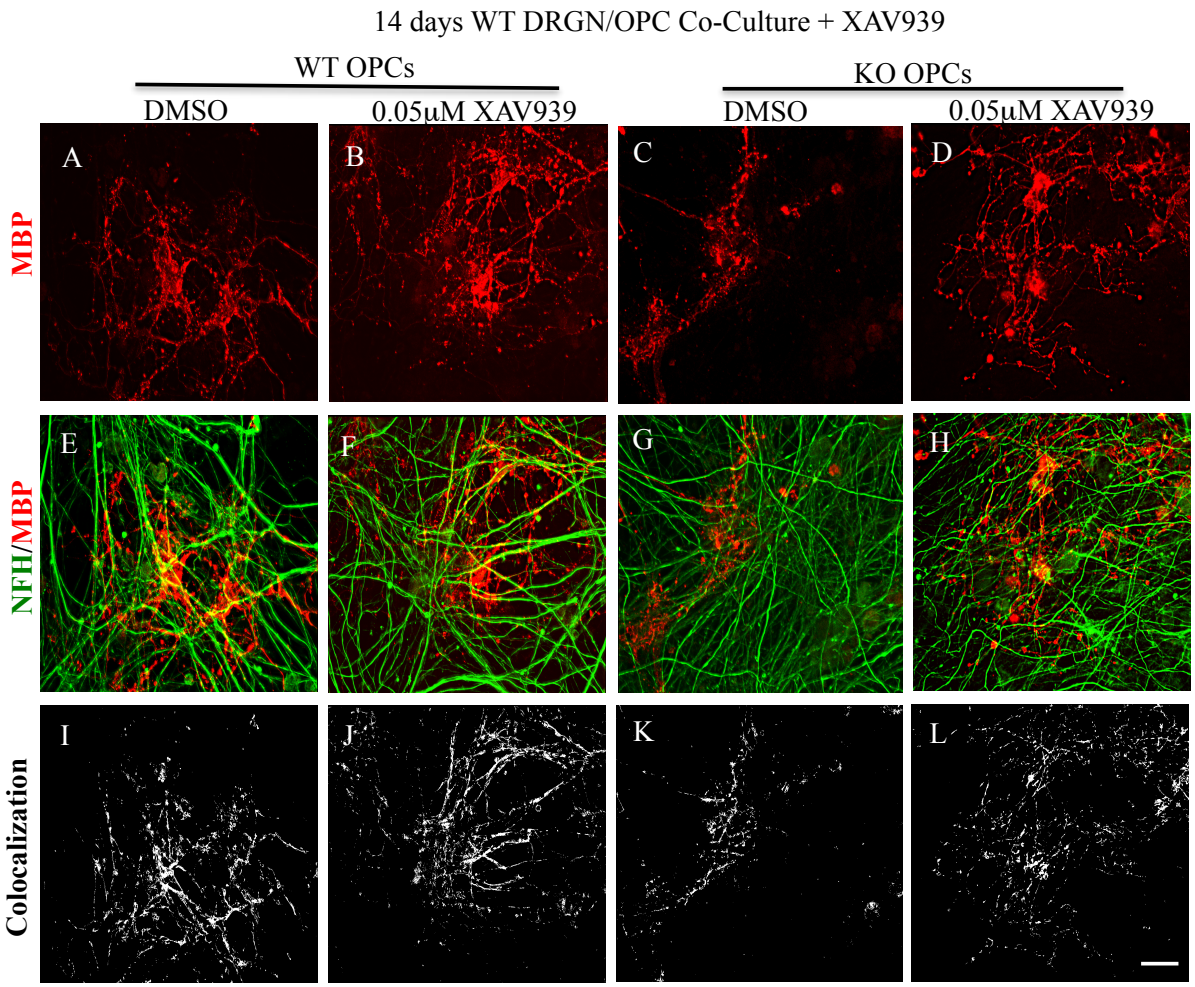


**Figure 4.3. XAV939 increases expression and co-localization of MBP along neurites during OPC/DRGN co-culture.** OPCs from independent cultures of oligospheres, WT (A,B,E,F,I,J) and PTPα null (KO) mice (C,D,G,H,K,L) were plated onto DRGNs derived from WT mice grown on laminin-2 coated chamber slides at day 14 of the neuronal culture. DRGNs and OPCs were co-cultured for 14 days in the presence of XAV939 at various doses (0, 0.05, 0.5 and 2.5mM). Immunostaining for neurofilament (NFH) marks the neurite bed (not shown). (A-D) Myelin basic protein (MBP) marks mature OLs and (N) MBP signal per visual field was used as an indicator of OPC differentiation. (E-H) NFH and MBP channels were merged. (I-L) Co-localized points were identified and as an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined using ImageJ. Scale bar 25μm.

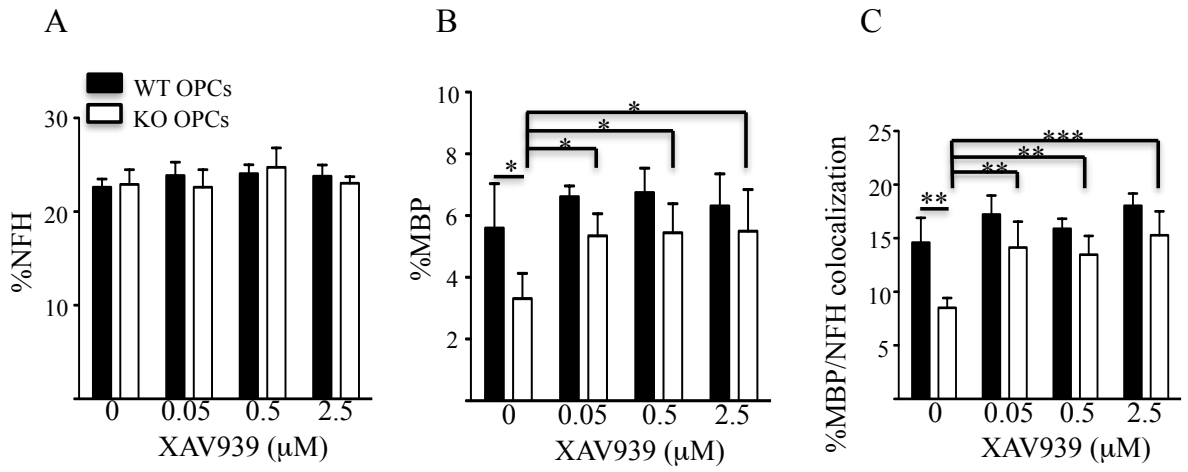


**Figure 4.4. XAV939 increases expression and co-localization of MBP along neurites during OPC/DRGN co-culture.** Quantitative analysis of OPC/WT DRGN co-cultures treated with XAV939. (A) a binary mask was formed to quantify neurite bed density based on NFH signal per visual field. (B) MBP signal per visual field was used as an indicator of OPC differentiation. (C) As an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined using ImageJ. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using two-way ANOVA followed by Bonferroni post-tests. (*P<0.05, **P<0.01, ***P<0.001) Data are from three independent experiments.

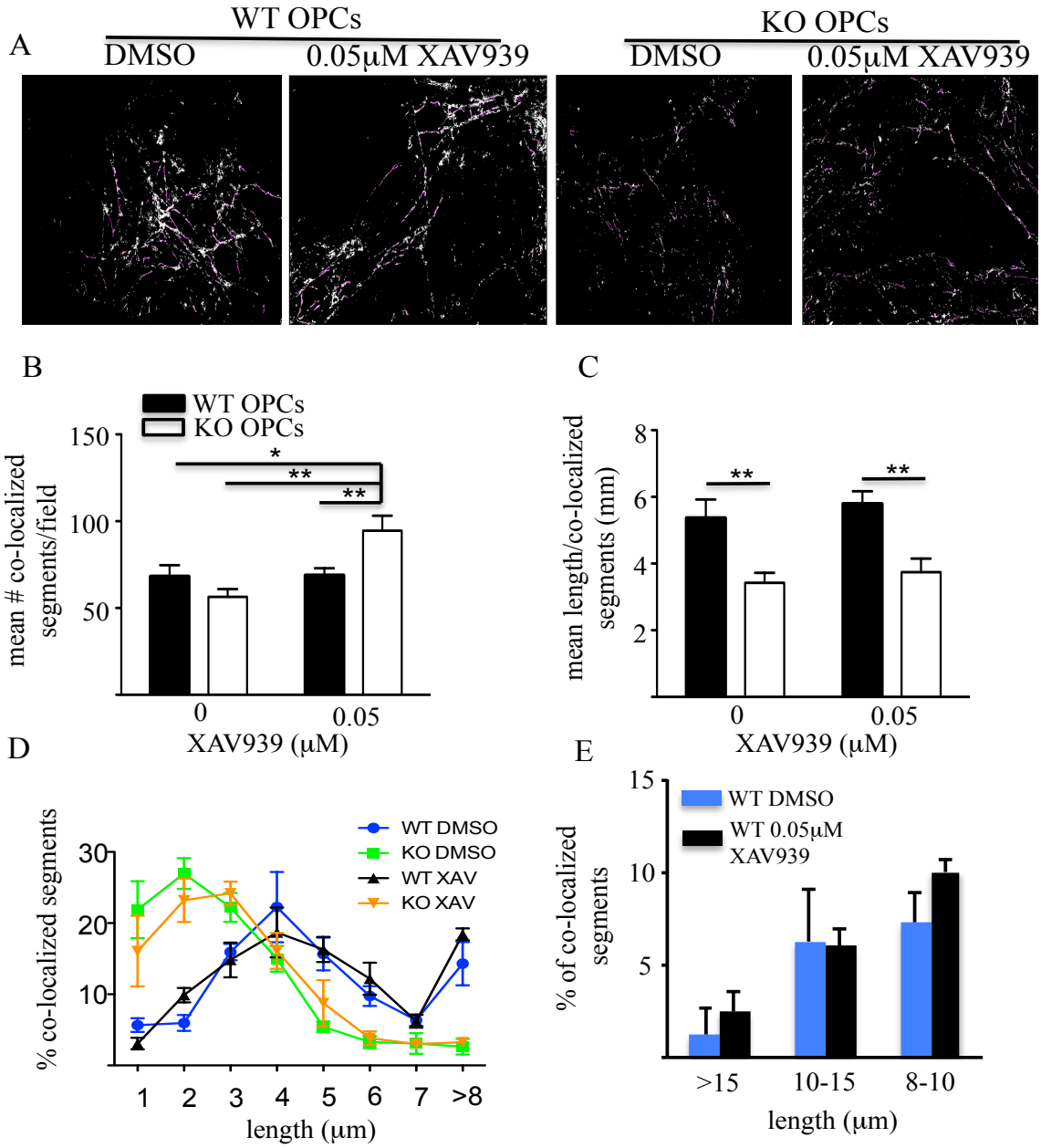


**Figure 4.5**. **XAV939 increases the number of MBP$^+$/NFH segments, but not the average length per co-localized segment.** (A) For each of three independent experiment, three representative images were used for quantification. (B) The number of MBP$^+$/NFH segments were compared for WT or KO OPC/WT DRGN co-cultures treated without, or with 50nM XAV939. (C) The average length per co-localized segment was measured using NeuronJ. (D) Distribution representing the percentage of co-localized segments at each length. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using two-way ANOVA followed by Bonferroni post-tests. (*P<0.05, **P<0.01) Data are from three independent experiments.

