CHAPTER 1

GENERAL INTRODUCTION

1.1. Organization of Nervous System

The nervous system is responsible for gathering information from the environment, processing this information, and attributing a specific signal that will lead to an adequate response. The mammalian nervous system is composed of two distinct morphological and functional entities that are the central nervous system (CNS), that includes the brain and the spinal cord and the peripheral nervous system (PNS), that contents the cranial nerves, spinal nerves and connections between the CNS and the target organs of the body. CNS is a control center that integrates all input signals from PNS and CNS to evoke an appropriate response. PNS, on the other hand, provides the sensory and motor wiring to convey and elicit the responses to the rest of the body in a voluntary (somatic nervous system) or involuntary modality (autonomic nervous system) (Kandel et al., 2000; Tortora and Derrickson, 2008).

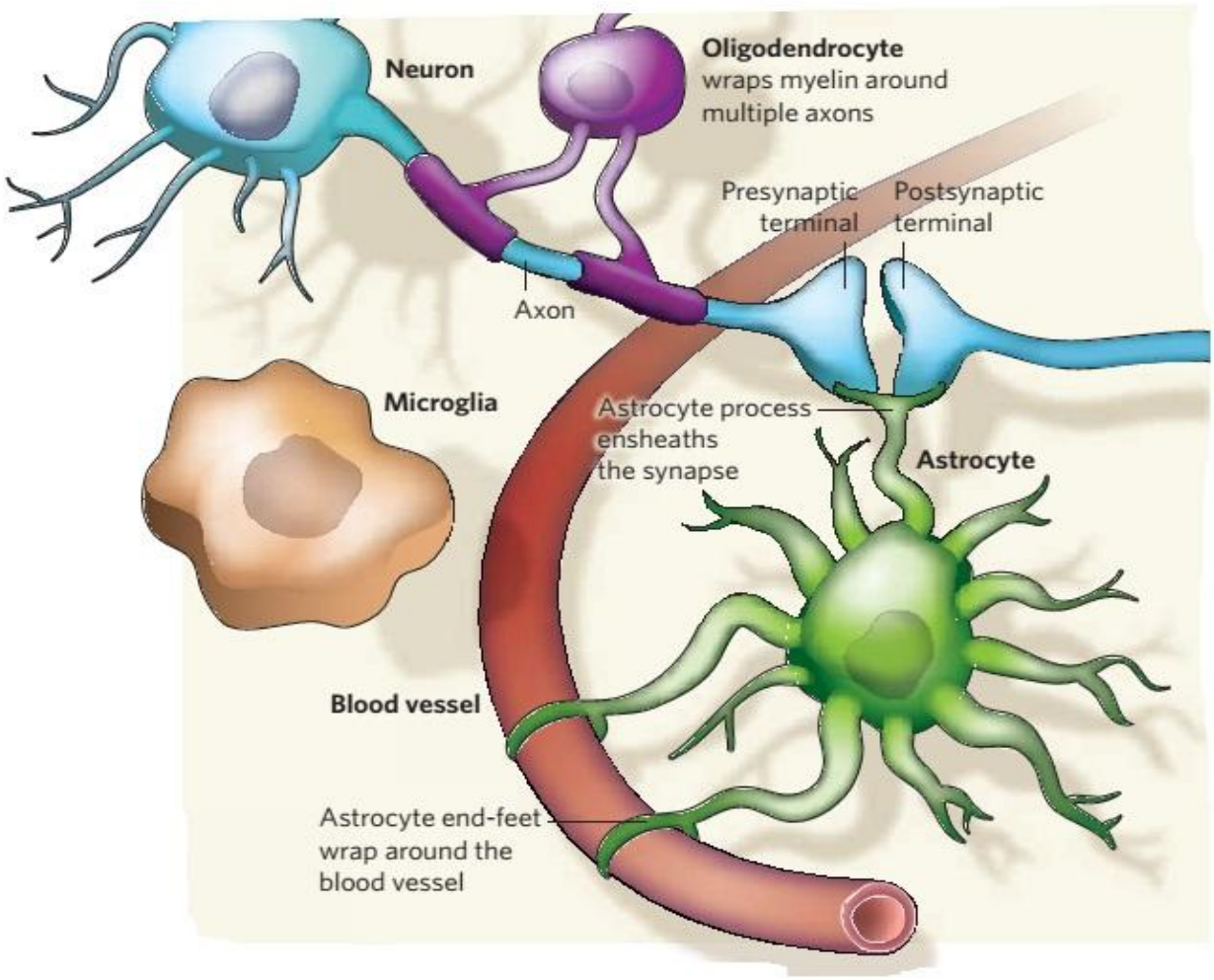
1.2. Cells of Central Nervous System

Central nervous system is an intricate and highly organized network of two main types of cells: Neurons and glial cells. Neurons process information by transmitting the electric impulses to other neurons via synaptic communication. Neurons are responsible for gathering information from sensory receptors, processing that information and generating action potentials (Tortora and Derrickson, 2008).Neurons communicate with each other by transmitting the electrical or chemical signals via synapse. On other hand glial cells provide metabolic, structural and trophic support to the surrounding neurons.

1.2.1 Glial cells

As the Greek name implies, Glia are commonly known as the glue of the nervous system. The great German pathologist Rudolf Virchow first described neuroglia about 150 years ago. Since their discovery, glia was thought for a long time to function only to support neurons passively. However, considerable amount of recent data has changed classical idea that brain function results exclusively from neuronal activity. We now appreciate the glia function as master regulators of the nervous system, controlling numerous aspects of nervous system development, plasticity and disease (Barres, 2008; Greener, 2015). Glial cells constitute a larger part of the nervous tissue and their proportion increases with the degree of evolution, from 25% in fruit flies to 90% in the human brain (Pfrieger and Barres, 1995). However, a recent review comments that

these estimates seem 'to be incorrect'. Stereological and nuclear counts suggest that, overall; the human brain has 'roughly equal' numbers of neurons and non-neuronal cells (Verkhratsky and Butt, 2013). CNS glial cells (Figure 1.1) comprises of microglia, astrocytes and the myelin forming cells (oligodendrocytes in the CNS and Schwann cells in the PNS).



(Allen and Barres, 2009)

Figure 1.1: Glia–neuron interactions. Oligodendrocytes form myelin sheath around axons to speed up neuronal transmission. Astrocytes extend processes that ensheath blood vessels and synapses. Microglia, immune cells keep the brain under surveillance for damage or infection.

1.2.1.1 Astrocytes

Astrocytes are the most abundant cell type in the central nervous system which have start shaped morphology and extend numerous processes that interweave in complex and intimate ways between neuronal cell bodies and fibers. Astrocytes are commonly identified by the presence of

intermediate filaments (glial fibrils).The major component of glial fibrils, glial fibrillary acidic protein (GFAP), is thought to be specific for astrocytes in the CNS. Astrocytes are known to play important roles in numerous processes of the development and physiology of the central nervous system such as trophic and metabolic support for neurons, neuronal survival and differentiation, neuronal guidance, neurite outgrowth and synaptogensis (Kettenmann and Ransom, 2004; Araque and Navarrete, 2010). Astrocytes contribute to homeostasis in the brain by providing neurons with energy and substrates for neurotransmission. They act as physical barriers between the synaptic connections of neighbouring neurons, and remove excess neurotransmitter and ions from the extracellular space. Astrocytes process wrapped around the blood capillaries and form a blood brain barrier which separate the CNS from systemic circulation.

However, more recent evidence shows that astrocytes can actively contribute to synaptic plasticity and activity by releasing neurotransmitters and affecting blood flow. These emerging functions suggest that astrocytes are active participants in brain activity rather than passive elements in maintaining the extracellular space (Barres, 2008). They also regulate the synapse formation and function by forming tripartite synapse (astrocytes projection envelope the synapse), where they monitor and respond the neuronal activity by expressing many of the same neurotransmitter receptors as neurons, and neurotransmitter released by neurons activates calcium-based signaling cascades in astrocytes. Astrocytes then release neuroactive substances, signaling back to neurons to form a feedback loop (Pfrieger and Barres, 1997; Ventura and Harris, 1999; Wang and Bordey, 2008; Allen and Barres, 2009). Astrocytes control neuronal maturation and survival by releasing the growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) (Rudge et al., 1992), and fibroblast growth factor(FGF) (Ojeda et al., 1999). Astrocytes respond to many form of injury: trauma, inflammation, multiple sclerosis, infection, neurodegeneration by reactive astrogliosis, which involve changes in gene expression and morphology, causing scar formation (Eddleston and Mucke, 1993; Pekny and Nilsson, 2005; Maragakis and Rothstein, 2006).

