THE UNIVERSITY OF CHICAGO

THE ROLE OF ZFP191 IN OLIGODENDROCYTE MATURATION AND MYELINATION

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES

AND THE PRITZKER SCHOOL OF MEDICINE

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

COMMITTEE ON NEUROBIOLOGY

BY

JOSHUA DAVID AAKER

CHICAGO, ILLINOIS

JUNE 2016

Table of Contents

xiii. Transcripts with increased expression in (*Mbp*^{shi}) *shiverer* oligodendrocytes

List of Figures:

List of Tables

Acknowledgements

I would like to start by thanking Dr. Brian Popko. You gave me an amazing project that expanded my scientific horizons and challenged me in many ways. Thank you for your guidance and your input throughout my years in your lab. I would also like to thank my committee members Drs. Christoper Gomez, Sarah London, Ernesto Bongarzone, and Ruth Anne Eatock, you contributed to seeing this project through and making it rigorous yet enjoyable.

I would also like to thank everyone in the Popko lab especially Benny and Ben. Benny you have been critical in moving this project forward and our discussions have been invaluable as I wrote my thesis. I very much appreciate you Ben, you have kept me grounded throughout this experience and I cannot wait to see what you do next.

To my previous science mentors Dr. Naoko Koyano-Nakagawa and Dr. Lin Aanonsen I would like to thank you also. Naoko you provided a nurturing environment in which I got to hone my craft and bring forth great science. Lin, there is a reason we call you 'Mom', you inspired me from day one and I hope this document is a reflection of the science you taught all of your students.

I need to thank John Babler. Mr. Babler you saw me as a young kid enthralled with science and math. Thanks for nurturing me and letting me grow, you are the reason I produced this work.

To my friends, thanks for helping me through this, I know it was tough. You have stood by me and pushed me when I needed it. My cohort at the UofC (Ben, Celia, Mike, Melissa, Aya, Alex, other Alex (which one is which?!), Jillian, and Lorenzo) we stuck through and got through this together. A big shout out also to all my Tims, Michael, Kevin and Jeremy, you suffered through my ravings, thanks.

And finally I would like to thank my family. You have been the ground upon which I grew and the rock upon which I stood tall. Thank you all for helping me to finish this. To my Mom thanks for suffering through late night phone calls and letting me vent when I needed to. To my Dad thanks for showing peace and grace throughout this endeavor.

I dedicate this to and thank my Grandmother Esther. You are still the paragon of the person I hope to be in life.

Abstract

Myelination is required for the faithful conduction of action potentials in the vertebrate nervous system. Oligodendrocytes are cells that produce myelin, a large extension of plasma membrane, which is composed of specialized lipids and proteins, and wrap neurons during myelination in the central nervous system (CNS). Our knowledge of myelin production, that is the production of specialized lipids and proteins in the myelin sheath, and myelination, how oligodendrocytes wrap neurons, however is incomplete particularly with respect to which transcription factors are required for myelin production. Recently our lab characterized a mouse mutant, *hypomyelinated in the central nervous system,* which was mapped to a disruption in the gene zinc-finger protein 191 (*Zfp191)*. Characterization of a *Zfp191* deficient mouse (*Zfp191*^{null}*)* has previously been shown that the absence of ZFP191 results in hypomyelination.

ZFP191 belongs to a protein family that is well characterized for its role in transcriptional modulation. The transcriptional program that controls oligodendrocyte maturation and CNS myelination has not been fully characterized. Our previous analysis of the *Zfp191*^{null} mouse demonstrated that a normal number of oligodendrocyte precursor cells trafficked correctly and displayed immunohistochemical reactivity for markers associated with proper maturation into oligodendrocytes. However, these oligodendrocytes had reduced levels of myelin basic protein (MBP), a required factor for myelin, and animals lacking ZFP191 did not possess myelinated neurons in the CNS. To explore how loss of ZFP191 results in hypomyelination I examined the role of ZFP191 in modulating the transcriptome and potential mechanisms for how ZFP191 functions in this potential modulation. *I hypothesized that ZFP191 is functioning to modulate the transcription of key components that control myelin production and myelination*.

Previous work has shown that the loss of ZFP191 results in decreased expression of key myelin related transcript levels in the whole brain when compared to a wild-type animal. Using massively parallel sequencing I examined how the loss of ZFP191 modulates the transcriptome of the whole brain, oligodendrocyte precursor cells and mature oligodendrocytes. The transcriptome could also have been perturbed due to the loss of myelin therefore I also examined the similar transcriptomes in another model of hypomyelination. This other model of hypomyelination is the result of the loss of MBP, which has previously shown is diminished in *Zfp191*^{null} mice. The analysis described in Chapter 2 will help to parse out the role of ZFP191 in hypomyelination and what changes are and are not independent of the loss of MBP.

We currently know that loss of ZFP191 results in hypomyelination, but how this loss results in this phenotype is unclear. ZFP191 belongs to a family of proteins that are known to play key roles in transcriptional regulation. In Chapter 3 I utilized an unbiased approach to interrogate where ZFP191 is interacting at the genomic level. Using this methodology I also determined a ZFP191 DNA binding sequence. Sequences that were found were used in biochemical assays to confirm this interaction and the role of ZFP191 in transcriptional regulation.

Knowing how loss of ZFP191 disrupts the transcriptome is critical to understanding the underlying transcriptional networks that play a role in oligodendrocyte development and myelination. When transcriptome data was combined with locations in the genome where ZFP191 bound I began to determine the primary downstream targets of ZFP191. Chapter 4 compares the perturbed transcriptomes characterized in Chapter 2 with the identified ZFP191 binding sites in Chapter 3.

Chapter 5 synthesizes our current understanding of the role of ZFP191 and its role in oligodendrocytes. I present a model of how ZFP191 may affect transcriptional regulation in the oligodendrocyte. I also propose potential avenues of further experimentation and analysis that will deepen and clarify our understanding of oligodendrocyte maturation and myelination.

The Appendix includes supplemental files, available online, for primers used in my experiments and also the processed files for all of the parallel sequencing files used in Chapters 2-4.

Chapter 1. Introduction

1.1 Overview

Myelination is required for the faithful and rapid conduction of action potentials in the vertebrate nervous system by ensheathing neuronal axons. Non-neuronal glial cells are responsible for producing myelin, including both oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system. In addition to expediting action potentials, the myelin sheath has been shown to provide trophic support and contributes to neuronal survival (Nave 2010). Most oligodendrocyte precursor cells (OPCs) in the CNS originate from neural progenitor cells (NPCs) from the subventricular zone (Richardson, Kessaris, and Pringle 2006). There are three waves of OPC generation in the forebrain, of which the second and third wave give rise to the pool of OPCs that eventually populate the brain (Kessaris et al. 2006).

1.2 Specification of the oligodendrocyte lineage

Extrinsic factors play a key role in orchestrated NPC fate choice in the CNS, including OPC fate specification. Sonic hedgehog (SHH) is a protein secreted by the floor plate and the notochord in the developing embryo (Pringle et al. 1996). SHH binds the Patched (PTCH1) receptor on the surface of NPCs which activates the co-receptor, Smoothened. When activated,

this SHH-driven pathway is meditated through the GLI-Kruppel family member (GLI) proteins, promoting the activator, GLI2, and inhibiting the repressive activity of the GLI3 (Qi et al. 2003, Yu, McGlynn, and Matise 2013). OPC specification is subsequently determined by GLI2-driven expression of early oligodendrocyte specification genes, homeobox protein Nkx-6.1 (*Nkx6*) and oligodendrocyte transcription factor 2 (*Olig2*). In mouse experiments where *Shh* is absent, the first wave of OPC generation is also absent in the forebrain (Tekki-Kessaris et al. 2001). To balance the fate commitment to OPCs, SHH is antagonized by wingless-related integration site/beta-catenin (Wnt/β-cat) and bone morphogenetic proteins (BMPs) that are secreted dorsally. The interplay of these factors is critical as blockade of either the Wnt/β-cat or BMP pathways results in increased OPC specification from NPCs due to the non-opposed signaling of SHH and continued stimulation of *Olig2* (Langseth et al. 2010, Hsieh et al. 2004).

There are several other mechanisms driving OPC specification that are SHH-independent. Cultured NPCs from *Shh*-null mice are still able to generate OPCs *in vitro* (Chandran et al. 2003). In addition, fibroblast growth factor-2 (FGF2) can independently specify OPC commitment and does so in the dorsal forebrain and spinal cord. It is believed that this FGF2 signaling is mediated through the mitogen-activate protein kinase (MAPK) pathway, particularly through phosphorylation of extracellular kinases 1/2 (ERK1/2) (Chandran et al. 2003, Furusho et al. 2011). OPC specification through this pathway results from downregulation of mothers against decapentaplegic homolog (SMAD) signaling (the main effector of the BMP blockade), which allows the stimulation of *Olig2* and commitment to OPC specification (Bilican et al. 2008). Therefore *Olig2* is activated both through SHH-dependent and independent pathways.

OLIG2 has been extensively studied in its role in OPC and oligodendrocyte development and function (Lu et al. 2000, Maire et al. 2010, Zhou, Choi, and Anderson 2001, Liu et al. 2007). The OLIG family members are basic helix-loop-helix transcription factors that are critical for OPC development, as *Olig2* null mice fail to produce OPCs in most areas of the CNS (Lu et al. 2002, Zhou and Anderson 2002, Ligon et al. 2006). There is not a strict requirement for OLIG2, however as some OPCs are generated in the hindbrain by a different transcription factor. It has been reported that instead of OLIG2, OPC generation is mediated through oligodendrocyte transcription factor 1 (OLIG1), which is closely related to OLIG2 (Lu et al. 2002). OLIG1, however, is not likely the primary driver toward OPC differentiation because in the absence of OLIG1 only a minor population of OPCs is absent (Lu et al. 2002, Zhou and Anderson 2002). Mice in which *Olig1* and *Olig2* were both deleted were completely devoid of OPCs. This highlights the critical nature of these proteins and their potential overlapping role in specification although they clearly have distinct functions (Lu et al. 2002, Zhou and Anderson 2002).

1.3 Factors in OPC identity maintenance

When NPCs commit to the OPC fate they begin to express the transcription factors OLIG2 and OLIG1 (Zhou and Anderson 2002, Lu et al. 2002). Upon upregulation of these two transcription factors, other critical transcription factors such as SRY-determining transcription factor (SOX10), paired-box protein 6 (PAX6), homeobox protein Nk6-1 and -2 (NKX6-1/6-2), homeobox protein Nk2-2 (NKX2-2) and achaete-scute family bHLH transcription factor 1 (ASCL1/MASH1) also participate in driving OPC function and maturation (Pringle et al. 1996, Cai et al. 2005, Sugimori et al. 2008).

SOX10 is a direct downstream target of OLIG2 (Kuspert et al. 2011, Yu et al. 2013). SOX10 is a member of the high-mobility-group domain family of transcription factors. In particular, SOX10, along with SRY-determining transcription factor 8 (SOX8) and SRY-determining transcription factor 9 (SOX9) are a highly related subgroup termed SoxE proteins. SOX10 along with SOX8 and SOX9 are coexpressed during the transition from NPC to OPC. SOX10 acts to block both nuclear factor 1 A-type (NFIA) activity and function, helping to maintain the OPC state (Glasgow et al. 2014). SOX10 however is not explicitly required for OPC development as *Sox10* null mice are populated with a relatively normal population of OPCs (Stolt et al. 2002). This may be due to the overlapping function of the other members of the SoxE family members as double deletion of *Sox10/Sox9* results in a dramatic reduction in OLIG2+ cells (Finzsch et al. 2008). The loss of OLIG2+ cells in *Sox10/Sox9* null mice is probably due to decreased platelet-derived growth factor receptor alpha (*Pdgfra*) expression that results in an increase in apoptosis and aberrant migration (Finzsch et al. 2008). Chromatin immunoprecipitation experiments demonstrated that *Pdgfra* is a direct target of both proteins and therefore loss of *Sox10/Sox9* fails to upregulate *Pdgfra*. Although SOX8 appears to play a lesser role in OPC specification and commitment, mice lacking *Sox8/Sox9* also resulted in a lack of OPCs (Stolt et al. 2002, Stolt et al. 2005). With *Sox9* inducing NFIA and *Sox10* counteracting NFIA, this suggests that SoxE proteins have many distinct but also overlapping functions and together drive differentiation to an OPC fate (Stolt et al. 2002).

Despite the fact that *Sox10*-deficient mice have a normal population of OPCs, SOX10 still appears to be the major effector in the SoxE family and loss of SOX10 leads to a near complete loss of developmental myelination (Stolt et al. 2002). The few oligodendrocytes that are able to develop and express mature myelin markers may have been able to due to

compensation by the remaining expression of SOX8 (Stolt et al. 2004). Indeed, a deletion of *Sox10* along with mice that did or did not express *Sox8* demonstrated that a dual loss of these proteins resulted in a total loss myelin gene expression.

1.4 Proliferation and migration of OPCs

OPCs remain proliferative throughout their lifespan, both during OPC development and in the adult CNS. In addition, OPCs increase in proliferation following demyelination or CNS injury (Kang et al. 2010). Mitogenic factors involved in proliferation often have an additional role in preventing differentiation that helps ensure the proper timing and distribution of myelination in the CNS (Wang, Colognato, and Ffrench-Constant 2007). OPCs are also highly dependent on these factors for their survival as these factors help prevent apoptosis (Baron, Colognato, and ffrench-Constant 2005). However, there is a limited pool of these mitogens, and some estimates have suggested in normal development between 20-50% of OPCs undergo apoptosis before they mature into oligodendrocytes in the developing brain (Barres et al. 1992a, b, Trapp et al. 1997). When OPCs are specified they begin to express PDGF receptors and are dependent on the secretion of the mitogen PDGFA from neurons and astrocytes to survive and proliferate (Noble et al. 1988, Hart et al. 1989, Fruttiger, Calver, and Richardson 2000). Deletion of *Pdgfa* results in moderate-to-severe loss of OPC development that is region-specific in the brain. Fruttinger et al. reported that there is a near 99% and 88% loss of OPCs at birth in the optic nerve and spinal cord, respectively. The same group also reported an OPC loss of ~80% in the cortex (Fruttiger et al. 1999). PDGFA requires the participation of several extracellular

matrix proteins along with their respective receptors to promote survival (Baron, Shattil, and ffrench-Constant 2002). The neural/glial antigen 2 (NG2) proteoglycan, which is also expressed following specification, has also been shown to play a key role in determining OPC proliferation by coparticipation with PDGFA (Nishiyama et al. 1996). When stimulated PDGFA interacts with αvβ3 integrin, and through its activation, αvβ3 integrin stimulates OPCs proliferation via the phosphoinositide 3-kinase/protein kinase C (PI3K/PKC) pathway (Baron, Shattil, and ffrench-Constant 2002).

The fibroblast growth factor (FGF) family also plays a key role in regulating OPC proliferation. Fibroblast growth factor-2 signals through fibroblast growth factor receptor 1/2 whereas fibroblast growth factor 18 signals using fibroblast growth factor receptor 2/3. Both cascades promote OPC proliferation and inhibit differentiation (Chandran et al. 2003, Fortin et al. 2005). Signaling through fibroblast growth factor receptor-3 by either fibroblast growth factor 8 or 17 appears to solely inhibit differentiation of OPCs into mature oligodendrocytes (Fortin et al. 2005, Furusho et al. 2011). FGF2 also helps to promote and maintain adequate expression of *Pdgfra* that further promotes OPC proliferation while also preventing differentiation (McKinnon et al. 1990, Baron et al. 2000). In addition to its expression in OPCs, fibroblast growth factor receptor-3 is expressed on neural stem cells and astrocytes (Pringle et al. 2003, Young et al. 2010). Several labs have attempted using genetic strategies removing either the effector molecules or their receptors together or solely in the oligodendrocyte lineage but their results have been inconclusive (Furusho et al. 2011, Murtie et al. 2005). With over 20 known members of the FGF family, at least 18 of which are expressed in the CNS, purely genetic approaches are insufficient to parse out the contributions of individual members of this family (Ford-Perriss,

Abud, and Murphy 2001). For instance, gene deletions may not result in a phenotype if there are compensatory mechanisms by highly related family members.

There are also axonal-OPC interactions that appear to be able to stimulate proliferation and survival. One major protein of focus is neuregulin 1 (NRG1). NRG1 is known to be either axonally bound or secreted, and it interacts with epidermal growth factor receptor (ErbB) tyrosine kinases on OPCs by stimulating the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) to promote OPC survival and proliferation (Canoll et al. 1999, Fernandez et al. 2000, Flores et al. 2000). However, NRG1 switches, in the presence of α6β1 integrins to a MAPK-driven pathway that also promotes survival but now drives differentiation as well (Colognato et al. 2002, Colognato et al. 2004). Genetic deletion of NRG1 or its receptors have not clarified the function of this protein, as the deletions do not appear to greatly perturb OPC survival, proliferation or mature myelination puzzling the field (Brinkmann et al. 2008).

Beyond mere proliferation, migration of OPCs is important for them to fulfill their function of producing mature myelin in axons throughout the entire CNS. PDGFA and FGF2 are both factors that help fuel OPC movement. These factors cooperate together for migration but activate different and non-overlapping pathways (Wolswijk and Noble 1992, Baron et al. 2000). The extracellular matrix (ECM) and cell adhesion molecules also play a key role in propelling and dispersing OPCs. Proteins as diverse as laminins and fibronectins (Chun et al. 2003, Relucio et al. 2009, Relucio et al. 2012) along with polysialylated neuronal cellular adhesion molecule and claudin-11 (Decker et al. 2000, Zhang et al. 2004, Tiwari-Woodruff et al. 2001, Tiwari-Woodruff et al. 2006) play complex roles in directing OPC trafficking through the strength of their interactions. Chemotactic elements can attract or repulse OPCs during migration. Both PDGFA and FGF2 again act to direct OPC migration by serving as chemoattractants (Noble et

al. 1988, Zhang et al. 2004, Bribian et al. 2006). The chemorepellent effects of secreted semaphorpins by OPCs and astrocytes including those of Semaphorin-3a and membrane-bound Semaphorin-4D/F has also been investigated (Spassky et al. 2002, Taniguchi et al. 2009, Armendariz et al. 2012).

1.5 Terminal differentiation of OPCs into mature oligodendrocytes

1.5.1 Prodifferentiation signals

When OPCs have correctly trafficked to their destination and begin induction of myelination, they terminally differentiate into premyelinating post-mitotic cells, a step that requires a large change in cellular behavior and gene expression. In mice failure to make this change results in apoptosis (Barres, Lazar, and Raff 1994, Trapp et al. 1997). Studies utilizing OPCs cultured *in vitro* demonstrated that withdrawal of mitogenic signaling and addition of triiodohyorine/thyroid hormone 3 (T3) halted proliferation and promoted terminal differentiation simultaneously (Barres, Lazar, and Raff 1994, Gao, Durand, and Raff 1997, Durand and Raff 2000). When single-cell cultures of OPCs are incubated with both PDGFA and T3, they divide for a predictable number of rounds before finally differentiating into oligodendrocytes (Temple and Raff 1985). One hypothesis is that there is an intrinsic timer in OPCs that requires PDGFA to continue dividing and T3 to terminally differentiate when that timer terminates (Temple and Raff 1985, Durand and Raff 2000). Studies in the live mouse do not show this synchronized differentiation, which is probably due to contextual cues from neighboring cells (Zerlin, Milosevic, and Goldman 2004, Kang et al. 2010, Zhu et al. 2011). Studies in rodents have shown that signaling from the thyroid may play a key role in oligodendrocyte differentiation as

hypothyroid and hyperthyroid mice have delayed or accelerated myelination respectively (Baas et al. 2002, Marta et al. 1998). The area of intrinsic and extrinsic timer in oligodendrocyte lineage cells is still a topic of much conversation in the field.

In addition to the extrinsic factors like those mentioned above, there are several well-characterized factors that *intrinsically* affect terminal differentiation. OLIG2 has long been known to play a key role in oligodendrocyte lineage development. As mentioned previously, OLIG2 has a pivotal role in OPC specification and fate commitment. However, studies have been hard to perform to elucidate the later roles of OLIG2 (e.g. differentiation) due to the absence of OPCs in *Olig2* null mice. One group however used an inducible conditional knockout approach that showed a stage-specific effect on levels of apoptosis where early deletion of *Olig2* driven by the oligodendrocyte-specific CNPase-promoter, resulted in the known phenotype of severe hypomyelination, while a later deletion of *Olig2* by an inducible proteolipid protein promoter (which is induced after CNPase), resulted in an acceleration and enhancement of myelination occurring through OLIG1 (Mei et al. 2013). How OLIG2 is able to function differently at two time points was recently characterized. OLIG2 is able to recruit the key chromatin-remodeling enzyme SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (BRG1/SMARCA4), a SWI/SNF chromatin-remodeling enzyme that plays a key role in the control of oligodendrocyte lineage development and maturation (Yu et al. 2013). The Lu lab demonstrated that OLIG2 functioned as a pre-patterning factor that directed BRG1 to the enhancers of *Sox10*, zinc finger homeobox 1b (*Zfhx1b)*, and myelin regulatory factor *Myrf*, as well as *Zfp191* at the onset of oligodendrocyte differentiation (Yu et al. 2013). As oligodendrocytes mature, OLIG2 and BRG1 redistribute to genes involved in cytoskeleton reorganization and curvature-dependent actin polymerization (both required for

morphogenesis during myelination). Taken together, these data suggest that OLIG2 functions to prepare the OPC for terminal differentiation possibly as a "pioneer factor" (Zaret and Carroll 2011).

Chromatin remodeling has increasingly become an area of active and rapid exploration in the area of oligodendrocyte biology. The protein ying-yang 1 (YY1) is a member of the zinc-finger family of proteins that act as either transcriptional activators and/or repressors through their interaction with DNA (He et al. 2007). YY1 most likely affects oligodendrocyte lineage development through interaction with histone deactylase 1 (HDAC1). It has been shown that inhibition of HDAC activity prevents the proper terminal differentiation both *in vitro* and *in vivo* (Marin-Husstege et al. 2002, Shen, Li, and Casaccia-Bonnefil 2005). However, there are reports that YY1, and thus HDAC activity, is also an activator and is important for oligodendrocyte development (Berndt et al. 2001). The use of conditional ablation of YY1 in post-mitotic OPCs demonstrated that YY1 also acts as a pro-differentiation molecule by repressing the transcription of known repressors of differentiation (discussed below) (He et al. 2007, Liu et al. 2015). Through its interacting with HDAC1, YY1 is also therefore able to act to repress-the-repressors, or it relieves that final break to allow for proper differentiation.

1.5.2 Antidifferentiation signals

In opposition to the many signals that promote differentiation there are many factors that inhibit differentiation, that likely serve as a mechanism to maintain homeostasis of the oligodendrocyte population in the CNS (Hughes et al. 2013). Notch signaling is a key pathway that inhibits oligodendrocyte differentiation. The axonally bound ligands Jagged1/JAG1 and Delta1/DLK1 bind and activate the NOTCH1 receptor generating the notch intracellular domain

(NICD). NICD functions as a transcription factor and increase the expression of hes family bHLH transcription factor 1 and 5 (*Hes1* and *Hes5*) (Wang et al. 1998, Kondo and Raff 2000). HES5 inhibits differentiation by binding to and competing with SOX10 at the *Mbp* promoter (Liu et al. 2006).

Notch signaling, surprisingly, can also promote OPC differentiation in a different context. Binding of axonally-bound F3/contactin to Notch signals the cleavage and liberation of NICD, which then interacts with deltex1. Rather than increasing *Hes1/5* expression NICD activates myelin associated glycoprotein (*Mag*), a key component of the myelin sheath (Hu et al. 2003). In this way Notch signaling is context specific and is able to modulate its role in inhibition.

In addition to the extracellular signaling pathway of Notch, the Wnt/β-cat pathway plays a key role in inhibition of differentiation. Activation of the receptor Frizzled by a Wnt signaling molecule allows β-cat to traffic to the nucleus and recruit transcription factor 7 like 2 (TCF7L2/TCF4) (Fu, Kesari, and Cai 2012). Although the direct targets of the TCF7L2/β-cat complex are unknown in oligodendrocytes this complex has been shown to upregulate inhibitor of differentiation 2 (*Id2)*, a potent oligodendrocyte differentiation inhibitor, in colon carcinoma and keratinocytes (Rockman et al. 2001, Memezawa et al. 2007). Surprisingly the deletion of *Tcf7l2* blocked oligodendrocyte differentiation rather than promote differentiation. While currently there is no model to explain this potential contradictory role of TCF7L2, one explanation is that HDAC1 and HDAC2 interact with TCF7L2 and displace the activating β-cat complex (Ye et al. 2009). The recruitment of HDAC1/2 via TCF7L2 to genes involved in blocking differentiation, such as *Id2*, therefore represses the repressors. The role of TCF7L2/β-cat signaling is a complex one as the presence of TCF7L2 is transient and tightly controlled with

its ultimate downregulation required to allow for proper differentiation (Fu, Kesari, and Cai 2012, Dai et al. 2014).

In addition to extracellular signaling, oligodendrocytes are prevented from differentiating by intrinsic transcriptional regulators. The ID2 and ID4 proteins are potent inhibitors of differentiation and are downstream of BMP signaling (Cheng et al. 2007). The ID proteins are helix-loop-helix (HLH) proteins but lack the basic domain for DNA binding (Samanta and Kessler 2004). Although they cannot bind DNA the ID proteins still have the ability to form heterodimers with other HLH proteins. ID2 and ID4 can interact with prodifferentiation proteins such as OLIG1 and OLIG2 and sequester them along with the cofactors used for transcriptional activation, which then prevent activation and differentiation. ID4, and to a lesser extent ID2, is also able to change the localization of the OLIG proteins from nuclear to cytoplasmic (Samanta and Kessler 2004). The use of siRNA against *Id4* also demonstrated premature oligodendrocyte differentiation (Marin-Husstege et al. 2006).

The G-protein-coupled receptor 17 (GPR17) also inhibits oligodendrocyte differentiation through ID2 and ID4 (Chen et al. 2009). Currently the ligand for GPR17 is underdetermined and is under investigation. GPR17 is an orphan oligodendrocyte-specific receptor that is transiently expressed in the transition form late stage OPC to early differentiated oligodendrocytes (Chen et al. 2009, Cahoy et al. 2008). The timing of GPR17 needs to be tightly controlled as sustained expression results in dysmyelination and stalled oligodendrocyte differentiation. Conversely mice lacking GPR17 had accelerated differentiation and myelination. The synchrony of time in oligodendrocyte lineage cells is complex and is still an area of study.

1.6 Transcriptional regulation of myelination

When oligodendrocytes commit to myelination they undergo an extraordinary change in cell morphology with an accompanying change in their transcriptional profile. As genes involved in the cell-cycle are downregulated, genes controlling myelination are induced in "waves" that are temporally distinct (Cahoy et al. 2008, Dugas et al. 2006). Importantly the major components of myelin such as proteolipid protein (PLP), MAG, and myelin basic protein (MBP) are strongly induced during differentiation (Dubois-Dalcq et al. 1986). In addition to the components of myelin, genes involving lipid synthesis, cytoskeletal regulators, and elements involved in the axoglial junction are induced. The use of genetic knockout animals has been integral to our understanding of the transcriptional program required for these sweeping changes. Interestingly, the animal models that lack the transcription factors *Olig1*, *Nkx2-2*, *Sox10*, *Myrf* and *Zfp191* all stall at either differentiation or early myelination (Xin et al. 2005, Qi et al. 2001, Stolt et al. 2002, Emery et al. 2009, Howng et al. 2010). The severe phenotype caused by the absence of these transcription factors implies that these factors are nonredundant and either are downstream from one another or control distinct and critical elements of the myelination transcriptional program. Understanding this transcriptional network is vital in our understanding of oligodendrocyte structure and function.

Although OLIG1 is expressed and maintained after oligodendrocyte specification, OLIG1 is thought to play a relatively small role in transcriptional control early on. Deficits from the lack of OLIG1 appear in the post-mitotic oligodendrocyte at the onset of myelination. *Olig1* deficient mice are able to upregulate surface markers associated with early stage oligodendrocytes (Xin et al. 2005). The oligodendrocytes are also able to initiate changes to their cell morphology and generate the stereotyped complex branching seen in normal mature oligodendrocytes, however these mice ultimately fail to myelinate. Although the downstream targets of OLIG1 are not

characterized, it along with SOX10, is able to activate the MBP promoter (Xin et al. 2005, Li et al. 2007). The lack of compensation by OLIG2 is another piece of evidence that these two provide have unique functions.

Like OLIG1, NKX2-2 is expressed early in the OPC lineage and maintained. Also, like OLIG1, deficits are seen with mice lacking *Nkx2-2* expression only during the later stages of oligodendrocyte development (Qi et al. 2001). In this instance the phenotype for the lack of NKX2-2 is a severe delay in expression of MBP and PLP in the CNS. One possibility is that NKX2-2 functions to promote differentiation both by causing direction repression of *Pdgfra* and, as one experiment demonstrated, activation of the PLP promoter (Zhu et al. 2014, Gokhan et al. 2005). Paradoxically NKX2-2 has been shown to repress transcription at the MBP promoter (Wei, Miskimins, and Miskimins 2005). The exact role that NKX2-2 plays is therefore unclear, as it displays both transcriptional activation and repression function *in vitro*.

SOX10 is another transcription factor that has been studied in the context of its ability to induce activation of PLP and MBP (Stolt et al. 2002). Although loss of SOX10 can be compensated (as mentioned above) early on in the lineage the lack of this transcription factor ultimately results in stalled differentiation (Stolt et al. 2002, Hornig et al. 2013). In addition to being a transcriptional activator SOX10 is known to recruit the mediator complex that modulates global transcriptional regulation (Vogl et al. 2013). The recruitment of the mediator complex is necessary for oligodendrocyte differentiation as demonstrated by the conditional ablation of the SOX10 interacting portion stalling the cell at the same stage as the SOX10 null mouse (Vogl et al. 2013). SOX10 in is also able to induce *Myrf* which is necessary for proper differentiation and myelination (Emery et al. 2009). Once MYRF is induced it partners with SOX10 to control the

induction of necessary myelin-specific genes (Hornig et al. 2013). While these proteins can function together they do segregate in the genome to control distinct groups of genes.