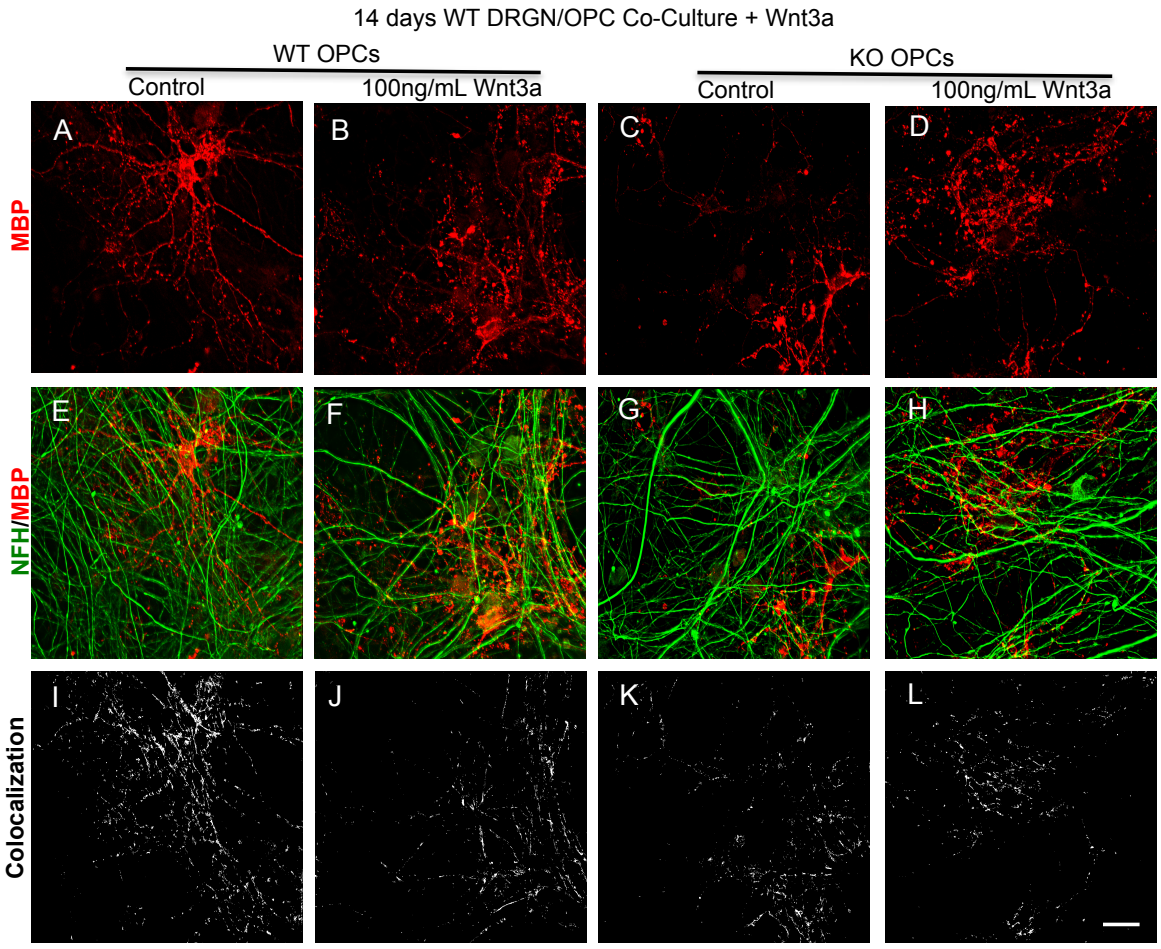


**Figure 4.6. Wnt3a decreases expression and co-localization of MBP along neurites during OPC/DRGN co-culture.** OPCs from independent cultures of oligospheres, WT (A,B,E,F,I,J) and PTPα null (KO) mice (C,D,G,H,K,L) were dissociated onto the DRGNs derived from WT mice at day 14 of the neuronal culture. DRGNs and OPCs were co-cultured for 14 days in the presence or absence of 100ng/mL of Wnt3a. Immunostaining for neurofilament (NFH) marks the neurite bed (not shown). (A-D) Myelin basic protein (MBP) marks mature OLs and (N) MBP signal per visual field was used as an indicator of OPC differentiation. (E-H) NFH and MBP channels were merged. (I-L) Co-localized points were identified and as an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined using ImageJ. Scale bar 25μm.

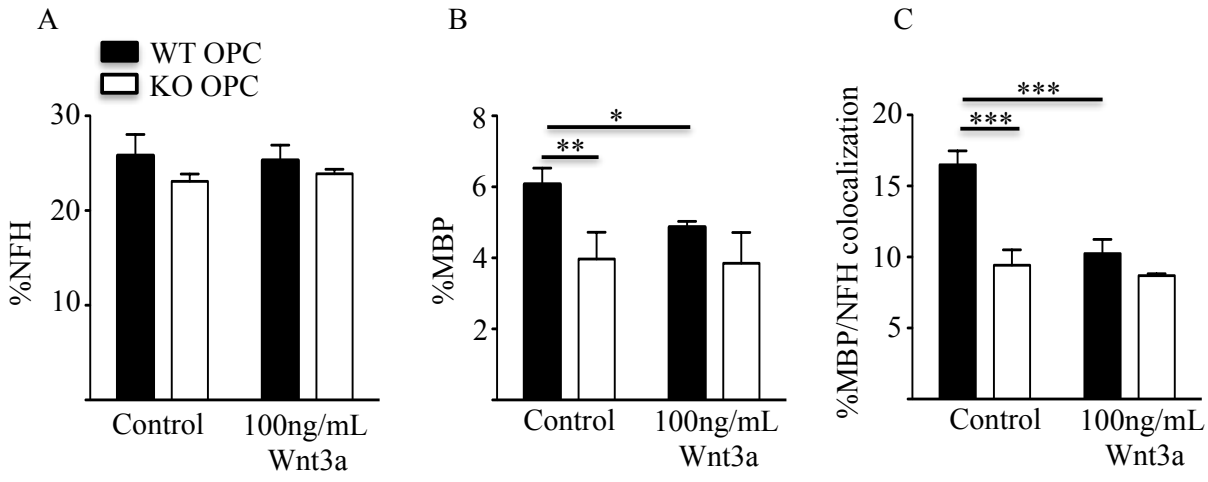


**Figure 4.7. Co-localization of MBP along neurites during OPC/DRGN co-culture is reduced by Wnt3a.** Quantitative analysis of OPC/WT DRGN co-cultures treated with 100ng/mL Wnt3a. (A) A binary mask was formed to quantify neurite bed density based on NFH signal per visual field. (B) MBP signal per visual field was used as an indicator of OPC differentiation. (C) As an indicator of potential myelination, the co-localization of MBP/NFH co-staining per NFH signal was determined using ImageJ. The bars in the graphs show the mean ± S.D. and the asterisks show significant differences as determined using two-way ANOVA followed by Bonferroni post-tests. (*P<0.05, **P<0.01, ***P<0.001) Data are from three independent experiments.

## Chapter 5: General discussion

I have shown using an *in vitro* myelinating co-culture system that PTPα is involved in at least two stages of myelination: to promote MBP expression and OL differentiation; and to promote the elongation of axo-glial contacts, indicated by co-localized $MBP^+$/NFH segments. Additionally, down-regulation of canonical Wnt signaling can remediate PTPα associated deficiencies in OL differentiation; however, PTPα dependent impairments in elongation of co-localized $MBP^+$/NFH segments do not appear to be rescued by down-regulating Wnt signaling. Furthermore, Wnt3a treatment represses both differentiation and MBP/NFH co-localization in WT OPC/DRGN co-cultures.

### 5.1 The role of PTPα in myelination

Our laboratory has previously reported that oligodendroglial PTPα plays at least two roles in regulating oligodendrocyte development: it serves as a negative regulator of OPC proliferation (Wang *et al.,* 2012); and activates the Src family kinase Fyn, which promotes morphological changes associated with differentiation by mediating the activity of several cytoskeleton associated molecules (Wang *et al.,* 2009) (Section 1.2.2.1.1). I have confirmed that PTPα promotes OL differentiation using an improved *in vitro* differentiation cell culture model. I demonstrated that PTPα promotes OL differentiation in an *in vitro* neuron-glia myelinating co-culture capable of myelination and furthermore showed that both oligodendroglial and neuronal PTPα expression promote the formation of long co-localized $MBP^+$/NFH segments. Therefore, it is imperative to identify signals that may regulate PTPα activity, and delineate PTPα dependent

interactions and signaling cascades to determine how PTPα may function during differentiation and myelination.

### 5.1.1 PTPα in axo-glial interactions

Maturation of OLs is a complex multi-stage process that precedes CNS myelination. OLs are derived from bipolar, proliferative, migratory progenitors that pass through a series of developmental stages before becoming mature myelinating OLs (descried in section 1.1.3.3). Mature OLs express myelin-associated proteins, extend multiple branched processes that contact and ensheath axons before compaction of the newly formed myelin sheath. Myelination also involves the selection of axons for myelination, initiation of axo-glial contact, and the establishment of stable intercellular axo-glial contact (Sherman and Brophy, 2005).

The paranodes are major sites of physical interactions between oligodendrocytes and the axon. The neural cell adhesion molecule F3/contactin interacts with Caspr in *cis* and together, forms a neuronal complex that becomes localized to the paranodal domain of axons during myelination (Peles *et al.,* 1997; Rios *et al.,* 2000; Tait *et al.*, 2000; Charles *et al.,* 2002). PTPα can associate with F3/contactin to form a neuronal receptor complex (Zeng *et al.,* 1999). The neuronal Caspr/contactin receptor complex binds oligodendroglial ligands such as neurofascin 155 (NF155) (Tait *et al.,* 2000; Charles *et al.,* 2002; Falk *et al.,* 2002). This interaction anchors the neuronal contactin/Caspr complex to the cytoskeleton through interactions with the scaffolding protein 4.1B (Denisenko-Nehrbass *et al.,* 2003). These interactions may be involved in forming the strong adhesion complex located at the paranodes (Schafer *et al.,* 2004). Whether neuronal PTPα signaling promotes the formation of stable axo-glial junctions through anchoring

the neuronal Caspr/contactin complex to the axonal cytoskeleton, or elongation of axo-glial contacts along the axon warrants further investigation.