1.2.1.2 Microglia

Microglia, the resident macrophages in the central nervous system (CNS), is of haematopoietic origin (Ginhoux et al., 2010).Microglia plays an important role in proper development and function of mammalian CNS. Microglia function as phagocytes, play an essential role during development by removing the debris that arises from neural apoptosis during development

(Ferrer et al., 1990; Egensperger et al., 1996). In addition to clearing of debris, phagocytosis function of microglial also play a role in synaptic homeostasis. Microglia are involve in neuronal pruning during development. Microglia secrets trophic factors like insulin-like growth factor-1 , basic fibroblast growth factor, hepatocyte growth factor, epidermal growth factor, platelet-derived growth factor, nerve growth factor, and brain-derived neurotrophic factor that support the neuronal development and function (Nayak et al., 2014; Ueno et al., 2013) Furthermore, microglia are activated and proliferate extensively in response to CNS damage, during trauma (Jensen et al., 1994), stroke (Lehrmann et al., 1997), inflammation (Gonzalez-Scarano and Baltuch, 1999) or autoimmune attack (Ponomarev et al., 2005).Microglia play an essential role in pathogenesis of diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, multiple sclerosis, Rett syndrome, X-linked adrenoleukodystrophy (X-ALD) and Lysosomal storage diseases (LSD) (Cartier et al., 2014).

1.2.1.3 Oligodendrocytes

Oligodendrocytes (OLs) are smaller cells with fewer processes, found by Rio Hortega using silver carbonate impregnation. The main function of oligodendrocytes is to produce the myelin sheath around most of the axons in CNS for providing rapid conduction of nerve impulses. Schwann cells are the myelinating cells of PNS, which ensheath the axons of peripheral nerves. Unlike Schwann cells which myelinate a single axon at a time, a single oligodendrocytes can myelinate 30-40 axons (Perez-Cerda et al., 2015; Baumann and Pham-Dinh, 2001; Jessen, 2004**).** Oligodendrocytes also support axonal energy metabolism and long-term integrity. However, OLs also form synaptic connections with neurons, and contribute actively and directly to neural signaling (Lin and Bergles, 2004). In addition, the myelin sheath is critical for the normal health of axon, regulating the homeostasis of the periaxonal space (Dyer, 2002). During development, OPCs (Oligodendrocytes Precursor cells) arise from neural precursors located within discrete regions in the CNS. The newly generated OPCs proliferate and migrate long distances in order to populate the entire CNS, before differentiating into myelinating oligodendrocytes. In vertebrates, the majority of myelination occurs postnatal and is thought to continue throughout life. Myelination requires sequential progression of oligodendrocytes through different development stages which includes OPCs that differentiates into immature OLs and then finally to mature myelinating oligodendrocytes which form myelin sheath around axons. Each stage of

oligodendrocytes development is marked by expression of stage specific cell surface antigens. Oligodendrocytes differentiation involves dramatic changes in their gene expression and in morphology from bipolar (OPCs) to extensive network of branches (Mature Oligodendrocytes) (Baumann and Pham-Dinh, 2001; Fields, 2008).

1.3. Oligodedendrocyte Development

1.3.1 Origin and specification

Oligodendrogenesis occurs within restricted regions of the brain and spinal cord during the late gestational and early postnatal periods, after neuronal differentiation has taken place (Altman and Bayer, 1984). In the developing spinal cord oligodendrocytes are produced in two separate waves. Initially, from E12.5, OPCs, identified by their expression of PDGFRα, arise from the VZ (ventral ventricular zone) of the pMN domain of the spinal cord (Zhou et al., 2001). pMN domain first gives rise to motor neuron precursors, and then, after the neurogenic/gliogenic switch, to OPCs. Shh signaling, acting through activation of Olig2, is essential for motor neuron and OL development (Figure 1.2). In the spinal cord, most oligodendrocytes (~85%) derived from pMN domain (Cai et al., 2005; Fogarty et al., 2005; Vallstedt et al., 2005; Tripathi et al., 2011). From there, OPCs migrate laterally and dorsally to occupy all area of the spinal cord and finally differentiate into myelin-forming oligodendrocyte. This initial specification of oligodendrocytes in the embryonic spinal cord is also dependent on the ventral signalling molecule Sonic hedgehog (Shh) (Tekki-Kessaris et al., 2001) secreted from the notochord and floor plate, which is thought to induce cell type specific expression of *Olig* genes (Lu et al., 2000; Zhou et al., 2001) and to enhance the survival of oligodendrocytes (Davies and Miller, 2001). Later, an additional source of OPCs arises in the dorsal spinal cord independent of Shh signaling (Figure 1.2), contributing to 10–15% of the final oligodendrocyte population in the spinal cord (Bradl and Lassmann, 2010).

In the forebrain, multiple waves of OPC production occur from embryonic to postnatal stages emerging in a ventral-to-dorsal progression (Figure 1.2). The first OPCs originate in the medial ganglionic eminence (MGE) and anterior entopeduncular (AEP) area of the ventral forebrain. These OPCs populate the entire embryonic telencephalon including the cerebral cortex, and are then joined by a second wave of OPCs derived from the lateral and/or caudal ganglionic eminences (Pringle and Richardson, 1993; Timsit et al., 1995; Richardson et al., 2000; Spassky

et al., 2000). However, this first population of OP cells originating from the ventricular germinal layer are completely lost and are replaced by dorsally originating OP cells (Vallstedt et al., 2005).The third wave of OPCs, finally, arises within the postnatal cortex (Kessaris *et al.*, 2006).Sonic Hedgehog signalling (Shh) has also been demonstrated to be important in oligodendrocyte specification in the embryonic ventral forebrain (Nery et al., 2001; Spassky et al., 2001; Tekki-Kessaris et al., 2001). OPCs exhibit multidirectional migration from their origins to distant sites under control of both repulsive and attractive cues. OPCs are marked by expression of platelet-derived growth factor receptor alpha (PDGFRα), Nkx2.2, and NG2, whereas mature OLs express proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte glycoprotein (MOG) (Simpson & Armstrong 1999; Tsai et al. 2002, 2003; Fancy et al., 2011).