Unlike OLIG1, NKX2-2, and SOX10, *Myrf* is not induced until differentiation and is just before *Plp* and *Mbp* (Emery et al. 2009). The absence of MYRF does not affect early specification or initial differentiation of oligodendrocytes but in the later stages of development the cells fail to upregulate myelin-related genes and undergo apoptosis. Although initially unclear how MYRF functions there was an assumption that it functioned as a transcriptional activator, because forced expression in OPCs rapidly induced *Mbp* and myelin oligodendrocyte glycoprotein (*Mog*). In a ChIP-seq experiment it was demonstrated that there is overlap of the OLIG2, MYRF and SOX10 binding sites (Bujalka et al. 2013, Yu et al. 2013). These binding sites are in regions adjacent to myelin genes and transcripts involved in generation of the myelin sheath (Bujalka et al. 2013, Cahoy et al. 2008). Although these transcription factors can work synergistically most of the regulatory regions are selectively targeted by one of the individual factors (Bujalka et al. 2013, Hornig et al. 2013). One model to explain this transcriptional network is that OLIG2 acts as prepatterning factor and recruits BRG1 to make regulatory elements available during differentiation for factors such as SOX10 and MYRF. These factors also bind near *Zfp191* and may have a role in its transcriptional regulation (Yu et al. 2013, He et al. 2016, Bischof et al. 2015).

ZFP191 is a zinc-finger protein that is required for proper maturation and myelination in the CNS (Howng et al. 2010). Although *Zfp191* is expressed from the OPC stage and maintained throughout the lineage deficits in $Zfp191^{null}$ mice were only seen during myelination. The oligodendrocytes were unable to induce *Mbp*, *Mog*, or *Mag* and this resulted in a hypomyelinated CNS. The mice displayed normal numbers of OPCs that trafficked correctly and upregulated the

surface marker CC1, an indicator of late stage oligodendrocytes, something that was not seen in OLIG1, SOX10 and MYRF deficient mice (Xin et al. 2005, Stolt et al. 2002, Emery et al. 2009). A microarray analysis provided evidence that the loss of ZFP191 was able to perturb genes required for differentiation and myelination implying that ZFP191 may be a transcription factor (Howng et al. 2010). This was not unexpected, as zinc-finger proteins are known to play a role in transcriptional regulation.

Many zinc-finger proteins play a role in transcriptional regulation (Sander et al. 2003). Zinc-finger proteins get their name because they use zinc ions to coordinate interaction with other factors including DNA, RNA and other proteins. ZFP191 contains 4 C_2H_2 zinc-finger domains with which it is thought to interact with DNA (Howng et al. 2010). The protein also contains a SCAN domain whose name is derived from the founding members of the motif family, SREZBP, CTFIN51, AW-1 and NUMBER 18 domain, and is thought to mediate protein-protein interaction (Williams, Blacklow, and Collins 1999). The SCAN domain family of zinc-finger domains is widely conserved among vertebrates and currently 23 mouse zinc-finger proteins are orthologous to human proteins (Sander et al. 2003). In humans there are estimated to be over 700 C_2H_2 zinc-finger proteins with most having not yet been investigated or fully characterized (Collins, Stone, and Williams 2001). The SCAN domains mediate protein-protein interaction through dimerization with other SCAN domains. These interactions can ultimately assemble transcriptional regulatory systems. The myeloid zinc finger gene for example was found to be able to interact with a novel SWI2/SNF2-related protein called Domino. Domino contains an ATP-dependent chromatin-remodeling complex that regulates gene expression in myeloid cells (Ogawa et al. 2003). The potential binding partners of ZFP191 or its human ortholog ZNF24 are not yet known. We also have not yet characterized what the role of ZFP191

in oligodendrocyte maturation and myelination. Recently there was a report of humans who had a region of chromosome 18 containing *Zfp191* that is deleted which results in childhood leukodystrophies lending clinical relevance to our understanding of ZFPP191 (Cody et al. 2015).

It is important to understand oligodendrocyte structure and function in order to learn and provide potential treatment for diseases such as multiple sclerosis and leukodystrophies. Currently the elucidation of factors involved in proper differentiation and myelin is incomplete. ZFP191 is known to be required for proper myelination and is well suited to act as a transcriptional regulator. Based on its structure and the reported perturbation of the transcriptome I plan to investigate the role of ZFP191 in the oligodendrocyte lineage and its affect on the transcriptome. I will also interrogate where ZFP191 binds in the genome and its role in transcriptional regulation. *I hypothesized that ZFP191 is functioning as a regulator of transcriptional activity of key genes that control oligodendrocyte maturation and myelination*.

Chapter 2: An analysis of the effect ZFP191 has on oligodendrocyte lineage transcriptomes

2.1 Introduction

Myelin is required for faithful conduction of action potentials and provides trophic support to axons (Nave 2010). Schwann cells in the peripheral nervous system and oligodendrocytes in the CNS synthesize myelin and ensheathe neurons. In the CNS most of the OPCs are derived from NPCs in the subventricular zone (Richardson, Kessaris, and Pringle 2006). Commitment to the oligodendrocyte lineage occurs when NPCs express the critical transcription factor OLIG2 (Lu et al. 2002). OLIG2 and the closely related protein OLIG1 are upregulated in response to extracellular signals such as Shh or FGF2 along with transcription factors such as PAX6, NKX6.1/6.2, NKX2.2 and ASCL1/MASH1 (Pringle et al. 1996, Cai et al. 2005, Sugimori et al. 2008).

OPCs are able to proliferate until they transit to their target axons where they undergo terminal differentiation (Menn et al. 2006). These terminally differentiated oligodendrocytes then produce specialized lipids and proteins to create the multilayered myelin sheath that wraps

axons. Terminal differentiation is achieved through a delicate balance of both inhibitors and promoters of differentiation. The G-protein-coupled receptor 17 is an oligodendrocyte specific receptor that has been shown to strongly inhibit differentiation and may work by increasing the expression of ID2/ID4 (Chen et al. 2009, Wang et al. 2001). ID2/ID4 along with HES1/5 have been shown to enforce the proliferating OPC state and to repress terminal differentiation (Wu et al. 2003, Liu et al. 2006). In addition, the Notch and Wnt signaling pathways have also been implicated in suppressing oligodendrocyte differentiation (Wang et al. 1998, Fancy et al. 2009, Nakatani et al. 2013).

Despite the inhibitory effect of the Wnt signaling pathway on oligodendrocyte differentiation, the Wnt effector TCF4 promotes oligodendrocyte differentiation (Fancy et al. 2009, Hammond et al. 2015). OLIG2 promotes differentiation of oligodendrocytes by recruiting the chromatin-remodeling enzyme SMARCA4/BRG1 to regulatory elements of key genes, including *Zfp191*, during differentiation (Yu et al. 2013). SOX10 is additionally required for the generation of myelinating oligodendrocytes and is a direct activator of several myelin related genes (Li et al. 2007). One of the genes controlled by SOX10 is *Myrf,* a necessary transcription factor for oligodendrocyte maturation and myelination (Hornig et al. 2013). SOX10 binds in the first intron of the *MYRF* gene and induces its expression during oligodendrocyte differentiation. Following its induction, MYRF mediates the progression of premyelinating oligodendrocytes to a mature, myelinating state (Emery et al. 2009). MYRF and SOX10 target many of the same myelin gene enhancers and promoters but also appear to target individual enhancers independently of the other (Bujalka et al. 2013, Hornig et al. 2013).

Lack of myelin either from inherited diseases (leukodystrophies) or from more complex diseases such as multiple sclerosis results in severe physical and cognitive disabilities. Due to

their importance in health and disease, oligodendrocytes have been well characterized particularly with respect to their transcriptional network (Fulton et al. 2011, Swiss et al. 2011). The generation and maintenance of myelin is sensitive to subtle changes in gene expression. Several diseases such as spastic paraplegia type 2 (Saugier-Veber et al. 1994) and Pelizaeus-Merzbacher disease are caused by perturbation of gene dosage (Lupski et al. 1992). Additionally, previous work has shown that the graded loss of *Mbp* results in a dose-dependent diminution of myelination in the mouse (Popko et al. 1987).

Zinc-finger protein 191 (ZFP191) was previously shown to be required for CNS myelin production and maturation (Howng et al. 2010). Lack of ZFP191 results in hypomyelination but does not change the number of OPCs or mature oligodendrocytes. ZFP191 mouse mutants express a number of genes critical to the myelination process at reduced transcript levels, which results in a decrease of these proteins. ZFP191 is a member of the C_2H_2 zinc-finger protein family, many members of which are known to be DNA binding proteins that function as transcriptional regulators (Edelstein and Collins 2005). In addition to the four Zn-finger domains in ZFP191, a SCAN domain is present that may mediate protein-protein interaction (Williams, Blacklow, and Collins 1999).

Using massively parallel RNA sequencing, we investigate the change in the transcriptome due to the loss of ZFP191 in the whole brain as well as in isolated primary OPCs and oligodendrocytes. We find that loss of ZFP191 in the whole brain primarily affects the oligodendrocyte transcriptome. To examine if the transcriptome perturbation was either solely or partially due to hypomyelination in the ZFP191-deficient mouse, we compared these results with whole brain and isolated oligodendrocyte lineage cells derived from the *shiverer* mouse. The *shiverer* mouse is also a model of hypomyelination that resulted from a spontaneous null

mutation in the gene encoding myelin basic protein (MBP) (Roach et al. 1985). These comparisons allow us to show that ZFP191 plays a key role in regulating transcripts that are required for oligodendrocyte maturation and myelination and this is largely independent of loss of myelin. The loss of myelin in both mouse mutants, however, does result in the perturbation of the cholesterol biosynthesis pathway.

2.2 Materials and Methods

2.2.1 Animal Work

Generation of *Zfp191*^{null} mice on the C57BL/6J background has been previously described (Howng et al. 2010). The C3Fe.SWV-*Mbp*^{shi}/J **"***shiverer"* strain of mice was purchased from The Jackson Laboratory (stock 001428) and has been previously described (Readhead et al. 1987). All animal procedures were conducted in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

2.2.2 Cell Culture

Primary OPCs were isolated from brain and enzymatically and mechanically dissociated as previously described (Emery and Dugas 2013). Briefly, both cortices were removed and cells were dissociated by enzymatic and mechanical methods. The single cell suspension was then immunopanned on two plates coated with 50mM Tris-HCl pH 9.5 and goat-anti-mouse IgG+IgM (Jackson ImmunoResearch, 115-055-044) and one plate coated in 50mM Tris-HCl with goat-anti-mouse IgM, Mu chain specific (Jackson ImmunoResearch, 115-005-020). These plates were

then incubated with rat neural antigen 2 (Ran-2), galactocerebroside (GC) and oligodendrocyte marker O4 (O4) hybridomas respectively in 0.2% bovine serum albumin and Dulbeccos phosphate buffered saline (D-PBS) (Life Technologies #14040133). The plates served as two sequential negative selection plates coated with Ran-2 and GC hybridomas followed by a positive selection plate coated with O4 hybridoma. The Ran-2 immunopanning captures type 1 astrocytes and meningeal cells (Bartlett et al. 1981). The GC immunopanning captures differentiated oligodendrocytes (Sommer and Schachner 1981). The O4 hybridoma is able to capture O-2A progenitor cells (Sommer and Schachner 1981). Cells were then trypsinized (0.25% in Earle's balanced salt solution, Life Technologies #14155063) and plated on poly-D-lysine coated plates. Cells were maintained in SATO serum-free Dulbecco's modified Eagle medium (Life Technologies #11960069) as previously described with the addition of B27 supplement (Life Technologies #17504044) (Dugas et al. 2006). For proliferation media PDGF-AA (platelet-derived growth factor-AA) (10 ng/mL, PeproTech #100-13A), NT-3 (neurotrophin-3) (1 ng/mL, PeproTech #450-03), forskolin (Sigma Aldrich (0.01mM) and CNTF (ciliary neurotrophic factor) (10 ng/mL, PeproTech #450-13) were added. To stimulate differentiation PDGF-AA was removed and T3 (triiodothyronine) (40ng/mL, Sigma # T6397) was added. Media was changed every other day and differentiated cells were collected after five days.

2.2.3 RNA sequencing

For whole brain RNA sequencing, mice were anesthetized with avertin (0.5% 2,2,2-tribromoethanol (Sigma-Aldrich #T48402) (w/v), 0.5% tert-amyl alcohol (Fluka #PHR1667) (v/v) in MillQ water) used at 100uL per 10g body weight, and the cerebral hemispheres were removed and snap frozen at -80C at post-natal day 21. Total RNA was isolated using Aurum

Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad #732-6830) following the manufacturer's protocol. OPC and oligodendrocyte total RNA was collected using Aurum Total RNA Kit (Bio-Rad #732-6820). Two biological replicates were used for all samples. The quality of the RNA was assessed using an Agilent 2100 Bioanalyzer. RNA sequencing libraries were prepared using Illumina TruSeq RNA sample Kit (#RS-122-2001) and sequenced by HiSeq 2000 and HiSeq 2500 sequencers using HiSeq SBS Kit (Illumina #FC-401-4002) at the University of Chicago Functional Genomics Facility. Sequencing data has been uploaded to Sequence Read Archive from the National Center for Biotechnology Information through the National Library of Medicine under accession PRJNA295245.

2.2.4 Bioinformatics

RNA sequencing reads were mapped to the mm9 genome using BowTie v.1 and the ExpressionPlot pipeline (Langmead et al. 2009, Friedman and Maniatis 2011). Differential expression was performed using DEseq in the ExpressionPlot software package (Anders and Huber 2010). The Bonferroni correction was used within DEseq to control for multiple comparisons. Only transcripts ≥0.5 Reads per Kilobase per Million mapped reads (RPKM), ≥1.5-fold change and $p<0.001$ were analyzed. KEGG pathway analysis was performed using DAVID (Huang da, Sherman, and Lempicki 2009b, a, Kanehisa et al. 2014, Kanehisa and Goto 2000). The Galaxy environment was used to compare datasets (Goecks et al. 2010, Blankenberg et al. 2010, Giardine et al. 2005). Cellular identity stratification was done using the online RNA-Seq transcriptome and splicing database (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html) (Zhang et al. 2014). The top 500

transcripts for each cell identity from the database were compiled to in order to allow for cell-type comparison and grouping from our results.

2.2.5 Real-time PCR

cDNA from RNA isolated for RNA sequencing was generated using iScript cDNA Synthesis Kit (Bio-Rad # 1708890**)** following the manufacturer's protocol. Real-time PCR was performed on the cDNA using iQ SYBR Green Supermix (Bio-Rad #1708882) using a CFX96 Touch Deep Well Real-Time PCR Detection System (Bio-Rad #1854095). Each sample was done in triplicate. Relative expression from amplified cDNA samples was determined using the $2^{-\Delta\Delta}{}_{CT}$ method (Pfaffl 2001). The primers sequences for all the transcripts that were selected for analysis are in Table 2.2.1. The expression of the selected transcripts data was normalized to hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) cDNA.

Transcript	Forward	Reverse
Hprt	TCAGACCGCTTTTTGCCGCGA	ATCGCTAATCACGACGCTGGGAC
Sp7	ACCCCAAGATGTCTATAAGCCC	AGTTGCCCACTATTGCCAACC
Pax7	AATGGCCTGTCTCCTCAGGT	TCTCCTGGCTTGATGGAGTC
Sc4mol	GGCTCCCTGATAGTTCACGA	TGGCATTCTTTCCCAATCAT
Lss	TGGTGATTATGGTGGTCCGC	TTGTCCTCGATGTGCAAGCC
Hmgcr	GTACGGAGAAAGCACTGCTGAA	TGACTGCCAGAATCTGCATGTC
Kcnip1	TCTAAAGACATCGCCTGGTGG	GGTGAAGTTCGTCTGTGCCT
Wnt7a	CGGATCGGTGGCTTCTCTTC	TACAGATGATGCTCGCACCC
Dll3	GGGCAGCTGTAGTGAAACCT	CTTCACCGCCAACACACAAG

Table 2.2.1. Primers used in real-time PCR for ChIP-seq verification.

2.3 Results

2.3.1 Loss of *Zfp191* expression perturbs the transcriptome and the steroid biosynthesis pathway in the whole brain

Previous work has shown that ZFP191 is required for CNS myelination, however the role that ZFP191 plays in normal myelin production by oligodendrocytes is not well understood (Howng et al. 2010). To examine the potential role of ZFP191 in transcriptional regulation of CNS myelination we used RNA sequencing to examine changes in the transcriptome. RNA sequencing was performed on post-natal day 21 (P21) whole brain RNA samples from $Zfp191^{null}$ and $Zfp191^{+/+}$ littermates. The levels of 394 transcripts were determined to be significantly different in the $Zfp191^{null}$ brains compared to $Zfp191^{+/+}$ brains (Appendix 1-2). The majority of the perturbed $Zfp191^{null}$ brain transcripts displayed reduced levels of expression in the mutant animals.

The loss of ZFP191 resulted in the decreased expression of 327 transcripts when compared to the $Zfp191^{+/+}$ CNS transcriptome (Appendix 1). Several transcripts that encode key myelin proteins, such as MBP and myelin oligodendrocyte glycoprotein (MOG), were identified

as having decreased transcript levels (Matthieu and Amiguet 1990). We also observed that transcripts that encode key oligodendrocyte transcriptional factors such as SOX10 and MYRF had reduced expression with loss of ZFP191 along with a downstream target of MYRF, the transcript for ring finger and FYVE-like domain containing E3 ubiquitin protein ligase (*Rffl)* (Bujalka et al. 2013).

Tables 2.3.1A and 2.3.1B show the top 10 transcripts based on RPKM values and fold changes respectively that have reduced expression in the *Zfp191*^{null} whole brain. Transcripts with reduced expression in the *Zfp191*^{null} brain were analyzed for changes in characterized pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000, Kanehisa et al. 2014). The steroid biosynthesis pathway is significantly disturbed due to disruption of transcripts that encode key biosynthetic pathway proteins such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), fatty acid 2-hydroxylase (FA2H), and 24-dehydrocholesterol reductase (DHCR24) (Saher and Simons 2010) (Appendix 1). Proper synthesis of cholesterol is known to be required for production of compact myelin in oligodendrocytes (Saher et al. 2005).

A. Genes sorted by *Zfp191*$^{+/+}$ transcript RPKM

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Plp1	8.39	1210.00
Mbp	6.06	749.00
Cldn11	6.62	258.00
Mal	9.57	222.00
Cnp	3.33	207.00
Mobp	13.82	129.00
Mag	10.87	117.00
Mog	7.27	111.00
Ugt8a	10.56	111.00
Tspan2	5.20	110.00

B. Genes sorted by fold change for *Zfp191*$^{+/+}$

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Gjb1	15.08	14.90
Mobp	13.82	129.00
Gjc2	11.53	12.20
Mag	10.87	117.00
Plekhh1	10.63	7.72
Ugt8a	10.56	111.00
A230069A22Rik	9.73	24.90
Mal	9.58	222.00
Ermn	9.12	35.10
Plp1	8.39	1210.00

Table 2.3.1. Transcripts that have reduced expression in *Zfp191*^{null} whole brains
RNA sequencing was performed on *Zfp191*^{null} and *Zfp191*$^{+/+}$ whole brain RNA samples (n=2 for each genotype). Transcripts listed for *Zfp191*$^{+/+}$ whole brains in A had a $p<0.001$ fold change

when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} whole brains and are sorted by RPKM value. Transcripts listed for *Zfp191*$^{+/+}$ whole brains in B had a RPKM value ≥7, a $p<0.001$ fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} whole brains, and are sorted by fold changes.

2.3.2 Loss of *Zfp191* expression mainly affects cell-specific transcripts in the oligodendrocyte lineage along with key signaling cascades

Using the dataset from Zhang et al. we stratified transcripts into cell specific identities (oligodendrocyte lineage, neuron, astrocyte, microglia, endothelia or non-cell specific) (Zhang et al. 2014). Of the 327 transcripts that had lower expression levels with loss of ZFP191, 221 transcripts were categorized into being from "oligodendrocyte lineage" cells (Fig. 2.3.1)

Loss of *Zfp191* expression increased the mRNA levels of 67 transcripts in the mutant CNS; ten transcripts with the most abundant RPKM are listed in Table 2.3.2 (also Appendix 2). One transcript that was increased encodes glial fibrillary acidic protein (*Gfap),* which may suggest astrogliosis (Bignami et al. 1972). This is supported by a 2.5-fold increase in serine peptidase inhibitor, clade A, member 3N (*Serpina3n),* which has been show to increase in reactive astrogliosis (Zamanian et al. 2012). In addition, there were increases in multiple transcripts that encode complement factors and chemokine ligands that are expressed by microglia that account for 39% of the elevated transcripts (Zhang et al. 2014). KEGG pathway analysis indicated that complement, chemokine signaling, and Toll-like receptor pathways are

increased with loss of ZFP191 in the whole brain. Unlike the transcripts that had decreased expression, which were mostly associated with oligodendrocyte lineage cells (Fig. 2.3.1A), transcripts that have higher levels with loss of ZFP191 were more often non-cell specific or associated with gliosis (Fig. 2.3.1B). This may suggest that loss of *Zfp191* expression may perturb cells indirectly by disrupting normal oligodendrocyte maturation and function

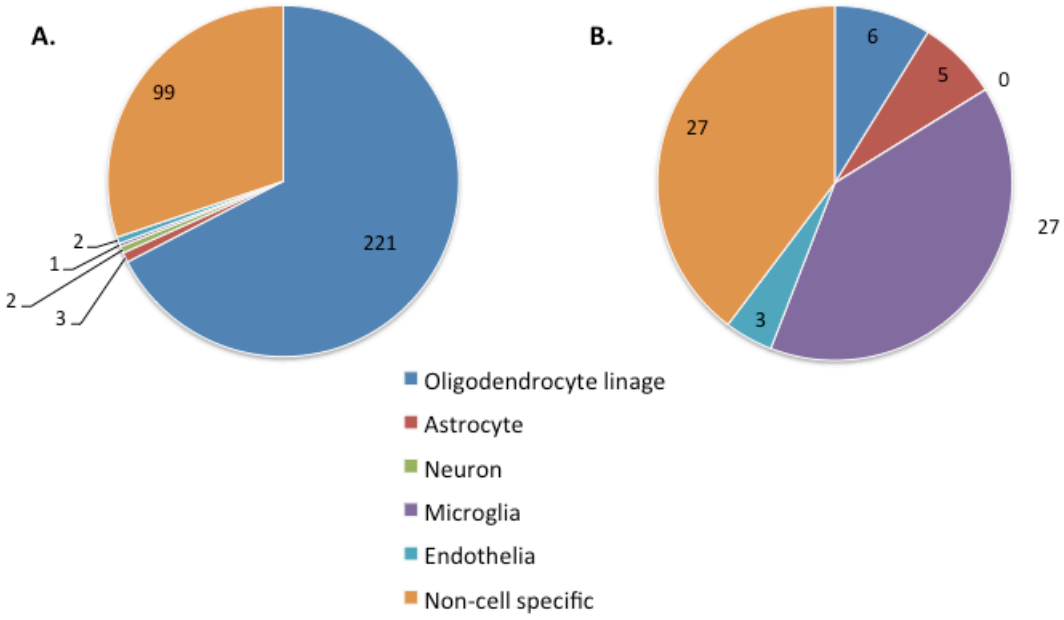

Figure 2.3.1. Loss of *Zfp191* expression mainly perturbs transcripts associated with the oligodendrocyte lineage in the whole brain. Number of transcripts with decreased expression by loss of ZFP191 (A) or increased expression (B) sorted into cellular identity categories using data from Zhang et al. (Zhang et al. 2014).

Gene	Fold change	*Zfp191*^{null} RPKM
Apoe	1.60	1050.00
Mt1	1.61	452.00
Mt2	1.53	303.00
Fam107a	1.70	161.00
Gfap	1.97	106.00
Ctss	1.69	33.90
C1qa	1.66	29.00
C1qb	1.70	28.20
Gabra2	1.62	21.40
C1qc	1.82	16.20

Table 2.3.2. Transcripts that have increased expression in *Zfp191*^{null} whole brains. RNA sequencing was performed on *Zfp191*^{null} and *Zfp191*$^{+/+}$ whole brains (n=2 for each genotype). Transcripts listed for *Zfp191*^{null} whole brains had a $p<0.001$ fold change when comparing *Zfp191*^{null} versus *Zfp191*$^{+/+}$ whole brains and are sorted by RPKM value.

2.3.3 Loss of ZFP191 perturbs the transcriptome of oligodendrocyte progenitor cells

Because the loss of ZFP191 in the whole brain predominantly disrupts oligodendrocyte lineage specific transcripts we wanted to further explore this affect in purified primary cells. We therefore isolated and cultured OPCs from both *Zfp191*^{null} and *Zfp191*$^{+/+}$ brains. Half of these cells were allowed to differentiate into mature oligodendrocytes. Both cell types from each genotype were then collected for RNA sequencing analysis. We discovered that the absence of ZFP191 during the OPC stage perturbs 444 transcripts (Appendix 3-4). Loss of ZFP191 results in lower expression levels of 160 transcripts. Tables 2.3.3A and 2.3.3B lists the transcripts that are most abundant in the *Zfp191*$^{+/+}$ brains and their corresponding reduction with the loss of ZFP191. Transcripts involved in many signal transduction pathways were disturbed including: delta-like 1 and 3 (*Dll1*, *Dll3),* which are involved in the Notch pathway and SRY (sex determining region Y)-box 9 (*Sox9),* which is involved in cAMP signaling (Grandbarbe et al. 2003, Tsuda et al. 2003). KEGG analysis demonstrated that Notch, mitogen-activated protein kinase (MAPK), calcium signaling and focal adhesion pathways were disrupted with loss of ZFP191 in OPCs.

A. Genes sorted by *Zfp191*$^{+/+}$ transcript RPKM

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Ptn	1.77	265.00
Bcan	1.99	178.00
Gpr56	2.17	119.00
Add3	2.18	101.00
Dll1	1.82	61.50
Tubb6	2.78	63.40
Rev3l	1.98	43.90
Chrna4	2.33	47.10
Pnmal2	1.75	35.30
Slc38a1	1.78	34.70

B. Genes sorted by fold change for *Zfp191*$^{+/+}$

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Matn4	27.70	27.40
Pstpip2	15.27	16.70
Pcdh11x	5.77	9.14
9030025P20Rik	5.24	10.90
Dll3	4.92	38.60
Col18a1	4.71	10.20
Hddc3	4.15	30.10
Tmem179	4.13	11.70
Kifc3	3.98	59.10
Lmo3	3.92	15.30

Table 2.3.3. Transcripts that have reduced expression in *Zfp191*^{null} OPCs
RNA sequencing was performed on RNA samples isolated from *Zfp191*^{null} and *Zfp191*$^{+/+}$ OPCs cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Zfp191*$^{+/+}$ OPCs in A had a $p<0.001$ fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} OPCs and are sorted by RPKM value. Transcripts listed for *Zfp191*$^{+/+}$ OPCs in B had a RPKM value ≥ 7, a $p<0.001$ fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} OPCs, and are sorted by fold changes.

Loss of ZFP191 results in 284 transcripts being expressed at a higher level than in *Zfp191*$^{+/+}$ OPCs (Tables 2.3.4A and 2.3.4B). Transcripts such as *Id2*, SRY (sex determining region Y)-box 5 (*Sox5)*, and *Nkx6.2* are known to be down-regulated when OPCs stop proliferating (Wang et al. 2001). In addition, in the absence of ZFP191, histone deactylase 1 (*Hdac1)* is highly expressed along with wingless-type MMTV integration site family, member 7A (*Wnt7a)*, both of which code for proteins that have been shown to be involved in the maintenance of the progenitor state (Ye et al. 2009, Yuen et al. 2014). Loss of ZFP191 causes several transcripts associated with disruption to the MAPK and Wnt signaling pathways to be more abundant.

2.3.4 Loss of ZFP191 perturbs key cell signaling pathways in mature oligodendrocytes

The absence of ZFP191 perturbs the mRNA levels of 5,564 transcripts in mature oligodendrocytes (Appendix 5-6). Over 45%, or 2,513, transcripts have decreased abundance with loss of ZFP191 in oligodendrocytes. Transcripts for *Sox10*, *Myrf*, *Mbp*, *Mog*, *Nkx6.2* and G protein-coupled receptor 37 (*Gpr37)*, all known to code for proteins that play a role in myelination, are decreased with loss of *Zfp191* expression (Tables 2.3.5A and 2.3.5B) (Emery 2010b). Many KEGG identified pathways are perturbed in the mutant oligodendrocytes including: regulation of actin cytoskeleton, receptor tyrosine-protein kinase erbB-3 (Erbb), MAPK, sphingomyelin metabolism and focal adhesion. Transcripts that were more abundant

(3,051) in the *Zfp191*^{null} oligodendrocytes compared to *Zfp191*$^{+/+}$ oligodendrocytes include platelet-derived growth factor receptor alpha polypeptide (*PDGFra)*, sex determining region Y-box 4 (*Sox4)*, and myelocytomatosis oncogene (*c-Myc)* along with the known differentiation repressors *Id2/4* and *Hes1/5* (Tables 2.3.6A and 2.3.6B).

A. Genes sorted by *Zfp191*^{null} transcript RPKM

Gene	Fold change	*Zfp191*^{null} RPKM
Fscn1	1.82	360.00
Rps26	1.91	249.00
Npy	64.28	214.00
Vgf	24.33	214.00
Olfm1	8.11	131.00
Id2	3.80	131.00
Wnk2	20.09	123.00
Tgfa	1.76	116.00
Elfn1	3.90	89.80
Mn1	50.44	79.80

B. Genes sorted by fold change for *Zfp191*^{null}

Gene	Fold change	*Zfp191*^{null} RPKM
Pax7	24760.00	15.40
Tmem119	936.10	7.82
Epha2	934.90	41.10
Rps3a	261.00	44.90
Rps18	240.60	42.10
Ebi3	72.15	17.10
Npy	64.28	214.00
Mn1	50.44	79.80
Kcnip1	33.72	73.00
Tspan18	31.19	16.60

Table 2.3.4. Transcripts that have increased expression in *Zfp191*^{null} OPCs
RNA sequencing was performed on RNA samples isolated from *Zfp191*^{null} and *Zfp191*$^{+/+}$ OPC cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Zfp191*^{null} OPCs in A had a $p<0.001$ fold change when comparing *Zfp191*^{null} versus *Zfp191*$^{+/+}$ OPCs, and are sorted by

RPKM value. Transcripts listed for *Zfp191*^{null} OPCs in B had a RPKM value ≥7, a p<0.001 fold change when comparing *Zfp191*^{null} versus *Zfp191*$^{+/+}$ OPCs, and are sorted by fold changes.