### 5.1.2 Activators of PTPα activity in oligodendrocyte development and myelination

Contactin is also expressed on the oligodendroglial surface (Kramer, *et al.,* 1997) and may mediate homophilic neuron-oligodendrocyte interactions (Faivre-Sarrailh and Rougon, 1997; Salzer, 2003). Oligodendroglial contactin interacts in *cis* with α6β1 integrin to form a receptor complex (Laursen *et al*., 2009). Oligodendroglial contactin can interact in *trans* with the extracellular component of the axonally expressed adhesion molecule L1 (Laursen *et al.,* 2009) whereas α6β1 integrin binds laminin 2 (Colognato *et al.,* 2004). Collectively, these interactions increase the overall activity of the SFK Fyn as binding of axonal L1 to oligodendroglial contactin increases phosphorylation of the activating Tyr-420 residue, whereas laminin-2 engagement of α6β1 on OLs results in dephosphorylation of the inhibitory Tyr-531 residue (Laursen *et al.,* 2009; Colognato *et al.,* 2004). This integrin/contactin complex has been proposed to integrate signals from the axonal surface and ECM to regulate Fyn activity, OL survival and myelination (Laursen *et al.,* 2009). I have shown that PTPα promotes laminin-2, therefore integrin-dependent OL differentiation and the formation of $MBP^{+}$/NFH segments. PTPα activates Fyn by dephosphorylating the inhibitory c-terminal tyrosine residue (Ponniah *et al*., 1999), and this dephosphorylation promotes OL differentiation (Wang *et al.,* 2009). These findings are consistent with previous observations from our laboratory, which demonstrated that PTPα is required for integrin dependent activation of SFKs in fibroblasts (Chen *et al.,* 2006). Additionally increased cell death was observed in oligodendrocytes with perturbed α6β1 integrin

signaling (Frost *et al.,* 1999; Colognato *et al.,* 2002; Benninger *et al.,* 2006). Therefore, it is likely that laminin 2/α6β1 integrin interaction requires PTPα activation of Fyn to facilitate downstream survival signals, and to promote OL differentiation and myelination.

Mice deficient in oligodendroglial β1 integrin form myelin that appears normal in the brain and spinal cord (Benninger *et al.,* 2006); however, abnormal myelination was observed in the spinal cord and optic nerve, but not in the corpus callosum (Lee *et al.*, 2006). The differential myelination phenotypes of β1 integrin mice may be attributable to inefficient myelination of small caliber axons during initial stages of myelination. Mice expressing a dominant negative form of β1 integrin in oligodendrocytes exhibited reduced efficiency in myelination in small-diameter axons; however by 28 days, no difference in myelin morphology and g-ratio (the ratio of axon diameter: diameter of myelinated axon) was observed between WT mice and mice expressing dominant negative β1 integrin (Camara *et al.*, 2009). This suggests that additional laminin receptors may exist and may contribute to OL differentiation myelination. Dystroglycan has been identified as a non-integrin oligodendroglial laminin-binding receptor that promotes laminin-2 dependent OL differentiation and myelination (Colognato *et al.*, 2007). Dystroglycan functional blocking antibodies reduced formation of myelin membrane sheets when OLs were cultured on laminin-2 substrate (Colognato *et al.,* 2007); therefore, it is likely that both α6β1 integrin and dystroglycan contribute to OL survival, differentiation, and myelination. While it is likely Fyn activation through laminin 2/α6β1 integrin interaction requires PTPα activity, it is unknown whether dystroglycan dependent OL differentiation and myelination signals through the PTPα/Fyn axis.

Laminin-2 is an extracellular component expressed on the surface of premyelinated CNS axonal tracts (Colognato *et al.,* 2002). Laminin-2 engagement of OPC α6β1 integrin can enhance myelin membrane formation, and laminin-2 deficient mice exhibit defective CNS myelination (Buttery and ffrench-Consant, 1999; Chun *et al.,* 2003). α6β1 integrin and dystroglycan are two laminin-2 receptors expressed by OPCs (Colognato *et al.,* 2002; 2007). I have shown laminin-2 promotes PTPα dependent OPC differentiation, axo-glial contact, and myelination. It is likely that PTPα facilitates α6β1 integrin signaling during OPC differentiation. Differentiating WT OPCs in the presence of β1 integrin targeted function blocking antibodies can be used to address this. To my knowledge, it has never been investigated whether PTPα plays a role in mediating dystroglycan signaling in oligodendrocytes. Addition of dystroglycan targeted function-blocking antibodies to OPC differentiation cultures can be used to investigate this hypothesis.

It has also been reported that the laminin family ECM protein netrin-1 activates the oligodendroglial receptor deleted in colorectal carcinoma (Dcc) and promotes process branching in OL through activation of Fyn activity (Rajasekharan *et al.,* 2009). It would be interesting to investigate whether PTPα plays a role in netrin-1 mediated activation of Fyn. Differentiating PTPα KO OPCs in the presence of netrin-1 can provide insight into a potential role for PTPα in netrin-1 mediated OL differentiation.

### 5.1.3 PTPα dependent intracellular signaling mechanisms in oligodendrocyte differentiation and myelination

Our laboratory has previously demonstrated that PTPα is an upstream activator of Fyn (Wang *et al.,* 2009). Fyn stimulates transcription of the MBP gene by activating transcription factors that bind to the Fyn response element in the MBP promoter region (Umemori *et al.,*

1999). Fyn also regulates MBP expression at the level of protein translation. QKI is a RNA binding protein that maintains MBP mRNA stability and can be phosphorylated by Fyn (Lu *et al.,* 2005). C-terminal phosphorylation of QKI by SFKs such as Fyn inhibits QKI binding to MBP mRNA (Zhang *et al.,* 2003). MBP mRNA is translated locally in oligodendroglial processes at points of axon-glia contact; therefore, repression of MBP mRNA translation is required until it arrives at its destination via transport in RNA granules (White *et al.,* 2008). The 3'UTR region of MBP mRNA contains an A2 response element (Ainger *et al.,* 1997), which binds heterogeneous nuclear ribonuclear protein A2 (hnRNP A2). hnRNP A2 can recruit and interact with hnRNP E1 in RNA granules to repress translation of mRNA sequences containing the A2 response element, such as MBP mRNA (Kosturko *et al.,* 2006). Tyrosine phosphorylation of hnRNP A2 in OLs by Fyn, in response to neuronal L1 binding to contactin, releases MBP mRNA from RNA granules thereby alleviating translation repression (White *et al.,* 2008). Therefore, PTPα is likely a major regulator of Fyn dependent MBPs expression, which is in concert with our laboratory's previous findings, which revealed forebrain hypomyelination in PTPα KO mice during development (Wang *et al.,* 2009).

### 5.2 Canonical Wnt signaling in PTPα-dependent OPC differentiation and myelination

Wnt signaling pathways are evolutionarily conserved and regulate developmental processes such as proliferation, stem cell renewal, cell fate commitment and developmental patterning (van Amerongen and Nusse, 2009). Aberrant activation of Wnt signaling promotes cell growth and survival may ultimately be involved in carcinogenesis (Barker and Clevers, 2006). Down-regulation of Wnt signaling effectively inhibits colon cancer cell proliferation *in vitro* and stimulates differentiation (Tetsu and McCormick, 1999; van de Wetering *et al.,* 2002).

The canonical Wnt signaling pathway has recently been implicated in OPC differentiation and myelination. Down-regulation of canonical Wnt signaling promotes OPC differentiation and myelination (Fancy *et al.,* 2011; Ye *et al.,* 2009). Following neonatal hypoxic ischemic white matter injury, OPCs express markers indicative of high Wnt activity, similar to markers expressed in colon cancer (Fancy *et al.,* 2014).

### 5.2.1 Down-regulation of Wnt signaling remediates MBP expression in PTPα KO oligodendrocytes

Our laboratory has demonstrated that in an *in vitro* cell culture of differentiating OPCs, addition of the small molecule Wnt signaling inhibitor XAV939 increases MBP expression, and thus OPC differentiation by PTPα KO OPCs. While down-regulating Wnt signaling remediates MBP expression by PTPα KO OPCs to levels comparable with untreated WT OPCs, XAV939 also significantly improves differentiation by WT OPCs (Section 4.2.2, Figure 4.2B). These data suggest that PTPα may be functioning independently of Wnt signaling to regulate MBP expression and OL differentiation. If PTPα and Wnt signaling regulated MBP expression and OL differentiation through a common signaling molecule, one would not expect to observe significant difference in MBP expression between WT and PTPα KO OPCs and Wnt when treated with equivalent doses of XAV939. However, despite being cultured at equivalent doses of XAV939, WT OPCs differentiated significantly better than PTPα KO OPCs which lends support to the possibility of differential regulation of MBP expression and OL differentiation by PTPα and Wnt signaling.

MBP/NFH co-localization in PTPα KO OPC/DRGN co-cultures increased following XAV939 addition, likely by increasing the number of $MBP^+$/NFH segments in PTPα KO OPC/DRGN co-cultures (Figure 4.5); however, the average length of co-localized segments in PTPα KO OPC/DRGN co-cultures was not increased by XAV939 addition. This suggests that down-regulation of Wnt signaling can remediate PTPα–dependent impairments in OL differentiation and formation of axo-glial contacts, both of which are prerequisite for myelination. However, subsequent stages of myelination, such as the stabilization and elongation of axo-glial contacts appear to be PTPα-dependent, as XAV939 addition did not significantly increase the average length of $MBP^+$/NFH segments in PTPα KO OPC/DRGN co-cultures. As described in section 1.1.2, myelination organizes the axon into specific domains: the nodes of Ranvier, the paranodes, and the juxtaparanodes. This distinct reorganization is indicative of proper myelination as various molecules become restricted to particular domains following myelination (Figure 1.2). Using an *ex vivo* cerebellar slice culture model, our laboratory has established that MBP expression and MBP/NFH co-localization are increased by XAV939 treatment in cerebellar slices derived from P1-P2 PTPα KO murine pups. However, immunostaining for Caspr, an axonal molecule that becomes restricted to the paranode following myelination, revealed that the number of paranodal structures did not increase with XAV939 addition (Dr. Philip Ly, personal communication). This supports that XAV939 addition improves OPC differentiation and axo-glial contact; however, the PTPα-dependent myelination deficiency in cerebellar slices was not rescued by XAV939 treatment, as no increase in the number of paranodal structures was observed.

In the OPC/DRGN myelinating co-culture system, I also showed that Wnt3a addition to WT OPC/DRGN co-cultures reduces differentiation and MBP/NFH co-localization; but, differentiation and MBP/NFH co-localization were not further reduced in Wnt3a treated KO OPC/DRGN co-cultures. This indicates OPC differentiation is already strongly inhibited in PTPα KO OPCs and activating Wnt signaling cannot further inhibit differentiation; therefore, activation of Wnt signaling does not have an additive effect on repressing OPC differentiation in PTPα KO OPCs. These observations also support that XAV939 and Wnt3a target the Wnt signaling pathway and have opposing effects on OPC differentiation.