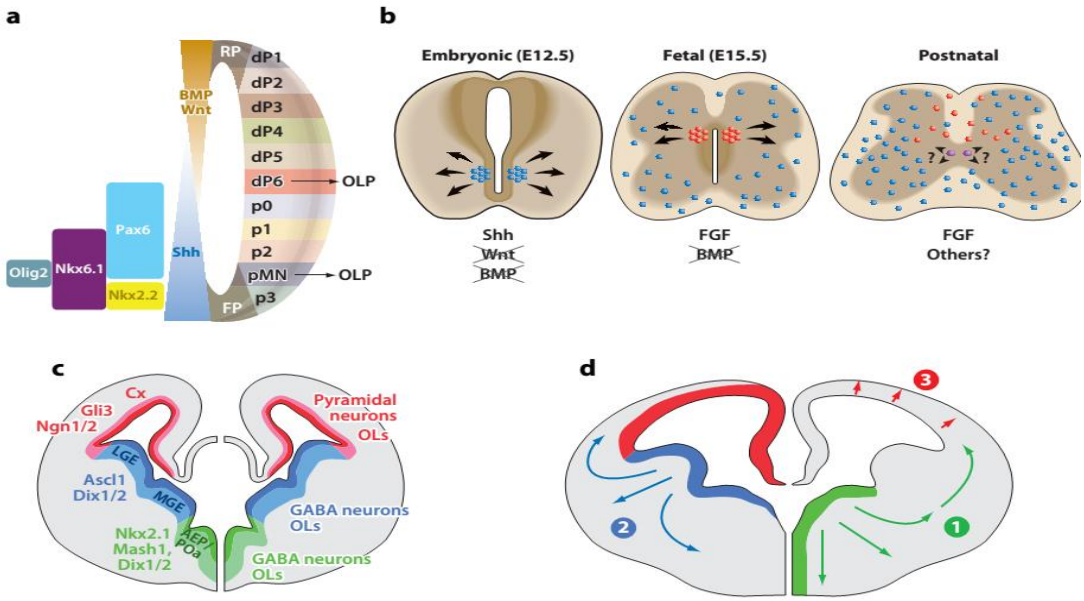
1.3.2 Migration

OPCs arise from discrete areas of spinal cord and brain must migrate long distance before differentiating into myelin forming oligodendrocytes after they reach their neuronal targets. OPC migration is guided by many soluble and membrane bound cues (Miller, 2002) to ensure the cells reach their final destination. Secreted molecules involve in regulation of OPCs migration are growth factors like PDGF, FGF or hepatocyte growth factor, chemotropic molecules like netrins and secreted semaphorins, and the chemokine CXCL1.OPCs migration is also controlled by contact mediated mechanisms involving many different extracellular matrix proteins and cell surface molecule (Bradl and Lassmann, 2010). Both PDGF and FGF have been shown to be potent motogens for OPCs in vitro (Armstrong et al., 1990; Frost et al., 1996; Milner et al., 1997). Activation of PDGFRα receptors, by the growth factor PDGF-AA, enhances OPC migration and proliferation. PDGFRα is a tyrosine kinase receptor which activates numerous signalling pathways. A recent study has demonstrated that the ERK signalling pathway mediates PDGF-AA dependent OPC migration (Vora et al., 2011). Hepatocyte growth factor (HGF), an established motogenic factor for neuronal precursors (Powell et al., 2001) induces a significant 2.5- fold increase in the migration rate of oligodendrocyte precursor cells in vitro, as well as correlative changes in the cytoskeleton of these cells (Yan and Rivkees, 2002). Vascular endothelial growth factor (VEGF) has been recently reported to promote OPC migration by reorganization of the actin cytoskeleton within the leading-edge processes of migrating OPCs

(Hayakawa et al., 2011).Netrin-1 can act either as a chemorepellent or -attractant for oligodendrocyte precursor cells (De Castro, 2003).Tenascin-C is considered as a general inhibitor of the migration of oligodendrocyte precursor cells in both an adhesion-dependent and independent manner (Kiernan et al., 1996). CXCL1 inhibits the migration of neonatal oligodendrocyte precursor cells and enhances their interaction with substrates. CXCL1inhibits the migration of neonatal oligodendrocyte precursor cells by antagonizing the PDGF-A-induced chemotactic effect on these cells (Tsai et al., 2002).

1.3.3 Proliferation

OPC proliferation is regulated by several signals released from neuron and astrocytes, in order to produce sufficient OPCs to differentiate into myelinating oligodendrocytes. Several growth factors released from neurons act as potent mitogens for OPCs. One of the best-characterized mitogens for oligodendrocyte precursors (OPCs) is the growth factor PDGF-AA (the active homodimer of PDGF-A), which binds to its receptor PDGFRα. PDGFRα receptors are abundantly expressed by OPCs, and become down-regulated as they differentiate into myelinating oligodendrocytes (Miller, 2002). Over expression of PDGF-A results in substantial increase in the number of spinal cord oligodendrocyte precursors (Calver et al., 1998), while in PDGF-A knockouts the number of oligodendrocyte precursors is dramatically reduced (Fruttiger et al., 1999). The chemokine CXCL1 enhances the proliferation of oligodendrocyte precursors to PDGF (Robinson et al., 1998; Wu et al., 2000) in a concentration-dependent manner as well as regulating the migration of immature precursor cells (Tsai et al., 2000). Another important growth factor fibroblast growth factor (FGF), which appears to be widely distributed throughout the embryonic CNS, also enhances OPC proliferation. FGF induced OPC proliferation is thought to extend the time period over which OPCs are proliferative and prevent their differentiation into myelinating oligodendrocytes (Gard and Pfeiffer, 1993; Mayer et al., 1993). Several other growth factors including neurotrophin-3 (NT-3) (Barres et al., 1994b), Brain derived neurotrophic factor (BDNF) (Van't Veer et al., 2009) and neuregulin (Roy et al., 2007; Brinkmann et al., 2008; Taveggia et al., 2008) also act as mitogens to increase OPC proliferation. Some messengers have been reported to inhibit OPC proliferation. In particular, retinoic acid, glucocorticoid and thyroid hormone are all suggested to cause OPCs to exit the cell cycle and differentiate (Barres et al., 1994a).



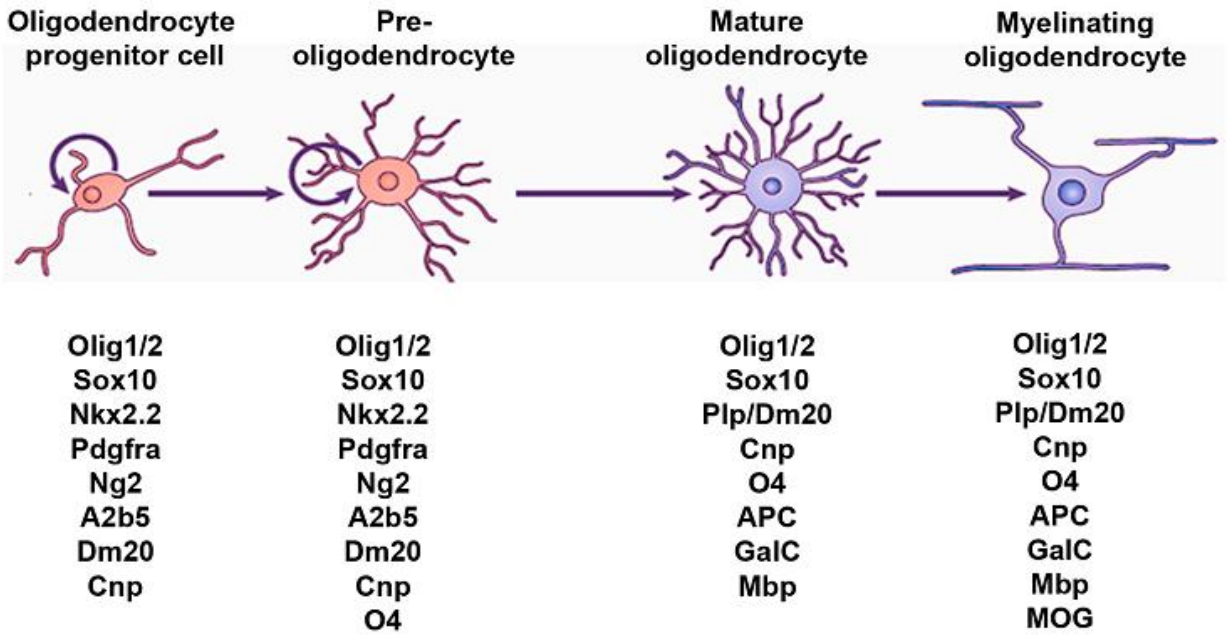
(Fancy et al., 2011)

Figure 1.2: Oligodendrocytes origin and specification in Spinal cord and brain. (a) Patterning of the embryonic neural tube by organizing signals (BMP, Shh and Wnt) and transcriptional factors (Olig2,Nkx2.2, Nkx6.1, and Pax6) in progenitor domains that give rise to specific neuron and glial subtypes. (b) Embryonic OPCs arise from the ventral pMN domain, whereas at fetal stages, a dorsal OPC-generating domain is active. OPCs from ventral and dorsal regions are intermixed in the spinal cord at birth. (c) In the forebrain, distinct domains demarcated by expression of specific transcription factors, such as Dlx1/2, Ascl1, and Olig2, give rise to neurons and glia. (d) Multiple waves of OPC production occur from embryonic to postnatal stages, emerging in a ventral-to-dorsal progression. The first wave originate in the medial ganglionic eminence (MGE) and anterior entopeduncular (AEP) (Green area), Second wave originate in lateral ganglionic eminences (LGE) (Blue area) and third from post natal cortex (Red area).