A. Genes sorted by *Zfp191*$^{+/+}$ transcript RPKM

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Plp1	6.64	8140.00
Tubb4	3.53	3790.00
Cldn11	5.27	3730.00
Mbp	4.82	3560.00
Mag	12.04	2670.00
Cnp	4.07	2380.00
Fth1	2.03	1740.00
Mal	13.77	1180.00
Aplp1	3.01	852.00
Mobp	17.51	841.00

B. Genes sorted by fold change for *Zfp191*$^{+/+}$

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Gjb1	381.34	181.00
Ly6a	132.40	19.30
Clmn	102.45	47.10
Vamp5	72.49	18.70
Rab37	64.52	7.69
Esyt3	63.85	7.10
C030030A07Rik	60.74	14.90
Pls1	57.19	54.70
Padi2	56.62	88.90
Pdlim2	46.26	180.00

Table 2.3.5. Transcripts that have reduced expression in *Zfp191*^{null} oligodendrocytes
RNA sequencing was performed on RNA samples isolated from *Zfp191*^{null} and *Zfp191*$^{+/+}$ oligodendrocytes cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Zfp191*$^{+/+}$ oligodendrocytes in A had a p<0.001 fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} oligodendrocytes, and are sorted by RPKM value. Transcripts listed for *Zfp191*$^{+/+}$

oligodendrocytes in B had a RPKM value ≥7, a p<0.001 fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} oligodendrocytes, and are sorted by fold changes.

A. Genes sorted by *Zfp191*^{null} transcript RPKM

Gene	Fold change	*Zfp191*^{null} RPKM
mt-Nd2	1.81	1610.00
Gfap	14.52	989.00
Fabp7	7.48	832.00
Vim	15.77	805.00
Apoe	5.10	795.00
Mt3	21.71	740.00
Ckb	2.02	612.00
Nnat	7.80	491.00
Mt1	2.50	348.00
Marcks	3.30	344.00

B. Genes sorted by fold change for *Zfp191*^{null}

Gene	Fold change	*Zfp191*^{null} RPKM
Rps18	2185.00	14.60
Rps3a	390.50	16.40
Npy	299.90	14.00
Acta2	253.00	33.40
Sod3	180.50	14.10
Ccnd2	166.80	46.50
Myl9	139.40	30.30
Ctgf	137.20	8.62
Glycam1	127.60	26.10
Gpc4	116.10	26.20

Table 2.3.6. Transcripts that have increased expression in *Zfp191*^{null} oligodendrocytes

RNA sequencing was performed on RNA samples isolated from *Zfp191*^{null} and *Zfp191*$^{+/+}$ oligodendrocytes cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Zfp191*^{null}

oligodendrocytes in A had a $p<0.001$ fold change when comparing *Zfp191*^{null} versus *Zfp191*$^{+/+}$ oligodendrocytes, and are sorted by RPKM value. Transcripts listed for *Zfp191*^{null} oligodendrocytes in B had an RPKM value ≥7, a $p<0.001$ fold change when comparing *Zfp191*^{null} versus *Zfp191*$^{+/+}$ oligodendrocytes, and are sorted by fold changes.

Transcripts that are decreased due to loss of ZFP191 in cultured primary mature oligodendrocytes represent nearly 77% of transcripts that are also decreased in the whole brain (327 total transcripts decreased in the whole brain, 252 of which are also decreased in cultured primary mature oligodendrocytes) (Fig. 2.3.2). The transcripts that are decreased in both datasets include critical myelin associated transcripts such as 2',3'-cyclic nucleotide 3' phosphodiesterase (*Cnp*), aspartoacylase (*Aspa*), myelin and lymphocyte protein T cell differentiation protein (*Mal1*), proteolipid protein 1 (*Plp1*), myelin-associated oligodendrocyte basic protein (*Mobp*), *Mag*, and *Mbp* along with key transcription factors *Sox10*, *Myrf*, and *Nkx6-2* (Emery 2010b, a). In addition, transcripts for key factors regulating transcription such as *Gpr37,* mothers against decapentaplegic homolog 7 *(Smad7)*, receptor tyrosine-protein kinase erbB-3, survival of motor neuron protein-interacting protein 1 (*Sip1/Zeb2)* along with transcripts for enzymes that play a key role in lipid biogenesis, such as elongation of very long chain fatty acids protein 7 (*Elovl7)* and ceramide synthase 2 (*Lass2)*, are decreased with loss of ZFP191 (Weng et al. 2012, Brinkmann et al. 2008). In contrast very few (14) transcripts have increased mRNA levels with loss of ZFP191 in both the whole brain and mature oligodendrocytes; these include inhibitor of differentiation 3 (*Id3)*, lunatic fringe (*Lfng)*, and apolipoprotein E (*Apoe)*.

There are 177 transcripts that are increased in both OPCs and oligodendrocytes that do not express ZFP191 (Fig. 2.3.3). Several of these transcripts encode proteins that play a role in the control of oligodendrocyte development by repressing maturation such as *Id2*, *Wnt5a/7a*, *Hdac1*, *Sox5* and paired-box 7 (*Pax7*) (Emery 2010b, a, Fulton et al. 2011, Kuspert and Wegner 2015, Liu et al. 2006). Elevation of these transcripts may inappropriately maintain transcripts that promote the OPC identity and/or prevent maturation. We noted that 1,522 transcripts that are normally decreased upon differentiation from the OPC to oligodendrocyte (Zhang et al. 2014) are not suppressed in mature oligodendrocytes that lack ZFP191. These transcripts include *Dll1*, *Ascl1/Mash1*, *c-Myc*, *Notch1*, *Pdgfra*, and *Sox2/4/6/9/21*. Many of these factors are known to play a role in transcriptional regulation.

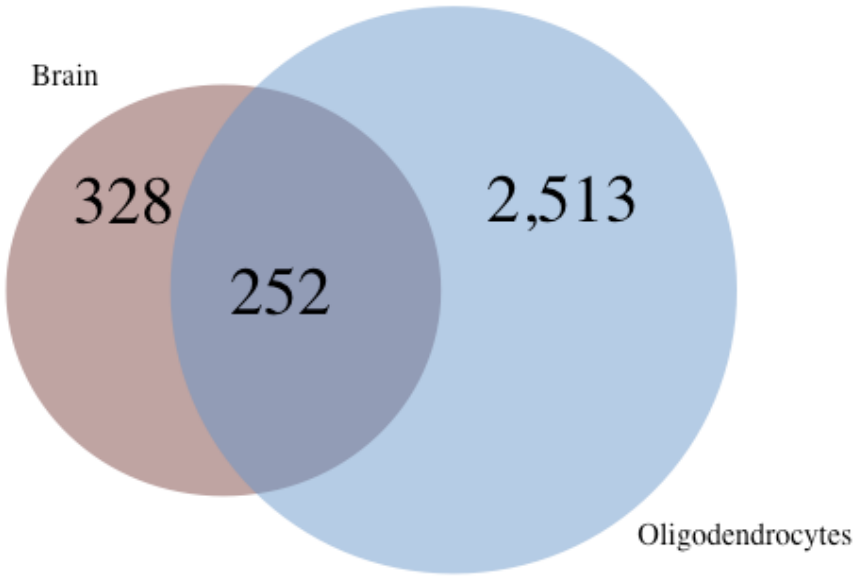

Figure 2.3.2. A Venn diagram comparing the number of transcripts with decreased expression in *Zfp191*$^{-/-}$ whole brain compared to *Zfp191*$^{+/+}$ whole brain (red) and transcripts with decreased expression in *Zfp191*$^{-/-}$ oligodendrocytes compared to *Zfp191*$^{+/+}$ oligodendrocytes (blue).

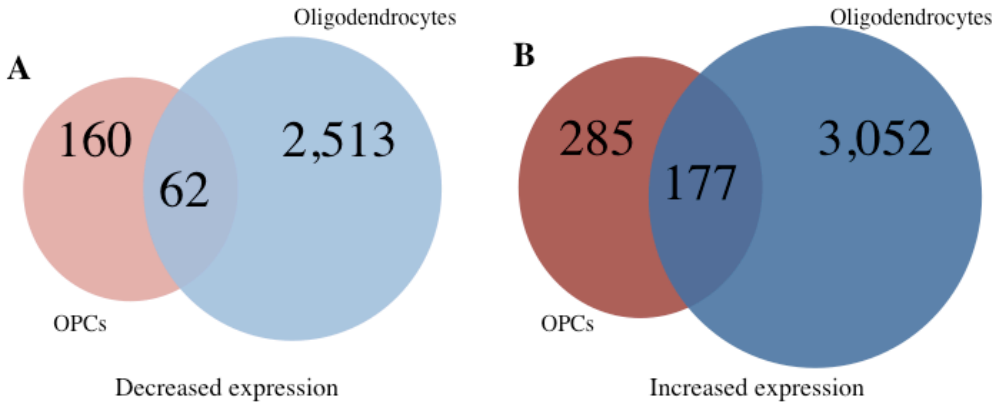

Figure 2.3.3. A Venn diagram comparing the number transcripts (A) with decreased expression in *Zfp191*$^{-/-}$ OPCs compared to *Zfp191*$^{+/+}$ OPCs (light red) and *Zfp191*$^{-/-}$ oligodendrocytes compared to *Zfp191*$^{+/+}$ oligodendrocytes (light blue). A Venn diagram comparing transcripts (B) with increased expression in *Zfp191*$^{-/-}$ OPCs compared to *Zfp191*$^{+/+}$ OPCs (dark red) and *Zfp191*$^{-/-}$ oligodendrocytes compared to *Zfp191*$^{+/+}$ oligodendrocytes (dark blue).

2.3.5 Transcription factor expression is perturbed with loss of *Zfp191*

Loss of *Zfp191* expression in the brain perturbs 25 transcripts identified as transcription factors from the Animal Transcription Factor DataBase (AnimalTFDB) and Riken Transcription Factor Database (TFdb) (Zhang et al. 2012, Kanamori et al. 2004). A majority of these transcripts (21) have reduced expression in $Zfp191^{null}$ brain. Table 2.3.7 lists the 10 transcripts that have the greatest fold change in $Zfp191^{null}$ oligodendrocytes. Our data demonstrate that loss of ZFP191 reduces expression of *Myrf*, *Nkx6-2* and *Olig1* all of which play a vital role in oligodendrocyte differentiation and myelination (Emery 2010b, a).

Gene	Fold change	*Zfp191*$^{+/+}$ RPKM
Scx	26.29	8.14
Onecut2	20.52	4.06
Zfp457*	18.23	1.11
Irx5	15.74	1.98
Pou3f1	15.52	19.3
Utf1	14.07	1.17
Mafa	11.69	1.07
Tle6*	10.19	3.43
Hes3*	8.96	11.3
Rbpjl*	8.40	2.23

Table 2.3.7. Transcription factor transcripts with reduced expression in *Zfp191*^{null} cultured oligodendrocytes

RNA sequencing was performed on RNA samples isolated from *Zfp191*^{null} and *Zfp191*$^{+/+}$ oligodendrocytes cultured *in vitro* (n=2 for each genotype). Transcription factor transcripts were compiled from AnimalTFDB and TFdb (Kanamori et al. 2004, Zhang et al. 2012). Transcription factor transcripts listed for *Zfp191*$^{+/+}$ whole brains had a RPKM value ≥0.5, a ≥1.5-fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} oligodendrocytes, $p<0.001$, and sorted by RPKM value. Transcription factor transcripts were compared to the following datasets: *=Gene expression is also reduced in *Zfp191*^{null} OPCs and oligodendrocytes.

In isolated and cultured OPCs, loss of *Zfp191* expression perturbs 58 transcription factor transcripts. In contrast to the whole brain data, the majority of these transcripts have increased expression (41) with loss of ZFP191. *Pax7* has an ~24,000 fold increase in *ZFP191*^{null} OPCs (this high fold change is due to the absence of detectable *Pax7* mRNA in *Zfp191*$^{+/+}$ cells). In cultured oligodendrocytes we identified 558 transcripts that encode putative transcription factors that have perturbed expression with the loss of ZFP191. As with the OPCs, loss of ZFP191 in oligodendrocytes results in an increase in more transcription factor transcripts (333) than their decrease. Similar to what was seen with OPCs *Pax7* has the largest fold change (241.4-fold) in *Zfp191*^{null} oligodendrocytes.

2.3.6 The perturbation of the transcriptome is primarily due to loss of ZFP191 rather than the absence of myelin

The lack of ZFP191 results in profound CNS hypomyelination, which might have a secondary effect on the transcriptome. To distinguish transcripts that are affected primarily through the loss of ZFP191 from those that are perturbed due to the loss of myelin, we examined the transcriptome of the well-characterized hypomyelinated *shiverer* mouse, in which the hypomyelination is the result of a spontaneous null mutation in the *Mbp* gene (Readhead et al. 1987). We performed RNA-sequencing on P21 *shiverer* whole brain RNA samples, as well as RNA isolated from primary OPCs and oligodendrocytes similar to the *Zfp191*$^{-/-}$ datasets. The loss of *Mbp* in P21 whole-brains results in the perturbation of 93 transcripts (Appendix 7-8). The majority of these transcripts were expressed at a higher level in *shiverer* mutant mice (67 transcripts) (Tables 2.3.8A and 2.3.8B also 2.3.9A and 2.3.9B). Transcripts that were more abundant in the *shiverer* brain include *Nkx6-2*, *Erbb3* and *Apoe.* Transcripts that had a lower

expression level in the *shiverer* mouse brains compared to $Mbp^{+/+}$ littermates include *Mbp,* UDP galactosyltransferase 8A *(Ugt8a), Fa2h, Mal, gelsolin (Gsn),* lanosterol synthase (*Lss*)*,* squalene epoxidase *(Sqle), Hmgcr,* and *Plp1*. Examining the transcripts for KEGG pathway analysis revealed that the loss of MBP in the whole brain perturbs phosphatidylinositol signaling along with sterol biosynthesis.

A. Genes sorted by *Mbp*$^{+/+}$ transcript RPKM

Gene	Fold change	*Mbp*$^{+/+}$ RPKM
Plp1	1.61	1120.00
Mbp	33.68	506.00
Mal	2.23	178.00
Ugt8a	3.19	122.00
Trf	2.31	69.30
Scd1	1.83	44.00
Apod	2.17	40.30
Gsn	1.81	33.60
Hmgcr	1.61	29.90
Fa2h	2.74	26.40

B. Genes sorted by fold change for *Mbp*$^{+/+}$ transcripts ≥7 RPKM

Gene	Fold change	*Mbp*$^{+/+}$ RPKM
Mbp	33.68	15.00
Ugt8a	3.19	38.20
Fa2h	2.74	9.66
Trf	2.31	30.00
Mal	2.23	79.90
Apod	2.17	18.60
Anln	2.113	8.02
Sc4mol	2.012	14.1

Table 2.3.8. Transcripts that have decreased expression in *shiverer* whole-brains
RNA sequencing was performed on *Mbp*^{shi} and *Mbp*$^{+/+}$ whole brain RNA samples (n=2 for each genotype). Transcripts listed for *Mbp*$^{+/+}$ whole brains in A had a $p<0.001$ fold change when comparing *Mbp*$^{+/+}$ versus *Mbp*^{shi} whole brains, and are sorted by RPKM value. Transcripts listed for *Mbp*$^{+/+}$ whole brains in B had an RPKM value ≥7, a $p<0.001$ fold change when comparing *Mbp*$^{+/+}$ versus *Mbp*^{shi} whole brains, and are sorted by fold changes.

A. Genes sorted by *Mbp*^{shi} transcript RPKM

Gene	Fold change	*Mbp*^{shi} RPKM
Apoe	1.60	752.00
Ptms	1.62	228.00
Ptgds	1.98	138.00
Csrp1	1.72	87.80
Sirt2	1.84	70.90
S100a1	1.76	55.80
Nfasc	1.71	53.90
Map4k4	1.53	45.60
P4hb	1.52	41.00
Nudc	1.53	38.20

B. Genes sorted by fold change for *Mbp*^{shi} transcripts ≥7 RPKM

Gene	Fold change	*Mbp*^{shi} RPKM
Gsbs	3.39	28.10
Car14	2.77	14.80
Pls1	2.70	7.77
Tmem2	2.15	8.09
Phlda1	2.05	14.10
H2afj	2.04	17.60
Adamts4	2.04	15.00
Rras2	2.02	17.60
Ptgds	1.98	138.00
Hsd17b7	1.98	10.10

Table 2.3.9. Transcripts that have increased expression in *shiverer* whole-brains
RNA sequencing was performed on *Mbp*^{shi} and *Mbp*$^{+/+}$ whole brain RNA samples (n=2 for each genotype). Transcripts listed for *Mbp*^{shi} whole brains in A had a $p<0.001$ fold change when comparing *Mbp*^{shi} versus *Mbp*$^{+/+}$ whole brains, and are sorted by RPKM value. Transcripts listed for *Mbp*^{shi} whole brains in B had an RPKM value ≥7, a $p<0.001$ fold change when comparing *Mbp*^{shi} versus *Mbp*$^{+/+}$ whole brains, and are sorted by fold changes.

Mbp expression loss in OPCs resulted in the perturbation of 10 transcripts all with low RPKM values and low changes, this is expected as *Mbp* is up-regulated upon exit of the OPC stage (Appendix 9-10). The loss of MBP in mature oligodendrocytes perturbs 387 transcripts, 311 of those transcripts are expressed at a lower abundance with the absence of MBP (Appendix 11-12) (Tables 2.3.10A and 2.3.10B along with 2.3.11A and 2.3.11B).

A. Genes sorted by *Mbp*$^{+/+}$ transcript RPKM

Gene	Fold change	*Mbp*$^{+/+}$ RPKM
Mbp	99.90	3150.00
Ugt8a	1.52	721.00
Ddr1	1.52	517.00
Mog	1.78	498.00
Phyhipl	1.54	417.00
Glul	1.56	415.00
Ptgds	2.92	377.00
Nrbp2	1.56	268.00
Cryab	2.72	252.00
Qdpr	1.70	239.00

B. Genes sorted by fold change for *Mbp*$^{+/+}$ transcripts ≥7 RPKM

Gene	Fold change	*Mbp*$^{+/+}$ RPKM
Mbp	99.90	3150.00
Stac2	5.75	7.18
Aqp1	3.95	9.78
Hrk	3.94	8.30
Eef1a2	3.54	7.88
Ly6a	3.36	17.10
Atp1a3	3.14	10.90
Ptgds	2.92	377.00
A830039N20Rik	2.85	12.70
Kcnma1	2.85	7.28

Table 2.3.10. Transcripts that have reduced expression in *shiverer* oligodendrocytes
RNA sequencing was performed on RNA isolated from *Mbp*^{shi} and *Mbp*$^{+/+}$ oligodendrocytes cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Mbp*$^{+/+}$ oligodendrocytes in A had a $p<0.001$ fold change when comparing *Mbp*$^{+/+}$ versus *Mbp*^{shi} in oligodendrocytes, and are sorted by RPKM value. Transcripts listed for *Mbp*$^{+/+}$ oligodendrocytes in B had a RPKM value ≥7, a $p<0.001$ fold change when comparing *Mbp*$^{+/+}$ versus *Mbp*^{shi} in oligodendrocytes, and are sorted by fold changes.

A. Genes sorted by *Mbp*^{shi} transcript RPKM

Gene	Fold change	*Mbp*^{shi} RPKM
Cyb5	1.72	153.00
Rap2a	1.76	85.80
Chst2	1.53	84.40
Sema6a	1.59	82.20
Sema6d	1.68	60.40
Mmd2	1.67	54.80
Dusp15	1.58	49.50
Cntn1	1.56	45.70
Mapre2	1.71	39.30
Fam107b	2.17	34.50

B. Genes sorted by fold change for *Mbp*^{shi} transcripts >7 RPKM

Gene	Fold change	*Mbp*^{shi} RPKM
Dct	6.32	16.50
Alox5	2.80	14.60
Fam107b	2.17	34.50
Daam2	1.93	9.02
Tmprss5	1.83	11.20
Capn5	1.80	24.70
Rap2a	1.76	85.80
Ndrg2	1.75	17.80
Kcna2	1.74	20.00
Id4	1.72	8.26

Table 2.3.11. Transcripts that have increased expression in shiverer oligodendrocytes

RNA sequencing was performed on RNA samples isolated from *Mbp*^{shi} and *Mbp*$^{+/+}$ oligodendrocytes cultured *in vitro* (n=2 for each genotype). Transcripts listed for *Mbp*^{shi} oligodendrocytes in A had a $p<0.001$ fold change when comparing *Mbp*^{shi} versus *Mbp*$^{+/+}$ in oligodendrocytes, and are sorted by RPKM value. Transcripts listed for *Mbp*^{shi} oligodendrocytes in B had an RPKM value ≥ 7, a $p<0.001$ fold change when comparing *Mbp*^{shi} versus *Mbp*$^{+/+}$ in oligodendrocytes, and are sorted by fold changes.

It is striking that approximately 75% of the transcripts that are reduced with loss of *Mbp* expression are also reduced with the loss of *Zfp191* expression (Fig. 2.3.4). Transcripts that have reduced expression include *Mbp*, *Mog*, *Ugt8a*, and *Fasn,* all key transcripts involved in oligodendrocyte development and maturation. When assessing the transcriptional differences detected in total brain RNA the comparison is even more striking as 25 out of 26 transcripts that have decreased expression with loss of *Mbp* expression are also decreased with loss of *Zfp191*$^{-/-}$ expression (Fig. 2.3.5A). Nevertheless, there are only 4 transcripts that have increased expression in both mouse models of hypomyelination in the whole brain (Fig. 2.3.5B).

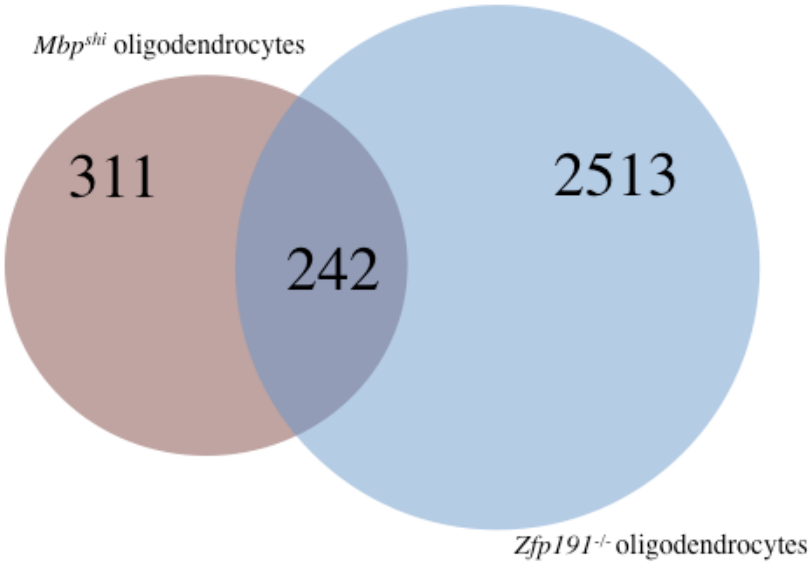

Figure 2.3.4. A Venn diagram comparing transcripts with decreased expression in *Mbp*^{shi} *oligodendrocytes* brain compared to *Mbp*$^{+/+}$ oligodendrocytes (red) and transcripts with decreased expression in *Zfp191*$^{-/-}$ oligodendrocytes compared to *Zfp191*$^{+/+}$ oligodendrocytes (blue).

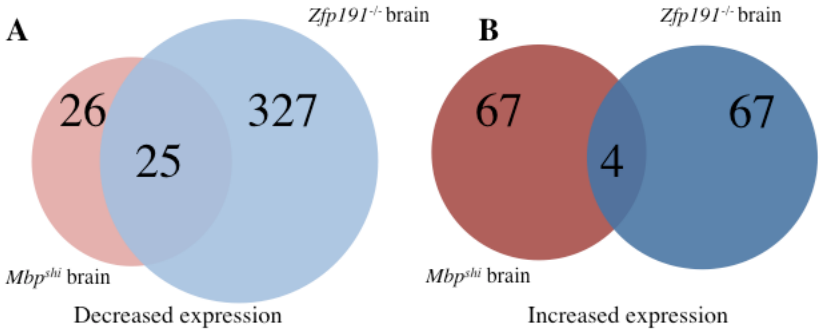

Figure 2.3.5. A Venn diagram comparing transcripts (A) with decreased expression in *Mbp*^{shi} whole brain compared to *Mbp*$^{+/+}$ whole brain (red) and *Zfp191*$^{-/-}$ whole brain compared to *Zfp191*$^{+/+}$ whole brain (blue). A Venn diagram comparing transcripts (B) with increased expression in *Mbp*^{shi} whole brain compared to *Mbp*$^{+/+}$ whole brain (red) and *Zfp191*$^{-/-}$ whole brain compared to *Zfp191*$^{+/+}$ whole brain (blue).

2.3.7 Disrupted cholesterol biosynthesis is a transcriptional fingerprint of hypomyelination

The cholesterol biosynthetic pathway is disrupted in the *Zfp191* and *Mbp* mutants examined here. Table 2.3.12 shows that both mouse models have reduced expression of a number of transcripts required for proper cholesterol biosynthesis, including the rate-limiting enzyme HMG-CoA reductase (HMGCR). The disruption of the cholesterol biosynthesis pathway in these two models suggests that this is a transcriptional signature of hypomyelination (Fig. 2.3.6). Interestingly, the expression level of these transcripts was not perturbed in isolated *Zfp191*^{null} or *shiverer* OPCs or oligodendrocytes, further suggesting that the observed perturbation in the cholesterol biosynthetic pathway is secondary to the lack of myelin production.

To confirm the expression levels of key cholesterol biosynthetic transcripts are perturbed (along with validation of other perturbed transcripts in both genotypes), reverse-transcriptase polymerase chain reaction (RT-PCR) was performed (Fig 2.3.7).

	Zfp191$^{-/-}$ brain		*shiverer* brain	
Gene	**Fold change**	**RPKM for *Zfp191*$^{+/+}$**	**Fold change**	**RPKM for *Mbp*$^{+/+}$**
Lss	2.13	7.50	1.72	6.62
Dhcr7	1.71	9.78	1.60	8.53
Sc5d	1.82	29.50	1.59	19.30
Sqle	1.82	26.50	1.68	24.90
Cyp51	1.99	23.20	1.93	20.90
Sc4mol	2.21	16.80	2.01	14.10
Hmgcr	1.66	28.20	1.60	29.90
Fdft1	1.80	19.00	1.61	14.30
Mvd	1.76	9.34	N.S.	7.47
Nsdhl	1.70	7.97	N.S.	9.17

Table 2.3.12. Genes perturbed in fatty acid degradation for cholesterol production. RNA sequencing was performed on *Zfp191*$^{+/+}$ and *Zfp191*^{null} whole brain RNA samples along with *Mbp*$^{+/+}$ and *Mbp*^{shi} whole brain RNA samples (n=2 for each genotype). Transcripts listed had a RPKM value ≥0.5, a ≥1.5-fold change when comparing *Zfp191*$^{+/+}$ versus *Zfp191*^{null} and *Mbp*$^{+/+}$ versus *Mbp*^{shi} in whole brains, and $p<0.001$. N.S. = not significant.

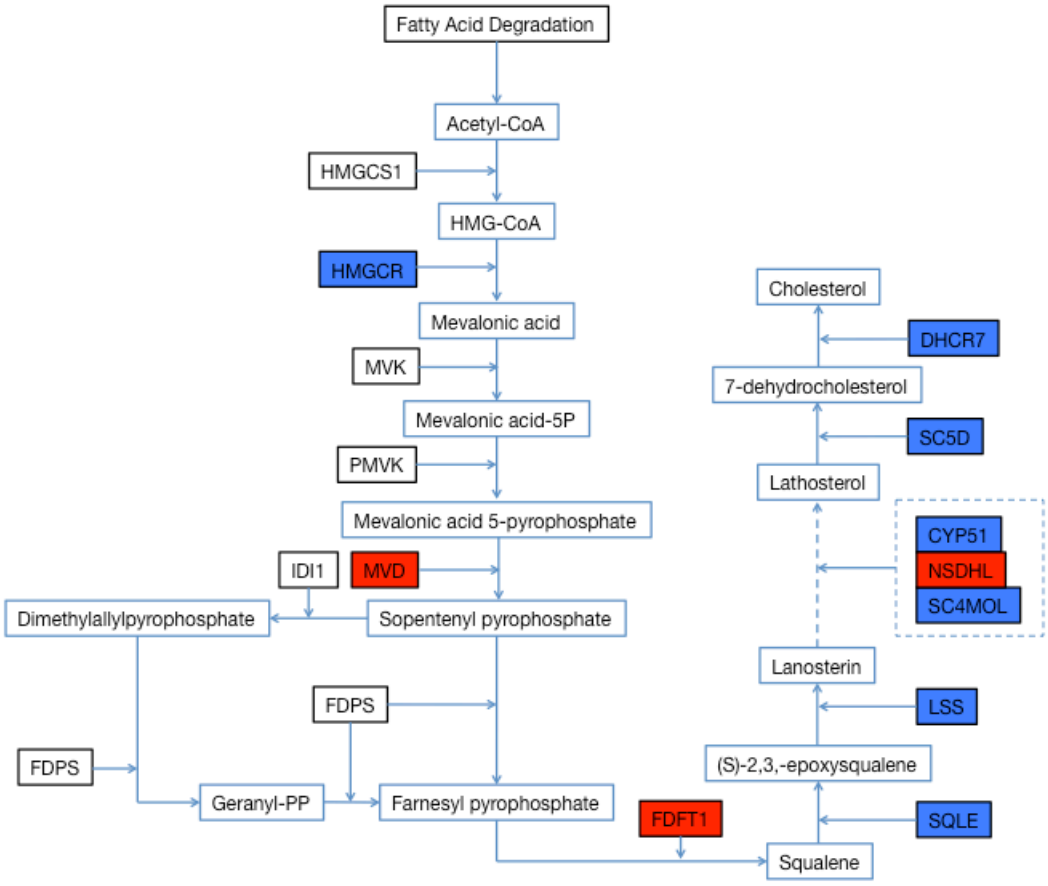

Figure 2.3.6. Cholesterol biosynthesis pathway transcripts are disrupted in hypomyelinated brains. Boxes outlined in black are the transcripts involved in this pathway. Boxes outlined in blue are the chemical compounds involved in this pathway. Boxes filled in with red are transcripts that have reduced expression in the *Zfp191*^{null} whole brain. Boxes filled in with blue are transcripts that have reduced expression in both the *Zfp191*^{null} and *shiverer* whole brains.