Dysregulated activation of canonical Wnt signaling delays myelination through repression of OL differentiation by inhibiting MBP expression (Feigenson *et al.,* 2009) and significantly delays remyelination (Fancy *et al.,* 2009). While down-regulation of canonical Wnt signaling promotes OPC differentiation and myelination (Fancy *et al.,* 2011; Ye *et al.,* 2009), others have reported that active Wnt signaling is required to promote OPC differentiation (Dai *et al.,* 2014; Azim and Butt, 2011). The seemingly paradoxical effects of canonical Wnt signaling, which may vary with the state of OL development, age, and signaling activity in the brain or spinal cord (Guo *et al.,* 2015), suggest more complex roles of this signaling pathway in OL development and myelination.

## 5.3 Future directions

As described in section 5.1.1, neuronal PTPα can associate with F3/contactin, which along with Caspr, becomes localized to the paranodes during myelination. It would be interesting to address whether neuronal PTPα, like the Caspr/contactin complex, also becomes enriched at the paranodes during myelination. Currently, there are no known commercially available PTPα

specific antibodies available that are suitable for immunostaining. Therefore, to investigate, DRGNs from PTPα KO mice can be transfected with an expression plasmid containing a fluorescently labeled form of PTPα (GFP or m-Cherry), followed by co-culture with OPCs to visualize potential changes in the localization of neuronal PTPα during myelination.

The "recapitulation hypothesis of remyelination" postulates that mechanisms of myelination and remyelination are conserved (Franklin and Hinks, 1999; Fancy *et al.,* 2011); therefore, it would be interesting to investigate whether the roles of PTPα in OL differentiation and myelination are recapitulated during remyelination. This hypothesis can be addressed by using the *ex vivo* cerebellar slice culture model. Treating cerebellar slices derived from WT or PTPa KO mice with lysolecithin can induce focal demyelination. The extent of remyelination can be determined to address whether remyelination, like myelination, is also PTPα-dependent.

The potential role of PTPα in remyelination can also be investigated *in vivo* through lysolecithin-induced demyelination. Lysolecithin can be injected into the spinal cord, or corpus callosum of WT or PTPα KO mice. Locally demyelinated lesions usually appear one-week post-injection and rapid remyelination typically follows (Merrill, 2009). The extent and progress of remyelination between WT and PTPα KO mice can be monitored by immunostaining for markers of differentiation and myelination at specific time points.

## 5.4 Summary and significance

CNS myelination is critical for the molecular organization, protection, and maintenance of axons and normal CNS function. As OLs are the only source of CNS myelin, absence or dysfunction of OLs give rise to a spectrum of myelin disorders (described in section 1.1.1)

frequently characterized by aberrant neuronal impulse transmission, leading to various symptoms that may consequently result in impaired cognitive and motor functions. Currently, there are no available therapies that promote myelin formation or repair. In MS, current therapies aim to modulate the disease course by targeting the inflammation associated with MS. Therefore, an opportunity exists to remediate the effects of dysmyelination or demyelination through therapeutic enhancement of myelin repair; however the molecular events that orchestrate OL differentiation and myelination are complex and poorly understood. The research described focused on investigating the function and actions of two particular signaling cascades, the PTPα and Wnt signaling cascades, and their roles in these processes.

Collectively, the findings described suggest that PTPα has at least two distinct roles during oligodendrocyte development: to promote OL differentiation by regulating MBP expression; and the formation and elongation of axo-glial contacts. During OL development, PTPα likely associates with oligodendroglial α6β1 integrin to promote integrin-dependent OL differentiation and myelination by regulating Fyn activity. PTPα-dependent impairments in OL differentiation and formation of axo-glial contacts can be remediated by down-regulating Wnt signaling; however subsequent stages of myelination remain dependent on PTPα as down-regulating Wnt signaling did not result in elongation of axo-glial contacts in the OPC/DRGN co-culture model, nor the formation of paranodal structures that represent the architecture of a myelinated axon in the *ex vivo* cerebellar slice culture model (Dr. Philip Ly, personal communication). These findings provide valuable insight into understanding the complex mechanisms that regulate distinct phases of myelination. Further understanding of these mechanisms will enhance our understanding of the cellular and molecular events that regulate

myelination and provide opportunities for the development of targeted therapies for myelin formation and repair.

**Bibliography**


Aberle, H., Bauer, A., Stappert, J., Kispert, A., and R. Kemler. (1997). Beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J, 16*, 3797-3804.

Ainger, K., Avossa, D., Diana, A.S., Barry, C., Barbarese, E., and J.H. Carson. (1997). Transport and localization elements in myelin basic protein mRNA. *J Cell Biol, 138*(1077-87).

Azim, K., and A.M. Butt. (2011). Gsk3beta negatively regulates oligodendrocyte differentiation and myelination in vivo. *Glia, 59*, 540-553.

Bansal, R., Stefansson, K., and S.E. Pfeiffer. (1992). Proligodendroblast antigen (POA). a developmental antigen expressed by A007/O4-positive oligodendrocyte progenitors prior to the appearance of sulfatide and galactocerebroside. *J Neurochem, 58*(6), 2221-2229.

Barker, N., and H. Clevers. (2006). Mining the Wnt pathway for cancer therapeutics. *Nat Rev Drug Discovery, 5*(997-1014).

Baron, W., Shattil, S.J., and C. ffrench-Constant. (2002). The oligodendrocyte precursor mitogen PDGF stimulates proliferation by activation of avb3 integrins. *EMBO J, 21*(8), 1957-1966.

Baron, W., Decker, L., Colognato, H., and C. ffrench-Constant. (2003). Regulation of integrin growth factor interactions in oligodendrocytes by lipid raft domains. *Curr Biol, 13*, 151-155.

Baron, W., Colognato, H., and C. ffrench-Constant. (2005). Integrin-growth factor interactions as regulators of oligodendroglial development and function. *Glia, 49*, 467-479.

Barres, B.A., Hart, I.K., Coles, H.S.R., Burne. J.F., Voyvodic, J.T., Richardson, W.D., and M.C. Raff. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. *Cell, 70*(1), 31-46.

Barres, B.A., Lazar, M.A., and M.C. Raff (1994). A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development *Development, 120*, 1097-1108

Benninger, Y., Colognato, H., Thurnherr, T., Franklin, R.J., Leone, D.P., Atanasoski, S., Nave, K.A., ffrench-Constant, C., Suter, U., and J.B. Relvas. (2006). Beta1-integrin signaling mediates premyelinating oligodendrocyte survival but is not required for CNS myelination and remyelination. *J Neurosci, 26*, 7665-7673.

Bernard, F., Moreau-Fauvarque, C., Heitz-Marchland, C., Zagar, Y., Dumas, L., Fouquet, S., Lee, X., Shao, Z., Mi, S., and A. Chedotal. (2012). Role of transmembrane semaphorin Sema6A in oligodendrocyte differentiation and myelination. *Glia, 60*, 1590-1604.

Bhandari, V., Lim, K.L., and C.J. Pallen. (1998). Physical and functional interactions between receptor-like protein-tyrosine phosphatase alpha and p59 fyn. *J Biol Chem, 273*, 8691-8698.

Bin, J.M., Leong, S.Y., Bull, S.J., Antel, J.P., and T.E. Kennedy. (2012). Oligodendrocyte precursor cell transplantation into organotypic cerebellar shiverer slices: a model to study myelination and myelin maintenance *PLoS One, 7*(7), e41237.

Blakemore, W.F., and R.J. Franklin. (2008). Remyelination in experimental models of toxin-indcued demyelination. *Curr Top Microbiol Immunol, 318*, 193-212.

Bogler, O., Wren, D., Barnett, S.C., Land, H., and M. Noble. (1990). Cooperation between two growth factors promotes extended self-renewal and inihibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proc Natl Acad Sci U S A, 87*, 6368-6372.

Buttery, P.C., and C. ffrench-Constant. (1999). Laminin-2/integrin interactions enhance myelin membrane formation by oligodendrocytes. *Mol Cell Neurosci, 14*, 199-212.

Cai, J., Qi, Y., Hu, X., Tan, M., Liu, Z., Zhang, J., Li, Q., Sander, M., and M. Qiu. (2005). Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. *Neuron, 45*, 41-53.

Calver, A.R., Hall, A.C., Yu, W., Walsh, F.S., Heath, J.K., Betsholtz, C., and W.D. Richardson. (1998). Oligodendrocyte population dynamics and the role if PDGF in vivo. *Neuron, 20*(5), 869-882.

Camara, J., Wang, Z., Nunes-Fonseca, C., Friedman, H.C., Grove, M., Sherman, D.L., Komiyama, N.H., Grant, S.G., Brophy, P.J., Peterson, A., and C. ffrench-Constant. (2009). Integrin-mediated axoglial interactions initiate myelination in the central nervous system. *J Cell Biol, 185*(4), 699-712.

Cartwright, C.A., Eckhart, W., Simon, S., and P.L. Kaplan. (1987). Cell transformation by pp60c-src mutated in the carboxy-terminal regulatory domain. *Cell, 49*(1), 83-91.

Casaccia-Bonnefil, P., Tikoo, R., Kiyokawa, H., Friedrich, V. Jr., Chao, M.V., and A. Koff. (1997). Oligodendrocyte precursor differentiation is perturbed in the absence of the cyclin-dependent kinase inhibitor p27Kip1. *Genes Dev, 11*, 2335-2346.

Casaccia-Bonnefil, P., Hardy, R.J., Teng, K.K., Levine, J.M., Koff, A., and M.V. Chao. (1999). Loss of p27Kip1 function results in increased proliferative capacity of oligodendrocyte progenitors but unaltered timing of differentiation. *Development, 126*, 4027-4037.

Cavallo, R.A., Cox, R.T., Moline, M.M., Roose, J., Polevoy, G.A., Clevers, M., Peifer, M., and

A. Bejsovec. (1998). Drosophila Tcf and Groucho interact to repress Wingless signaling activity. *Nature, 395*, 604-608.

Charles, P., Hernandez, M.P., Stankoff, B., Aigrot, M.S., Colin, C., Rougon, G., Zalc, B., and C. Lubetzki. (2000). Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molcule. *Proc Natl Acad Sci U S A, 97*, 7585-7590.

Charles, P., Tait, S., Faivre-Sarrailh, C., Barbin, G., Gunn-Moore, F., Denisenko-Nehrbass, N., Gunnoc, A.M., Girault, J.A., and C. Lubetzki. (2002). Neurofascin is a glial receptor for the paranodin/Caspr-contactin axonal complex at the axoglial junction. *Curr Biol., 12*, 217-220.

Chen, M., Chen, S.C., and C.J. Pallen. (2006). Integrin-induced tyrosine phosphorylation of protein-tyrosine-phosphatase-alpha is required for cytoskeletal reorganization and cell migration. *J Biol Chem, 281*(17), 11972-11980.

Chen, Y., Balasubramaniya, V., Peng, J., Hurlock, E.C., Tallquist, M., Li, J., and Q. R. Lu. (2007). Isolation and culture of rat and mouse oligodendrocyte precursor cells. *Nat Protoc, 2*, 1044-1051.