1.3.4 Differentiation

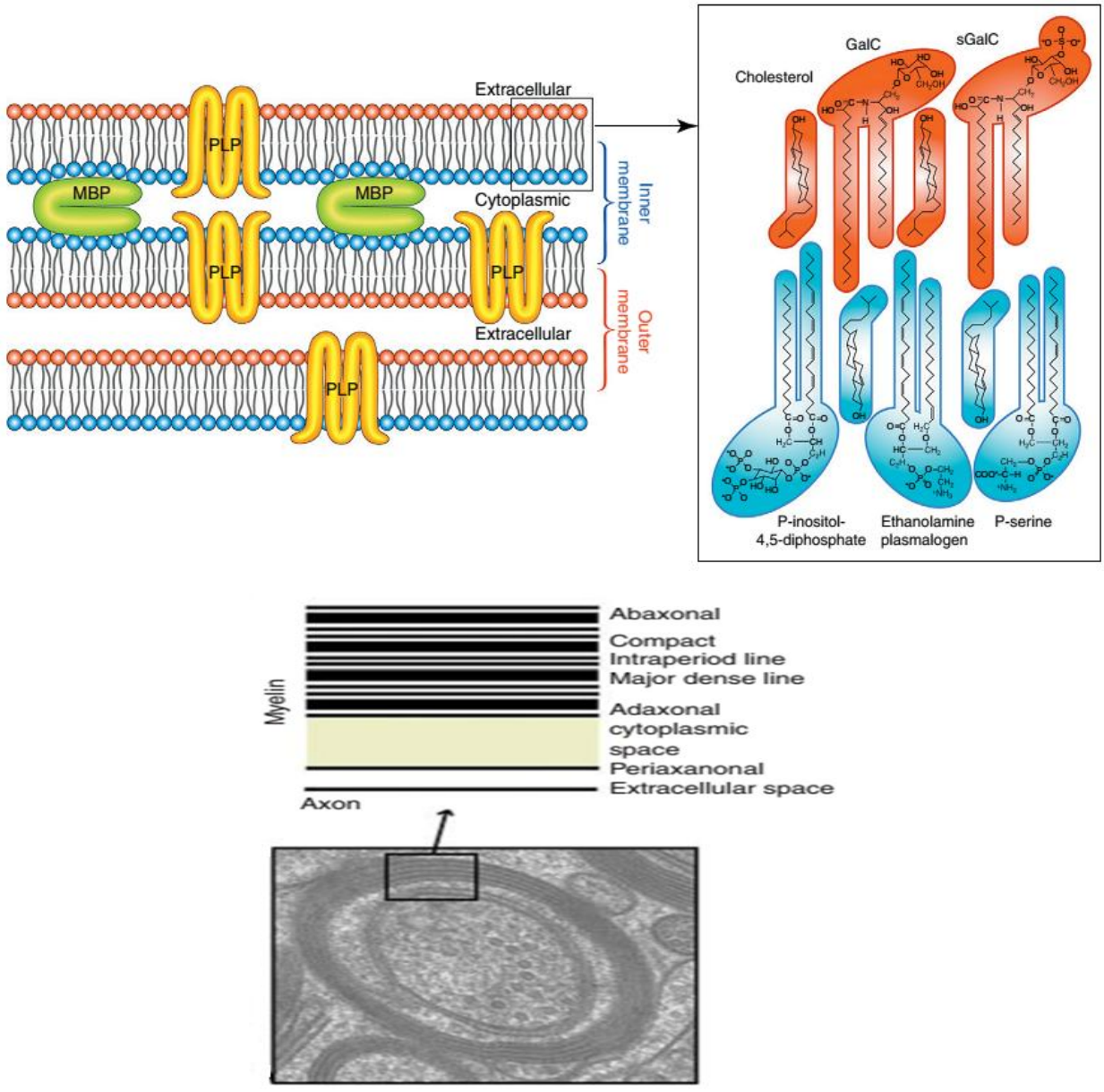
Developmentally, large proportion of OPCs differentiate into premyelinating oligodendrocytes which, given the appropriate environmental cues, will further mature into myelinating oligodendrocytes and myelinate nearby receptive axons (Kukley et al., 2010; Emery, 2010.) Oligodendrocytes differentiation from OPCs to mature myelinating cells are characterized by dynamic change in expression pattern of molecular markers and a dramatic change in cell morphology from bipolar to multipolar (Figure 1.3). OPCs are characterized by the expression of the ganglioside A2B5, the chondroitin sulfate proteoglycan NG2 and the platelet-derived growth factor receptor α (PDGFRα), but as they undergo terminal differentiation, they lose the these OPCs specific markers and begin to express the immature marker O4, followed by PLP, galactocerebroside and myelin basic protein (MBP) (Nishiyama et al., 2009). Morphometric analysis by single cell dye-filling has revealed that premyelinating oligodendrocytes have larger somas and longer processes which cover a greater area, when compared with OPCs (Kukley et al., 2010). However, not all OPCs differentiate into myelinating oligodendrocytes: a large proportion of these cells persist as OPCs in the adult, where they represent 5% of the cells in the brain (Dawson et al., 2003). In order to maintain a constant number of OPCs and produce new oligodendrocytes, OPC divide asymmetrically giving rise to one OPC and one differentiating cell (Sugiarto et al., 2011; Zhu et al., 2011a). OPC cell number is controlled by regulating both cell proliferation and cell death. The final number of oligodendrocytes is thought to be regulated by competition for local survival factors (Barres et al., 1992; Barres and Raff, 1994). In addition, only OPCs that make the appropriate contacts with axons are thought to survive and initiate myelination (Trapp et al., 1997), suggesting that axon derived signals are also important for oligodendrocyte survival. In vitro analyses suggest that maturation of oligodendrocytes from the precursor stage to the mature cell is identical in culture, even without neurons, as in intact tissue. Thus the capacity of oligodendrocyte progenitors to differentiate into oligodendrocytes is intrinsic to the lineage. In the absence of neurons, oligodendrocytes can clearly make a myelin-like membrane; nevertheless, coculture with neurons increases myelin gene expression, such as PLP, MBP, and MAG (Baumann and Pham-Dinh, 2001).Progression through the oligodendrocyte lineage is tightly regulated by a various of intrinsic and extrinsic cues, which regulate myelination both spatially and temporally during development and after demyelination. These signals include growth factors, protein kinases, and

extracellular matrix molecules, all of which influence epigenetic modifications, transcriptional and translational regulation, and the actin cytoskeleton in oligodendrocytes. Differences in temporal expression of these factors and signals in the developing CNS result in early lineage progression and myelination in the spinal cord, and later myelination of cortical regions. Increasing evidence indicates that there are regionally diverse OPC populations that may be generated by distinct localized signaling mechanisms (Bauer et al., 2009; Emery, 2010; Kessaris et al., 2008; Mitew et al., 2014; Bercury and Macklin, 2015).



(Traiffort et al., 2016)

Figure 1.3: Developmental stages of oligodendrocytes lineage development. Morphological and antigenic expressions progression form progenitor to mature myelinating oligodendrocytes.



(Aggarwal et al., 2011; Simons and Nave, 2016)

Figure 1.4: Structure of myelin sheath. Schematic representation of the major structural components of myelin which include myelin proteins and lipid. Compact myelin is formed by the apposition of the external surfaces and internal surfaces of the myelin bilayer that constitute the intraperiodic line and the major dense line, respectively.

1.4. Myelination

The rapid impulse propagation is required for motor, sensory, and cognitive functions of the nervous system, which in vertebrates is facilitated by myelination. Myelination involves the wrapping of axon segments by multilayered, insulating structure, the myelin sheath produced by extended processes of oligodendrocytes. The ensheathment of axons by myelin provides the structural basis for saltatory action potential propagation which accelerates nerve conduction 20–100-fold compared with non myelinated axons of the same diameter (Nave and Werner, 2014). In humans, myelination starts during the second half of gestation and continues until at least 20 years of age (Minkowski, 1967; Knaap and Valk, 1995; Giedd, 2004). Interestingly, myelination occurs caudal to rostral in the brain and rostral to caudal in spinal cord (Brody et al., 1987; Kinney et al., 1988; Baumann and Pham-Dinh, 2001).The myelin sheath has a unique composition with low water content of only 40% and a high proportion of lipids with 70-85% (highly enriched in glycosphingolipids and cholesterol) of the dry mass, which contributes to its ability to be a good insulator. Consequently, the amount of protein in this structure is particularly low compared to normal membrane composition with only 15-30% of the dry mass (Waxman et al. 1995).