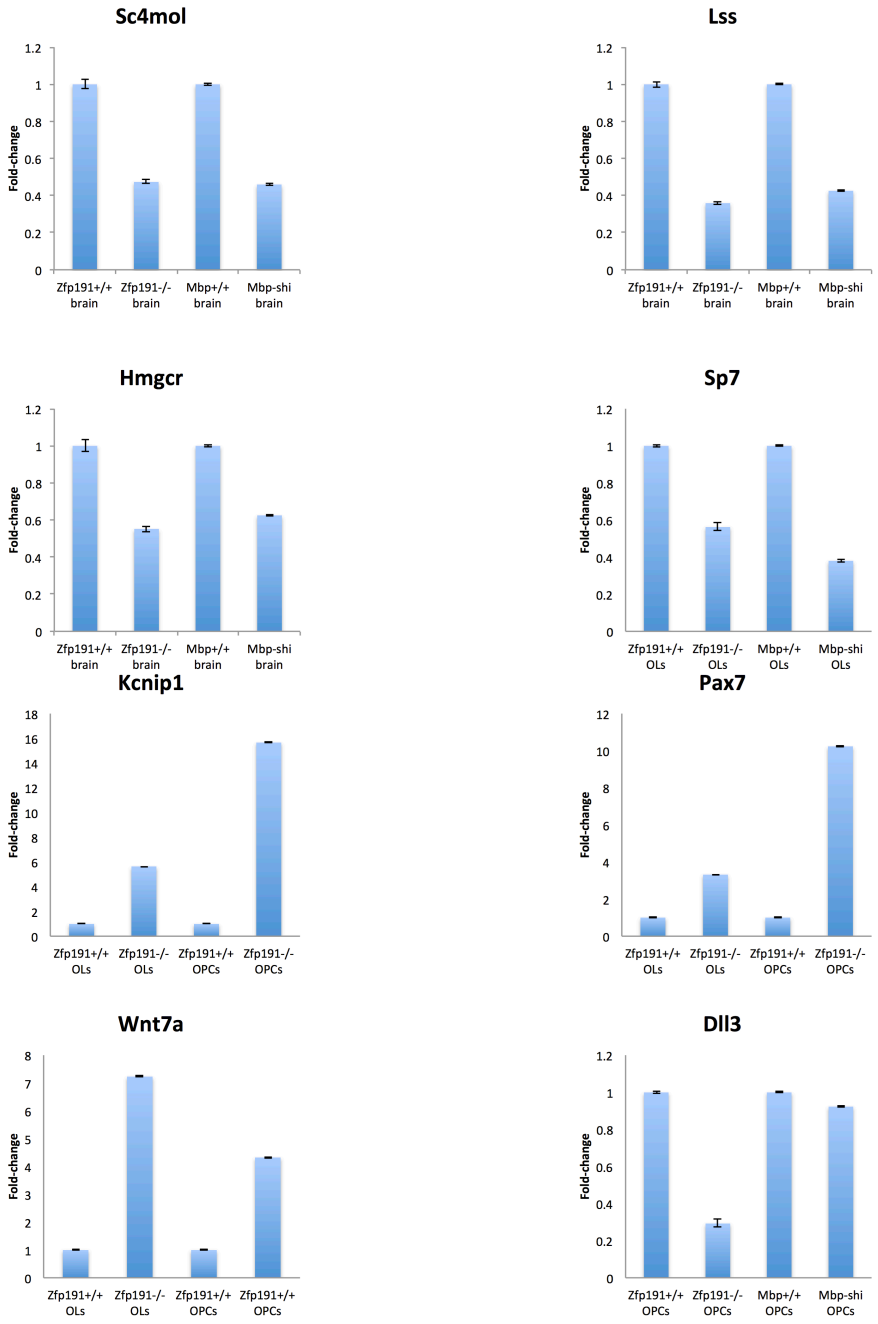

Figure 2.3.7. Relative fold-changes of selected transcripts for verification. Relative fold-changes from RT-PCR. N = 2 for isolated cells. N = 4 for isolated whole brains. Error bars are standard error of the mean. *Hprt* was used as baseline.

2.4 Discussion

In the current study I examined the role of the putative transcription factor ZFP191 on the transcriptome using massively parallel sequencing of RNA isolated from total brain, as well as isolated oligodendrocyte lineage cells (Howng et al. 2010). I found that the loss of ZFP191 on the P21 whole mouse brain was able to significantly perturb the transcriptome. A majority of the transcripts that had decreased expression with loss of *Zfp191* are associated with the oligodendrocyte lineage cells, which is in agreement with previous work showing that *in vitro* ZFP191 deficient cells are unable to myelinate properly and the CNS itself is hypomyelinated (Howng et al. 2010). The transcripts that increased with the loss of ZFP191, fewer than those that decreased, are transcripts associated with gliosis. The gliosis signature could be from the loss of *Zfp191* in astrocytes and/or microglia and/or it could reflect a secondary response improper oligodendrocyte function and hypomyelination.

The transcriptome of OPCs was also perturbed with loss of ZFP191 that implies that ZFP191 has a function early on in the generation and specification of OPCs. The loss of *Zfp191* expression in cultured oligodendrocytes results in a perturbation of ~5,500 transcripts. Many transcripts involved in the generation of myelin were decreased with loss of *Zfp191* expression such as *Mbp*, *Mog, Mobp* and *Cnp.* The majority of transcripts that had lower expression in the *Zfp191*$^{-/-}$ whole brain were also lower in *Zfp191*$^{-/-}$ oligodendrocytes suggesting that perturbation of the oligodendrocytes is the primary reason for the perturbation in the whole brain.

Multiple transcription factors required for proper maturation and myelination were disrupted with loss of ZFP191. Due to the decreased levels of *Sox10* and *Myrf* this data raises the possibility that ZFP191 functions, at least in part, upstream of *Sox10* and *Myrf*. In addition there was a maintained expression of factors that should be turned off for proper differentiation such

as *Id2/4* and *Hes1/5* in *Zfp191*^{null} oligodendrocytes. ZFP191 may play a direct role in downregulating these transcripts or this may represent a secondary effect associated with the absence of ZFP191.

In addition to disrupting transcriptional networks, the loss of ZFP191 perturbs several signaling cascades such as Notch, Erbb, MAPK and Wnt signaling cascades. One striking example is that the loss of *Zfp191* allows for elevated expression of *Wnt7a* compared to the cells with ZFP191. WNT7A has been shown to affect the developing brain as both an autocrine and a paracrine signaling molecule (Qu et al. 2013). WNT7a is able to function to ensure maintenance of the progenitor state and prevent differentiation. In this way ZFP191 may be affecting other cell types by perturbing excreted signals along with the proper cell-cell signaling cascades.

Although the loss of MBP, and by extension myelin, perturbs the transcriptome of the samples I investigated, this perturbation was of a lower magnitude than the loss of ZFP191. In keeping with this smaller perturbation transcriptional networks and signaling cascades were not as affected as the loss of the transcription factor ZFP191. One caveat is that *Id2/Id4* had inappropriately high expression with loss of MBP suggesting there may be a feedback mechanism to communicate the state of the myelin sheath or at least its initiation. It is important to note that overall a majority of the transcripts that are perturbed with loss of *Mbp* expression are also perturbed with loss of *Zfp191* expression which may imply that ZFP191 acts upstream of *Mbp*.

The absence of myelin in the *Zfp191*^{null} and *shiverer* mouse whole brains results in the reduction of several transcripts that encode key proteins in the cholesterol and lipid biosynthetic pathways. The loss of either ZFP191 or MBP results in reductions of *Hmgcr*, which encodes the rate-limiting enzyme in cholesterol biosynthesis. This reduction along with reduced expression

of other genes that are key in the biosynthetic pathway suggest that oligodendrocytes have a feedback system that allows them to regulate myelin lipid synthesis depending on their myelinating state. The control of cholesterol homeostasis is critical for myelination (Saher et al. 2005, Verheijen et al. 2009), and defects in lipid synthesis have been linked to demyelination (Rolyan et al. 2015). The decrease of key lipid biosynthesis transcripts in these models of hypomyelination may be due to a direct effect on oligodendrocytes, or it may reflect a secondary effect of oligodendrocyte perturbation on the homeostatic production of cholesterol by the CNS.

ZFP191 is a putative transcription factor that plays a required role in CNS myelination (Emery 2010b, a, Howng et al. 2010, Yu et al. 2013, Kuspert and Wegner 2015). My experiments demonstrate that ZFP191 plays a key role in the transcriptional networks that controls oligodendrocyte development and myelination. Loss of ZFP191 disrupts the entire oligodendrocyte lineage but oligodendrocytes in particular. ZFP191 may function upstream of *Sox10* and *Myrf* to help modulate the transcriptional network and by extension the signaling cascades used to generate myelin. ZFP191 also has a demonstrated role in allowing the correct amount of cholesterol transcripts to be upregulated. Therefore ZFP191 is a central transcriptional regulator of oligodendrocyte development and myelination.

Chapter 3. Identification of ZFP191 binding sites and function

3.1 Introduction

ZFP191 is required for myelination and is a putative transcription factor due to its zinc-finger protein domain and its ability to bind DNA *in vitro* (Howng et al. 2010, Liu et al. 2012, Wang et al. 2008). Although zinc-finger domains are widely studied, there are currently no high quality algorithms for predicting the DNA binding site in the genome based on the protein's amino acid sequence. To begin to determine how ZFP191 modulates oligodendrocyte maturation and myelin production it is critical that we understand where in the genome the protein binds as this may reveal genes under its transcriptional control. Currently we have no known downstream targets of ZFP191. Previous studies have used ZFP191 mutants or truncations of ZFP191 for *in vitro* analysis of synthetic sequences (Harper et al. 2007). With the ability to query binding in the whole genome using chromatin immunoprecipitation with massively parallel sequencing (ChIP-seq) it has become easier to interrogate where ZFP191 is able to bind in the genome. Using this data it is also possible to discover sequences that may represent ZFP191's binding motif.

To determine where ZFP191 binds *in vivo*, ChIP-sequencing was performed on cultured oligodendrocyte lineage cells. Sequences identified were examined using a "motif-finder" to discover DNA sequences that were present at a higher degree than chance. This DNA sequence likely corresponds to the nucleic acid sequence where ZFP191 binds in the genome. Both OPCs and oligodendrocytes of wild-type cells were examined to explore the occupancy of ZFP191 throughout the cell lineage. This data will also be compared to our currently acquired data from Chapter 2 to determine bona fide downstream targets of ZFP191 (in Chapter 4). In Chapter 2 many genes in oligodendrocytes lacking ZFP191 failed to be transcribed at appropriate levels

when compared to *Zfp191*$^{+/+}$ oligodendrocyte mRNA levels. This fact along with the understanding that many zinc-finger proteins have a stereotyped binding motif may mean that ZFP191 has a discrete binding sequence and may function (at least partially) as a transcriptional activator.

3.2 Materials and Methods

3.2.1 Chromatin Isolation and Immunoprecipitation

Oligodendrocyte precursor cells or mature oligodendrocytes from wild-type mice along with *Zfp191*$^{-/-}$ mature oligodendrocytes were isolated as before (section 2.2.2)(Emery and Dugas 2013). 15 million OPCs were fixed by adding 1/10 of a formaldehyde solution (37% formaldehyde (Sigma #F-8775), 5M NaCl (Sigma S-7113), 0.5M EDTA, pH 8.0 (Cellgro 46-034-Cl), 1M HEPES, pH 7.9 (Gibco #15630)) to existing media. Plates were then agitated for 15 minutes at room temperature. The fixation was stopped by adding 1/20 volume glycine solution (2.5M glycine (Sigma G-7403)) to the existing media in each plate. The plates then sat for 5 minutes without agitation and then scrapped into a 50mL conical tube on ice. Tubes were spun at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells. The supernatant was removed and the pellet was resuspended in 10 ml chilled PBS with 0.5% Igepal CA-630 (Sigma I-8896) per tube by pipetting and cells were combined into one tube. Cells were again centrifuged at 800 x g in a refrigerated centrifuge for 10 minutes to pellet the cells. The supernatant was again removed and the pellet was resuspended in 10 ml chilled PBS with 0.5% Igepal CA-630 (Sigma I-8896). To inhibit proteases, 1mM PMSF (Sigma P-7626) in was added. Cells were centrifuged again for a third time at 800 x g in a refrigerated centrifuge for 10 minutes and then snap frozen on dry ice. Cells were then processed by Active Motif (Carlsbad, CA) for

FactorPath ChIP-sequencing in a proprietary manner. For precipitation a rabbit anti-ZNF24 polyclonal antibody was used (Sigma HPA024062).

3.2.2 ChIP-seq Peak Detection and Analysis

The Model-based Analysis for ChIP-seq (MACS) version 1.4 tool was used to identify regions of the genome, henceforth called peaks, which were enriched for precipitated DNA (Feng et al. 2012, Feng, Liu, and Zhang 2011). MACS options were: tag size 36, band width 300, MFold 20 and build model to get primary binding regions. Primary regions were further filtered: the p value cutoff was set to $<1x10^{-5}$ and an enrichment of 5. *Zfp191*$^{+/+}$ ChIP OPC and OL were inputs and *Zfp191*$^{-/-}$ served as control. MACS removed redundant peaks, regions of high enrichment compared to the genomic background using 1000bp scrolling window around highly enriched regions for comparison. From this, peaks of specified p-value will be "called" as significant. Peaks with a p-value $< 1x10^{-5}$ were deemed significant. MACS assigned a false discovery rate (FDR) of which 5% or less was considered acceptable. A summary of the peak significance and quality is shown in Table 3.3.1. Browser Extensible Data (BED) files of the intervals, peak coordinates and summits were compiled for viewing the data on the UCSC browser.

3.2.3 ChIP-seq Peak Analysis

Multiple analyses were performed to assess the potential of ChIP-seq peaks to act as enhancers. Genomic distribution analysis was performed by the Cis-regulatory Element Annotation System (CEAS) (Shin et al. 2009). CEAS compares ChIP-seq data tag base-pair distribution to that of the normal genome using the UCSD mm9 genome for comparison. CEAS

outlined the distribution of peaks at 1000, 2000, and 3000bp upstream of the transcriptional start site, introns, exons and UTRs. ChIP-seq tag counts were uploaded and visualized using the UCSC genome browser (Rosenbloom et al. 2015). Plots were generated using the CEAS toolkit on the Galaxy/Cistrome module (Blankenberg et al. 2010, Goecks et al. 2010, Liu et al. 2011). Peak2gene in Cistrome was used to identify genes.

3.2.4 Transcription Factor Motif Analysis

Peaks from both ChIP-seq experiments were analyzed for *de novo* and known motifs using the MEME-ChIP suite from the National Biomedical Computation Resource (Bailey et al. 2009, Bailey et al. 2015, Machanick and Bailey 2011). MEME-ChIP utilized MEME, DREME and Centrimo to identify motifs that are enriched in each dataset (Bailey and Machanick 2012, Machanick and Bailey 2011, Bailey et al. 2010). Each motif identified was given an expect value (E-value), and an E-value of 0.01 or lower was considered significant. Significant motifs were then scanned through the TOMTOM database to identify known motif matches (Gupta et al. 2007). Each motif was given a p-value match to an identified motif, and those lower than 0.01 were considered significant. Finally, FIMO or MAST was used to identify the genomic locations of individual motifs within ChIP-seq peaks using 100bp sequences centered around the summits of called peaks (Grant, Bailey, and Noble 2011, Bailey and Gribskov 1998).

3.2.5 ChIP-Real-time PCR

Liftover was performed using the UCSD genome databases to convert the identified mouse genome sequences (mm9) into rat genome sequences (rn4). For validation of peaks found in ChIP-seq, ChIP-RT-PCR was used performed using rat oligodendrocytes cultured *in vitro*

transfected with a FLAG-tagged ZFP191 expression construct (Origene MR205667) (similar to previously described in Section 2.2.2). To isolate and amplify immunoprecipitated DNA ChIP-IT Express (Active Motif #53008) was used with minimal modification to manufacturer's protocol. Briefly, cells were fixed and pelleted following the same procedure used for ChIP-seq above. The use of hypertonic solutions, agitation and centrifugation was used to isolate the chromatin. The cells were then sonicated for 45 minutes on ice with 30 seconds of sonication and 30 seconds of rest for each cycle. The sheared chromatin was centrifuged for 10 minutes at 18,000 x g at 4C. The supernatant was transferred to an ice-cold fresh 1.7mL microcentrifuge tube and stored at -80C until use. The chromatin was quantified and shearing was verified. The precipitation was according to the manufacture's protocol using non-precipitated chromatin as input. 5ug of anti-FLAG (Sigma F1804 or anti-OLIG2 antibody (R & D Systems AF2418) was used. For real-time PCR 2uL of precipitated DNA and "input DNA" was amplified using iQ SYBR Green Supermix (BioRad #170-8882) in a BioRad C1000 thermocycler with a CFX96 real-time system attachment. Primers are listed in a Table 3.2.1. p21 was used as a positive control (Ligon et al. 2007).

Transcript	Forward	Reverse
Sox10 - Distal	GCAGAGGACATGGTTGTGGA	CTGCCTCAGACTGCTGACAA
Sox10 - Peak 1	CATACCCAAGGGCTCTCTGC	TCCACAACCATGTCCTCTGC
Sox10 - Peak 2	GGGTAGGTCACAACCCACTG	TGCCATCCTTTGTGTGTGGT
Zfp536	CGGCTCCTCTAACGTGACTG	CCTCGCCGATGTCTGAAGAA
Elovl7	TGCTTATACAGCGTGCACCA	TGGACTGAGGATTGAACTCAGA
Smad7	AGGATCTTGTCCCCGAGCAG	CTGCGTCTCAGGCAGCTCTC
p21	AGGTGTCTAGACTCCAGATT	AAAATCAAGGCTTTGCTGG

Table 3.2.1. Primers used for qPCR validation of ChIP-seq peaks. Summary of the primers used for ChIP-qPCR validation of peaks identified by MACS in ChIP-seq. Each peak produced an amplicon of 115-120 base pairs from sonicated DNA.

3.2.6 EMSA

Nuclear extract was prepared from HEK cells transfected with a ZFP191 expression plasmid with a GFP tag (Origene MG205667) using the NE-PER nuclear protein extraction kit (Pierce, Rockford, IL #78833), according to manufacturer's instructions. Briefly, cells were lysed in hypotonic CER buffer with added protease inhibitors and the cytoplasmic fraction was separated by centrifugation. The nuclear pellet was resuspended in hypertonic NER buffer with protease inhibitors and incubated on ice for 40 minutes and vortexed every 10 minutes. For nuclear extracts, the soluble proteins in the lysate were separated by centrifugation. Biotinylated probes were purchased from IDT DNA. Probes were purchased with the complimentary strand:

TCAT – TCATTCATTCATTCATTCATTCATCAT.

Scramble – AGAGTGGTCAATACCCCCTCTG.

Mbp – GTT TTG TTA ATG CAT TTA ATT CCT CAC AGT ATA GCT GTT GTC TTC TCT CCT CAT CTG CGT TCG TTT CTG ACA GCT ACA GAA TTT AT.

SOX10 – GGT AGT TGC TTC ATT CAT TCA TTC ACT CAT TCA TTC ATT CAT TCA TTC ATT CAT TCA TTC ATC TTG TTG GAG TTT CCT TTA GTA CA.

The LightShift Chemiluminenscent EMSA kit was used for all reactions (Pierce 20148). Briefly buffer, water, 2ug dI/DC were mixed with 5ug of nuclear extract. 1.5pmol of biotinylated probe was added and mixed. For reactions that contained the unlabeled “cold” probe this was added in 200-fold excess and mixed before the labeled probe was added. All 20uL were incubated for 20 minutes at room temperature. During incubation 5% TBE PAGE gels were run at 100V in 0.5X TBE (Bio-Rad #456-5015). After incubation 5uL of DNA running dye as added to each sample and 20uL was added to the gel. The samples were run for 60-90 minutes at 100V in 0.5X TBE. The gels were then transferred to Biodyne B nylon membranes (Thermo Scientific #77016) in

0.5V at 380mA for 45-60 minutes. Membranes were either immediately developed or sealed in a bag with 0.5X TBE. Membranes were developed using LightShift Chemiluminiscent EMSA kit (same as above). The protocol consisted of blocking for 15 minutes, incubated with a supplied primary streptavidin antibody attached to horseradish peroxidase for 15 minutes, washed, equilibrated and then exposed to a peroxidase solution. The membranes were then viewed on a Bio-Rad Gel Doc.

3.2.7 Cloning and Luciferase Assays

Putative enhancers (*Sox10* and *Mbp* related sequences) were amplified by PCR and inserted in to the pGL3-promter (Promega, E841A), with luciferase driven by a strong SV-40 promoter. Primers: Sox10 forward 5'-GGA CAT ATC CAA GGG CTC TG-3', Sox10 reverse 5'-TGC CAT GCA CAT CAG TAT CC-3', MBP forward 5'-TGT GCC TTT CCA GCA CTG T-3', MBP reverse 5'-CTG AGG TGC TGT GGA AAG GT-3'. MBPshort-For 5' – ctctcctcatctgcgttcgtt – 3' MBPshort-For 5'-aggaacagtgccagcaaaca-3' insertions were sequenced to check for PCR error. The pGL3-control was used as a positive control.

Transfection protocol was adapted from Amaxa. Subconfluent cells were grown in 24-well tissue culture plate, and were co-transfected using Nucleofection Amaxa O-17 with 320 fmol of enhancer-pGL3 vector and 50fmolof control cGFP. Cells were allowed to grow to recover in proliferation media for 24 hours and then switched to differentiation media for 48 hours. Cells were then lysed and assayed with the Dual-Luciferase Assay kit from Promega (E1910) . Relative light was measured using the BioTek Synergy plate reader. *GFP* activity was assayed using HORIBA FluoroLog-3 Spectrofluorometer. Standard deviation was calculated and statistical comparison was done with Students t-test.

3.3 Results

3.3.1 ChIP-sequencing of ZFP191 in *Zfp191*$^{+/+}$ OPCs and OLs

Currently there are no known downstream targets of ZFP191. One method of examining potential downstream targets of potential transcription factors is to use ChIP-sequencing (Todeschini, Georges, and Veitia 2014). Cultured mouse *Zfp191*$^{+/+}$ OPCs, *Zfp191*$^{+/+}$ oligodendrocytes, and *Zfp191*$^{-/-}$ oligodendrocytes were isolated and ChIP-seq was performed using an antibody to ZFP191. Precipitated DNA was then aligned to the mouse genome from UCSD. After these sequences were collected and aligned, software was used to assess (also termed "calling") significant regions of precipitated DNA where ZFP191 likely bound.

Model-based Analysis for ChIP-Seq (MACS) was used to call for regions of ZFP191 immunoprecipitated DNA enrichment, termed as peaks. MACS was able to call 3,998 peaks for *Zfp191*$^{+/+}$ OPCs using *Zfp191*$^{-/-}$ oligodendrocytes as a control (this comparison will be referred to as the OPC dataset) and 765 peaks for *Zfp191*$^{+/+}$ oligodendrocytes using *Zfp191*$^{-/-}$ oligodendrocytes (this comparison will be referred to as the oligodendrocyte dataset), with a p-value $<1x10^{-5}$ (Table 3.1) (Feng et al. 2012, Feng, Liu, and Zhang 2011). Using *Zfp191*$^{-/-}$ oligodendrocytes as a control allowed us to account for any off-target binding of the antibody due to these cells lacking ZFP191. *Zfp191*$^{-/-}$ oligodendrocytes was used as a control rather than *Zfp191*$^{-/-}$ OPCs because there was a greater perturbation with loss of ZFP191 in oligodendrocytes than in OPCs meaning that ZFP191 is likely binding to more areas in the genome in oligodendrocytes. Therefore this control was used to ensure that at the potential maximum about of ZFP191 binding that any peaks seen in the *Zfp191*$^{-/-}$ oligodendrocytes were removed. There were more ZFP191 peaks called in the OPC dataset potentially due to the increased number of

unique reads and possibly due to a "sono-seq" effect in OPCs. The OPCs are actively dividing cells and therefore have more "open" chromatin than post-mitotic cells therefore allowing OPC DNA to be fragmented by sonication more easily which could explain the greater number of called peaks (Auerbach et al. 2009). Regardless of the difference in the detected peaks between the two samples, only peaks that have a p-value $<1x10^{-5}$ were used.

Cis-regulatory Element Annotation System (CEAS) was used to analyze identified peaks in both datasets (Shin et al. 2009, Ji et al. 2006). CEAS reported there was a significant enrichment of ZFP191 binding at gene promoters, defined in this instance as 3000bp from the transcription start site, when compared with the normal mouse genomic distribution. The annotated mouse genome from the UCSD genome browser calculates this region as representing ~1.1% of the total genome. ZFP191 identified peaks found in the OPC dataset were in the annotated promoter regions of the genome for 14.5% of the total peaks (Figure 3.1A) (p-values of $<1x10^{-322}$). For the oligodendrocyte dataset, ZFP191 peaks were detected 6.3% in the annotated promoter region (Figure 3.1B) (p-value $<1x10^{-62}$). Consistent with this finding both datasets have their distribution of MACS called peaks slightly upstream of the transcriptional start site (Figure 3.1C and 3.1D). This increase in occupancy for ZFP191 suggests it is near regions where it could have a role in regulation as a transcription factor. In both datasets no other region of the genome was as enriched for ZFP191 detected peaks as compared to the 3000bp region upstream of the transcriptional start site. However ZFP191 may play a role in exerting transcriptional control further from the transcriptional start site so all MACS called peaks will be examined as potential areas for ZFP191 interaction.

Sample	Number of Reads	Number of Alignments	Unique Alignments	Peaks
WT OPCs	32,751,168	19,839,196	15,591,084	3,834
WT OLs	23,788,263	19,744,582	7,417,927	653
KO OLs	26,399,656	15,914,969	5,499,174	

Table 3.3.1 Summary of ChIP-seq reads, mapping and MACS peak analysis

ChIP-seq reads were aligned to the mm9 genome by the Eland Alignment tool and only uniquely mapped reads were retained. MACS was used to determine regions of enrichment of ChIP-seq reads. OLs = oligodendrocytes. WT = *Zfp191*$^{+/+}$ KO = *Zfp191*$^{-/-}$

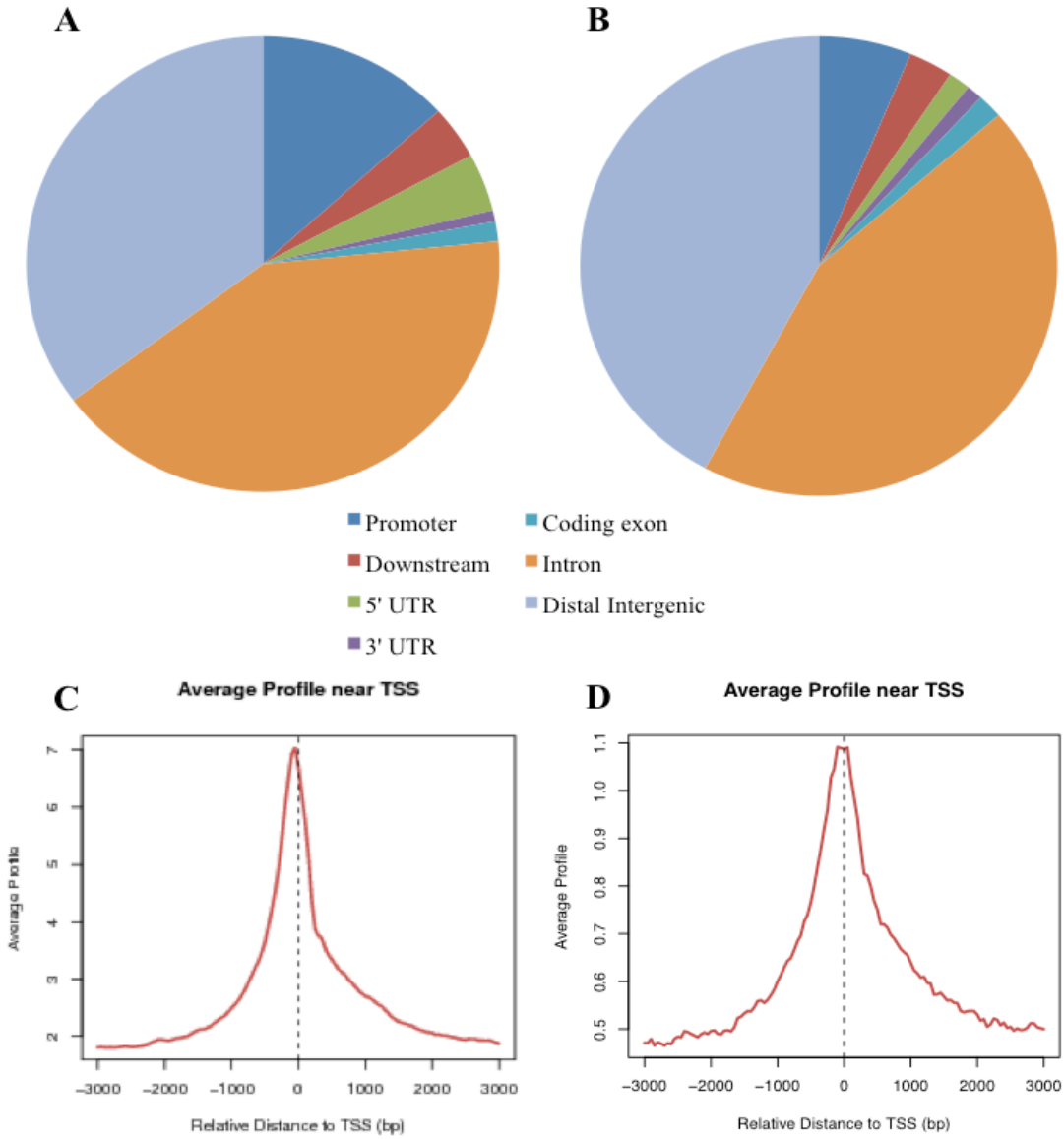

Figure 3.3.1. Cis-regulatory Element Annotation System analysis for ZFP191 MACS identified peaks.