Chen, Y., Wu, H., Wang, S., Koito, H., Li, J., Ye,F., Hoang, J., Escobar, S.S., Gow, A., Arnett, H.A., Trapp, B.D., Karandikar, N.J., Hsieh, J., and Q.R. Lu. (2009). The oilgodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. *Nat Neurosci, 12*(1398-1406).

Chong, S.Y.C., and J.R. Chan. (2010). Tapping into the glial reservoir: cells committed to remaining uncommitted. *J Cell Biol, 188*(3), 305-312.

Chong, S.Y.C., Rosenberg, S.S., Fancy, S.P.J., Zhao, C., Shen, Y.A., Hahn, A.T., Mcgee, A.W., Xu, X., Zheng, B., Zhang, L.I., Rowitch, D.H., Franklin, R.J.M., Lu, Q.R., and J.R. Chan. (2012). Neurite outgrowth inhibitors Nogo-A establishes spatial segregation and extent of oligodendrocyte myelination. *Proc Natl Acad Sci U S A, 109*(4), 1299-1304.

Chun, S.J., Rasband, M.N., Sidman, R.L., Habib, A.A., and T. Vartanian. (2003). Integrin-linked kinase is required for laminin-2-induced oligodendrocyte cell spreading and CNS myelination. *J Cell Biol, 163*(2), 397-408.

Clevers, H., and R. Nusse. (2012). Wnt/beta-catenin signaling and disease. *Cell, 149*(6), 1192-1205.

Colognato, H., Baron, W., Avellana-Adalid, V., Relvas, J.B., Baron-Van Evercooren, A., Georges-Labouesse, E., and C. ffrench-Constant. (2002). CNS integrins switch growth factor signalling to promote target-dependent survival. *Nat Cell Bio, 4*(11), 833-841.

Colognato, H., Ramachandrappa, S., Olsen, I.M., and C. ffrench-Constant. (2004). Integrins

direct Src family kinases to regulate distinct phases of oligodendrocyte development. *J Cell Biol, 167*, 365-375.

Colognato, H., Galvin, J., Wang, Z., Relucio, J., Nguyen, T., Harrison, D., Yurchnco, P.D., and C. ffrench-Constant. (2007). Identification of dystroglycan as a second laminin receptor in oligodendrocytes, with a role in myelination. *Development, 134*, 1723-1736.

Compston, A., and A. Coles. (2002). Multiple sclerosis. *Lancet, 359*, 1221-1231.

Compston, A., and A. Coles. (2008). Multiple sclerosis. *Lancet, 372*, 1502-1517.

Constantinescu, C.S., Farooqi, N., O'Brien, K., and B. Gran. (2011). Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *British Journal of Pharmacology, 164*, 1079-1106.

Cowan-Jacob, S.W., Fendrich, G., Manley, P.W., Jahnke, W., Fabbro, D., Liebetanz, J., and T. Meyer. (2005). The crystal structure of a c-Src complex in active conformation suggests possible steps in c-Src activation. *Structure, 13*(6), 861-871.

Crawford, A.H., Stockley, J.H., Tripathi, R.B., Richardson, W.D., and R.J.M. Franklin. (2014). Oligodendrocyte progenitors: adult stem cells of the central nervous system? *Experimental Neurology, 260*, 50-55.

Cross, R.H., and R.T. Naismith. (2014). Established and novel disease-modifying treatments in multiple sclerosis. *Journal of Internal Medicine, 275*(4), 350-363.

Cui, L.C., Fang, J., Kennedy, T.E., Almazan, G., and J.P. Antel. (2014). Role of p38MAPK in S1P receptor-mediated differentiation of human oligodendrocyte progenitors. *Glia, 62*, 1361-1375.

Cui, QL, and G. Almazan. (2007). IGF-1 induced oligodendrocyte progenitor proliferation requires PI3K/Akt, MEK/ERK, and Src-like tyrosine kinases. *J Neurochem, 100*, 1480-1493.

Dai, J., Bercury, K.K., and W.B. Macklin. (2014). Interaction of mTOR and Erk1/2 signaling to regulate oligodendrocyte differentiation. *Glia, 62*, 2096-2109.

Dai, Z.M., Sun, S., Wang, C., Huang, H., Hu, X., Zhang, Z., Lu, Q.R., and M. Qiu. (2014). Stage-specific regulation of oligodendrocyte development by Wnt/beta-catenin signaling. *J Neurosci, 34*, 8467-8473.

Dawson, M.R., Levine, J.M., and R. Reynolds. (2000). NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? *J Neurosci Res, 61*(5), 471-479.

Dawson, M.R., Polito, A., Levine, J.M., and R. Reynolds. (2003). NG2-expressing glial

progenitor cels: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci, 24*, 476-488.

de Castro, F., and Bribian, A. . (2005). The molecular orchestra of the migration of oligodendrocyte precursors during development. *Brain Research Reviews, 49*, 227-241.

Decker, L., Avellana-Adalid, V., Nait-Oumesmar, B., Durbec, P., and A. Baron-van Evercooren. (2000). Oligodendrocyte precursor migration and differentiation: combined effects of PSA residues, growth factors, and substrates. *Mol Cell Neurosci, 16*(4), 422-439.

den Hertog, J., Pals, C.E., Peppelenbosch, M.P., Tertoolen, L.G., de Laat, S.W., and W. Kruijer. (1993). Receptor protein tyrosine phosphatase alpha activates pp60 c-src and is involved in neuronal differentiation. *EMBO J*(12), 3789-3798.

Denisenko-Nehrbass, N., Oguievetskaia, K., Goutebroze, L., Galvez, T., Yamakawa, H., Ohara, O., Carnaud, M., and J.A. Girault. (2003). Protein 4.1B associates with both Caspr/paranodin and Caspr2 at paranodes and juxtaparanodes of myelinated fibres. *Eur J Neurosci, 17*, 411-416.

Dupree, J.L., Girault, J-A., and B. Popko. (1999). Axo-glial interactions regulate the localization of axonal paranodal proteins. *J Cell Biol, 147*(6), 1145-1151.

Durand, B., Gao, F.B., and M. Raff. (1997). Accumulation of the cyclin-dependent kinase inhibitor p27/Kip1 and the timing of oligodendrocyte differentiation. *EMBO J, 16*, 306-317.

Dutta, D.J., Zameer, A., Mariani, J.N., Zhang, J., Asp, L., Huynh, J., Mahase, S., Laitman, B.M., Agraw, A.T., Mitiku, N., Urbanski, M., Melendez-Vasquez, C.V., Casaccia, P., Hayot, F., Bottinger, E.P., Brown, C.W., and G.R. John. (2014). Combinatorial actions of Tgf-beta and Activin ligands promote oligodendrocyte development and CNS myelination. *Development, 141*, 2414-2428.

Ebner, S., Dunbar, M., and R.D. McKinnon. (2000). Distinct roles for PI3K in proliferation and survival of oligodendrocyte progenitor cells. *J Neurosci Res, 84*, 1692-1702.

Engelman, J.A., Luo, J., and L.C. Cantley. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet, 7*, 606-619.

Faivre-Sarrailh, C., and G. Rougon (1997). Axonal molecules of the immunoglobulin superfamily bearing a GPI anchor: their role in controllig neurite outgrowth. *Mol. Cell. Neurosci., 9*, 109-115.

Falk, J., Bonnon, C., Girault, J., and C. Faivre-Sarrailh. (2002). F3/contactin, a neuronal cell adhesion molecule in axogenesis and myelination. *Biol of the Cell, 94*, 327-334.

Fancy, S.P., Barazini, S.E., Zhao, C., Yuk, D.I., Irvine, K.A., Kaing, S., Sanai, N., Franklin, R.J., and D.H. Rowtich. (2009). Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes and Development, 23*(1571-85).

Fancy, S.P., Chan, J.R., Baranzani, S.E., Frankling, R.J., and D.H. Rowitch. (2011). Myelin regeneration: a recapitulation of development? *Ann Rev Neurosci, 34*, 21-43.

Fancy, S.P.J., Harrington, E.P., Yuen, T.J., Silbereis, J.C., Zhao, C., Baranzini, S.E., Bruce, C.C., Otero, J.J., Huang, E.J., Nusse, R., Franklin, R.J.M., and D.H. Rowitch. (2011). Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. *Nat Neurosci, 14*, 1009-1016.

Fancy, S.P.J., Harrington, E.P., Baranzini, S.E., Silbereis, J.C., Shiow, L.R., Yuen, T.J., Hunag, E.J., Lomvardas, S., and D.H. Rowitch. (2014). Parallel states of pathological Wnt signaling in neonatal brain injury and colon cancer. *Nat Neurosci, 17*, 506-512.

Feigenson, K., Reid, M., See, J., Crenshaw III, E.B., and J.B. Grinspan. (2009). Wnt signaling is sufficient to perturb oligodendrocyte maturation. *Mol. Cell. Neurosci., 42*, 255-265.

Fernandez, P.A., Tang, D.G., Cheng, L., Prochiantz, A., Mudge, A.W. and M.C. Raff. (2000). Evidence that axon-derived neuregulin promotes oligodendrocyte survival in the developing rat optic nerve. *Neuron, 28*, 81-90.

Fewou, S.N., Ramakrishnan, H., Bussow, H., GIeselmann, V., and M. Eckhardt. (2007). Down-regulation of polysialic acid is required for efficient myelin formation. *J Biol Chem, 282*(22), 16700-16711.

Fields, R.D., and K. Itoh. (1996). Neural cell adhesion molecules in activity-dependent development and synaptic plasticity. *Trends Neurosci, 19*(11), 473-480.

Flores, A.I., Mallon, B.S., Atsui, T.,m Ogawa, W., Rosenzweig, A., Okamoto, T., and W.B. Macklin. (2000). Akt-mediated survival of oligodedrocytes induced by neuregulins. *J Neurosci, 20*, 7622-7630.

Flores, A.I., Narayanan, S.P., Morse, E.N., Shick, H.E., Yin, X., Kidd, G., Avila, R.L., Kirschner, D.A., and W.B. Macklin. (2008). Constitutively active Akt induces enhanced myelination in the CNS. *J Neurosci, 28*, 7174-7183.

Franklin, R.J., and G.L. Hinks. (1999). Understanding CNS remyelination: clues from developmental and regeneration biology. *J Neurosci Res, 58*, 207-213.

Franklin, R.J.M., and C. ffrench-Constant. (2008). Remyelination in the CNS: from biology to therapy. *Nat Rev Neurosci, 9*, 839-855.

Frost, E.E., Buttery, P.C., Milner, R., and C. ffrench-Constant. (1999). Integrins mediate a neuronal survival signal for oligodendrocytes. *Curr Biol, 9*, 1251-1254.

Fumagalli, M., Daniele, S., Lecca, D., Lee, P.R., Parravicini, C., Fields, R.D., Rosa, P., Antonucci, F., Verderio, C., Trincavelli, M.L., Bramanti, P., Martini, C., and M.P. Abbracchio. (2011). Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. *J Biol Chem, 286*, 10593-10604.