1.4.1 Myelin structure and composition

Myelin is a multilayered stack of uniformly thick membrane with a characteristic periodic structure of alternating electron-dense and -light layers (the major dense line and the intraperiod line, respectively) as revealed by electron microscopy (EM) (Hartline, 2008). The major dense line represents the closely condensed cytoplasmic myelin membranes, whereas the interperiod line consists of the tightly apposed outer membranes (Figure 1. 4). The compaction between the membranes in each of these layers is tight and results in a periodicity of about 12 nm. The myelinated segments of the axons are around 150 mm in length and are only interrupted by small gaps, the nodes of Ranvier, which contain sodium channels at a high density. This organization allows energy-efficient saltatory propagation of action potentials. Within the myelin internodes, there are small regions of uncompacted membranes: namely, the innermost and outermost tongues of myelin membranes, the paranodes, and the Schmidt–Lanterman incisures in the peripheral nervous system. This complicated architecture appears much simpler if myelin is depicted in its

unwrapped state as a large sheet surrounded with channel-like tubes at the borders (Mobius et al., 2010; Aggarwal et al., 2011). The g-ratio is the ratio of the axonal diameter divided by the diameter of the axon and its myelin sheath, used to assess axonal myelination. Most myelinated axons in any given animal have the same g-ratio and this value is usually between 0.6 and 0.7. This means that the thickness of the myelin sheath varies according to the diameter of the axon: bigger axons have thicker myelin, and vice versa (Sherman and Brophy, 2005).

Myelin encompasses ~40-50% of the CNS on the dry weight basis (O'Brien and Sampson, 1965). The structural and molecular composition of myelin is unique which confer its insulating property (Figure 1.4). Compared with other plasma membranes, myelin consists of 70% lipids (highly enriched in glycosphingolipids and cholesterol) and 30% proteins (Morell et al., 1994).None of the lipids is unique for myelin, but certain lipids are clearly enriched. Among these lipids are cholesterol, cerebrosides (e.g. galactosylcerebroside, GalC), sulfatides, and galactolipids in general, while the overall amount of lecithin and ethanolamine is reduced (Siegel, 1998).

1.5. CNS Myelin Proteins

Even though myelin consists to only 30% of proteins, these proteins are rather specific and important for the function of the myelin sheath. Major proteins in myelin (Figure 1.4) are the proteolipid protein (PLP1) and its smaller splicing isoform DM20 (together 30-45%), the different isoforms of the myelin basic protein (MBP, 22-35%), and 2′,3′-Cyclic nucleotide 3′-phospho-diesterase (CNP, 4-15%). The remaining 5-25% is thought to be composed of all other myelin proteins: among these is the myelin oligodendrocyte glycoprotein (MOG), myelin oligodendrocyte basic protein (MOBP), the isoforms of the myelin associated glycoprotein (MAG) and Claudin11.The function of these proteins will be shortly summarized in the following paragraphs.

1.5.1 Proteolipid protein (PLP)

The PLP gene produces two main isoforms,30 kDa tetraspan in PLP1 and 26 kDa DM-20 together constitute up to 50 % of all CNS myelin proteins. DM20 protein expression in the CNS is highest during embryonic development, whereas PLP protein expression increases in relation

to DM20 during postnatal development (LeVine et al., 1990).PLP play important role in stabilizing the intraperiod line of CNS myelin has generally been assumed, based largely on the fact that the extracellular loops of this protein are present at this location. Furthermore, the CNS intraperiod line is abnormally condensed both in the PLP knockout mice and in spontaneously occurring PLP mutants (Campagnoni et al., 2001) confirming a structural role for PLP in determining the membrane spacing at the intraperiod line. PLP enhance the myelin stability possibly by forming a 'zipper-like' structure after it is compacted, while myelin in the PLP-null mutant is extra sensitive to osmotic shock during fixation, suggesting that PLP importance in stability of myelin. Furthermore, in older PLP/DM20knockout mice, there is significant axonal degeneration, suggesting that while myelin can form in the absence of PLP/DM20, CNS myelin devoid of PLP/DM20 cannot sustain normal axonal function. Despite the apparent similarity of the PLP and DM20, DM20 cannot replace PLP in transgenic mice (Stecca et al., 2000) the same long-term axonal degeneration occurs in mice expressing exclusively DM20 protein (Quarles et al., 2006).Mutations in the PLP1 gene have been described in patients with Pelizaeus-Merzbacher disease (PMD). The disease usually starts in early childhood and is characterized by developmental retardation, tremor, general weakness and ataxia (Nave et al., 1986). Giving the severity of these phenotypes, it is surprising that PLP1 knockout mice are fully myelinated, but have only a reduced level of cholesterol in their myelin. Double knockout mice lacking both PLP1 and the PLP-related glycoprotein M6B are severely hypo-myelinated. These finding support the model that PLP1 sequesters cholesterol to enable proper myelination (Werner et al., 2013). Apart from PLP1 mutant and knockout mice, mice that overexpress PLP1 have been generated and these mice show dysmyelination (Readhead et al., 1994) and are being used as a model for PMD. In a study using these mice, it could be shown that cholesterol-feeding drastically improves morphological and clinical outcome of these animals (Saher et al., 2012). The presence of PLP proteins in non-myelinating cells suggests an involvement in other functions unrelated to myelination. PLP has been linked to the regulation of several cellular processes including ion exchange, cell migration and programmed cell death. As a membrane protein, PLP also participate in the transduction of signals between the ECM (extracellular matrix) and the interior of the cell. PLP forms a complex in OLs containing αvβ5 integrin and calreticulin, a Ca2+-binding protein (Gudz et al., 2002). The consequences of this complex for integrin function were revealed by studies showing enhanced levels of binding of OLs to the

ECM protein fibronectin. Interestingly these actions were stimulated by the activation of mAchRs (muscarinic acetylcholine receptors), suggesting a role for neuronal signaling in controlling integrin/PLP signalling in OLs. The sensitivity of PLP–αvβ5 activity to neurotransmitters such as acetylcholine may provide a means by which neuronal activity may regulate OL maturation and subsequent myelination. Further work by Gudz et al. (2006) identified a similar complex in OPCs, this time involving an interaction between PLP andavb3 integrin which is involved in regulating the migration of these cells.

In addition to impairment of myelin sheath formation in PLP gene mutations, these mutations are also associated with a substantial decrease in the survival of OLs (Knapp et al., 1986; Boison and Stoffel, 1989; Gow et al., 1998), which has been associated with an accumulation of PLP product in the ER (endoplasmic reticulum) of OLs, leading to apoptosis (Gow et al., 1998). Further evidence linking PLP and OLs viability has emerged from cell culture studies showing opposing effects on survival when PLP protein expression is either enhanced, leading to a reduction in survival (Bongarzone et al., 2001), or reduced, leading to an increase in survival (Yang and Skoff, 1997). A study by Skoff et al. (2004a) shows that PLP is expressed in a number of developing non-neural cell types, and that the levels of PLP in these cells correlates with the degree of apoptosis. Since apoptosis is facilitated under low pH conditions, and expression of PLP appeared to acidify the medium, it seems possible that this influence on pH may contribute to the enhanced level of cell death associated with PLP expression.