A. Genomic distribution of called peaks in OPCs B. Genomic distribution of called peaks in oligodendrocytes. C. Average peak profile score over the +/- 3000bp span of annotated transcriptional start sites in OPCs. D. Average peak profile score over the +/- 3000bp span of annotated transcriptional start sites in oligodendrocytes.

3.3.2 Validation of MACS called peaks

For verification of peaks identified by MACS, ChIP-qPCR was performed. 7 peaks were selected from regions identified in MACS from both the OPC and oligodendrocyte datasets. To determine which peaks to use and which genes may interact with ZFP191 to control oligodendrocyte maturation and myelination, regions were chosen that have been shown play a key role in oligodendrocyte maturation and function. The MACS identified regions were near genes that include: *Sox10*, *Zfp536*, *Elovl7*, and *Smad7*. A region that was distal to a putative *Sox10* enhancer and not identified by MACS was used as a negative control. The *p21* sequence, which has been shown to interact with OLIG2, was used as a positive control (Ligon et al. 2007). Cultured OPCs and oligodendrocytes from rats were nucleofected with an expression vector for ZFP191 that also included a FLAG tag. ChIP was then performed using an antibody directed to the FLAG sequence or an antibody against OLIG2. In most cases there was a significant increase in fold-change over input DNA using qPCR on the ChIP-ed samples (Figure 3.3.2). However, the peak *Sox10-Peak 1* did not show an increase over input DNA. In addition it appears the region around the *Smad7* peak was not as robust as the other regions although it did show a fold-change increase compared to input DNA. This analysis may not fully reflect the ChIP-seq data, as the areas used for ChIP-qPCR were significantly shorter than the identified ChIP-seq peaks. Despite this, these regions were verified, and a set of these peaks was used for further analysis. These sequences were also analyzed for common DNA motifs to examine if they were candidates for biochemical and gene reporter assays.

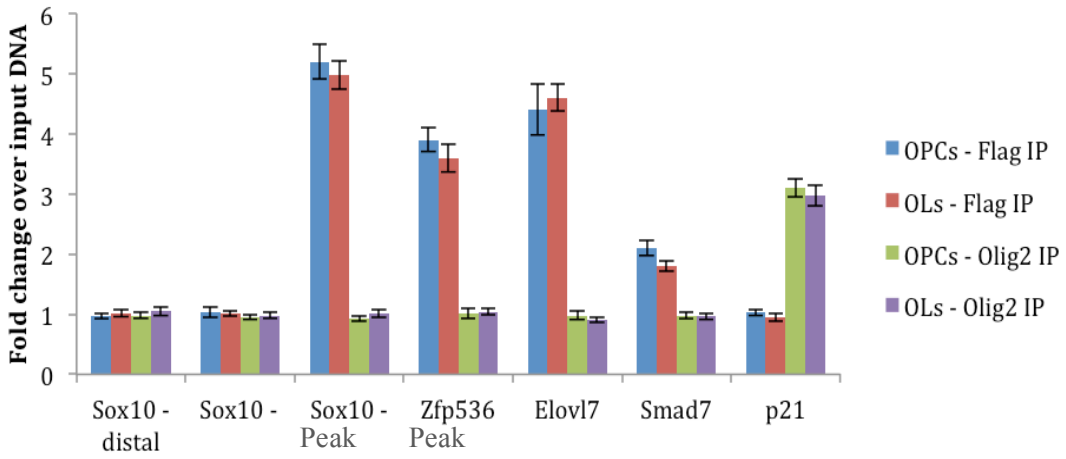

Figure 3.3.2 ChIP-qPCR of peaks identified by MACS. ChIP was repeated both rat OPCs and OLs. Samples were normalized to input chromatin and comparative CT analysis was performed. Error bars are standard deviation. N = 3. Flag IP = immunoprecipitation using an anti-FLAG antibody. Olig2 IP = immunoprecipitation using an anti-OLIG2 antibody.

3.3.3 Determination of ZFP191 binding sequence

Zinc-finger transcription factors bind to DNA that often contains a specific sequence of base pairs. To date there have been no good tools to *a priori* predict binding sites of a protein based on its amino acid sequence (Persikov et al. 2014, Persikov and Singh 2014). One method to characterize a potential binding site is to examine multiple sequences in regions where the protein of interest or transcription factor is known to interact. Using the data collected from ChIP-seq, peaks were analyzed to determine ZFP191's DNA binding sequence. Sequences that were called by MACS were pruned to 100bp centrally-enriched around the summit of the sequence. The summit of the sequence is the area of the peak that had the deepest level of sequencing and is therefore the area where the transcription factor is thought to predominantly bind. Sequences from the ZFP191 ChIP-seq from both OPCs and oligodendrocytes were used separately with the sequences from the *Zfp191*$^{-/-}$ oligodendrocytes as a negative control to account for any off-target antibody interaction. Sequences were then bioformatically analyzed using MEME SUITE. MEME SUITE (Bailey et al. 2015, Bailey et al. 2009) is a suite of software tools used to analyze, discover and characterize potential motifs in a variety of sequences including DNA or RNA. MEME SUITE is able to identify known and *de novo* sequences. Using this software, a potential ZFP191 binding motif was determined (Figure 3.3.3). The sequence characterized is an 8-21bp repeat of the nucleic acids thymine-cytosine-adenine-thymine (abbreviated as TCAT). In both the OPC and oligodendrocytes datasets this was the highest ranking and most significant sequence. To further confirm that this was a potential ZFP191 binding site the 600 sequences with the strongest MACS scores from the OPC dataset and the 100 sequences with the strongest MACS scores in oligodendrocytes were re-analyzed. The sequence was again confirmed as a TCAT repeat in both cases. Submission of the TCAT

Figure 3.3.3. MEME motif generated from MACS called peaks analyzed by MEME SUITE. The predicted motif, in 2-bit format sequence logo format, was found in motifs ranging from 43-8bp all with the TCAT repeat (Schneider and Stephens 1990). P-value <0.0001.

repeat sequence to TOMTOM, software that compares motifs to known sequences in the UniPROBE and JASPAR databases, found that this sequence is uncharacterized (Gupta et al. 2007). To determine which sequences were likely candidates for further analysis, of the top 600 sequences with the most enriched MACS values in the OPC data set, 165 sequences had a perfect match on a 21bp TCAT repeat with an E-value of $1.4^{10^{-1076}}$. Using the 8bp TCAT repeat and the same sample set 263 sequences were determined. Of the 100 sequences with the most enriched MACS values in the oligodendrocyte dataset, 48 had the 21bp TCAT repeat with an E-value of $1.6^{10^{-217}}$. (E-values less than 10 and with a p-value <0.0001 are significant). The TCAT repeat motif is therefore highly reproducible in two different datasets with multiple changes in stringencies. Sequences that contain the TCAT repeat will be further examined in assays to determine ZFP191's ability to interact with these genomic sequences *in vitro* and *in vivo.*

Figure 3.4 shows tracks assembled from the UCSD genome browser of potential candidates. *Sox10*, *Myrf*, and *Elovl7* have multiple identified peaks by MACS (green bars) and each contain at least one TCAT repeat (red bars). Near *Sox10* the sequence of interest is 7 kilobase pairs (kbp) upstream of the transcriptional start site and there were 3 additional peaks identified ranging from 1-11kb upstream of the transcriptional start site; however, those peaks lacked the TCAT repeats. In *Myrf* the sequence of interest that contains a TCAT positive peak is in the first intron, which is a region that has been shown to be critical in the regulation of this gene (Hornig et al. 2013). *Elovl7* has multiple MACS identified both before the transcriptional start site and within intronic regions. The MACS identified peak that contains the TCAT repeat is 1kbp upstream of *Elovl7*'s transcriptional start site. Although these sites may not be bona fide

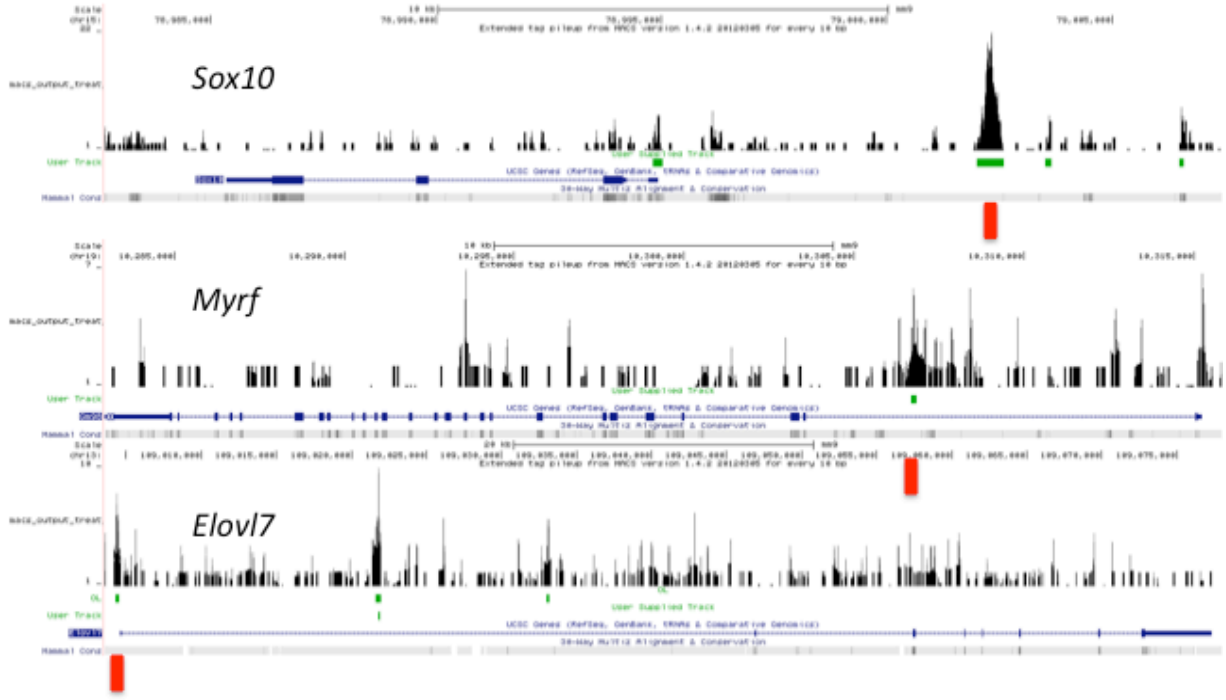

Figure 3.3.4. Track visualization of MACS peaks in selected genes. Gene tracks were visualized using the UCSD genome browser and overlaid with custom tracks corresponding to peaks identified by MACS peak calling (green lines). Some of these peaks have stretches of the TCAT repeat identified by MEME SUITE (red boxes).

ZFP191 binding sites, they imply that ZFP191 may not be functioning at promoter sequences (or at least exclusively) and may be impacting transcriptional regulation by interacting in enhancer regions possibly through interacting with other factors through the SCAN domain.

3.3.4 Validation of ZFP191 binding site biochemically

Due to the lack of known downstream targets it was important to verify that ZFP191 can bind sequences characterized from the ChIP-seq and MACS/MEME SUITE analysis. One methodology to confirm biochemical interaction with a protein and a nucleic acid sequence is an electromobility shift assay (EMSA). An EMSA relies on the principle that protein-DNA complexes migrate slower than free DNA molecules when subjected to non-denaturing electrophoresis (Hellman and Fried 2007). If the DNA and the protein of interest interact they move slower through the gel matrix and the complex appears to be a shift from the free nucleic acids. Often DNA probes will be labeled with radioisotopes or biotin for detection. A double-stranded DNA probe of TCAT repeats was labeled with biotin as a positive control. The protein sample that was used was the nuclear fraction of HEK cells over-expressing a GFP-tagged ZFP191 expression construct. This lysate was subjected to western blot to ensure that ZFP191 was correctly translated. The protein lysate and labeled probe were allowed to interact and electrophoresed on a non-denaturing polyacrylamide gel. Next to this, free probe was allowed to run in its own lane so there would be confirmation to show the ZFP191-TCAT complex shift. Another reaction with the ZFP191and biotin-labeled TCAT mixture was incubated with 200-fold molar excess of unlabeled TCAT probe to verify that the shift seen was sequence specific. A probe that was known to not interact with any known proteins was also used as a negative

control in a separate reaction to account for any non-specific probe binding to ZFP191. Figure 3.5A validates that indeed ZFP191 is able to bind a 26bp biotin-labeled DNA probe composed of TCAT repeats. This interaction is sequence specific because the unlabeled probe outcompetes the ZFP191 interaction as shown by the absence of a shift in lane 3. In figure 3.5A lane 2 shows a shift of a band compared to lane 1. In addition, lane 5 in figure 3.5A is unable to interact with the ZFP191 lysate. This experiment demonstrates the ZFP191-TCAT interaction is not due solely to ZFP191 interacting with any DNA probe and it is specific to the TCAT repeat. Other labs have performed similar experiments albeit with the ZFP191 protein truncated to remove the SCAN domain and this work is in agreement with those results (Liu et al. 2012).

After validation that ZFP191 was able to interact with TCAT repeats, two genomic regions were selected that had peaks as determined by MACS and contained stretches of the TCAT repeat. The regions were 7kbp upstream of the transcriptional start site of *Sox10* and a region 5kbp upstream of the transcriptional start site of *Mbp*. Both probes were subjected to EMSA after being incubated with the isolated nuclear fraction of HEK cells overexpressing a GFP-tagged ZFP191. Figure 3.5B shows that in lane 2 (for the *Sox10* probe) and lane 6 (for the *Mbp* probe) there is a noticeable shift compared to the free probe for each, lane 1 and 5 respectively. Lanes 3 and 7, along with lanes 4 and 8 show that each probe could be outcompeted by an excess amount of the unlabeled TCAT probe or the unlabeled probe with the same sequence as the labeled probe. This experiment verifies that identified peaks by MACS and that contain TCAT repeats are able to interact with ZFP191 *in vitro*. These sequences were then further used to examine ZFP191's ability to interact *in vivo* and function as a transcriptional activator.

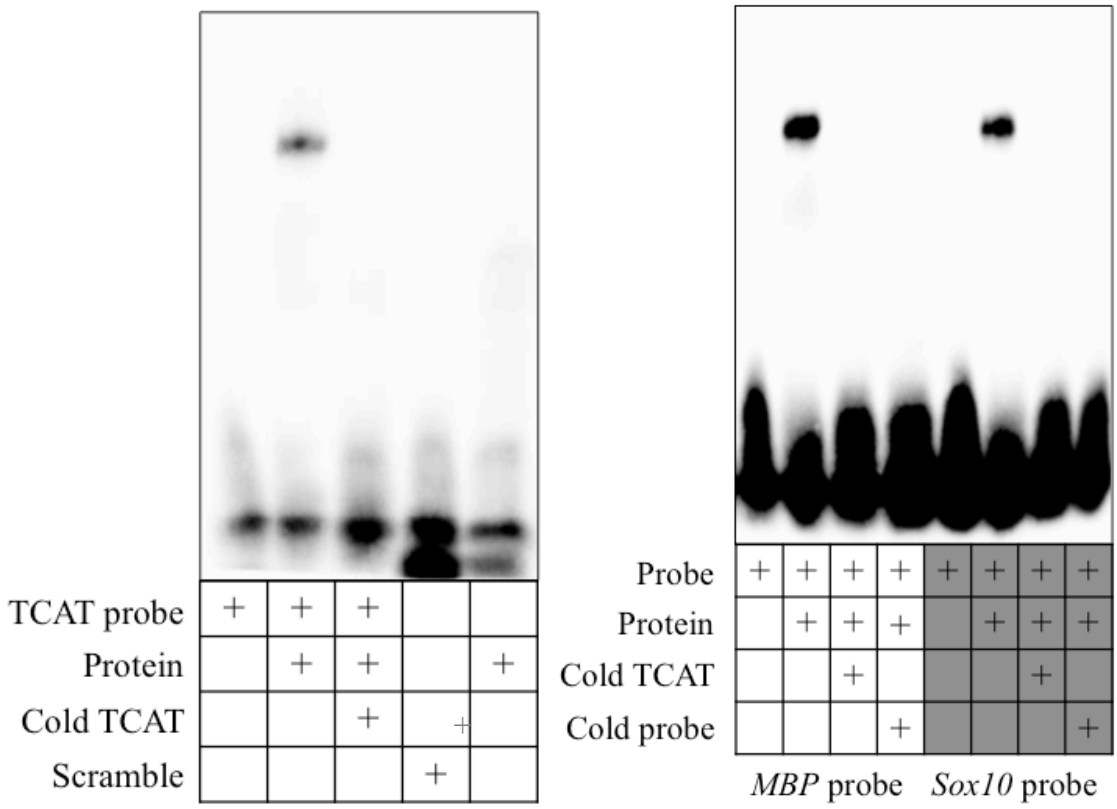

Figure 3.3.5. Electromobility shift assay of ZFP191 probes. ZFP191 tagged with GFP was transfected into HEK cells and the nuclear lysate was collected and used as "Protein". The "TCAT probe" is a 26bp double stranded DNA probe labeled with biotin and consisting of TCAT repeats. A. The lysate containing ZFP19 is able to shift the TCAT probe but not the biotin labeled "Scramble" probe. The addition of 200-fold excess molar of the an unlabeled "Cold Probe" 26bp TCAT repeat probe demonstrated that ZFP191 binds to TCAT in a sequence specific manner. B. Double stranded DNA probes for regions near *Mbp* and *Sox10* ("Probe") identified by MACS were labeled with biotin and incubated with the nuclear lysate over-expressing ZFP191 "Protein". "Cold TCAT", an unlabeled 26bp TCAT probe was used in 200-fold molar excess to outcompete ZFP191-probe interaction. Unlabeled probes for the same *Mbp* and *Sox10* ("Cold Probe") were also used to outcompete the ZFP191-probe shift.

3.3.5 Validation of ZFP191 acting as a transcriptional activator

Although ZFP191 has been shown to bind DNA, it is important to analyze the ability of ZFP191 to modulate transcription of factors involved in oligodendrocyte maturation and myelination. With confirmation of interaction between ZFP191 and regions around *Sox10* and *Mbp in vitro*, these sequences were used to construct plasmids for gene reporter assays. Gene reporter assays are a useful method to examine if the protein of interest can function as a transcriptional modulator. One such method involves cloning a putative sequence of interest into a reporter plasmid that produces luciferase, an enzyme that can be easily visualized with a table top luminometer, when it is transcribed (Arnone, Dmochowski, and Gache 2004). This method allows screening of sequences that can act as a promoter, an enhancer, an activator or repressor based on the plasmid used (Whitfield et al. 2012). Based on the failure to upregulate key genes involved in oligodendrocyte maturation and myelination when ZFP191 is absent (Chapter 2), ZFP191 may (at least in some contexts) act as a transcriptional activator. The use of the pGL3-promoter plasmid, which contains the early SV40 promoter, can be used to examine if the *Sox10* and *Mbp* sequences used above can act as enhancers or more broadly speaking as cis-acting regulatory elements.

In order to show that it is the presence of ZFP191 that was able to function as an activating transcriptional regulator, both mouse wild type and *Zfp191*$^{-/-}$ cells were used. The constructs were nucleofected, along with appropriate controls, into OPCs of both genotypes. After nucleofection the OPCs were allowed to proliferate for 24 hours and then differentiated for 48 hours. The cells were allowed to differentiate to ensure that any co-factors that might be required would be present in the OPC stage or the intermediate to oligodendrocyte stage. Another reason the cells were allowed to differentiate was due to the data from Chapter 2, which

demonstrated that a majority of transcripts are perturbed as the OPCs differentiate, which suggest ZFP191 may have maximal effect directly after the proliferation stage.

Figure 3.3.6 illustrates that the presence of either the *Mbp* or *Sox10* region was able to increase luciferase activity above the baseline of the pGL3-promoter (pGL3-p) plasmid that did not contain an enhancer element. This increase in activity is present only in cells that contain ZFP191 indicating that ZFP191 is able to function as a transcriptional regulator. The pGL3-control (pGL3-c) plasmid contains both the early SV40 promoter (exactly like pGL3-p) and the SV40 enhancer sequence that allows for robust expression and served as a positive control. To verify that it is the TCAT region that interacts with ZFP191, the *Mbp* element was reduced from 483bp to 103bp centered on the MACS identified summit. Figure 3.3.6 demonstrates that pGL3-*Mbp*-For is still able to produce an increase in activity though not as robust as the larger element. The smaller *Mbp*-For fragment was also cloned into the pGL3-p reporter plasmid in the opposite orientation (*Mbp*-Rev) to examine if this would have any effect on transcription. The pGL3-*Mbp*-Rev plasmid was also able to enhance luciferase activity in OLs containing ZFP191 compared to cells that were lacking the protein potential indicating that ZFP191 acts orientation independent in its transcriptional control. It is worth noting that there is a statistical difference between *Mbp*-Rev and *Mbp*-For and that this may indicate that while ZFP191 can function independent of orientation, there may be an orientation effect for controlling expressions. This gene reporter assay demonstrates that ZFP191 is able to bind to previously identified sequences and in doing so acts as a transcriptional activator in the context of OPCs transitioning to oligodendrocytes.

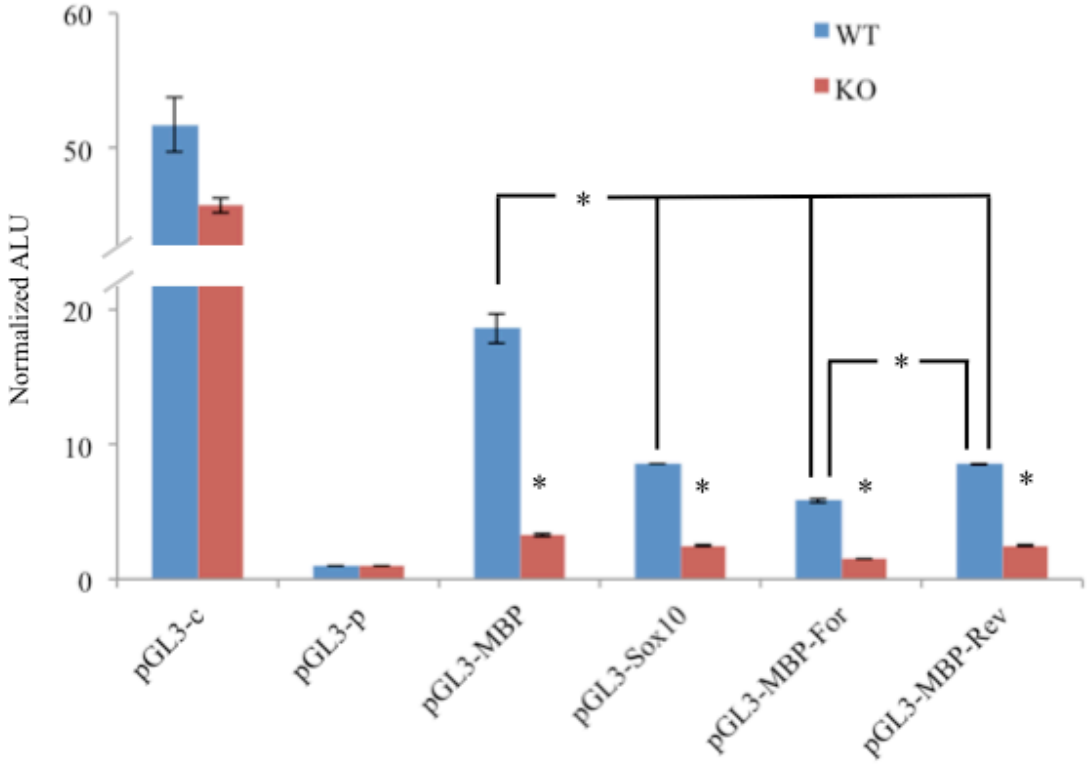

Figure 3.3.6 Luciferase reporter assay in wild-type and *Zfp191*$^{+/+}$ oligodendrocytes. A luciferase assay was performed to assess activity of enhancers. MACS identified regions were cloned into a pGL-3-p luciferase gene report plasmid. The two regions tested were around a *Mbp* (pGL3-MBP) peak and around a *Sox10* (pGL3-Sox10) peak. Bars in blue indicate OLs that are *Zfp191*$^{+/+}$ and bars in red indicate OLs that are *Zfp191*$^{-/-}$. The regions near *Mbp* and *Sox10* function in the presence of ZFP191 show an increase in luciferase activity demonstrating that ZFP191 can function as a transcriptional activator. The *Mbp* region was truncated around the TCAT motif and placed in a forward (pGL3-MBP-For) and reverse (pGL3-MPB-Rev) orientation. Shortening the probe or changing the orientation did not affect the ability of ZFP191 to increase luciferase activity. All samples were co-transfected with GFP. Each sample is a ratio of the luciferase signal by the GFP signal. Samples were then normalized to the pGL3-p plasmid that does not contain an enhancer. Error bars are standard deviation. * = $p < 0.05$, ALU = arbitrary luciferase units.

3.4 Discussion

This chapter outlines the identification of a putative ZFP191 binding element utilizing ChIP-seq and bioinformatics. Proliferating OPCs and mature oligodendrocytes were subjected to ChIP-seq to examine areas within the genome that ZFP191 can bind. Earlier studies had not characterized any potential downstream targets of ZFP191 (Liu et al. 2012, Wang et al. 2008). ChIP-seq analysis using MACS in OPCs identified 3,834 peaks that were significant and 653 peaks in oligodendrocytes. Importantly there were 318 peaks in common, suggesting that ZFP191 may be regulating the same genes throughout the maturation process. This also implies the converse, that ZFP191 may have distinct roles in transcriptional regulation during differentiation. The ChIP-seq was verified by ChIP-qPCR showing that ZFP191 can consistently bind these elements. Of note is that the confirmed transcripts all are known to play critical roles in oligodendrocyte lineage maturation and function.

To help better understand the role of ZFP191's role in potential transcriptional regulation it is important to understand where in the genome ZFP191 is bound. The use of the UCSD annotated mouse genome allows for a comparison of known genes, gene elements, and areas of the genome. There was a 10-fold increase of ZFP191 binding peaks in the OPC ChIP-seq upstream of the mouse genome relative to expected. Although not as enriched, there were also more peaks than would be expected in the oligodendrocyte ChIP-seq in the same area. In both cases, this places ZFP191 binding sites around areas that have cis-regulatory elements, which suggests that ZFP191 participates in transcriptional regulation. It is interesting to speculate why there were fewer peaks identified near the transcriptional start sites in the oligodendrocyte ChIP-seq. One reason may be due to an experimental artifact as there were fewer overall reads and fewer peaks detected in the oligodendrocyte dataset. A more interesting reason may be that

ZPF191 exerts its transcriptional control more distally. As cells differentiate and begin to condense their DNA, elements that were once distal are now brought into close apposition (possibly through DNA looping) (Petrascheck et al. 2005).

Along with the location of where ZFP191 is bound in the genome, it is critical to understand its nucleic acid binding site. Using motif recognition a TCAT-repeat was determined in sequences identified by ChIP-seq. This sequence was particularly enriched in sequences that had increased ChIP-seq read depth. Other labs have generated artificial probes and shown that truncated forms of ZFP191 are able to bind this sequence but no one has reported that full-length ZPF191 is able to interact with this sequence within the context of genomic DNA. Verification was done to ensure that, in the EMSA experiment, full length ZFP191 did indeed bind the TCAT-repeat. In order to establish biological relevance, two sequences near the genes *Sox10* and *Mbp* were chosen as they contained sequences identified bioinformatically to contain TCAT-repeats. Both sequences were able to interact with ZFP191 demonstrating that ZFP191 is able to bind TCAT sites in a genomic context. It was also established that ZFP191 binding is sequence-specific as another probe was unable to interact with ZFP191. Due to ZFP191's ability to interact with the *Mbp* and *Sox10* adjacent sequences, these regions were used to examine the role of ZFP191 as a transcriptional activator in a gene reporter assay.

As mentioned previously the zinc-finger protein YY1 (He and Casaccia-Bonnefil 2008) is able to be both a transcriptional activator and a repressor depending on its context. This may also be true for ZFP191. One reason to initially investigate that ZFP191 is in some contexts a transcriptional activator is that the both the *Sox10* and *Mbp* transcripts are not produced at wild-type levels in oligodendrocytes that are lacking ZFP191. This could imply that ZFP191 is either directly or indirectly activating these two genes. In addition, ZFP191 may function by directly

outcompeting or removing a repressor on these genes. Given that ZFP191 can interact with these tested regions in the in EMSA and a gene reporter assay, ZFP191 appears to be directly regulating the activation of these genes.

The results outlined in this chapter demonstrate that ZFP191 binds in the genome in areas that play a role in transcriptional control and regulation. ZFP191 also has a stereotyped nucleic acid binding sequence that was detected through two time points in the oligodendrocyte cell lineage. ZFP191 is able to bind biologically relevant sequences *in vitro* and is able to act as a transcriptional activator *in vivo.* The use of the regions around *Sox10* and *Mbp* in these experiments provides evidence that ZFP191 has a role in their transcriptional regulation and thus is a critical factor in oligodendrocyte maturation and myelination via at least these two key downstream factors. It will be important to examine the stringency of ZFP191 in detected regions that do not contain TCAT repeats as this has not yet been characterized. In addition an examination of other regions in different contexts will allow us to study which genes ZFP191 controls and if it can act as a direct repressor in some instances.