Gahwiler, B.H., Capogna, M., Debanne, D., Mckinney, R.A., and S.M. Thompson. (1997). Organotypic slice cultures: a technique has come of age. *Trends in neurosci, 20*, 471-477.

Goebbels, S., Oltrogge, J.H., Kemper, R., Heilmann, I., Bormuth, I., Wolfer, S., Wichert, S.P., Mobius, W., Liu, X., Lappe-Siefke, C., Rossner, M.J., Groszer, M., Suter, U., Frahm, J., Boretius, S., and K.A. Naves. (2010). Elevated phosphatidylinositol 3,4,5-triphosphate in glia triggers cell autonomous membrane wrapping and myelination. *J Neurosci, 30*, 8953-8964.

Gold, R., Linington, C., and H. Lassmann. (2006). Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain, 129*.

Goldman, S. (2005). Stem and progenitor cell-based therapy of the human central nervous system. *Nature Biotechnology, 23*, 862-871.

Gow, A., and R.A. Lazzarini. (1996). A cellular mechanism governing the severity of Pelizaeus-Merzbahcer disease. *Nat Genet, 13*, 422-428.

Guardiola-Diaz, H.M., Ishii, A., and R. Bansal. (2012). Erk1/2 MAPK and mTOR signaling sequentially regualtes progression through distinct stages of oligodendrocyte differentiation. *Glia, 60*, 476-486.

Guo, F., Lang, J., Sohn, J., Hammond, E., Chang, M., and D. Pleasure. (2015). Canonical Wnt signaling in the oligodendroglial lineage-puzzles remain. *Glia*. Advance online publication. doi: 10.1002/glia.22813.

Harder, K.W., Moller, N.P., Peacock, J.W. and F.R. Jirik. (1998). Protein-tyrosine phosphatase alpha regulates Src family kinases and alters cell-substratum adhesion. *J Biol Chem, 273*, 31890-31900.

Haynes, R.L., Baud, O., Li, J., Kinney, H.C., Volpe, J.J., and D.R. Folkerth. (2005). Oxidative and nitrative injury in periventricular leukomalacia: a review. *Brain Pahol, 2005*(15), 3.

Heller, B.A., Ghidinelli, M., Voelkl, J., Einheber, S., Smith, R., Grund, E., Morahan, G., Chandler, D., Kalatdjieva, L., Giancotti, F., King, R.H., Fejes-Toth, A.N., Fejes-Toth, G., Feltri, M.L., Lang, F., and J.L. Salzer. (2014). Functionally distinct PI 3-kinase pathways

regulate myelination in the peripheral nervous system. *J Cell Biol, 204*(7), 1219-1236.

Hoshina, N., Tezuka, T., Yokoyama, K., Kozuka-Hata, H., Oyama, M., and T. Yamamoto. (2007). Focal adhesion kinase regulates laminin-induced oligodendroglial process outgrowth. *Genes to Cells, 12*, 1245-1254.

Houser, S.L., and J.R. Oksenberg. (2006). The neurobiology of multiple sclerosis: Genes, inflammation, and neurodegeneration. *Neuron, 52*(1), 61-76.

Hu, Q.D., Ang, B.T., Karsak, M., Hu, W.P., Cui, X.Y., Duka, T., Takeda, Y., Chia, W., Sankar, N., Ng, Y.K., Ling, E.A., Maciag, T., Small, D., Trifonova, R., Kopan, R., Okano, H., Nakafuku, M., Chiba, S., Hirai, H., Aster, J.C., Schachner, M., Pallen, C.J., Watanabe., K and Z.C. Xiao. (2003). F3/contactin acts as a functional ligand for Notch during oligodendrocyte myelination. *Cell, 115*, 163-175.

Huang, J.K., Jarjour, A.A., Nait Oumesmar, B., Kernion, C., Willams, A., Krenzel, W., Kagechika, H., Bauer, J., Zhao, C., Evercooren, A.B., Chambon, P., ffrench-Constant, C., and R.J. Franklin. (2011). Retnoid X receptor gamma signaling accelerates CNS remyelination. *Nat Neurosci, 14*, 45-53.

Huang, S.A., Mishina, Y.M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G.A., Charlat, O., Willette, E., Zhang, Y., Wiessner, S., Hild, M., Shi, X., Wilson, C.J., Mickanin, C., Myer, V., Fazal, A., Tomlinson, R., Serluca, F., Shao, W., Cheng, H., Shultz, M., Rau, C., Schirle, M., Schlegl, J., Ghidelli, S., Fawell, S., Lu, C., Curtis, D., Kirschner, M.W., Lengauer, C., Finan, P.M., Tallarcio, J.A., Bouwmeester, T., Porter, J.A., Bauer, A., and F. Cong. (2009). Tankyrase inhibition stabilizes axin and antagonizes Wnt signaling. *Nature, 461*, 614-620.

Hudson, L.D., Berndt, J., Puckett, C., Kozak, C.A., and R.A. Lazzarini. (1987). Aberrant splicing of hte proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. *Proc Natl Acad Sci U S A, 84*, 1454-1458.

Hynes, R.O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell, 110*(6), 673-687.

Igarashi, M., Schaumburg, H.H., Powers, J., Kishmoto, Y., Kolodny E., and K. Suzuki. (1976). Fatty acid abnormality in adrenoleukodystrophy. *J Neurochem, 26*, 851-860.

Ishibashi, T., Dakin, K.A., Stevens, B., Lee, P.R., Kozlov, S.V., Stewart, C.L., and R.D. Fields. (2006). Astrocytes promote myelination in response to electrical impulses. *Neuron, 49*, 823-832.

Janda, C.Y., Waghray, D., Levin, A.M., Thomas, C., and K.C. Garcia. (2012). Structural basis of Wnt recognition by Frizzled. *Science, 337*, 59-64.

Jarjour, A.A., Bull, S.J., Almasieh, M., Rajasekharan, S., Baker, K.A., Mui, J., Antel, J.P., Di Polo, A., and T.E. Kennedy. (2008). Maintenance of axo-oligodendroglial paranodal junctions requires DCC and netrin-1. *J Neurosci, 28*(43), 11003-11014.

Jarjour, A.A., Zhang, H., Bauer, N., ffrench-Constant, C., and A. Williams. (2012). In vitro modeling of central nervous system myelination and remyelination. *Glia, 60*(1), 1-12.

Jepson, S., Vought, B., Gross, C.H., Gan, L., Austen, D., Frantz, J.D., Zwahlen, J., Lowe, D., Markland, W., and R. Krauss. (2012). LINGO-1, a transmembrane signaling protein, inhibits oligodendrocyte differentiation and myelination through intercellular self-interactions. *J Biol Chem, 287*(26), 22184-22195.

Kalani, M.Y., Cheshier, S.H., Cord, B.J., Bababeygy, S.R., Vogel, H., Weissman, I.L., Palmer, T.D., and R. Nusse. (2008). Wnt-mediated renewal of neural stem/progenitor cells. *Proc Natl Acad Sci U S A, 105*, 16970-16975.

Kaplan, R., Morse, B., Huebner, K., Croce, C., Howk, R., Ravera, M., Ricca, G., Jaye, M., and J. Schlessinger. (1990). Cloning of three human tyrosine phosphatses reveals a multigene family of receptor-linked protein-tyrosine-phosphatases expressed in brain. *Proc Natl Acad Sci U S A, 87*, 7000-7004.

Katoh, M., and M. Katoh. (2007). Wnt signaling pathway and stem cell signaling network. *Clin Cancer Res, 13*(14), 4042-4045.

Kaye, E.M. (2001). Update on genetic disorders affecting white matter. *Pediatric Neurology, 24*(1), 11-24.

Kessaris, K., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., and W.D. Richardson. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elmination of an embryonic lineage. *Nat Neurosci, 9*, 173-179.

Kim, L.C., Song, L., and E.B. Haura. (2009). Src kinases as thearpeutic targets for cancer. *Nat Rev Clin Oncol, 6*, 587-595.

Kiss, J.Z., and G. Rougon. (1997). Cell biology of polysialic acid. *Curr Opin Neurobiol, 7*(5), 640-646.

Knapp, P.E., Skoff, R.P., and D.W. Redstone. (1986). Oligodendroglial cell death in jimpy mice: an explanation for the myelin deficit. *J Neurosci, 6*(10), 2813-2822.

Kondo, T., and M. Raff. (2000). Basic helix-loop-helix proteins and the timing of oligodendrocyte differentiation. *Development, 127*, 2989-2998.

Kosturko, L.D., Mggipinto, M.J., Korza, G., Lee, J.W., Carson, J.H., and E Barbarese. (2006). Heterogenous nuclear ribonuclear protein E1 binds to hnRNP A2 and inhibits translation

of A2 response relement mRNAs. *Mol Biol Cell, 17*(8), 3521-3533.

Kramer, E.M., Koch, T., Niehaus, A., and J. Trotter. (1997). Oligodendrocytes direct glycosyl phosphatidyinositol-anchored proteins to the myelin sheath in glycosphingolipid-rich complexes. *J Biol Chem, 272*, 8397-8345.

Laursen, L.S., Chan, C.W., and C. ffrench-Constant. (2009). An integrin-contactin complex regulates CNS myelination by differential Fyn phosphorylation. *J Neurosci, 29*(29), 9174-9185.

Lee, K.K., de Repentigny, Y., Saulnier, R., Rippstein, P., Macklin, W.B., and R. Kothary. (2006). Dominant-negative beta1 integrin mice have region-specific myelin defects accompanied by alterations in MAPK activity. *Glia, 53*, 836-844.

Lehmann, H.C., Kohne, A., Bernal, F., Jangouk, P., Meyer zu Horste, G., Dehmel, T., Hartung, H., Previtali, S.C., and B.C. Kieseier. (2009). Matrix metalloproteinase-2 is involved in myelination of dorsal root ganglia neurons. *Glia, 57*, 479-489.

Levison, S.W., Rothstein, R.P., Romanko, M.J., Snyder, M.J., Meyers, R.L. and S.J. Vannucci. (2001). Hypoxia/ischemia depletes the rat perinatal subventricular zone of oligodendrocyte progenitors and neural stem cells. *Dev Neurosci, 23*, 234-247.

Li, V.S.W., Ng, S.S., Boersema, P.J., Low, T.Y., Karthaus, W.R., Gerlach, J.P., Mohammed, S., Heck, A.J.R., Maurice, M.M., Mahmoudi, T., and H. Clevers. (2012). Wnt signaling through inhibition of b-catenin degradation in an intact Axin1 complex. *Cell, 149*, 1245-1256.

Liang, X., Draghi, N.A., and M.D. Resh. (2004). Signaling from integrins to fyn to Rho family GTPases regulates morphologic differentiation of oligodendrocytes. *J Neurosci, 24*(32), 7140-7149.

Liu, P., Cheng, H., Roberts, T.M., and J.J. Zhao. (2009). Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discovery, 8*, 629-644.