1.5.2 Myelin Basic Proteins (MBP)

All members of the MBP family are produced by alternative splicing. The MBP gene is part of a larger gene complex, called Golli, which regulates the expression of the Golli and MBP transcripts. In mice, five different classical MBP-isoforms are known; these can be distinguished into the 21.5 kDa, 18.5 kDa, 17.22 kDa, 17.24 kDa and 14 kDa isoform. The expression of these isoforms varies during brain development, with 18.5 and 14 kDa isoforms being most abundant in adult mice (Siegel, 1998). As the name indicates, MBP is highly basic; at physiological pH the protein has a net charge of +19, which allows MBP to bind to negatively charged lipids and probably to anionic proteins. This binding to biological membranes seems to induce the formation of stable conformations of MBP, while the protein lacks a well-defined 3D structure in solution. Negatively charged lipids, such as Phosphatidyl-inositol (4,5)- diphosphate (PIP2), seems to be essential for proper folding of MBP (Boggs, 2006). MBP is important for the

compaction of myelin membranes. The high-affinity binding to membranes allows MBP to zip together two membranes and to squeeze out the cytoplasm. During this process, MBP molecules interact with each other to form a meshwork that hinders other proteins from entering into compacted regions (Aggarwal et al., 2013). One interesting feature of MBP is that its mRNA is transported in form of granules and that MBP is translated locally. Due to the tight binding of MBP to membranes, the local translation is probably necessary to enable a correct localization of the protein. The importance of MBP is highlighted by the naturally occurring Shiverer mutant mice. These animals carry a mutation in the MBP gene and lack most MBP isoforms. As a result, myelin in homozygous shiverer mice cannot be compacted properly and these mice suffer from convulsions and die at a very young age (Chernoff, 1981).

1.5.3 Cyclic nucleotide phosphodiesterase (CNP)

Constituting 4% of the protein content of the CNS myelin and expressed earlier than any other myelin protein. Two isoforms of the 2', 3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) are known; the 45 kDa protein CNP1 and the 47 kDa protein CNP2. CNP exhibits enzymatic activity and was shown to hydrolyze artificial substrates. However, neither the natural substrate nor the biological function in oligodendrocytes has been identified so far (Sprinkle, 1989).CNP is localized in non-compacted myelin and stays associated with the membrane by an isoprenyl-anchor. CNP1-deficient mice appear healthy and myelin structure seems normal, but upon aging, these animals develop axonal degeneration (Lappe-Siefke et al.; 2003; Rasband et al.; 2005; Edgar et al., 2009). Interestingly, CNP1 deficient mice seem to be highly susceptible to further damages. Recent studies also show defective paranodal loop formation with cytoplasm filled extensions of OL on either side of the node (Rasband et al., 2005).Overexpression of CNPase leads to abnormal myelin formation with aberrant OL membrane expansion (Gravel et al., 1996). Despite the uncertainty regarding the contribution of CNP catalytic activity to myelination, there has been significant progress in identifying functions for CNP beyond the myelin sheath, including roles in OL process outgrowth and RNA transport (Fulton et al., 2009).

1.5.4 Myelin-oligodendrocyte glycoprotein (MOG)

The myelin-oligodendrocyte-glycoprotein (MOG) is a single-pass transmembrane protein that belongs to the immunoglobulin super family of cell adhesion molecules. It was first identified as an antigen responsible for the demyelination in animals injected with CNS homogenate (Birling

et al., 1993).This 28 kDa protein is specifically expressed on the surface of OLs and on the outermost lamellae of myelin sheath. MOG is expressed by OLs in their later stage of differentiation, and is widely used as a surface marker of OL maturation (Scolding et al., 1989; Coffey and McDermott, 1997).The biological function of MOG is not yet known, but it might have adhesive functions. MOG-deficient mice appear healthy and show no pathological abnormalities (Delarasse et al., 2003).

1.5.5 Myelin-associated glycoprotein (MAG)

Similar to MOG, the Myelin-associated glycoprotein (MAG) is a transmembrane protein of the immunoglobulin super family and shows significant homology to the neural cell adhesion molecule (NCAM). Two different isoforms are known; the small S-MAG and the larger L-MAG. Theoretically, these proteins have a molecular weight of 64 kDa and 69 kDa, but are much heavier due to a high degree of glycosylation.MAG knockout mice show only mild neurological pathologies, in particular, CNS myelination is delayed and the animal exhibit abnormal formation of paranodal loops (Montag et al., 1994; Li et al., 1994). Early expression of MAG suggests its role in the initial interaction of OL processes with axons before myelination. However, relatively high levels of MAG in the postnatal brain may play an important role in maintenance of myelin and myelinated axons. Acting as a bifunctional cue, MAG is known to promote embryonic and neonatal neurite outgrowth. It also acts as a major inhibitor of postnatal neurite outgrowth (Johnson et al., 1989; McKerracher et al., 1994; DeBellard et al., 1996; Turnley and Bartlett, 1998). MAG null mice show significant neurological deficits (Li et al., 1994; Montag et al., 1994; Yin et al., 1998; Pan et al., 2005). It displays structural abnormalities in the periaxonal areas of the myelin sheath, more specifically in the periaxonal cytoplasmic collar. The myelin compaction seems to be relatively normal, but there is a significant delay in the onset of CNS myelination.

1.5.6 Myelin-associated oligodendrocytic basic protein (MOBP)

The myelin-associated oligodendrocytic basic protein (MOBP) family constitutes the third most abundant protein in CNS myelin. The mouse Mobp gene comprises eight exons and pre-mRNA processing gives rise to at least seven Mobp splice variants which are expressed solely in the oligodendrocyte. Like MBP, MOBP localizes to major dense line (MDL) of compact myelin. It has been proposed that MOBP has an intimate role in the stabilization of the radial component

characterized by a band of claudin -/oligodendrocyte-specific protein (OSP)-rich tight junctions crossing the myelin sheath. However, despite significant effort, the normal physiological function of MOBP remains unknown. It has been widely suggested that MOBP and MBP have a similar role in the compaction and/or maintenance of the myelin sheath. However, Mobp homozygous null mice display no apparent clinical phenotype and no defect in the process of myelination. MOBP can induce experimental allergic encephalomyelitis in mice and has been proposed to have a role in the pathogenesis of multiple sclerosis (Yamamoto et al., 1999; Gow et al., 1999; Montague et al., 2006).

1.5.7. Minor myelin proteins

Several other proteins are highly enriched in myelin, but show lower abundance.Tmem10 (Opalin), a type I single pass transmembrane protein, is one of these proteins (Kippert et al., 2008,Yoshikawa et al., 2008, Golan et al., 2008).Tmem10 is highly enriched in oligodendrocytes, it might interact with the actin cytoskeleton, but the physiological function of this protein is unknown (Kippert et al., 2008).Tmem10-knockout mice do not exhibit any obvious phenotype. Other myelin proteins have four transmembrane domains and belong to the family of tetraspanins. Examples are Claudin 11, MAL, CD81, and CD9.Claudin 11 has a molecular weight of 22 kDa and belongs to the tight junction proteins. It is expressed in the brain, but also in testis. Knockout mice lack tight junctions in CNS myelin and between sertoli cells, these animals have mild neurological deficits such as hind limb weakness and are infertile (Gow et al., 1999). The mice suffer from deafness due to the lack of tight junctions between basal cells (Gow et al., 2004, Kitajiri et al., 2004). Claudin 11 and PLP1 are both tetraspanins and knockout of both of these genes alone results in relatively mild phenotypes. Therefore, Chow et al. (2005) generated double knockout mice for PLP1 and Claudin 11 had severe neurological problems including abnormal myelin compaction. The myelin and lymphocyte protein (MAL) is a lipid-raft-associated protein and predominantly expressed by Schwann cells and oligodendrocytes. An interesting point about this protein is that it seems to have different functions in CNS and PNS. Overexpression of MAL induces hypo-myelination in the PNS, while knockout animals show accelerated myelination, but nerves appear normal (Buser et al., 2009). The morphology of CNS nerves, however, seems to be altered in MAL knockout mice (Schaeren-Wiemers et al., 2004).