Chapter 4 Comparison of transcriptome analysis and ZFP191 ChIP-seq binding analysis

4.1 Introduction

With the increased use and access of large datasets many labs now have the ability to compare, contrast and more importantly combine this information to aid in discovery. One striking example of this is the ENCyclopedia Of DNA Elements (ENCODE) project (Weinstock 2007). ENCODE has begun to assemble vast datasets that include ChIP-seq and RNA-seq data (along with other assays) from different cell types and different species. The goal is to identify all the functional elements of the (human) genome. Already the group has used the method of functional genomics to characterize that chromatin accessibility and histone modification patterns are highly predictive of the presence of transcriptional start sites and most importantly that it is possible to quantitatively correlate RNA sequence production and processing with transcription factor binding at promoters. The use of functional genomics can therefore be a tool to begin to analyze and integrate ChIP-seq data and RNA-seq data for previously uncharacterized transcriptional regulators like ZPF191. This chapter will compare, contrast and combine elements from RNA-seq datasets from Chapter 2 and ChIP-seq datasets from Chapter 3 to assemble a clearer role for ZFP191 in oligodendrocyte maturation and myelination.

4.2 Materials and Methods

4.2.1 Datasets and analysis

Datasets from the RNA-seq experiments in Chapter 2 and the ChIP-seq experiments in Chapter 3 were used. All comparisons were done in Cistrome (Liu et al. 2011).

4.3 Results

4.3.1 Comparison of whole brain RNA-seq dataset with OPC ChIP-seq dataset

The ZFP191 ChIP-sequencing of wild-type OPCs in Chapter 3 located 3,834 peaks after analysis with MACS. Of the those identified peaks, 1,310 (34%) were identified to contain TCAT repeats. To characterize where the peaks were in the genome and if they were located near genes of interest, peak2gene was used to identify annotated genes 30kbp upstream or downstream of the identified peaks. Using the peak2gene software package there were 2,074 genes within 30kbp of ZFP191 peaks in wild-type OPCs. This 30kbp window upstream and downstream will be the only region referred to in this chapter only unless otherwise noted. These identified genes were then compared to RNA-seq datasets generated in Chapter 2. In the RNA-seq dataset, the loss of ZFP191 in the whole brain perturbed the levels of 397 transcripts, 327 of those transcripts were reduced with loss of ZFP191 compared to a *Zfp191*$^{+/+}$ brain. In comparing the OPC ChIP-seq analysis with decreased transcript levels with loss of ZFP191, there are 74 genes in common. Approximately 50% (38) of the peaks associated with those genes contain a TCAT repeat. One speculation is that these genes may have decreased mRNA levels because ZFP191, due to its absence, is unable to participate in transcriptional activation. Table 4.3.1 lists the top 10 transcripts by fold-change that have decreased expression levels in the whole brain with loss of ZFP191 and also have peaks within 30kbp from the OPC ChIP-seq dataset.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT	Oligodendrocyte
Gjc2	11.53	1.05	+	+
Mal	9.58	23.10		+
Tnni1	7.35	0.26	+	+
Mog	7.27	15.30	+	+
Cldn11	6.62	38.90	+	+
Mbp	6.07	123.00	+	+
Adamts4	5.70	2.47		+
Depdc1b	5.70	0.12	+	+
Tspan2	5.20	21.10	+	+
Ppp1r14a	5.11	3.79	+	+

Table 4.3.1. Transcripts with reduced expression in *Zfp191*^{null} whole brains near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat. The "Oligodendrocyte" column indicates if they are cell-specific to the oligodendrocyte lineage when classified using the Zhang et al. database (Zhang et al. 2014).

Many of the genes which had decreased transcript levels in the RNA-seq experiment, were located near identified peaks and could be stratified into cell-specific identity using the previously mentioned Zhang database (Zhang et al. 2014). Almost 80% of the identified genes (58) are characterized as being highly enriched in the oligodendrocyte cell lineage with the remaining genes having transcripts not associated with a specific cell type. Of the 58 oligodendrocyte cell-specific identified genes, 31 are near peaks that contain TCAT repeats (Table 4.3.1). With the loss of ZFP191 transcripts associated with the oligodendrocyte lineage had decreased levels and their genes are in a region containing ZFP191 binding sites.

In contrast to the 327 genes that have decreased expression in the brain with the loss of ZFP191, 67 genes had increased transcript level with the loss of ZFP191. Using the peaks identified in the OPC ChIP-seq experiment with this set of increased genes, there are 6 genes within 30kbp of these peaks. Of the peaks near these genes, 4 peaks contain a TCAT repeat and only one gene has a transcript that has a cell-specific identity for oligodendrocytes. Table 4.3.2 shows these genes along with their cell-specific identity as noted by the Zhang database.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT	Identity
Capg	2.32	0.867	+	Endo
Col9a2	1.741	1.77	+	Oligo
Fcgr3	1.67	7.42	+	Micro
Mt1	1.609	452	-	Pan
Mt2	1.527	303	-	Pan
Slc11a1	1.959	0.724	+	Micro

Table 4.3.2. Transcripts with increased expression in *Zfp191*^{null} whole brains near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat. The "Identity" column lists the cell-specific identity as determined by the Zhang et al. database. Endo = endothelial cells. Oligo = oligodendrocytes. Micro = microglia. Pan = transcripts that cannot be stratified into one cell-specific identity.

Because these peaks are near genes that have transcript levels that increase with the loss of ZFP191, this may mean that ZFP191 is able to in some instances function as a repressor. The only gene that has an oligodendrocyte cell-specific transcript is *Col9a2*. *Col9a2* is collagen, type IX, alpha 2 that is assocaited with the mouse optic senesory lineage cells (Hartman et al. 2015). Although it is enriched in wild type OLs, loss of this gene does not produce any known white matter deficits in humans or mice (Briggs and Chapman 2002). Far fewer genes were found near peaks that had transcript levels that increased with loss of ZFP191 in the whole brain. In this analysis it appears unlikely that ZFP191 is functioning as a repressor.

4.3.2 Comparison of OPC RNA-seq dataset with OPC ChIP-seq dataset

As the majority of genes with perturbed transcripts in the whole brain and associated peaks could be stratified into being oligodendrocyte cell-specific, RNA-seq from wild-type and *Zfp191*^{null} OPCs was combined with peaks identified from ZFP191 OPC ChIP-seq. In Chapter 2 it was noted that although the transcriptome of OPCs was perturbed with loss of ZFP191, this perturbation affects fewer transcripts within OLs. However it is possible that ZFP191 has a small role in maintaining the OPC identity due to 444 transcripts levels that were changed with the loss of ZFP191. There were 160 transcripts that have reduced levels due to loss of ZFP191 in OPCs identified in the RNA-seq experiment. When compared with the genes within 30kbp of peaks identified by the ZFP191 OPC CHIP-seq experiment, 25 genes were identified. Over half (13) of the peaks near these genes have regions that contain TCAT repeats. Table 4.3.3 lists the top 10 transcripts by fold-change of transcripts with decreased expression levels in OPCs with loss of ZFP191 that also have peaks within 30kbp from the OPC ChIP-seq experiment.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT
Matn4	27.70	0.99	-
Masp2	11.41	0.17	-
Rbpjl	10.29	0.35	-
Ccl17	6.33	0.17	-
Usp44	5.29	0.32	-
Dll3	4.92	7.83	+
Col18a1	4.71	2.17	-
Kifc3	3.98	14.90	+
Wdfy1	3.73	3.37	+
Gas7	3.64	2.06	-

Table 4.3.3. Transcripts with reduced expression in *Zfp191*^{null} OPCs near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat.

Of the 25 genes near identified peaks, 9 have transcripts that can stratified as being oligodendrocyte cell-specific. Of those genes, 5 of them are near peaks that contain TCAT repeats. Table 4.3.4 shows the 9 genes oligodendrocyte cell-specific genes that have transcripts with decreased levels in OPCs with the loss of ZFP191 and also have peaks in the examined region.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT
Dll3	4.92	7.83	+
Elfn2	2.22	9.86	+
Gas7	3.64	2.06	-
Matn4	27.70	0.99	-
Rbpjl	10.30	0.35	-
Slitrk3	1.80	13.40	+
Tle6	3.58	1.46	+
Tmem117	1.94	1.85	+
Xkr5	2.05	1.05	-

Table 4.3.4. Oligodendrocyte cell-specific transcripts with reduced expression in *Zfp191*^{null} OPCs near identified peaks. Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat.

Dll3 which is near a TCAT peak has an almost 5-fold decrease in transcript level with the loss of ZFP191. *Dll3* is a known Notch ligand where it may play a role in gliogenesis and neurogenesis (Ladi et al. 2005) Due to the proximity of the TCAT containing peak, ZFP191 may have a role in transcriptional activation of *Dll3* in OPCs.

Overall there were more genes in the OPCs that had increased mRNA levels with loss of ZFP191 compared to those with decreased levels. When these 284 genes were compared to peaks from the OPC ChIP-seq experiment, 66 genes were in common in both the RNA-seq and ChIP-seq experiments, over twice as many than those peaks near genes with decreased transcript levels. Although 32 of the 66 genes were near peaks with identified TCAT repeats, only 6 transcripts are oligodendrocyte cell-specific including *Sox5*. *Sox5* is known to play a role in repressing differentiation and is 2-fold higher in OPCs lacking ZFP191 than wild-type. Since 60 of the genes are not associated specifically with oligodendrocytes and nearly half have peaks that contain TCAT repeats it is puzzling why ZFP191 is binding in these regions. It may be that ZFP191 is acting as a repressor and/or that ZFP191 does not exert a critical role at the OPC stage compared with its role at the oligodendrocyte stage. The context, particularly of the stage of differentiation may affect the role of ZFP191.

4.3.3 Comparison of brain RNA-seq dataset with oligodendrocyte ChIP-seq dataset

ChIP-seq analysis during the oligodendrocyte stage identified 653 ZFP191 binding peaks. Using peak2gene there were 527 genes within 30kbp of the identified peaks, a higher percentage than the OPC ChIP-seq analysis. The genes identified at the oligodendrocyte stage are near peaks of which 203 have TCAT repeats, a similar percentage as the OPC stage. When comparing all 527 genes with transcripts with decreased levels in the *Zfp191*^{null} brain, there are

25 genes in common. Compared to the genes with decreased expression with loss of ZFP191 in the whole brain and the OPC ChIP-seq analysis above where 50% of the genes had peaks with TCAT repeats, only 8 genes had peaks near them that contained TCAT repeats near genes with transcripts that had decreased levels with loss of ZFP191 in the brain. As in the analysis above comparing the decreased transcript levels in the whole brain with the OPC ChIP-seq, a high number (18) of the genes with decreased transcript levels in the whole brain with loss of ZFP191 and peaks near them are associated as oligodendrocyte specific. Table 4.3.5 lists the top 10 transcripts by fold-change of transcripts with decreased expression level in the whole brain with loss of ZFP191 and also have peaks within 30kbp from the oligodendrocyte ChIP-seq dataset. In addition, 10 of the 25 genes associated with the oligodendrocyte ChIP-seq peaks were also found in the OPC ChIP-seq experiment. Only one transcript, *Lfng*, had increased levels in the brain with loss of ZFP191 and also had a ZFP191 peak near it. *Lfng* is known to play a role in Notch signaling and neurogenesis (Kato et al. 2010).

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT	OPC ChIP-seq
Mobp	13.82	129.00	-	-
Aspa	9.12	5.48	+	-
Tspan2	5.20	110.00	-	+
Padi2	5.01	8.28	+	+
Rab7b	4.59	2.90	-	+
Elovl7	4.36	12.70	-	+
Sox10	4.14	16.90	+	+
Litaf	3.43	7.20	+	+
Gjc3	3.38	25.80	-	+
St6galnac3	2.686	3.17	-	+

Table 4.3.5. Transcripts with reduced expression in *Zfp191*^{null} whole brains near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat. The "OPC ChIP-seq" column identifies genes that also had peaks from ZFP191 OPC ChIP-seq within 30kbp.

4.3.4 Comparison of the oligodendrocyte RNA-seq dataset to the oligodendrocyte ChIP-seq dataset

Comparing the 2,513 genes that have decreased expression in oligodendrocytes without ZFP191 to peaks detected in a ZFP191 expressing oligodendrocyte ChIP-seq, there are 134 genes that are within 30kbp of the peaks of which 55 contain TCAT repeats. Only 32 of the genes in common between the datasets are associated with oligodendrocyte specific transcripts, with the other 102 genes not associated with a specific cell type. Table 4.3.6 lists the top 10 transcripts by fold-change of transcripts with decreased expression levels in oligodendrocytes with loss of ZFP191 and that also have peaks within 30kbp from the oligodendrocyte ChIP-seq dataset.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT	OPC ChIP-seq
Rab37	64.52	0.12	+	-
Padi2	56.62	1.57	+	+
Aspa	50.12	0.11	+	-
Mobp	17.51	48.00	-	-
St6galnac3	15.36	1.52	-	+
Gipr	14.92	0.25	-	-
Plekhg3	13.09	4.53	-	-
Txndc17	12.14	54.80	+	+
Diras1	12.00	5.68	-	-
Slc34a3	11.26	0.12	-	+

Table 4.3.6. Transcripts with reduced expression in *Zfp191*^{null} oligodendrocyte near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat. The "OPC ChIP-seq" column identifies genes that also had peaks from ZFP191 OPC ChIP-seq within 30kbp.

Other genes with reduced expression and had peaks near them include *Tspan2* and *Mobp*, which both do not have peaks with a TCAT repeat and *Sox10* along with *Elovl7*, which do have peaks with TCAT, repeats. ZFP191 shares binding sites in both the OPC and oligodendrocyte ChIP-seq datasets near the same 87 genes, which could indicate that ZFP191 plays a role in their transcriptional regulation, particularly activation.

With the loss of ZFP191 in oligodendrocytes, 3,051 genes have increased levels of mRNA compared to wild-type oligodendrocytes. There were 70 genes within 30kbp of peaks detected in the ZFP191 oligodendrocyte ChIP that had increased transcript levels with loss of ZFP191 in oligodendrocytes. About 35% of those genes (24) had peaks associated with them that contained TCAT repeats. 44 of the genes also had peaks near them in the OPC ChIP-seq dataset. Only 9 genes had transcripts associated with oligodendrocytes along with nearby peaks according to the Zhang database. Table 4.3.7 lists the top 10 transcripts by fold-change of transcripts that have both increased expression level in oligodendrocytes with loss of ZFP191 and are within 30kbp of peaks from the oligodendrocyte ChIP-seq dataset.

Gene	Fold change	*Zfp191*^{null} RPKM	TCAT	OPC ChIP-seq
Acsf2	25.93	1.52	-	-
Col22a1	21.04	0.93	-	-
Tcea3	17.20	0.72	-	+
Gstm1	15.23	65.40	-	-
Lfng	14.11	25.50	-	-
Psd2	9.97	10.20	-	-
Six3os1	9.61	0.73	-	-
Uhrf1	7.40	3.42	-	+
Crtap	6.01	2.48	+	+
Racgap1	5.41	9.75	-	-

Table 4.3.7. Transcripts with increased expression in *Zfp191*^{null} OLs near identified peaks.

Genes are within 30kbp of peaks identified from the ZFP191 OPC ChIP-seq. The "TCAT" column indicates which peaks contain a TCAT repeat. The "OPC ChIP-seq" column identifies genes that also had peaks from ZFP191 OPC ChIP-seq within 30kbp.

4.3.5 Comparison of the oligodendrocyte RNA-seq dataset to the OPC ChIP-seq dataset

One possibility why oligodendrocytes lacking ZFP191 do not produce compact myelin may be that the OPCs are unable to properly differentiate. The lack of ZFP191 may mean that genes that should be activated are not (or not to the expression level that is needed) or possibly ZFP191 is acting as a repressor that fails to terminate transcription of genes that prevent differentiation. One way to examine this is to use the ZFP191 binding sites in the OPC ChIP-seq experiment and compare it to transcripts perturbed in the oligodendrocyte RNA-seq. If ZFP191 is acting solely as an activator than one could expect to find ZFP191 binding peaks only near genes of transcripts that fail to increase with the lack of ZFP191 in oligodendrocytes. Conversely if ZFP191 is acting as a repressor one would expect to find peaks identified in the ZFP191 OPC ChIP-seq experiment to be near genes of transcripts that are inappropriately increased with the lack of ZFP191 in oligodendrocytes. In this analysis it appears that ZFP191 may be able to operate as both. There are 523 genes that have reduced transcript levels with the loss of ZFP191 in oligodendrocytes that have peaks near them in the OPC ChIP-seq experiment. There are also 501 genes that have increased expression with the loss of ZFP191 and that have peaks detected near them in the OPC ChIP-seq experiment. Between the two datasets there are 318 genes that have peaks near them at both time points, suggesting ZFP191 exerts transcriptional regulation at some regions throughout maturation.

4.4 Discussion

The use of functional genomics can be a powerful tool to integrate the direct role transcription factor binding has on the transcriptome. Currently however this analysis is merely associative and is useful to narrow down potential interacting candidates. In this analysis RNA-

seq datasets from cells with or without ZFP191 in combination with ZFP191 ChIP-seq experiments were combined. This method can be used to identify downstream targets of ZFP191 and targets that would not be characterized due to experimental variability. Because ZFP191 peaks were found near the same genes in both the OPC and oligodendrocyte state it implies that ZFP191 is exerting a role in transcriptional regulation of these genes throughout the lineage. Some of these genes include *Sox10*, *Elovl7* and *Zfp488,* all of which are known to play key roles in oligodendrocyte maturation. All of these regions also contain TCAT-repeats. Many genes identified in this chapter can be classified as oligodendrocyte cell-specific (i.e. there are particularly enriched in the oligodendrocyte lineage cells), particularly those transcripts that are reduced in the brain or cells lacking ZFP191. This suggests that indeed ZFP191 is playing a direct role in transcriptional regulation of genes require for oligodendrocyte maturation and myelination. Due to the decrease of the transcript levels of these genes with absence of ZFP191 and the nearby binding of ZFP191, this suggests ZFP191 may have a primary role as a transcriptional activator. This was examined and confirmed with *Sox10* and *Mbp* in Chapter 3.

ZFP191 could also potentially have a role in being a transcriptional repressor. ZFP191 binding sites were in regions near genes that have increases in their transcript levels with loss of ZFP191. This will have to be further investigated to determine if ZFP191 is exerting repressive control or if other perturbed factors cause increased transcription. It will be critical to examine if binding in the OPC has a different role in transcriptional regulation than at the oligodendrocyte stage, particularly if its role changed from repressor to activator or vice versa. Tight control of transcription has been shown to be critical in proper differentiation of cells (Aaker et al. 2009, Aaker et al. 2010). There is the potential that while ZFP191 is binding near those sites it is not exerting any transcriptional control. This could be that the region in question does not function in

transcriptional control or that ZFP191 transcriptional regulation is weaker than other elements that exert a stronger signal in controlling transcription.

There should be some consideration that there were fewer detected peaks in the oligodendrocyte ChIP-seq experiment. As discussed in Chapter 3 this may be due to chromatin accessibility. It is curious to note that there are peaks detected near *Mbp* in the OPC ChIP-seq experiment but not the oligodendrocyte ChIP-seq experiment even though *Mbp* transcript increases during the later stage of maturation. This is also seen with the genes *Cldn11*, *Myrf, Mog*, and *Cnp*. It is likely that the current oligodendrocyte ChIP-seq experiment missed these binding events, either through the chromatin immunoprecipitation process or through bioinformatics analysis. Even with the potential lack of the full ZFP191 binding events in oligodendrocyte stage cells, functional genomics was useful to demonstrate that ZFP191 and its binding sites are near genes in an associative manner that may play a critical role in maturation and myelination.

Chapter 5. Discussion

5.1 Summary

Currently, we have incomplete knowledge about the mechanism(s) that allow an oligodendrocyte to mature and differentiate normally. Our understanding is also lacking of the factors that help initiate, coordinate, and ultimately produce proper myelination. It is safe to assume that in addition to transcriptional networks, correct synthesis of the components of myelin, appropriate cytoskeletal changes and appropriate cell-cell communication are all areas that require more study for the development and function of oligodendrocytes. This project focused primarily on one transcription factor, ZFP191, and the role it has in transcriptional control of the oligodendrocyte lineage cell.

ZFP191 is a member of a family of proteins that are known to play key roles in transcriptional regulation due to their ability to interact with the genome. Other labs have used portions of the ZFP191 to demonstrate that *in vitro* ZFP191 is able to bind DNA, and in some cases, function to increase transcription under artificial conditions. Our initial studies reported that ZFP191 was necessary for proper myelination of the CNS and that expression of genes required for proper maturation and myelination were decreased with loss of ZFP191. *I hypothesized that ZFP191 is functioning as a regulator of transcriptional activity of key genes that control oligodendrocyte maturation and myelination*.

5.2 ZFP191 plays a key role in oligodendrocyte development and myelination

In these current studies, I examined the role of the putative transcription factor ZFP191 in the integrity of the transcriptome in OPCs and mature oligodendrocytes using massively parallel

sequencing of RNA isolated from total brain, as well as isolated oligodendrocyte lineage cells (Howng et al. 2010). In examining how the loss of ZFP191 affects the transcriptome of the P21 whole mouse brain, I report that the transcriptome is significantly perturbed with loss of this transcription factor. This perturbation of the transcriptome was expected due our previous knowledge that there is a decrease in mRNA levels of some essential myelin transcripts such as *Mbp*, *Plp*, *Mog*, and *Mobp*, following the loss of ZFP191, along with the observation that these cells assume a hypomyelination phenotype. Although *Zfp191* is expressed in all cell types in the CNS, 70% of the genes that had decreased expression with loss of ZFP191 could be categorized specifically as belonging to the oligodendrocyte lineage. The majority of genes that had transcript levels perturbed by loss of ZFP191 were downregulated, suggesting that ZFP191 is involved with transcription activation. If ZFP191 was acting as a repressor, then in its absence genes that maintain the progenitor state would be relieved and able to increase their mRNA levels. However the transcripts with increased mRNA levels compared to cells that had ZFP191 were transcripts associated with gliosis, not oligodendrocyte specific transcripts. The gliosis associated transcripts are likely a result from the improper maturation of oligodendrocytes and the resulting hypomyelination. It remains possible that loss of ZFP191in OPCS and oligodendrocytes is spuriously associated with global changes in gene expression or that observed changes are unimportant for differentiation along the oligodendrocyte maturation axis. Loss of ZF191 in other cell types may affect their transcriptomes in equal magnitude but without biological relevance. The use of cell-type specific Cre transgenic mice would allow us to make a precise investigations of about this possibility.

To investigate the role of ZFP191 in the oligodendrocyte lineage, as a majority of the gene perturbed with loss of ZFP191 in the whole brain were associated with oligodendrocyte

lineage transcripts, primary OPCs and oligodendrocytes were isolated from both genotypes (*Zfp191*$^{+/+}$ and *Zfp191*$^{-/-}$). The deficits seen with lack of ZFP191 are noticeable when the OPC is post-mitotic and is attempting to upregulate factors required for myelin production. Even in mice that lack ZFP191 there is a normal number of OPCs produced, they are able to traffic correctly and can produce processes to attempt to produce wrap neurons. Therefore it is intriguing to note that loss of ZFP191 does perturb the transcriptome of the OPCs. This could suggest that ZFP191 has a role in the NPC to OPC transition or participates in OPC maintenance. The perturbed transcripts could also be an artifact from a small number of cells precociously differentiating. This would imply that ZFP191 has an immediate effect on the transcriptome on cells as they exit the cell-cycle placing ZFP191's activity right after the OPC stage.

The largest perturbation was in the transcriptome of oligodendrocytes that do not express ZFP191. ~75% of the transcripts with decreased levels in the *Zfp191*^{null} brain are also decreased in oligodendrocytes that do not express ZFP191. This is an indication that the absence of ZFP191 affects the cells autonomously and that effects were not a byproduct of being removed from the brain. In addition to decreased transcript levels for proteins that are integral to myelin, loss of ZFP191 in oligodendrocytes perturbs transcriptional networks and signaling cascades. The increased perturbation in oligodendrocytes compared to OPCs or the brain could be due to a direct action of ZFP191 or an indirect effect from the disruption of the normal transactional network and signaling cascades. Most likely it is a combination of both direct and indirect effects. In support of at least part of the disruption coming from the perturbation of transcription factors, I observed that both *Sox10* and *Myrf* have reduced levels of transcript in cells lacking ZFP191. As both of these transcription factors play key roles in transcriptional regulation in combination and distinct from one another it is likely their disruption accounts for some portion

of the perturbed transcriptome (Hornig et al. 2013). In the future it will be interesting to compare the identified genomic targets of SOX10 and MYRF and compare them to the perturbed genes. One interesting piece of data is that the lack of ZFP191 seemed to allow sustained expression of transcriptional repressors rather than the termination normally seen during differentiation (Dugas et al. 2006, Zhang et al. 2014). The lack of down regulation of these genes suggests that *Zfp191*^{null} oligodendrocytes have not fully differentiated into cells capable of myelinating axons.

With the lack of *Zfp191* expression there is also a decreased mRNA levels of *Mbp*. This lack of MBP is one key reason why hypomyelination is seen. To account for the role of the lack of myelin on the transcriptome I compared my data with the transcriptome of another hypomyelination mouse mutant. The *shiverer* mouse lacks MBP and was used to assess the direct effects of the loss of ZFP191 versus the secondary effect of the loss of myelin. The loss of MBP results in 75% fewer transcripts being perturbed in the whole brain than in the *Zfp191*^{null} mouse. In addition fewer transcription factors were perturbed with loss of MBP although repressors that were improperly elevated in the *Zfp191*^{null} cells were also elevated in the *shiverer* cells, raising the possibility that that the lack of myelin is feeding back to the oligodendrocyte cells and not terminating repressor activity.

The absence of myelin in the *Zfp191*^{null} and *shiverer* mouse whole brains results in the reduction of expression levels of genes involved in the cholesterol and lipid biosynthetic pathways. The loss of either ZFP191 or MBP results in a reduction of *Hmgcr*, which encodes for the rate-limiting enzyme in cholesterol biosynthesis. These data suggest that oligodendrocytes have a feedback system that allows them to regulate myelin lipid synthesis depending on their myelinating state. The control of cholesterol homeostasis is critical for myelination (Saher et al. 2005, Verheijen et al. 2009), and defects in lipid synthesis have been linked to demyelination

(Rolyan et al. 2015). Data from these models of hypomyelination may help identify novel sensors in oligodendrocytes for proper cholesterol and/or lipid biosynthesis. Lipid biosynthesis transcripts are also expressed abundantly in many cells in the CNS, particularly astrocytes, which play a key role in providing lipids and cholesterol for neurons (for review see (Pfrieger and Ungerer 2011)). The decrease of key lipid biosynthesis transcripts in these models of hypomyelination may be due to a direct effect on oligodendrocytes, or it may reflect a secondary effect of oligodendrocyte perturbation on the homeostatic production of cholesterol by other cells in the CNS.

5.3 ZFP191 binds in the genome, has an identifiable binding motif, and can function as a transcriptional activator

Proliferating OPCs and mature oligodendrocytes were subjected to ChIP-seq to determine binding regions and motifs for ZFP191. Earlier studies had not characterized any potential downstream targets of ZFP191 and instead relied on synthetic sequences (Liu et al. 2012, Wang et al. 2008). ChIP-seq analysis using MACS was able to detect significant peaks in both cell types. A significant finding was the 318 peaks in common in both the OPC and oligodendrocyte ChIP-seq experiments, which suggests that ZFP191 regulates the same genes throughout differentiation. Another explanation is that ZFP191 binds to these identified regions in OPCs and recruits factors that control transcription in the transition from OPC to oligodendrocytes.

There were fewer peaks detected in the oligodendrocytes than the OPCs. This may be due to a technical error or an artifact of the methodology. This may also be explained due to the more compact chromatin as the cell exits mitosis and differentiates making it more difficult to segment

the genome. Regardless of the lower reads, DNA regions from both cells could be replicated and verified by an alternative method.

I next determined the location of ZFP191 peaks in relation to the annotated genome. In doing so it is clear that ZFP191 preferentially binds in regions proximal to transcriptional start sites. The identification that ZFP191 binding sites are in areas that have a high likelihood of being cis-regulatory elements suggests that ZFP191 participates in transcriptional regulation. In comparing the sequences identified with ChIP-seq, I used motif recognition to characterize a TCAT-repeat, which was present in many of the regions. This sequence was characterized *de novo* and it is consistent with the current published literature (Liu et al. 2012, Wang et al. 2008). No other binding motif was detected which may mean that the TCAT-repeat is the only sequence ZFP191 can bind or there was a problem with collection and analysis. I cannot disprove that ZFP191 can bind another sequence but I did verify that full-length ZFP191 is able to interact with TCAT-repeat in two assays.

In order to establish biological relevance, two sequences near the genes *Sox10* and *Mbp* that contained TCAT-repeats were assayed. Both sequences interacted with ZFP191 in an electromobility shift assay. These sequences also were able to increase luciferase activity in a gene reporter assay. One reason to suggest that ZFP191 is acting to ensure transcriptional activation is that the both *Sox10* and *Mbp* fail to be expressed at wild type levels in cells that are lacking ZFP191. This could imply that ZFP191 is either directly or indirectly activating these two genes when interpreting the transcriptome data alone. Because ZFP191 can interact with these regions in two separate assays it appears that ZFP191 is directly regulating the activation of these genes. Additional experiments will have to be done on other identified regions, particularly those that do not contain a TCAT-repeat, to see if ZFP191 can interact with those sequences.

Other contexts should be investigated to examine if ZFP191's transcriptional regulation changes and to examine if the occupancy of ZFP191 changes.