Logan, C.Y., and R. Nusse. (2004). The Wnt signaling pathway in development and disease. *Ann Rev Cell Dev Biol, 20*, 781-810.

Lu, Q.R., Yuk, D., Alberta, J.A., Zhu, Z, Pawlitzky, I., Chan, J., McMahon, A.P., Stiles, C.D., and D.H. Rowitch. (2000). Sonic hedgehog - regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron, 25*, 317-329.

Lu, Z., Ku, L., Chen, Y., and Y. Feng. (2005). Developmental abnormalities of myelin basic protein expression in fyn knock-out brain reveal a role of fyn in posttranscriptional regulation. *J Biol Chem, 280*(1), 389-395.

Marino, F.E., Risbridger, G., and E. Gold. (2013). The therapeutic potential of blocking the activin signalling pathway. *Cytokine & Growth Factor Rev, 24*, 477-484.

Massague, J. (2012). TGF-beta signaling in context. *Nat Rev Mol Cell Bio, 13*, 616-630.

Matsushima, G.K., and P. Morell. (2001). The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol, 11*, 107-116.

Mckinnon, R.D., Matsui, T., Aranda, M., and M. Dubois-Dalcq. (1990). A role for fibroblast growth factor in oligodendrocyte development. *Ann. N. Y. Acad. Sci., 638*, 378-386.

Mckinnon, R.D., Matsui, T., Dubois-Dalcq, M., and S.A. Aaronson. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron, 5*, 603-614.

McKinnon, R.D., Piras, G., Ida, J.A., and M. Dubois-Dalcq. (1993). A role for TGF-beta in oligodendrocyte differentiation. *J Cell Biol, 121*(6), 1397-1407.

Menn, B., Garcia-Verdugo, J.M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., and A. Alvarez-Buylla. (2006). Origins of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci, 26*(30), 7907-7918.

Merrill, J.E. (2009). In vitro and in vivo pharmacological models to assess demyelination and remyelination. *Neuropsychopharmacology, 34*, 55-73.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R.L., McCoy, J.M., and R.B. Pepinsky (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat Neurosci, 7*, 221-228.

Mi, S., Miller, R.H., Lee, X., Scott, M.L., Shulag-Morskaya, S., Shao, Z., Chang, J., Thill, G., Levesque, M., Zhang, M., Hession, C., Sah, D., Trapp, B., He, Z., Jung, V., McCoy, J.M., and R.B. Pepinsky. (2005). LINGO-1 negatively regualtes myelination by oligodendrocytes. *Nat Neurosci, 8*(745-51).

Miller, R.H. (2002). Regulation of oligodendrocyte development in the vertebrate CNS. *Progress in Neurobiology, 67*, 451-467.

Miron, V.E., Boyd., A., Zhao, J.W., Yuen, T.J., Ruckh, J.M., Shadrach, J.L., van Wijngaarden, P., Wagers, A.J., Williams, A., Franklin, R.J., and C. ffrench-Constant. (2013). M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci, 16*(9), 1211-1218.

Mitew, S., Hay, C.M., Peckham, H., Xiao, J., Koenning, M., and B. Emery (2014). Mechanisms reguating the development of oligodendrocytes and central nervous system myelin. *Neuroscience, 276*, 29-47.

Mitra, S.K., Hanson, D.A., and D.D. Schlaepfer. (2005). Focal adhesion kinase: In command and control of cell motility. *Nat Rev Mol Cell Bio, 2005*(6), 56-68.

Molofsky, A.V., Krencik, R., Ullian, E.M., Tsai, H.H., Beneen, B., Richardson, W.D., Barres, B.A., and D.H. Rowitch. (2012). Astrocytes and disease: a neurodevelopmental perspective. *Genes and Development, 26*, 891-907.

Moser, A.B., Kreiter, N., Bezman, L., Lu, S., Raymond, G.V., Naidu, S., and H.W. Moser. (1999). Plasma very long chan fatty acids in 3000 peroxisome disease patients and 29000 controls. *Ann Neurol, 45*(1), 100-110.

Mueller, B.K., Mack, H., and N Teusch. (2005). Rho kinase, a promising drug target for neurological disorders *Nat Rev Drug Discovery, 4*, 387-398.

Nait-Oumesmar, B., Decker, L., Lachapelle, F., Avellana-Adalid, V., Bachelin, C., and A. Baron-van Evercooren. (1999). Progenitor cells of the adult mous subventricular zone proliferate, migrate, and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci, 11*, 4357-4366.

Narayanan, S.P., Flores, A.I., Wang, F., and W.B. Macklin. (2009). Akt stignlas through the mammalian target of rapamycin pathway to regulate CNS myelination. *J Neurosci, 28*, 7174-7183.

Nave, K.A., Lai, C., Bloom, F.E., and R.J. Milner. (1986). Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing *Proc Natl Acad Sci U S A, 83*(23), 9264-9268.

Nave, K.A. (2010). Myelination and support of axonal integrity by glia. *Nature, 468*, 244-252.

Noble, M., Murray, K., Stroobant, P., Waterfield, M.D., and P. Riddle. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte-type-2 astrocyte progenitor cell. *Nature, 333*, 560-562.

Noble, M., Mayer-Proschel, M., and R.H. Miller. (2005). *The oligodendrocyte* (M. S. R. a. M. Jacobson Ed. 4th ed.): Kluwer Academic/Plenum Publishers.

O'meara, R.W., Ryan, S.D., Colognato, H., and R. Kothary. (2011). Derivation of enriched oligodendrocyte cultures and oligodendrocyte/neuron myelinating co-cultures from post-natal murine tissues. *Journal of Visualized Experiments, 54*(e3324).

O'meara, R.W., Michalski, J.P., Anderson, C., B., Rippstein, P., and R. Kothary. (2013). Integrin-linked kinase regulates process extension in oligodendrocytes via control of actin cytoskeletal dynamics. *J Neurosci, 33*(23), 9781-9793.

Osterhout, D.J., Wolven, A., Wolf, R.M., Resh, M.D., and M.V. Chao. (1999). Morphological differentiation of oligodendrocytes requires activation of fyn tyrosine kinase. *J Cell Biol, 145*(6), 1209-1218.

Palazuelos, J., Lingenerm M., and A. Aguirre. (2014). TGF-beta signaling regulates the timing of CNS myelination by modulating oligodendrocyte progenitor cell cycle exit through SMAD3/4/FoxO1/Sp1. *J Neurosci, 34*(23), 7917-7930.

Pallen, C.J. (2003). Protein tyrosine phosphatase alpha (PTPa): A Src family kinase activator and mediator of multiple biological effects *Curr Top in Med Chem, 3*, 821-835.

Pang, Y., Zheing, B., Kimberly, S.L., Cai, Z., Rhodes, P.G., and R.C.S. Lin. (2012). Neuron-oligodendrocyte myelination co-culture derived from embryonic rat spinal cord and cerebral cortex. *Brain and Behavior, 2*(1), 53-67.

Pedraza, L., Huang, J.K., and D.R. Colman. (2001). Organizing principles of the axoglial apparatus. *Neuron, 30*(335-44).

Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G.D., and J. Schlessinger. (1997). Identification of a novel contactin-associated transmembrane receptor with mulitple domains implicated in protein-protein interactions. *EMBO J, 16*, 978-988.

Peles, E., and J.L. Salzer. (2000). Molecular domains of myelinated axons. *Current Opinion in Neurobiology, 10*, 558-565.

Petrone, A., Battaglia, F., Wang, C., Dusa, A., Su, J., Zagzag, D., Bianchi, R., Casaccia-Bonnefil, P., Arancio, O., and J. Sap. (2003). Receptor protein tyrosine phosphatase alpha is essential for hippocampal neuronal migration and long-term potentiation. *EMBO J, 33*(4121-31).

Pfeiffer, S.E., Warrington, A.E., and R. Bansal. (1993). The oligodendrocyte and its many cellular processes. *Trends Cell Biol, 3*, 191-197.

Ponniah, S., Wang, D.Z.M., Lim, K.L., and C.J. Pallen. (1999). Targeted disruption of the tyrosine phosphatse PTPa leads to constituitive downregulation of the kinases src and fyn. *Curr Biol., 9*, 535-538.

Rajasekharan, S., Baker, K.A., Horn, K.E., Jarjour, A.A., Antel, J.P., and T.E. Kennedy. (2009). Netrin 1 and Dcc regulate oligodendrocyte process branching and membrane extension via Fyn and RhoA. *Development, 136*, 415-426.

Readhead, C., Popko, B., Takahashi, N., Shine, H.D., Saavedra, R.A., Sidman, R.L., and L. Hood. (1987). Expression of a myelin basic gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. *Cell, 48*, 703-712.

Richardson, W.D., Pringle, N.P., Mosley, M.J., Wsetermark, B., and M. Dubois-Dalcq. (1988). A role for platelet-derived frowth factor in normal gliogenesis in the central nervous system. *Cell, 53*, 309-319.

Rios, J.C., Melendez-Vasquez, C.V., Einheber, S., Lustic, M., Grumet, M., Hemperly, J., Peles, E., and J.L. Salzer. (2000). Contactin-associated protein (Caspr) and contactin form a complex that is targeted to the paranodal junctions during myelination. *J Neurosci, 20*(22), 8354-8364.

Robinson, S., Petelenz, K., Li, Q., Cohen, M., Dechant, A., Tabrizi, N., Bucek, M., Lust, D., and R. Miller (2005). Developmental changes induced by graded prenatal systemic hypoxic-ischemic insults in rats. *Neurobiol Dis, 18*, 568-581.

Rowitch, D.H., and A.R. Kriegstein. (2010). Developmental genetics of vertebrate glial-cell specification. *Nature, 468*, 214-222.

Rutishauser, U., and L. Landmesser. (1996). Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. *Trends Neurosci, 19*(10), 422-427.

Salzer, J.L. (2003). Polarized domains of myelinated axons. *Neuron, 40*, 297-318.

Schafer, D.P., Bansal, R., Hedstrom, K.L., Pfeiffer, S.E., and M.N. Rasband. (2004). Does paranode formation and maintenance require partitioning of neurofascin 155 into lipid rafts? *J Neurosci, 24*(13), 3176-3185.

Schiffmann, R., Moller, J.R., Trapp, B.D., Shih, H.H., Farrer, R.G., Katz, D.A., Alger, J.R., Parker, C.C., Hauger, P.E., Kaneski, C.R., Heiss J.D., Kaye, E.M., Quarles, R.H., Bardy, R.O., and N.W. Barton. (1994). Childhood ataxia with diffuse central nervous system hypomyelination. *Ann Neurol, 35*(3), 331-340.

Schiffmann, R., and O. Elroy-Stein. (2006). Childhood ataxia with CNS hypomyelination/vanishing white matter disease - a common leukodystrophy caused by abnormal control of protein synthesis. *Mol Genet Metab, 88*(1), 7-15.

Schmierer, B., and C.S. Hill. (2007). TGFbeta-SMAD signal transduction: molcular specificity and functional flexibility. *Nat Rev Mol Cell Bio, 8*, 970-982.