1.6. Myelin Assembly

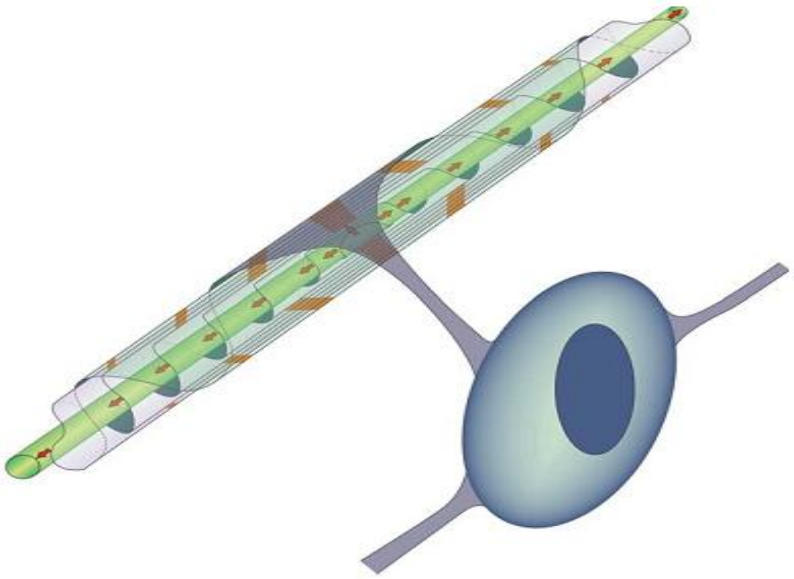
Most oligodendrocytes generate between 20 and 60 myelinating processes with internodal lengths of 20mm-200mm and up to 100 membrane turns (Matthews and Duncan 1971; Hildebrand et al., 1993; Chong et al., 2012). Myelination is a complex sequence of events which includes (1) proliferation and migration of oligodendrocyte precursor cells (OPCs) in white matter tracts, (2) recognition of target axons and axon– glia signaling, (3) differentiation of OPCs into myelinating oligodendrocytes, (4) membrane outgrowth and axonal wrapping, (5) trafficking of membrane components, (6) myelin compaction, and (7) node formation. First, OPCs migrate away from the neuroepithelium of the ventricular/subventricular zone of the brain into the developing white matter, in which they proliferate and form an evenly spaced network of processing-bearing cells. After OPCs have moved into their defined territories, their further paths can be very different. Although some OPCs remain in a precursor state, others will differentiate into myelin-forming oligodendrocytes. To ensure that the number of oligodendrocytes matches the number of axons to be myelinated, an excess of cells are produced, which are later eliminated by apoptosis (Raff et al., 1993; Barres and Raff 1994; Trapp et al., 1997; Simons and Nave, 2016). For oligodendrocytes, the threshold axonal diameter to myelinate is more variable (0.4 - 1.2μm) than for Schwann cells and may be as low as 0.2 μm (Nave and Werner, 2014). Although larger axons are preferentially myelinated in vivo, axons with small diameter 200– 300 nm can be myelinated in some regions of the CNS. It is likely that oligodendrocytes receive specific signals from small-caliber axons to initiate myelination. The non receptor tyrosine kinase Fyn could be one of these signals as loss of Fyn causes a severe hypomyelination of such small-caliber axons without affecting myelination of larger axons (Umemori et al., 1994). Once a particular axon has been engaged by a myelinating oligodendrocyte process, dramatic changes in plasma membrane architecture are induced and converted into flat sheets that spread and wind along the axons to generate a multilayered stack of membranes. Myelin grows by two distinct but coordinated motions: the wrapping of the leading edge at the inner tongue around the axon that is, underneath the previously deposited membrane, and the lateral extension of myelin membrane layers toward the nodal regions (Figure 1.5) (Snaidero et al., 2014).Thus, the lateral cytoplasmic-rich edges of each myelin layer always stay in close contact with the axonal surface and move in a continuous helical manner toward the future node in which they align and form the paranodal loops. The different myelin components are synthesized in oligodendrocytes at several sub

cellular localizations and are transported by various mechanisms to the growing myelin sheath. MBP mRNA is transported within cytoplasmic granule followed by local translation of MBP in growing myelin process which is in contact with axon (White et al., 2008; Laursen et al., 2011; Wake et al., 2011). Once MBP is bound to two adjacent cytoplasmic membrane surfaces, it appears to "polymerize" by lateral interactions with previously deposited MBP "monomers," thereby driving membrane zippering at the cytoplasmic surfaces of the myelin bilayer (Aggarwal et al., 2013).Compact myelin becomes a protein-poor membrane, lacking major glycoproteins at the extracellular leaflet, which is likely to uncover weak (generic) forces that promote the association of two bilayers at their extracellular surface (Bakhti et al., 2014). OPCs are covered by a dense layer of large and negatively charged self-repulsive oligosaccharides, the compacted myelin of fully matured oligodendrocytes lacks most of these glycoproteins and complex glycolipids. Such a conversion may contribute to the transformation of oligodendrocyte lineage cells from the self-avoiding and "repulsive" OPCs that tile the entire CNS to "sticky" oligodendrocytes, which ensheath axons with a multilayered stack of self-associating membranes (Simons and Nave, 2016).

The process of myelination requires the formation of small gaps in the myelin sheath, referred to as the nodes of Ranvier. These are highly complex structures, of 0.5-1 µm in length, which allow ion influx into the axon to generate the action potential. Early in the process of myelination, an axo-glial junction forms between the distal, uncompacted loops of myelin and the axolemma, this gives rise to the paranode that separates the node from the juxtaparanode (Salzer, 2003). Each of these domains contains specific membrane proteins which are highly organised. The paranode contains the axonal protein contactin-associated protein (Caspr) and contactin which form complexes with the glial 155 kDa isoform of neurofascin, that generate septate-like junctions between the myelin and the axon (Sherman and Brophy, 2005).The juxta paranodes lie just under the compact myelin sheath immediately adjacent to the paranodes and have high densities of voltage gated K+ channels and neuron-glial-related nodal axonal cell adhesion molecules (NrCAMs), Caspr2 and TAG-1 . These cell adhesion molecules form a complex and interact with TAG-1, which is expressed by the oligodendrocyte cell membrane (Poliak et al., 2003). The nodes of Ranvier are the sites for action potential regeneration, and are therefore highly enriched in voltage-gated Na^+ channels.

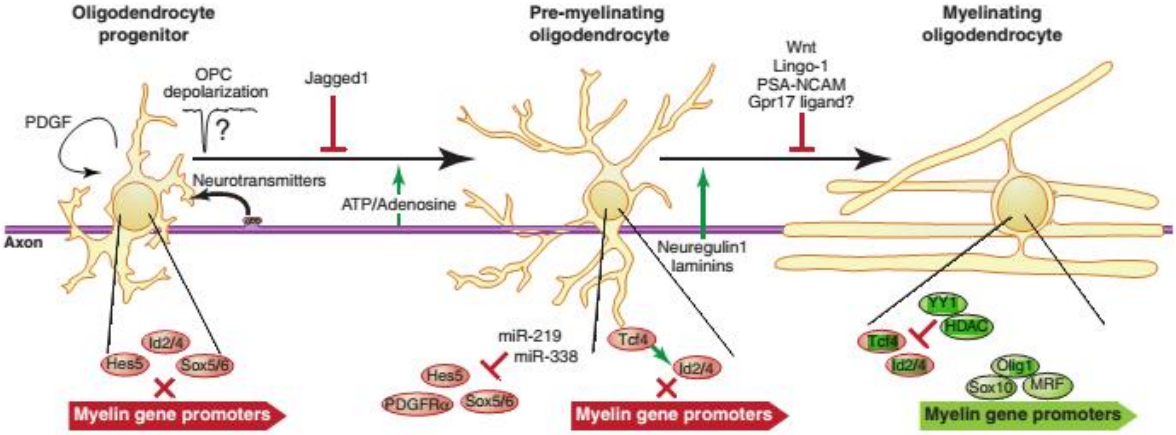
The Na^+ channels are clustered and anchored at the nodes via interactions with several cytoskeletal/scaffolding proteins, including transmembrane neurofascin-186 and NrCAM (which interact with extracellular matrix proteins), and intracellular ankyrin-G and βIV spectrin. Concentration of Na^+ channels at the nodes allows for the action potential to be regenerated at each node, as it propagates from one node to the next by saltatory conduction. The processes of myelination and node formation could be coordinated by axons, by myelinating glia, or by a mixture of both. Substantial evidence suggests that interactions with myelinating glia are vital for the assembly of nodal domains in the axon. Na^+ channels and ankyrin G, which are diffusely expressed on axons cultured in the absence of glia, cluster in the presence of glia (Kaplan et al., 1997).