5.4 ZFP191 binds in the genome near genes perturbed with the loss of ZFP191

The integration of the experiments presented in Chapters 2 and 3 can help establish the role of ZFP191 generally and also how ZFP191 affects oligodendrocyte maturation and myelination in particular. I combined RNA-seq datasets from cells with or without ZFP191 in combination with ZFP191 ChIP-seq experiments. Finding ZFP191 peaks near the same genes in both the OPC and OL experiments implies that ZFP191 plays a role in transcriptional regulation of those genes throughout differentiation. These genes include *Sox10*, *Elovl7* and *Zfp488,* all of which are known to play key roles in oligodendrocyte maturation and contain TCAT-repeats. Many of the genes perturbed with the loss of ZFP191 and with peaks within 30kbp are oligodendrocyte specific. This suggests that ZFP191 is proximal to areas of transcriptional regulation for genes required for oligodendrocyte maturation and myelination. With the limited number of biochemical assays performed it is unclear if ZFP191 always has a role as a transcriptional activator as it did with *Sox10* and *Mbp*. Future experiments will needed to determine if ZFP191's role is context specific. While this merging of these two datasets is only associative, it does narrow the future candidates for biochemical analysis. It also establishes groups of regions that that may function together based on their use of the same binding motif, occupancy through the lineage, behavior of the transcript in response to the loss of ZFP191, and gene ontology. In the presence of ZFP191, the selected regions around *Sox10* and *Mbp* were able to be responsive and increase levels of luciferase. In at least these two cases I believe that

ZFP191 has a role in the transcriptional activation of genes vital to oligodendrocyte maturation and myelination.

I hypothesized that ZFP191 is functioning as a regulator of transcriptional activity of key genes that control oligodendrocyte maturation and myelination.

5.5 A potential model for the role of ZFP191 in transcriptional regulation

Here I propose a more general model for how ZFP191 may be functioning to affect transcription, as ZFP191 has not yet be shown to recruit known effectors of transcription. ZFP191 can bind in the genome in a sequence specific manner at the OPC stage. This binding places ZFP191 near genes that play important roles in oligodendrocyte maturation and myelination. Similar to OLIG2, ZFP191 may have a function as a prepatterning element to recruit and/or interact with other elements near regions of transactional control. During differentiation, ZFP191 is able to dynamically change its binding partner and ZFP191 is in areas of the genome that can direct the necessary transcriptional regulator to come in close approximation to the appropriate gene. In this way ZFP191 can function by recruiting activators, repressors and/or chromatin remodeling agents. Therefore ZFP191 acts as a conduit for transcriptional modulation in regions near genes required for myelination.

5.6 Proposed experiments

The many genes of interest presented in Chapter 4 need to be assayed to show that they indeed are able to interact with ZFP191 and these binding events are able to affect transcription.

It will also be interesting to stratify regions identified between those that contain TCAT-repeat and those that do not. Ranking these separate categories based on their MACS scores may present regions that have the ability to interact with ZFP191 albeit to a lower affinity. (This may be the case of the *Smad7* region tested in Figure 3.3.2.) The current methodology for the gene reporter assays is to allow the cells to differentiate, but it may be of interest to examine regions that were only detected in the OPC ChIP-seq, the OL-ChIP-seq and peaks that were in both performing a gene reporter assay in non-differentiating cells. This may allow us to parse out how ZFP191 changes its occupancy, if it does, based on the stage of differentiation. It will also be of use to compare other ChIP-seq datasets with ours to see if there are regions of cooperation in transcriptional regulation similar to *Sox10* and *Myrf*. Comparing the binding sites of SOX10 and MYRF to the transcripts perturbed with loss of ZFP191, we may be able to parse out which transcripts lost are the direct result of the loss of ZFP191 versus a secondary effect. Other potential avenues of interest would be performing ChIP-seq at an intermediate stage as this is when it appears to be the most dynamic and exerting the most control. These experiments will lead us closer to understand the transcriptional network that is required for oligodendrocyte maturation and myelination.

References

Aaker, J. D., A. L. Patineau, H. J. Yang, D. T. Ewart, W. Gong, T. Li, Y. Nakagawa, S. C. McLoon, and N. Koyano-Nakagawa. 2009. "Feedback regulation of NEUROG2 activity by MTGR1 is required for progression of neurogenesis." *Mol Cell Neurosci* 42 (4):267-77. doi: 10.1016/j.mcn.2009.07.011.

Aaker, J. D., A. L. Patineau, H. J. Yang, D. T. Ewart, Y. Nakagawa, S. C. McLoon, and N. Koyano-Nakagawa. 2010. "Interaction of MTG family proteins with NEUROG2 and ASCL1 in the developing nervous system." *Neurosci Lett* 474 (1):46-51. doi: 10.1016/j.neulet.2010.03.004.

Anders, S., and W. Huber. 2010. "Differential expression analysis for sequence count data." *Genome Biol* 11 (10):R106. doi: 10.1186/gb-2010-11-10-r106.

Armendariz, B. G., A. Bribian, E. Perez-Martinez, A. Martinez, F. de Castro, E. Soriano, and F. Burgaya. 2012. "Expression of Semaphorin 4F in neurons and brain oligodendrocytes and the regulation of oligodendrocyte precursor migration in the optic nerve." *Mol Cell Neurosci* 49 (1):54-67. doi: 10.1016/j.mcn.2011.09.003.

Arnone, M. I., I. J. Dmochowski, and C. Gache. 2004. "Using reporter genes to study cis-regulatory elements." *Methods Cell Biol* 74:621-52.

Auerbach, R. K., G. Euskirchen, J. Rozowsky, N. Lamarre-Vincent, Z. Moqtaderi, P. Lefrancois, K. Struhl, M. Gerstein, and M. Snyder. 2009. "Mapping accessible chromatin regions using Sono-Seq." *Proc Natl Acad Sci U S A* 106 (35):14926-31. doi: 10.1073/pnas.0905443106 [pii].

Baas, D., C. Legrand, J. Samarut, and F. Flamant. 2002. "Persistence of oligodendrocyte precursor cells and altered myelination in optic nerve associated to retina degeneration in mice devoid of all thyroid hormone receptors." *Proc Natl Acad Sci U S A* 99 (5):2907-11. doi: 10.1073/pnas.052482299.

Bailey, T. L., M. Boden, F. A. Buske, M. Frith, C. E. Grant, L. Clementi, J. Ren, W. W. Li, and W. S. Noble. 2009. "MEME SUITE: tools for motif discovery and searching." *Nucleic Acids Res* 37 (Web Server issue):W202-8. doi: 10.1093/nar/gkp335.

Bailey, T. L., M. Boden, T. Whitington, and P. Machanick. 2010. "The value of position-specific priors in motif discovery using MEME." *BMC Bioinformatics* 11:179. doi: 10.1186/1471-2105-11-179.

Bailey, T. L., and M. Gribskov. 1998. "Combining evidence using p-values: application to sequence homology searches." *Bioinformatics* 14 (1):48-54.

Bailey, T. L., J. Johnson, C. E. Grant, and W. S. Noble. 2015. "The MEME Suite." *Nucleic Acids Res* 43 (W1):W39-49. doi: 10.1093/nar/gkv416.

Bailey, T. L., and P. Machanick. 2012. "Inferring direct DNA binding from ChIP-seq." *Nucleic Acids Res* 40 (17):e128. doi: 10.1093/nar/gks433.

Baron, W., H. Colognato, and C. ffrench-Constant. 2005. "Integrin-growth factor interactions as regulators of oligodendroglial development and function." *Glia* 49 (4):467-79. doi: 10.1002/glia.20132.

Baron, W., B. Metz, R. Bansal, D. Hoekstra, and H. de Vries. 2000. "PDGF and FGF-2 signaling in oligodendrocyte progenitor cells: regulation of proliferation and differentiation by multiple intracellular signaling pathways." *Mol Cell Neurosci* 15 (3):314-29. doi: 10.1006/mcne.1999.0827.

Baron, W., S. J. Shattil, and C. ffrench-Constant. 2002. "The oligodendrocyte precursor mitogen PDGF stimulates proliferation by activation of alpha(v)beta3 integrins." *EMBO J* 21 (8):1957-66. doi: 10.1093/emboj/21.8.1957.

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

Barres, B. A., I. K. Hart, H. S. Coles, J. F. Burne, J. T. Voyvodic, W. D. Richardson, and M. C. Raff. 1992b. "Cell death in the oligodendrocyte lineage." *J Neurobiol* 23 (9):1221-30. doi: 10.1002/neu.480230912.

Barres, B. A., M. A. Lazar, and M. C. Raff. 1994. "A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development." *Development* 120 (5):1097-108.

Bartlett, P. F., M. D. Noble, R. M. Pruss, M. C. Raff, S. Rattray, and C. A. Williams. 1981. "Rat neural antigen-2 (RAN-2): a cell surface antigen on astrocytes, ependymal cells, Muller cells and lepto-meninges defined by a monoclonal antibody." *Brain Res* 204 (2):339-51.

Berndt, J. A., J. G. Kim, M. Tosic, C. Kim, and L. D. Hudson. 2001. "The transcriptional regulator Yin Yang 1 activates the myelin PLP gene." *J Neurochem* 77 (3):935-42.

Bignami, A., L. F. Eng, D. Dahl, and C. T. Uyeda. 1972. "Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence." *Brain Res* 43 (2):429-35.

Bilican, B., C. Fiore-Heriche, A. Compston, N. D. Allen, and S. Chandran. 2008. "Induction of Olig2 precursors by FGF involves BMP signalling blockade at the Smad level." *PLoS One* 3 (8):e2863. doi: 10.1371/journal.pone.0002863.

Bischof, M., M. Weider, M. Kuspert, K. A. Nave, and M. Wegner. 2015. "Brg1-dependent chromatin remodelling is not essentially required during oligodendroglial differentiation." *J Neurosci* 35 (1):21-35. doi: 10.1523/JNEUROSCI.1468-14.2015.

Blankenberg, D., G. Von Kuster, N. Coraor, G. Ananda, R. Lazarus, M. Mangan, A. Nekrutenko, and J. Taylor. 2010. "Galaxy: a web-based genome analysis tool for experimentalists." *Curr Protoc Mol Biol* Chapter 19:Unit 19 10 1-21. doi: 10.1002/0471142727.mb1910s89.

Bribian, A., M. J. Barallobre, N. Soussi-Yanicostas, and F. de Castro. 2006. "Anosmin-1 modulates the FGF-2-dependent migration of oligodendrocyte precursors in the developing optic nerve." *Mol Cell Neurosci* 33 (1):2-14. doi: 10.1016/j.mcn.2006.05.009.

Briggs, M. D., and K. L. Chapman. 2002. "Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations." *Hum Mutat* 19 (5):465-78. doi: 10.1002/humu.10066.

Brinkmann, B. G., A. Agarwal, M. W. Sereda, A. N. Garratt, T. Muller, H. Wende, R. M. Stassart, S. Nawaz, C. Humml, V. Velanac, K. Radyushkin, S. Goebbels, T. M. Fischer, R. J. Franklin, C. Lai, H. Ehrenreich, C. Birchmeier, M. H. Schwab, and K. A. Nave. 2008. "Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system." *Neuron* 59 (4):581-95. doi: 10.1016/j.neuron.2008.06.028.

Bujalka, H., M. Koenning, S. Jackson, V. M. Perreau, B. Pope, C. M. Hay, S. Mitew, A. F. Hill, Q. R. Lu, M. Wegner, R. Srinivasan, J. Svaren, M. Willingham, B. A. Barres, and B. Emery. 2013. "MYRF is a membrane-associated transcription factor that autoproteolytically cleaves to directly activate myelin genes." *PLoS Biol* 11 (8):e1001625. doi: 10.1371/journal.pbio.1001625.

Cahoy, J. D., B. Emery, A. Kaushal, L. C. Foo, J. L. Zamanian, K. S. Christopherson, Y. Xing, J. L. Lubischer, P. A. Krieg, S. A. Krupenko, W. J. Thompson, and B. A. Barres. 2008. "A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function." *J Neurosci* 28 (1):264-78. doi: 10.1523/JNEUROSCI.4178-07.2008.

Cai, J., Y. Qi, X. Hu, M. Tan, Z. Liu, J. Zhang, Q. Li, M. Sander, and M. Qiu. 2005. "Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling." *Neuron* 45 (1):41-53. doi: 10.1016/j.neuron.2004.12.028.

Canoll, P. D., R. Kraemer, K. K. Teng, M. A. Marchionni, and J. L. Salzer. 1999. "GGF/neuregulin induces a phenotypic reversion of oligodendrocytes." *Mol Cell Neurosci* 13 (2):79-94. doi: 10.1006/mcne.1998.0733.

Chandran, S., H. Kato, D. Gerreli, A. Compston, C. N. Svendsen, and N. D. Allen. 2003. "FGF-dependent generation of oligodendrocytes by a hedgehog-independent pathway." *Development* 130 (26):6599-609. doi: 10.1242/dev.00871.

Chen, Y., H. Wu, S. Wang, H. Koito, J. Li, F. Ye, J. Hoang, S. S. Escobar, A. Gow, H. A. Arnett, B. D. Trapp, N. J. Karandikar, J. Hsieh, and Q. R. Lu. 2009. "The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination." *Nat Neurosci* 12 (11):1398-406. doi: 10.1038/nn.2410.

Cheng, X., Y. Wang, Q. He, M. Qiu, S. R. Whittemore, and Q. Cao. 2007. "Bone morphogenetic protein signaling and olig1/2 interact to regulate the differentiation and maturation of adult oligodendrocyte precursor cells." *Stem Cells* 25 (12):3204-14. doi: 10.1634/stemcells.2007-0284.

Chun, S. J., M. N. Rasband, R. L. Sidman, A. A. Habib, 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. doi: 10.1083/jcb.200304154.

Cody, J. D., C. Sebold, P. Heard, E. Carter, B. Soileau, M. Hasi-Zogaj, A. Hill, D. Rupert, B. Perry, L. O'Donnell, J. Gelfond, J. Lancaster, P. T. Fox, and D. E. Hale. 2015. "Consequences of chromsome18q deletions." *Am J Med Genet C Semin Med Genet* 169 (3):265-80. doi: 10.1002/ajmg.c.31446.

Collins, T., J. R. Stone, and A. J. Williams. 2001. "All in the family: the BTB/POZ, KRAB, and SCAN domains." *Mol Cell Biol* 21 (11):3609-15. doi: 10.1128/MCB.21.11.3609-3615.2001.

Colognato, H., W. Baron, V. Avellana-Adalid, J. B. Relvas, A. Baron-Van Evercooren, E. Georges-Labouesse, and C. ffrench-Constant. 2002. "CNS integrins switch growth factor signalling to promote target-dependent survival." *Nat Cell Biol* 4 (11):833-41. doi: 10.1038/ncb865.

Colognato, H., S. Ramachandrappa, I. M. Olsen, and C. ffrench-Constant. 2004. "Integrins direct Src family kinases to regulate distinct phases of oligodendrocyte development." *J Cell Biol* 167 (2):365-75. doi: 10.1083/jcb.200404076.

Dai, Z. M., S. Sun, C. Wang, H. Huang, X. Hu, Z. Zhang, Q. R. Lu, and M. Qiu. 2014. "Stage-specific regulation of oligodendrocyte development by Wnt/beta-catenin signaling." *J Neurosci* 34 (25):8467-73. doi: 10.1523/JNEUROSCI.0311-14.2014.

Decker, L., V. Avellana-Adalid, B. Nait-Oumesmar, P. Durbec, 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):422-39. doi: 10.1006/mcne.2000.0885.

Dubois-Dalcq, M., T. Behar, L. Hudson, and RA. Lazzarini. 1986. "Emergence of three myelin proteins in oligodendrocytes cultured without neurons." *J Cell Biochem* 102:384-392.

Dugas, J. C., Y. C. Tai, T. P. Speed, J. Ngai, and B. A. Barres. 2006. "Functional genomic analysis of oligodendrocyte differentiation." *J Neurosci* 26 (43):10967-83. doi: 10.1523/JNEUROSCI.2572-06.2006.

Durand, B., and M. Raff. 2000. "A cell-intrinsic timer that operates during oligodendrocyte development." *Bioessays* 22 (1):64-71. doi: 10.1002/(SICI)1521-1878(200001)22:1<64::AID-BIES11>3.0.CO;2-Q.

Edelstein, L. C., and T. Collins. 2005. "The SCAN domain family of zinc finger transcription factors." *Gene* 359:1-17. doi: 10.1016/j.gene.2005.06.022.

Emery, B. 2010a. "Regulation of oligodendrocyte differentiation and myelination." *Science* 330 (6005):779-82. doi: 10.1126/science.1190927.

Emery, B. 2010b. "Transcriptional and post-transcriptional control of CNS myelination." *Curr Opin Neurobiol* 20 (5):601-7. doi: 10.1016/j.conb.2010.05.005.

Emery, B., D. Agalliu, J. D. Cahoy, T. A. Watkins, J. C. Dugas, S. B. Mulinyawe, A. Ibrahim, K. L. Ligon, D. H. Rowitch, and B. A. Barres. 2009. "Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination." *Cell* 138 (1):172-85. doi: 10.1016/j.cell.2009.04.031.

Emery, B., and J. C. Dugas. 2013. "Purification of oligodendrocyte lineage cells from mouse cortices by immunopanning." *Cold Spring Harb Protoc* 2013 (9):854-68. doi: 10.1101/pdb.prot073973.

Fancy, S. P., S. E. Baranzini, C. Zhao, D. I. Yuk, K. A. Irvine, S. Kaing, N. Sanai, R. J. Franklin, and D. H. Rowitch. 2009. "Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS." *Genes Dev* 23 (13):1571-85. doi: 10.1101/gad.1806309.

Feng, J., T. Liu, B. Qin, Y. Zhang, and X. S. Liu. 2012. "Identifying ChIP-seq enrichment using MACS." *Nat Protoc* 7 (9):1728-40. doi: 10.1038/nprot.2012.101.

Feng, J., T. Liu, and Y. Zhang. 2011. "Using MACS to identify peaks from ChIP-Seq data." *Curr Protoc Bioinformatics* Chapter 2:Unit 2 14. doi: 10.1002/0471250953.bi0214s34.

Fernandez, P. A., D. G. Tang, L. Cheng, A. Prochiantz, A. W. Mudge, and M. C. Raff. 2000. "Evidence that axon-derived neuregulin promotes oligodendrocyte survival in the developing rat optic nerve." *Neuron* 28 (1):81-90.

Finzsch, M., C. C. Stolt, P. Lommes, and M. Wegner. 2008. "Sox9 and Sox10 influence survival and migration of oligodendrocyte precursors in the spinal cord by regulating PDGF receptor alpha expression." *Development* 135 (4):637-46. doi: 10.1242/dev.010454.

Flores, A. I., B. S. Mallon, T. Matsui, W. Ogawa, A. Rosenzweig, T. Okamoto, and W. B. Macklin. 2000. "Akt-mediated survival of oligodendrocytes induced by neuregulins." *J Neurosci* 20 (20):7622-30.

Ford-Perriss, M., H. Abud, and M. Murphy. 2001. "Fibroblast growth factors in the developing central nervous system." *Clin Exp Pharmacol Physiol* 28 (7):493-503.

Fortin, D., E. Rom, H. Sun, A. Yayon, and R. Bansal. 2005. "Distinct fibroblast growth factor (FGF)/FGF receptor signaling pairs initiate diverse cellular responses in the oligodendrocyte lineage." *J Neurosci* 25 (32):7470-9. doi: 10.1523/JNEUROSCI.2120-05.2005.

Friedman, B. A., and T. Maniatis. 2011. "ExpressionPlot: a web-based framework for analysis of RNA-Seq and microarray gene expression data." *Genome Biol* 12 (7):R69. doi: 10.1186/gb-2011-12-7-r69.

Fruttiger, M., A. R. Calver, and W. D. Richardson. 2000. "Platelet-derived growth factor is constitutively secreted from neuronal cell bodies but not from axons." *Curr Biol* 10 (20):1283-6.

Fruttiger, M., L. Karlsson, A. C. Hall, A. Abramsson, A. R. Calver, H. Bostrom, K. Willetts, C. H. Bertold, J. K. Heath, C. Betsholtz, and W. D. Richardson. 1999. "Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice." *Development* 126 (3):457-67.

Fu, H., S. Kesari, and J. Cai. 2012. "Tcf7l2 is tightly controlled during myelin formation." *Cell Mol Neurobiol* 32 (3):345-52. doi: 10.1007/s10571-011-9778-y.

Fulton, D. L., E. Denarier, H. C. Friedman, W. W. Wasserman, and A. C. Peterson. 2011. "Towards resolving the transcription factor network controlling myelin gene expression." *Nucleic Acids Res* 39 (18):7974-91. doi: 10.1093/nar/gkr326.

Furusho, M., Y. Kaga, A. Ishii, J. M. Hebert, and R. Bansal. 2011. "Fibroblast growth factor signaling is required for the generation of oligodendrocyte progenitors from the embryonic forebrain." *J Neurosci* 31 (13):5055-66. doi: 10.1523/JNEUROSCI.4800-10.2011.

Gao, F. B., B. Durand, and M. Raff. 1997. "Oligodendrocyte precursor cells count time but not cell divisions before differentiation." *Curr Biol* 7 (2):152-5.

Giardine, B., C. Riemer, R. C. Hardison, R. Burhans, L. Elnitski, P. Shah, Y. Zhang, D. Blankenberg, I. Albert, J. Taylor, W. Miller, W. J. Kent, and A. Nekrutenko. 2005. "Galaxy: a platform for interactive large-scale genome analysis." *Genome Res* 15 (10):1451-5. doi: 10.1101/gr.4086505.

Glasgow, S. M., W. Zhu, C. C. Stolt, T. W. Huang, F. Chen, J. J. LoTurco, J. L. Neul, M. Wegner, C. Mohila, and B. Deneen. 2014. "Mutual antagonism between Sox10 and NFIA regulates diversification of glial lineages and glioma subtypes." *Nat Neurosci* 17 (10):1322-9. doi: 10.1038/nn.3790.

Goecks, J., A. Nekrutenko, J. Taylor, and Team Galaxy. 2010. "Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences." *Genome Biol* 11 (8):R86. doi: 10.1186/gb-2010-11-8-r86.

Gokhan, S., M. Marin-Husstege, S. Y. Yung, D. Fontanez, P. Casaccia-Bonnefil, and M. F. Mehler. 2005. "Combinatorial profiles of oligodendrocyte-selective classes of transcriptional regulators differentially modulate myelin basic protein gene expression." *J Neurosci* 25 (36):8311-21. doi: 10.1523/JNEUROSCI.1850-05.2005.

Grandbarbe, L., J. Bouissac, M. Rand, M. Hrabe de Angelis, S. Artavanis-Tsakonas, and E. Mohier. 2003. "Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process." *Development* 130 (7):1391-402.

Grant, C. E., T. L. Bailey, and W. S. Noble. 2011. "FIMO: scanning for occurrences of a given motif." *Bioinformatics* 27 (7):1017-8. doi: 10.1093/bioinformatics/btr064.

Gupta, S., J. A. Stamatoyannopoulos, T. L. Bailey, and W. S. Noble. 2007. "Quantifying similarity between motifs." *Genome Biol* 8 (2):R24. doi: 10.1186/gb-2007-8-2-r24.

Hammond, E., J. Lang, Y. Maeda, D. Pleasure, M. Angus-Hill, J. Xu, M. Horiuchi, W. Deng, and F. Guo. 2015. "The Wnt Effector Transcription Factor 7-Like 2 Positively Regulates Oligodendrocyte Differentiation in a Manner Independent of Wnt/beta-Catenin Signaling." *J Neurosci* 35 (12):5007-22. doi: 10.1523/JNEUROSCI.4787-14.2015.

Harper, J., L. Yan, R. M. Loureiro, I. Wu, J. Fang, P. A. D'Amore, and M. A. Moses. 2007. "Repression of vascular endothelial growth factor expression by the zinc finger transcription factor ZNF24." *Cancer Res* 67 (18):8736-41. doi: 10.1158/0008-5472.CAN-07-1617.

Hart, I. K., W. D. Richardson, C. H. Heldin, B. Westermark, and M. C. Raff. 1989. "PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage." *Development* 105 (3):595-603.

Hartman, B. H., R. Durruthy-Durruthy, R. D. Laske, S. Losorelli, and S. Heller. 2015. "Identification and characterization of mouse otic sensory lineage genes." *Front Cell Neurosci* 9:79. doi: 10.3389/fncel.2015.00079.

He, D., C. Marie, C. Zhao, B. Kim, J. Wang, Y. Deng, A. Clavairoly, M. Frah, H. Wang, X. He, H. Hmidan, B. V. Jones, D. Witte, B. Zalc, X. Zhou, D. I. Choo, D. M. Martin, C. Parras, and Q. R. Lu. 2016. "Chd7 cooperates with Sox10 and regulates the onset of CNS myelination and remyelination." *Nat Neurosci*. doi: 10.1038/nn.4258.

He, Y., and P. Casaccia-Bonnefil. 2008. "The Yin and Yang of YY1 in the nervous system." *J Neurochem* 106 (4):1493-502. doi: 10.1111/j.1471-4159.2008.05486.x.

He, Y., J. Dupree, J. Wang, J. Sandoval, J. Li, H. Liu, Y. Shi, K. A. Nave, and P. Casaccia-Bonnefil. 2007. "The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation." *Neuron* 55 (2):217-30. doi: 10.1016/j.neuron.2007.06.029.

Hellman, L. M., and M. G. Fried. 2007. "Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions." *Nat Protoc* 2 (8):1849-61. doi: 10.1038/nprot.2007.249.

Hornig, J., F. Frob, M. R. Vogl, I. Hermans-Borgmeyer, E. R. Tamm, and M. Wegner. 2013. "The transcription factors Sox10 and Myrf define an essential regulatory network module in differentiating oligodendrocytes." *PLoS Genet* 9 (10):e1003907. doi: 10.1371/journal.pgen.1003907.

Howng, S. Y., R. L. Avila, B. Emery, M. Traka, W. Lin, T. Watkins, S. Cook, R. Bronson, M. Davisson, B. A. Barres, and B. Popko. 2010. "ZFP191 is required by oligodendrocytes for CNS myelination." *Genes Dev* 24 (3):301-11. doi: 10.1101/gad.1864510.

Hsieh, J., J. B. Aimone, B. K. Kaspar, T. Kuwabara, K. Nakashima, and F. H. Gage. 2004. "IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes." *J Cell Biol* 164 (1):111-22. doi: 10.1083/jcb.200308101.

Hu, Q. D., B. T. Ang, M. Karsak, W. P. Hu, X. Y. Cui, T. Duka, Y. Takeda, W. Chia, N. Sankar, Y. K. Ng, E. A. Ling, T. Maciag, D. Small, R. Trifonova, R. Kopan, H. Okano, M. Nakafuku, S. Chiba, H. Hirai, J. C. Aster, M. Schachner, C. J. Pallen, K. Watanabe, and Z. C. Xiao. 2003. "F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation." *Cell* 115 (2):163-75.

Huang da, W., B. T. Sherman, and R. A. Lempicki. 2009a. "Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists." *Nucleic Acids Res* 37 (1):1-13. doi: 10.1093/nar/gkn923.

Huang da, W., B. T. Sherman, and R. A. Lempicki. 2009b. "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources." *Nat Protoc* 4 (1):44-57. doi: 10.1038/nprot.2008.211.

Hughes, E. G., S. H. Kang, M. Fukaya, and D. E. Bergles. 2013. "Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain." *Nat Neurosci* 16 (6):668-76. doi: 10.1038/nn.3390.

Ji, X., W. Li, J. Song, L. Wei, and X. S. Liu. 2006. "CEAS: cis-regulatory element annotation system." *Nucleic Acids Res* 34 (Web Server issue):W551-4. doi: 10.1093/nar/gkl322.

Kanamori, M., H. Konno, N. Osato, J. Kawai, Y. Hayashizaki, and H. Suzuki. 2004. "A genome-wide and nonredundant mouse transcription factor database." *Biochem Biophys Res Commun* 322 (3):787-93. doi: 10.1016/j.bbrc.2004.07.179.

Kanehisa, M., and S. Goto. 2000. "KEGG: kyoto encyclopedia of genes and genomes." *Nucleic Acids Res* 28 (1):27-30.

Kanehisa, M., S. Goto, Y. Sato, M. Kawashima, M. Furumichi, and M. Tanabe. 2014. "Data, information, knowledge and principle: back to metabolism in KEGG." *Nucleic Acids Res* 42 (Database issue):D199-205. doi: 10.1093/nar/gkt1076.

Kang, S. H., M. Fukaya, J. K. Yang, J. D. Rothstein, and D. E. Bergles. 2010. "NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration." *Neuron* 68 (4):668-81. doi: 10.1016/j.neuron.2010.09.009.

Kato, T. M., A. Kawaguchi, Y. Kosodo, H. Niwa, and F. Matsuzaki. 2010. "Lunatic fringe potentiates Notch signaling in the developing brain." *Mol Cell Neurosci* 45 (1):12-25. doi: 10.1016/j.mcn.2010.05.004.

Kessaris, N., M. Fogarty, P. Iannarelli, M. Grist, M. Wegner, and W. D. Richardson. 2006. "Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage." *Nat Neurosci* 9 (2):173-9. doi: 10.1038/nn1620.

Kondo, T., and M. Raff. 2000. "Basic helix-loop-helix proteins and the timing of oligodendrocyte differentiation." *Development* 127 (14):2989-98.

Kuspert, M., A. Hammer, M. R. Bosl, and M. Wegner. 2011. "Olig2 regulates Sox10 expression in oligodendrocyte precursors through an evolutionary conserved distal enhancer." *Nucleic Acids Res* 39 (4):1280-93. doi: 10.1093/nar/gkq951.

Kuspert, M., and M. Wegner. 2015. "SomethiNG 2 talk about-Transcriptional regulation in embryonic and adult oligodendrocyte precursors." *Brain Res*. doi: 10.1016/j.brainres.2015.07.024.

Ladi, E., J. T. Nichols, W. Ge, A. Miyamoto, C. Yao, L. T. Yang, J. Boulter, Y. E. Sun, C. Kintner, and G. Weinmaster. 2005. "The divergent DSL ligand Dll3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands." *J Cell Biol* 170 (6):983-92. doi: 10.1083/jcb.200503113.

Langmead, B., C. Trapnell, M. Pop, and S. L. Salzberg. 2009. "Ultrafast and memory-efficient alignment of short DNA sequences to the human genome." *Genome Biol* 10 (3):R25. doi: 10.1186/gb-2009-10-3-r25.