Sherman, D.L., and P.J. Brophy. (2005). Mechanisms of axon ensheathment and myelin growth. *Nat Rev Neurosci, 6*, 683-690.

Shi, Y., and J. Massague. (2003). Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell, 113*, 685-700.

Shields, S.A., Gilson, J.M., Blakemore, W.F., and R.J. Franklin. (1999). Remyelination occurs as extensively but more slowly in old rat compared to young rats following gliotoxin-

induced CNS demyelination. *Glia, 28*, 77-83.

Shimizu, T., Kagawa, T., Wada, T., Muroyama, Y., Takada, S., and K. Ikenaka. (2005). Wnt signaling controls the timing of oligodendrocyte development in the spinal cord. *Dev Biol, 282*, 397-410.

Shock, L.P., Bare, D.J., Klinz, S.G., and P.F. Maness. (1995). Protein tyrosine phosphatase expressed in the developing brain and retinal Muller glia. *Brain Res Mol Brain Res, 28*, 110-116.

Skeleton, M.R., Ponniah, S., Wang, D.Z., Doetschman, T., Vorhees, C.V., and C.J. Pallen. (2003). Protein tyrosine phosphatase alpha (PTP alpha) knockout mice show deficits in morris water maze learning, decreased locomotor activity and decreases in anxiety. *Brain Res, 984*, 1-10.

Sperber, B.R., Boyle-Walsh, E.A., Engleka, M.J., Gadue, P., Peterson, A.C., Stein, P.L., Scherer, S.S., and F.A. McMorris. (2001). A unique role for Fyn in CNS myelination. *J Neurosci, 21*, 2039-2047.

Sriram, S., and I. Steiner. (2005). Experimental allergic encephalomyelitis: A misleading model of multiple sclerosis. *Ann Neurol, 58*, 939-945.

Stettner, M., Wolffram, K., Mausberg, A.K., Wolf, C., Heikaus, S., Derksen, A., Dehmel, T., and B.C. Kieseier. (2013). A reliable in vitro model for studying peripheral nerve myelination in mouse. *J Neurosci Methods, 214*(1), 69-79.

Stevens, B., Porta, S., Haak, L.L., Gallo, V., and R.D. Fields. (2002). Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. *Neuron, 36*, 855-868.

Su, J., Muranjan, M., and J. Sap. (1999). Receptor protein tyrosine phosphatse alpha activates src family kinases and controls integrin-mediated responses in fibroblasts. *Curr Biol., 9*, 505-511.

Tait, S., Gun-Moore, F., Collinson, J.M., Huang, J., Lubetzki, C., Pedraza, L., Sherman, D.L., Colman, D.R., and P.J. Brophy. (2000). An oligodendrocyte cell adhesion molecule at the site of assembly of the paranodal axo-glial junction. *J Cell Biol, 150*, 657-666.

Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T., and S. Takada. (2006). Monosaturated fatty acid modification of Wnt proteins: its role in Wnt secretion. *Dev Cell, 11*, 791-801.

Tanaka, K., Kitagawa, Y., and T. Kadowaki. (2002). Drosophila segment polarity gene products porcupine stimulates the posttranslational N-glycosylation of wingless in the endoplasmic reticulum. *J Biol Chem, 277*, 12816-12823.

Tetsu, O., and F. McCormick. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells *Nature, 398*, 422-426.

Thomas, S.M., and J.S. Brugge. (1997). Cellular functions regulated by Src family kinases. *Ann Rev Cell Dev Biol, 13*, 513-609.

Tiran, Z., Peretz, A., Sines, T., Shinder, V., Sap, J., Attali, B., and A. Elson. (2006). Tyrosine phosphatases epsilon and alpha perform specific and overlapping functions in regulation of voltage-gated potassium channels in Schwann cells. *Mol Biol Cell, 17*(10), 4330-4342.

Trapp, D.D., and P.K. Stys. (2009). Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurol, 8*(280-91).

Tyler, W.A., Gangoli, N., Gokina, P., Kim, H.A., Covey, M., Levison, S., and T.L. Wood. (2009). Activation of the mammalian target of rapamycin (mTOR) is essential for oligodendrocyte differntiation. *J Neurosci, 29*(19), 6367-6378.

Umemori, H., Sato, S., Yagi, T., Aizawa, S., and T. Yamamoto. (1994). Initial events of myelination involve Fyn tyrsoine signaling. *Nature, 367*, 572-576.

Umemori, H., Kadowaki, Y., Hirosawa, K., Yoshida, Y., Hironaka, K., Okana, H., and T. Yamamoto. (1999). Stimulation of myelin basic protein gene transcription by Fyn tyrosine kinase for myelination. *J Neurosci, 19*(4), 1393-1397.

van Amerongen, R., and R Nusse. (2009). Towards an integrated view of Wnt signaling in development. *Development, 136*, 3205-3214.

van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der horn, K., Batlle, E., Coudreuse, D., Haramis, A.P., Tjon-Pon-Fong, M., Moerer, P., van den Born, M., Soete, G., Pals, S., Eilers, M., Medema, R., and H. Clevers. (2002). The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell, 111*(2), 241-250.

Vivanco, I., and C.L. Sawyers. (2002). The phosphatidylinositol 3-kinase AKT pathway in human cancer *Nat Rev Cancer, 2*, 489-501.

Wang, P.S., Wang, J., Zheng, Y., and C.J. Pallen. (2009). Protein tyrosine phosphatase alpha acts as an upstream regulator of Fyn signaling to rpomote oligodendrocyte differentiation and myelination. *J Biol Chem, 284*, 33692-33702.

Wang, P.S., Wang, J., Zheng, Y., and C.J. Pallen. (2012). Loss of protein-tyrosine phosphatase alpha increases proliferation and delays maturation of oigodendrocyte progenitor cells *J Biol Chem, 287*(15), 12529-12540.

Wang, S., Sdrulla, A.D., diSibio, G., Bush, G., Nofziger, D., Hicks, C., Weinmaster, G., and

B.A. Barres. (1998). Notch receptor activation inhibits oligodendrocyte differentiation. *Neuron, 21*, 63-75.

Wang, Y., and C.J. Pallen. (1991). The receptor-like protein tyrosine phosphatase HPTPa has two active catalytic domains with distinct substrate specificities. *EMBO J, 10*(11), 3231-3237.

Watkins, T.A., Emery, B., Mulinyawe, S., and B.A. Barres. (2008). Distinct stages of myelination regulated by gamma-secretase and astrocyres in a rapidly myelinating CNS co-culture system. *Neuron, 60*(4), 555-569.

Weiss, A., and L. Attisano. (2013). The TGFbeta superfamily signaling pathway. *Wiley Interdiscip Rev Dev Biol, 2*(1), 47-63.

White, R., Consior, C., Kramer-Albers, E., Stohr, N., Huttelmaier, S., and J. Trotter. (2008). Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2-dependent RNA granules. *J Cell Biol, 181*(4), 579-586.

Willert, K., Brown, J.D., Danenberg, E., Duncan, A.W., Weissman, I.L., Reya, T., Yates, J.R., and R. Nusse. (2003). Wnt proteins are lipid modified and can act as stem cell growth factors. *Nature, 423*, 448-452.

Windrem, M.S., Nunes, M.C., Rashbaum, W.K., Schwartz, T.H., Goodman, R.A., McKhann, G., Roy, N.S., and S.A. Goldman. (2004). Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nat Med, 10*, 93-97.

Wolf, R.M., Wilkes, J.J., Chao, M.V., and M.D. Resh. (2001). Tyrosine phosphorylation of p190RhoGAP by Fyn regulates oligodendrocyte differentiation. *J Neurobiol, 49*, 62-78.

Wong, A.W., Xiao, J., Kemper, D., Kilpatrick, T.J., and S.S. Murray. (2013). Oligodendroglial expression of TrkB independently regulates myelination and progenitor cell proliferation. *J Neurosci, 33*(11 ), 4947-4957.

Wong, H., Bourdelas, A., Krauss, A., Lee, H., Shao, Y., Wu, D., Mlodzik, M., Shi, D., and J. Zheng. (2003). Direct binding of the PDZ domain of Dishevelled to a conserved sequence in the c-terminal region of Frizzled. *Mol Cell, 12*(5), 1251-1260.

Wood, P., Okada, E., and R. Bunge. (1980). The use of networks of dissociated rat dorsal root ganglion neurons to induce myelination by oligodendrocytes in culture. *Brain Research, 196*, 247-252.

Xu, W., Harrison, S.C., and M.J. Eck. (1997). Three-dimensional structure of the tyrosine kinase c-Src. *Nature, 385*, 595-602.

Yaffe, M.B. (2002). Phosphotyrosine-binding domains in signal transduction. *Nat Rev Mol Cell Bio, 3*, 177-186.

Yamada, T., Taniwaki, T., Shinnoh, N., Uchiyama, A., Shimozawa, N., Ohyagi, Y., Asahara, H., and J. Kira. (1999). Adrenoleukodystrophy protein enhances association of very long-chain acyl-coenzyme A synthetase with the peroxisome. *Neurology, 52*(3), 615.

Yandava, B.D., Billinghurst, L.L., and E.Y. Snyder. (1999). Global cell replacement is feasible via neural stem cell transplantation: eveidence from the dysmyelinated shiverer mouse brain. *Proc Natl Acad Sci U S A, 96*, 7029-7034.

Ye, F., Chen, Y., Hoang, T., Montgomery, R.L., Zhao, X.H., Bu, H., Hu, T., Taketo, M.M., van Es, J.H., Clevers, H., Hsieh, J., Bassel-Dudy, R., Olson, E.N., and Q.R. Lu. (2009). HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. *Nat Neurosci, 12*, 829-838.

Ye, H., Tan, Y.L. Ponniah, S., Takeda, Y., Wang, S.Q., Schachner, M., Watanabe, K., Pallen, C.J., and Z.C. Xiao. (2008). Neural recognition molecules CHL1 and NB-3 regulate apical dendrite orientation in the neocortex via PTP alpha. *EMBO J, 27*, 188-200.

Zeng, L., Alessandri, L.D., Kalousek, M.B., Vaughan, L., and C.J. Pallen. (1999). Protein tyrosine phosphatase alpha (PTPa) and contactin form a novel neuronal receptor complex linked to the intracellular tyrosine kinase fyn. *J Cell Biol, 147*(4), 707-713.

Zhang, Y., Lu, Z., Ku, L., Chen, Y., Wang, H., and Y. Feng. (2003). Tyrosine phosphorylation of AKI mediates deelopmental sginals to regulate mRNA metabolism. *EMBO J, 22*(8), 1801-1810.

Zheng, X.M., Wang, Y., and C.J. Pallen. (1992). Cell transformation and activation of pp60 c-src by overexpression of a protein tyrosine phosphatase. *Nature, 359*, 336-339.

Zoupi, L., Savvaki, M., and D. Karagogeos. (2011). Axons and myelinating glia: An intimate contact. *IUBMB Life, 63*(9), 730-735.