Langseth, A. J., R. N. Munji, Y. Choe, T. Huynh, C. D. Pozniak, and S. J. Pleasure. 2010. "Wnts influence the timing and efficiency of oligodendrocyte precursor cell generation in the telencephalon." *J Neurosci* 30 (40):13367-72. doi: 10.1523/JNEUROSCI.1934-10.2010.

Li, H., Y. Lu, H. K. Smith, and W. D. Richardson. 2007. "Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes." *J Neurosci* 27 (52):14375-82. doi: 10.1523/JNEUROSCI.4456-07.2007.

Ligon, K. L., E. Huillard, S. Mehta, S. Kesari, H. Liu, J. A. Alberta, R. M. Bachoo, M. Kane, D. N. Louis, R. A. Depinho, D. J. Anderson, C. D. Stiles, and D. H. Rowitch. 2007. "Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma." *Neuron* 53 (4):503-17. doi: 10.1016/j.neuron.2007.01.009.

Ligon, K. L., S. Kesari, M. Kitada, T. Sun, H. A. Arnett, J. A. Alberta, D. J. Anderson, C. D. Stiles, and D. H. Rowitch. 2006. "Development of NG2 neural progenitor cells requires Olig gene function." *Proc Natl Acad Sci U S A* 103 (20):7853-8. doi: 10.1073/pnas.0511001103.

Liu, A., J. Li, M. Marin-Husstege, R. Kageyama, Y. Fan, C. Gelinas, and P. Casaccia-Bonnefil. 2006. "A molecular insight of Hes5-dependent inhibition of myelin gene expression: old partners and new players." *EMBO J* 25 (20):4833-42. doi: 10.1038/sj.emboj.7601352.

Liu, G., S. Jiang, C. Wang, W. Jiang, Z. Liu, C. Liu, H. Saiyin, X. Yang, S. Shen, D. Jiang, P. Zhou, D. Han, X. Hu, Q. Yi, and L. Yu. 2012. "Zinc finger transcription factor 191, directly binding to beta-catenin promoter, promotes cell proliferation of hepatocellular carcinoma." *Hepatology* 55 (6):1830-9. doi: 10.1002/hep.25564.

Liu, J., L. Magri, F. Zhang, N. O. Marsh, S. Albrecht, J. L. Huynh, J. Kaur, T. Kuhlmann, W. Zhang, P. A. Slesinger, and P. Casaccia. 2015. "Chromatin landscape defined by repressive histone methylation during oligodendrocyte differentiation." *J Neurosci* 35 (1):352-65. doi: 10.1523/JNEUROSCI.2606-14.2015.

Liu, T., J. A. Ortiz, L. Taing, C. A. Meyer, B. Lee, Y. Zhang, H. Shin, S. S. Wong, J. Ma, Y. Lei, U. J. Pape, M. Poidinger, Y. Chen, K. Yeung, M. Brown, Y. Turpaz, and X. S. Liu. 2011. "Cistrome: an integrative platform for transcriptional regulation studies." *Genome Biol* 12 (8):R83. doi: 10.1186/gb-2011-12-8-r83.

Liu, Z., X. Hu, J. Cai, B. Liu, X. Peng, M. Wegner, and M. Qiu. 2007. "Induction of oligodendrocyte differentiation by Olig2 and Sox10: evidence for reciprocal interactions and dosage-dependent mechanisms." *Dev Biol* 302 (2):683-93. doi: 10.1016/j.ydbio.2006.10.007.

Lu, Q. R., T. Sun, Z. Zhu, N. Ma, M. Garcia, C. D. Stiles, and D. H. Rowitch. 2002. "Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection." *Cell* 109 (1):75-86.

Lu, Q. R., D. Yuk, J. A. Alberta, Z. Zhu, I. Pawlitzky, J. Chan, A. P. McMahon, C. D. Stiles, and D. H. Rowitch. 2000. "Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system." *Neuron* 25 (2):317-29.

Lupski, J. R., C. A. Wise, A. Kuwano, L. Pentao, J. T. Parke, D. G. Glaze, D. H. Ledbetter, F. Greenberg, and P. I. Patel. 1992. "Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A." *Nat Genet* 1 (1):29-33. doi: 10.1038/ng0492-29.

Machanick, P., and T. L. Bailey. 2011. "MEME-ChIP: motif analysis of large DNA datasets." *Bioinformatics* 27 (12):1696-7. doi: 10.1093/bioinformatics/btr189.

Maire, C. L., A. Wegener, C. Kerninon, and B. Nait Oumesmar. 2010. "Gain-of-function of Olig transcription factors enhances oligodendrogenesis and myelination." *Stem Cells* 28 (9):1611-22. doi: 10.1002/stem.480.

Marin-Husstege, M., Y. He, J. Li, T. Kondo, F. Sablitzky, and P. Casaccia-Bonnefil. 2006. "Multiple roles of Id4 in developmental myelination: predicted outcomes and unexpected findings." *Glia* 54 (4):285-96. doi: 10.1002/glia.20385.

Marin-Husstege, M., M. Muggironi, A. Liu, and P. Casaccia-Bonnefil. 2002. "Histone deacetylase activity is necessary for oligodendrocyte lineage progression." *J Neurosci* 22 (23):10333-45.

Marta, C. B., A. M. Adamo, E. F. Soto, and J. M. Pasquini. 1998. "Sustained neonatal hyperthyroidism in the rat affects myelination in the central nervous system." *J Neurosci Res* 53 (2):251-9.

Matthieu, J. M., and P. Amiguet. 1990. "Myelin/oligodendrocyte glycoprotein expression during development in normal and myelin-deficient mice." *Dev Neurosci* 12 (4-5):293-302.

McKinnon, R. D., T. Matsui, M. Dubois-Dalcq, and S. A. Aaronson. 1990. "FGF modulates the PDGF-driven pathway of oligodendrocyte development." *Neuron* 5 (5):603-14.

Mei, F., H. Wang, S. Liu, J. Niu, L. Wang, Y. He, A. Etxeberria, J. R. Chan, and L. Xiao. 2013. "Stage-specific deletion of Olig2 conveys opposing functions on differentiation and maturation of oligodendrocytes." *J Neurosci* 33 (19):8454-62. doi: 10.1523/JNEUROSCI.2453-12.2013.

Memezawa, A., I. Takada, K. Takeyama, M. Igarashi, S. Ito, S. Aiba, S. Kato, and A. P. Kouzmenko. 2007. "Id2 gene-targeted crosstalk between Wnt and retinoid signaling regulates proliferation in human keratinocytes." *Oncogene* 26 (35):5038-45. doi: 10.1038/sj.onc.1210320.

Menn, B., J. M. Garcia-Verdugo, C. Yaschine, O. Gonzalez-Perez, D. Rowitch, and A. Alvarez-Buylla. 2006. "Origin of oligodendrocytes in the subventricular zone of the adult brain." *J Neurosci* 26 (30):7907-18. doi: 10.1523/JNEUROSCI.1299-06.2006.

Murtie, J. C., Y. X. Zhou, T. Q. Le, and R. C. Armstrong. 2005. "In vivo analysis of oligodendrocyte lineage development in postnatal FGF2 null mice." *Glia* 49 (4):542-54. doi: 10.1002/glia.20142.

Nakatani, H., E. Martin, H. Hassani, A. Clavairoly, C. L. Maire, A. Viadieu, C. Kerninon, A. Delmasure, M. Frah, M. Weber, M. Nakafuku, B. Zalc, J. L. Thomas, F. Guillemot, B. Nait-Oumesmar, and C. Parras. 2013. "Ascl1/Mash1 promotes brain oligodendrogenesis during myelination and remyelination." *J Neurosci* 33 (23):9752-68. doi: 10.1523/JNEUROSCI.0805-13.2013.

Nave, K. A. 2010. "Myelination and the trophic support of long axons." *Nat Rev Neurosci* 11 (4):275-83. doi: 10.1038/nrn2797.

Nishiyama, A., X. H. Lin, N. Giese, C. H. Heldin, and W. B. Stallcup. 1996. "Interaction between NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells is required for optimal response to PDGF." *J Neurosci Res* 43 (3):315-30. doi: 10.1002/(SICI)1097-4547(19960201)43:3<315::AID-JNR6>3.0.CO;2-M.

Noble, M., K. Murray, P. Stroobant, M. D. Waterfield, 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 (6173):560-2. doi: 10.1038/333560a0.

Ogawa, H., T. Ueda, T. Aoyama, A. Aronheim, S. Nagata, and R. Fukunaga. 2003. "A SWI2/SNF2-type ATPase/helicase protein, mDomino, interacts with myeloid zinc finger protein 2A (MZF-2A) to regulate its transcriptional activity." *Genes Cells* 8 (4):325-39.

Persikov, A. V., E. F. Rowland, B. L. Oakes, M. Singh, and M. B. Noyes. 2014. "Deep sequencing of large library selections allows computational discovery of diverse sets of zinc fingers that bind common targets." *Nucleic Acids Res* 42 (3):1497-508. doi: 10.1093/nar/gkt1034.

Persikov, A. V., and M. Singh. 2014. "De novo prediction of DNA-binding specificities for Cys2His2 zinc finger proteins." *Nucleic Acids Res* 42 (1):97-108. doi: 10.1093/nar/gkt890.

Petrascheck, M., D. Escher, T. Mahmoudi, C. P. Verrijzer, W. Schaffner, and A. Barberis. 2005. "DNA looping induced by a transcriptional enhancer in vivo." *Nucleic Acids Res* 33 (12):3743-50. doi: 10.1093/nar/gki689.

Pfaffl, M. W. 2001. "A new mathematical model for relative quantification in real-time RT-PCR." *Nucleic Acids Res* 29 (9):e45.

Pfrieger, F. W., and N. Ungerer. 2011. "Cholesterol metabolism in neurons and astrocytes." *Prog Lipid Res* 50 (4):357-71. doi: 10.1016/j.plipres.2011.06.002.

Popko, B., C. Puckett, E. Lai, H. D. Shine, C. Readhead, N. Takahashi, S. W. Hunt, 3rd, R. L. Sidman, and L. Hood. 1987. "Myelin deficient mice: expression of myelin basic protein and generation of mice with varying levels of myelin." *Cell* 48 (4):713-21.

Pringle, N. P., W. P. Yu, S. Guthrie, H. Roelink, A. Lumsden, A. C. Peterson, and W. D. Richardson. 1996. "Determination of neuroepithelial cell fate: induction of the oligodendrocyte lineage by ventral midline cells and sonic hedgehog." *Dev Biol* 177 (1):30-42. doi: 10.1006/dbio.1996.0142.

Pringle, N. P., W. P. Yu, M. Howell, J. S. Colvin, D. M. Ornitz, and W. D. Richardson. 2003. "Fgfr3 expression by astrocytes and their precursors: evidence that astrocytes and oligodendrocytes originate in distinct neuroepithelial domains." *Development* 130 (1):93-102.

Qi, Y., J. Cai, Y. Wu, R. Wu, J. Lee, H. Fu, M. Rao, L. Sussel, J. Rubenstein, and M. Qiu. 2001. "Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor." *Development* 128 (14):2723-33.

Qi, Y., M. Tan, C. C. Hui, and M. Qiu. 2003. "Gli2 is required for normal Shh signaling and oligodendrocyte development in the spinal cord." *Mol Cell Neurosci* 23 (3):440-50.

Qu, Q., G. Sun, K. Murai, P. Ye, W. Li, G. Asuelime, Y. T. Cheung, and Y. Shi. 2013. "Wnt7a regulates multiple steps of neurogenesis." *Mol Cell Biol* 33 (13):2551-9. doi: 10.1128/MCB.00325-13.

Readhead, C., B. Popko, N. Takahashi, H. D. Shine, R. A. Saavedra, R. L. Sidman, and L. Hood. 1987. "Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype." *Cell* 48 (4):703-12.

Relucio, J., M. J. Menezes, Y. Miyagoe-Suzuki, S. Takeda, and H. Colognato. 2012. "Laminin regulates postnatal oligodendrocyte production by promoting oligodendrocyte progenitor survival in the subventricular zone." *Glia* 60 (10):1451-67. doi: 10.1002/glia.22365.

Relucio, J., I. D. Tzvetanova, W. Ao, S. Lindquist, and H. Colognato. 2009. "Laminin alters fyn regulatory mechanisms and promotes oligodendrocyte development." *J Neurosci* 29 (38):11794-806. doi: 10.1523/JNEUROSCI.0888-09.2009.

Richardson, W. D., N. Kessaris, and N. Pringle. 2006. "Oligodendrocyte wars." *Nat Rev Neurosci* 7 (1):11-8. doi: 10.1038/nrn1826.

Roach, A., N. Takahashi, D. Pravtcheva, F. Ruddle, and L. Hood. 1985. "Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice." *Cell* 42 (1):149-55.

Rockman, S. P., S. A. Currie, M. Ciavarella, E. Vincan, C. Dow, R. J. Thomas, and W. A. Phillips. 2001. "Id2 is a target of the beta-catenin/T cell factor pathway in colon carcinoma." *J Biol Chem* 276 (48):45113-9. doi: 10.1074/jbc.M107742200.

Rolyan, H., Y. Y. Tyurina, M. Hernandez, A. A. Amoscato, L. J. Sparvero, B. C. Nmezi, Y. Lu, M. R. Estecio, K. Lin, J. Chen, R. R. He, P. Gong, L. H. Rigatti, J. Dupree, H. Bayir, V. E. Kagan, P. Casaccia, and Q. S. Padiath. 2015. "Defects of Lipid Synthesis Are Linked to the Age-Dependent Demyelination Caused by Lamin B1 Overexpression." *J Neurosci* 35 (34):12002-17. doi: 10.1523/JNEUROSCI.1668-15.2015.

Rosenbloom, K. R., J. Armstrong, G. P. Barber, J. Casper, H. Clawson, M. Diekhans, T. R. Dreszer, P. A. Fujita, L. Guruvadoo, M. Haeussler, R. A. Harte, S. Heitner, G. Hickey, A. S. Hinrichs, R. Hubley, D. Karolchik, K. Learned, B. T. Lee, C. H. Li, K. H. Miga, N. Nguyen, B. Paten, B. J. Raney, A. F. Smit, M. L. Speir, A. S. Zweig, D. Haussler, R. M. Kuhn, and W. J. Kent. 2015. "The UCSC Genome Browser database: 2015 update." *Nucleic Acids Res* 43 (Database issue):D670-81. doi: 10.1093/nar/gku1177.

Saher, G., B. Brugger, C. Lappe-Siefke, W. Mobius, R. Tozawa, M. C. Wehr, F. Wieland, S. Ishibashi, and K. A. Nave. 2005. "High cholesterol level is essential for myelin membrane growth." *Nat Neurosci* 8 (4):468-75. doi: 10.1038/nn1426.

Saher, G., and M. Simons. 2010. "Cholesterol and myelin biogenesis." *Subcell Biochem* 51:489-508. doi: 10.1007/978-90-481-8622-8_18.

Samanta, J., and J. A. Kessler. 2004. "Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation." *Development* 131 (17):4131-42. doi: 10.1242/dev.01273.

Sander, T. L., K. F. Stringer, J. L. Maki, P. Szauter, J. R. Stone, and T. Collins. 2003. "The SCAN domain defines a large family of zinc finger transcription factors." *Gene* 310:29-38.

Saugier-Veber, P., A. Munnich, D. Bonneau, J. M. Rozet, M. Le Merrer, R. Gil, and O. Boespflug-Tanguy. 1994. "X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus." *Nat Genet* 6 (3):257-62. doi: 10.1038/ng0394-257.

Schneider, T. D., and R. M. Stephens. 1990. "Sequence logos: a new way to display consensus sequences." *Nucleic Acids Res* 18 (20):6097-100.

Shen, S., J. Li, and P. Casaccia-Bonnefil. 2005. "Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain." *J Cell Biol* 169 (4):577-89. doi: 10.1083/jcb.200412101.

Shin, H., T. Liu, A. K. Manrai, and X. S. Liu. 2009. "CEAS: cis-regulatory element annotation system." *Bioinformatics* 25 (19):2605-6. doi: 10.1093/bioinformatics/btp479.

Sommer, I., and M. Schachner. 1981. "Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system." *Dev Biol* 83 (2):311-27.

Spassky, N., F. de Castro, B. Le Bras, K. Heydon, F. Queraud-LeSaux, E. Bloch-Gallego, A. Chedotal, B. Zalc, and J. L. Thomas. 2002. "Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1." *J Neurosci* 22 (14):5992-6004. doi: 20026573.

Stolt, C. C., P. Lommes, R. P. Friedrich, and M. Wegner. 2004. "Transcription factors Sox8 and Sox10 perform non-equivalent roles during oligodendrocyte development despite functional redundancy." *Development* 131 (10):2349-58. doi: 10.1242/dev.01114.

Stolt, C. C., S. Rehberg, M. Ader, P. Lommes, D. Riethmacher, M. Schachner, U. Bartsch, and M. Wegner. 2002. "Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10." *Genes Dev* 16 (2):165-70. doi: 10.1101/gad.215802.

Stolt, C. C., S. Schmitt, P. Lommes, E. Sock, and M. Wegner. 2005. "Impact of transcription factor Sox8 on oligodendrocyte specification in the mouse embryonic spinal cord." *Dev Biol* 281 (2):309-17. doi: 10.1016/j.ydbio.2005.03.010.

Sugimori, M., M. Nagao, C. M. Parras, H. Nakatani, M. Lebel, F. Guillemot, and M. Nakafuku. 2008. "Ascl1 is required for oligodendrocyte development in the spinal cord." *Development* 135 (7):1271-81. doi: 10.1242/dev.015370.

Swiss, V. A., T. Nguyen, J. Dugas, A. Ibrahim, B. Barres, I. P. Androulakis, and P. Casaccia. 2011. "Identification of a gene regulatory network necessary for the initiation of oligodendrocyte differentiation." *PLoS One* 6 (4):e18088. doi: 10.1371/journal.pone.0018088.

Taniguchi, Y., M. Amazaki, T. Furuyama, W. Yamaguchi, M. Takahara, O. Saino, T. Wada, H. Niwa, F. Tashiro, J. Miyazaki, M. Kogo, T. Matsuyama, and S. Inagaki. 2009. "Sema4D deficiency results in an increase in the number of oligodendrocytes in healthy and injured mouse brains." *J Neurosci Res* 87 (13):2833-41. doi: 10.1002/jnr.22124.

Tekki-Kessaris, N., R. Woodruff, A. C. Hall, W. Gaffield, S. Kimura, C. D. Stiles, D. H. Rowitch, and W. D. Richardson. 2001. "Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon." *Development* 128 (13):2545-54.

Temple, S., and M. C. Raff. 1985. "Differentiation of a bipotential glial progenitor cell in a single cell microculture." *Nature* 313 (5999):223-5.

Tiwari-Woodruff, S., L. Beltran-Parrazal, A. Charles, T. Keck, T. Vu, and J. Bronstein. 2006. "K+ channel KV3.1 associates with OSP/claudin-11 and regulates oligodendrocyte development." *Am J Physiol Cell Physiol* 291 (4):C687-98. doi: 10.1152/ajpcell.00510.2005.

Tiwari-Woodruff, S. K., A. G. Buznikov, T. Q. Vu, P. E. Micevych, K. Chen, H. I. Kornblum, and J. M. Bronstein. 2001. "OSP/claudin-11 forms a complex with a novel member of the tetraspanin super family and beta1 integrin and regulates proliferation and migration of oligodendrocytes." *J Cell Biol* 153 (2):295-305.

Todeschini, A. L., A. Georges, and R. A. Veitia. 2014. "Transcription factors: specific DNA binding and specific gene regulation." *Trends Genet* 30 (6):211-9. doi: 10.1016/j.tig.2014.04.002.

Trapp, B. D., A. Nishiyama, D. Cheng, and W. Macklin. 1997. "Differentiation and death of premyelinating oligodendrocytes in developing rodent brain." *J Cell Biol* 137 (2):459-68.

Tsuda, M., S. Takahashi, Y. Takahashi, and H. Asahara. 2003. "Transcriptional co-activators CREB-binding protein and p300 regulate chondrocyte-specific gene expression via association with Sox9." *J Biol Chem* 278 (29):27224-9. doi: 10.1074/jbc.M303471200.

Verheijen, M. H., N. Camargo, V. Verdier, K. Nadra, A. S. de Preux Charles, J. J. Medard, A. Luoma, M. Crowther, H. Inouye, H. Shimano, S. Chen, J. F. Brouwers, J. B. Helms, M. L. Feltri, L. Wrabetz, D. Kirschner, R. Chrast, and A. B. Smit. 2009. "SCAP is required for timely and proper myelin membrane synthesis." *Proc Natl Acad Sci U S A* 106 (50):21383-8. doi: 10.1073/pnas.0905633106.

Vogl, M. R., S. Reiprich, M. Kuspert, T. Kosian, H. Schrewe, K. A. Nave, and M. Wegner. 2013. "Sox10 cooperates with the mediator subunit 12 during terminal differentiation of myelinating glia." *J Neurosci* 33 (15):6679-90. doi: 10.1523/JNEUROSCI.5178-12.2013.

Wang, H., R. Sun, G. Liu, M. Yao, J. Fei, and H. Shen. 2008. "Characterization of the target DNA sequence for the DNA-binding domain of zinc finger protein 191." *Acta Biochim Biophys Sin (Shanghai)* 40 (8):704-10.

Wang, S., A. D. Sdrulla, G. diSibio, G. Bush, D. Nofziger, C. Hicks, G. Weinmaster, and B. A. Barres. 1998. "Notch receptor activation inhibits oligodendrocyte differentiation." *Neuron* 21 (1):63-75.

Wang, S., A. Sdrulla, J. E. Johnson, Y. Yokota, and B. A. Barres. 2001. "A role for the helix-loop-helix protein Id2 in the control of oligodendrocyte development." *Neuron* 29 (3):603-14.

Wang, Z., H. Colognato, and C. Ffrench-Constant. 2007. "Contrasting effects of mitogenic growth factors on myelination in neuron-oligodendrocyte co-cultures." *Glia* 55 (5):537-45. doi: 10.1002/glia.20480.

Wei, Q., W. K. Miskimins, and R. Miskimins. 2005. "Stage-specific expression of myelin basic protein in oligodendrocytes involves Nkx2.2-mediated repression that is relieved by the Sp1 transcription factor." *J Biol Chem* 280 (16):16284-94. doi: 10.1074/jbc.M500491200.

Weinstock, G. M. 2007. "ENCODE: more genomic empowerment." *Genome Res* 17 (6):667-8. doi: 10.1101/gr.6534207.

Weng, Q., Y. Chen, H. Wang, X. Xu, B. Yang, Q. He, W. Shou, Y. Chen, Y. Higashi, V. van den Berghe, E. Seuntjens, S. G. Kernie, P. Bukshpun, E. H. Sherr, D. Huylebroeck, and Q. R. Lu. 2012. "Dual-mode modulation of Smad signaling by Smad-interacting protein Sip1 is required for myelination in the central nervous system." *Neuron* 73 (4):713-28. doi: 10.1016/j.neuron.2011.12.021.

Whitfield, T. W., J. Wang, P. J. Collins, E. C. Partridge, S. F. Aldred, N. D. Trinklein, R. M. Myers, and Z. Weng. 2012. "Functional analysis of transcription factor binding sites in human promoters." *Genome Biol* 13 (9):R50. doi: 10.1186/gb-2012-13-9-r50.

Williams, A. J., S. C. Blacklow, and T. Collins. 1999. "The zinc finger-associated SCAN box is a conserved oligomerization domain." *Mol Cell Biol* 19 (12):8526-35.

Wolswijk, G., and M. Noble. 1992. "Cooperation between PDGF and FGF converts slowly dividing O-2Aadult progenitor cells to rapidly dividing cells with characteristics of O-2Aperinatal progenitor cells." *J Cell Biol* 118 (4):889-900.

Wu, Y., Y. Liu, E. M. Levine, and M. S. Rao. 2003. "Hes1 but not Hes5 regulates an astrocyte versus oligodendrocyte fate choice in glial restricted precursors." *Dev Dyn* 226 (4):675-89. doi: 10.1002/dvdy.10278.

Xin, M., T. Yue, Z. Ma, F. F. Wu, A. Gow, and Q. R. Lu. 2005. "Myelinogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in Olig1-null mice." *J Neurosci* 25 (6):1354-65. doi: 10.1523/JNEUROSCI.3034-04.2005.

Ye, F., Y. Chen, T. Hoang, R. L. Montgomery, X. H. Zhao, H. Bu, T. Hu, M. M. Taketo, J. H. van Es, H. Clevers, J. Hsieh, R. Bassel-Duby, E. N. Olson, and Q. R. Lu. 2009. "HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction." *Nat Neurosci* 12 (7):829-38. doi: 10.1038/nn.2333.

Young, K. M., T. Mitsumori, N. Pringle, M. Grist, N. Kessaris, and W. D. Richardson. 2010. "An Fgfr3-iCreER(T2) transgenic mouse line for studies of neural stem cells and astrocytes." *Glia* 58 (8):943-53. doi: 10.1002/glia.20976.

Yu, K., S. McGlynn, and M. P. Matise. 2013. "Floor plate-derived sonic hedgehog regulates glial and ependymal cell fates in the developing spinal cord." *Development* 140 (7):1594-604. doi: 10.1242/dev.090845.

Yu, Y., Y. Chen, B. Kim, H. Wang, C. Zhao, X. He, L. Liu, W. Liu, L. M. Wu, M. Mao, J. R. Chan, J. Wu, and Q. R. Lu. 2013. "Olig2 targets chromatin remodelers to enhancers to initiate oligodendrocyte differentiation." *Cell* 152 (1-2):248-61. doi: 10.1016/j.cell.2012.12.006.

Yuen, T. J., J. C. Silbereis, A. Griveau, S. M. Chang, R. Daneman, S. P. Fancy, H. Zahed, E. Maltepe, and D. H. Rowitch. 2014. "Oligodendrocyte-encoded HIF function couples postnatal myelination and white matter angiogenesis." *Cell* 158 (2):383-96. doi: 10.1016/j.cell.2014.04.052.

Zamanian, J. L., L. Xu, L. C. Foo, N. Nouri, L. Zhou, R. G. Giffard, and B. A. Barres. 2012. "Genomic analysis of reactive astrogliosis." *J Neurosci* 32 (18):6391-410. doi: 10.1523/JNEUROSCI.6221-11.2012.

Zaret, K. S., and J. S. Carroll. 2011. "Pioneer transcription factors: establishing competence for gene expression." *Genes Dev* 25 (21):2227-41. doi: 10.1101/gad.176826.111.

Zerlin, M., A. Milosevic, and J. E. Goldman. 2004. "Glial progenitors of the neonatal subventricular zone differentiate asynchronously, leading to spatial dispersion of glial clones and to the persistence of immature glia in the adult mammalian CNS." *Dev Biol* 270 (1):200-13. doi: 10.1016/j.ydbio.2004.02.024.

Zhang, H. M., H. Chen, W. Liu, H. Liu, J. Gong, H. Wang, and A. Y. Guo. 2012. "AnimalTFDB: a comprehensive animal transcription factor database." *Nucleic Acids Res* 40 (Database issue):D144-9. doi: 10.1093/nar/gkr965.

Zhang, H., L. Vutskits, V. Calaora, P. Durbec, and J. Z. Kiss. 2004. "A role for the polysialic acid-neural cell adhesion molecule in PDGF-induced chemotaxis of oligodendrocyte precursor cells." *J Cell Sci* 117 (Pt 1):93-103. doi: 10.1242/jcs.00827.

Zhang, Y., K. Chen, S. A. Sloan, M. L. Bennett, A. R. Scholze, S. O'Keeffe, H. P. Phatnani, P. Guarnieri, C. Caneda, N. Ruderisch, S. Deng, S. A. Liddelow, C. Zhang, R. Daneman, T. Maniatis, B. A. Barres, and J. Q. Wu. 2014. "An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex." *J Neurosci* 34 (36):11929-47. doi: 10.1523/JNEUROSCI.1860-14.2014.

Zhou, Q., and D. J. Anderson. 2002. "The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification." *Cell* 109 (1):61-73.

Zhou, Q., G. Choi, and D. J. Anderson. 2001. "The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2." *Neuron* 31 (5):791-807.

Zhu, Q., X. Zhao, K. Zheng, H. Li, H. Huang, Z. Zhang, T. Mastracci, M. Wegner, Y. Chen, L. Sussel, and M. Qiu. 2014. "Genetic evidence that Nkx2.2 and Pdgfra are major determinants of the timing of oligodendrocyte differentiation in the developing CNS." *Development* 141 (3):548-55. doi: 10.1242/dev.095323.

Zhu, X., R. A. Hill, D. Dietrich, M. Komitova, R. Suzuki, and A. Nishiyama. 2011. "Age-dependent fate and lineage restriction of single NG2 cells." *Development* 138 (4):745-53. doi: 10.1242/dev.047951.

Appendices:

All files available online in Microsoft Excel format.

i. Primers used for RT-PCR

ii. Transcripts with reduced expression in *Zfp191*$^{-/-}$ whole brain

iii. Transcripts with increased expression in *Zfp191*$^{-/-}$ whole brain

iv. Transcripts with reduced expression in *Zfp191*$^{-/-}$ OPCs

v. Transcripts with increased expression in *Zfp191*$^{-/-}$ OPCs

vi. Transcripts with reduced expression in *Zfp191*$^{-/-}$ oligodendrocytes

vii. Transcripts with increased expression in *Zfp191*$^{-/-}$ oligodendrocytes

viii. Transcripts with reduced expression in (*Mbp*^{shi}) *shiverer* whole brain

ix. Transcripts with increased expression in (*Mbp*^{shi}) *shiverer* whole brain

x. Transcripts with reduced expression in (*Mbp*^{shi}) shiverer OPCs

xi. Transcripts with increased expression in (*Mbp*^{shi}) shiverer OPCs

xii. Transcripts with reduced expression in (*Mbp*^{shi}) *shiverer* oligodendrocytes

xiii. Transcripts with increased expression in (*Mbp*^{shi}) *shiverer* oligodendrocytes