Many axonal signals have been shown to regulate the myelination. For example, neurons are reported to change their patterns of expression of axonal proteins before the onset of, and during the early stages of myelination (Coman et al., 2005). In particular, polysialylated neural cell adhesion molecule (PSA-NCAM) and the adhesion molecule L1 CAM are expressed on the axonal surface at the beginning of myelination, but soon disappear as axons become myelinated (Coman et al., 2005), suggesting that these molecules promote myelination. In addition to axon-derived signals regulating myelination, there has been much interest in the possibility that electrical activity can provide instructive signals which promote myelination. Initial evidence of activity-dependent myelination came from studies demonstrating that when neuronal action potentials are blocked by TTX, myelination is impaired (Barres and Raff, 1993; Demerens et al., 1996).



(Simons and Nave, 2015)

Figure 1.5: Model showing myelin wrapping around axon. There are two motions: the wrapping of the leading edge at the inner tongue around the axon underneath the previously deposited membrane and the lateral extension of myelin membrane layers toward the nodal regions.



(Emery, 2010b)

Figure 1.6: Schematic of the oligodendrocyte lineage showing some of the intrinsic and extrinsic factors that regulates oligodendrocyte differentiation and the myelination of axons.

1.7. Myelin Function

Historically, myelin was considered be an inert membrane whose low capacitance and high resistance alone facilitated rapid and efficient propagation of action potentials. Current understanding, however, recognizes myelin as a dynamic participant in CNS physiology. In addition to insulating properties, myelin involve with clustering ion channels, which serve to increase conduction velocity up to 100-fold for myelinated neurons. Myelination also regulates the maturation, survival, and regenerative capacity of axons through trophic support and signaling molecules. Myelination affects the axon diameter through modification of its cytoskeleton (Soldan and Pirko, 2012).Loss of the normal fine structure of myelin can cause late-onset axonal degeneration and even premature death, as shown in mouse mutants that lack certain myelin-specific proteins (Griffiths et al., 1998) For example, axonal spheroids leading to axonal degeneration become a prominent feature in the white matter tracts of mice lacking the myelin proteins PLP (Monasterio-Schrader et al., 2013, Griffiths et al., 1998) or CNP (Edgar et al., 2009) or functional oligodendroglial peroxisomes (Kassmann et al., 2007). Recent studies suggests that oligodendrocytes and axons are metabolically coupled, oligodendrocyte that takes up blood-derived glucose and delivers glycolysis products (pyruvate/lactate) via mono carboxylate transporters (MCT1 and MCT2) to myelinated axons (Funfschil ling et al., 2012; Lee et al., 2012).Additionally, CNS myelin can inhibit neurite development and axonal sprouting, as well as stabilize existing synapses. Surface membrane proteins, such as Nogo-A, participate in these processes, whereas other members of the Nogo family have been shown to be proapototic (Soldan and Pirko, 2012).

1.8. Myelin Disorders

Several neurological disorders lead to defects in the myelin sheath resulting in abnormal functioning of the nervous system. Some of these disorders which destroy myelin sheath cause demyelination which results in degeneration of axons lying beneath. Conversely, other group of myelin disorders involves defective myelin sheath formation or maintenance is known as the dysmyelinating diseases. These disorders are mainly because of dysfunctional OL or Schwann cells. Several prominent myelin related disorders are discussed below

1.8.1 Multiple Sclerosis (MS)

MS is the most common demyelinating disorder and leading cause of neurological disability in young adults (Noseworthy et al., 2000). MS is a chronic, disabling inflammatory demyelinating disease of as-yet-unknown etiology. However, the most widely accepted theory is that of an autoimmune disease which leads to inflammation, followed by demyelination and OL death. The inability to repair the areas where myelin has eroded, results in astrocytic scar formation, further myelin degeneration and eventual axonal loss (Prineas and Raine, 1976; Adams et al., 1989; Ransohoff, 1999; Melanson et al., 2009). The immunopathological events can be divided into: (i) an initial T cell priming, (ii) activation phase in the periphery (i.e., thymus, lymph nodes), and a subsequent (iii) migration of the pro-inflammatory T cells and monocytes across the blood-brain barrier (BBB),(iv) amplification of local inflammation and activation of resident antigen presenting cells (APCs), such as microglia, (v) effector phase of the disease: invasion of CNS parenchyma resulting in damaging of oligodendrocytes, myelin sheath and axons. Induction of autoimmune responses against myelin or myelin related components in the peripheral blood, leads to activation of naive T cells to the inflammatory Th1 subtype that eventually cross the blood-brain barrier (BBB) to exert their targeted attack against CNS myelin. Within the CNS, these inflammatory TH1 cells start to proliferate and also secrete pro-inflammatory cytokines such as, but not limited to BDNF, TNFα, IFNγ etc., which results in the recruitment of B cells that ultimately damage the myelin (Zamvil and Steinman, 2003; Peterson and Fujinami, 2007).The resultant myelin damage impair the normal propagation of electrical impulses essential for neuronal functioning. As a result, patients with MS initially experience, weakness in limbs, pain insensitivity, gait instability and ataxia (Roxburgh et al., 2005). These clinical neurological deficits start to occur either in the form of discrete attacks (relapsing remitting MS) that the patient fully recovers from or progress slowly accumulating more neurological disabilities over time (progressive MS) from which the patient never recovers, thereby leaving them with a permanent disability. As the disease worsens, other physiological areas deteriorate such as bladder function, fatigue, cognitive deficits, memory loss ,impaired attention and slower information processing (Hauser and Oksenberg, 2006; Melanson et al., 2009; Melanson et al., 2010).

1.8.2 The Leukodystrophies

The leukodystrophies are group of genetically determined disorders characterized by demyelination/dysmyelination in the CNS as well as PNS. Each of these disorders is caused by a specific inherited biochemical defect in the metabolism of myelin-related proteins or lipids in the brain tissue. Currently, leukodystrophies appear to fall into three broad categories (1) hypomyelination, in which there is absent or diminished myelin production; (2) dysmyelination, in which there is abnormal myelin development; and (3) demyelination, in which there is loss and/or destruction of previously established myelin. However, certain leukodystrophies do not fit clearly into the above categories. For example, Rett syndrome and Batten disease can have white matter changes and clinical features that would initially suggest a leukodystrophy before subsequent testing leads to their ultimate diagnosis (Gordon et al., 2014). The major leukodystrophic conditions are listed below:

1.8.2.1 Adrenoleukodystrophies (ALD)

ALD is an X-linked genetic disorder characterized by mutation in the ATP-binding cassette, sub-family D (ALD), member 1(ABCD1) gene (Mosser et al., 1993). ALD affects 1 in 16800 individuals who are homozygous males and heterozygous females (Bezman et al., 2001). About 40% of these males, who have a mutation in their childhood, die within several years because of cerebral demyelination (Moser et al., 1980; Suzuki et al., 2005). The mutation in the ABCD1gene results in accumulation of a very long chained fatty acid (VLCFA) in the neural tissues and adrenal glands (Bezman et al., 2001). After exhibiting normal early development, impaired metabolism of VLCFA, which is the structural component of myelin, leads to myelin deterioration followed by loss of motor skills and regression (Moser et al., 1999).

1.8.2.2 Metachromatic leukodystrophies (MLD)

MLD is an autosomal recessive disorder caused by mutation in lysosomal hydrolase arylsulfatase A (ARSA) gene (Gieselmann et al., 1991). This leads to accumulation of ARSA substrate galactosylceramide I^3–sulfate (sulfatide), a major sphingolipid of myelin. It results in myelin deterioration in the CNS and PNS and accumulation of sulfatide in glial and neuronal cells. The characteristic symptoms of MLD are progressive neurological dysfunction,

including seizures and ataxia, leading to deformed cerebral cortex and death in infancy (Hagberg, 1971).

1.8.2.3 Globoid Cell leukodystrophies (GLD)

GLD, also known as Krabbe's disease, is a lysosomal storage disorder caused by mutation in the lysosomal enzymes cerebroside β-galactocerebrosidase (GALC), which catabolizes galactocerebrosides (Suzuki and Suzuki, 1970; Wenger et al., 1997). This manifestation leads to accumulation of galactocerebrosides and psychosine in oligodendrocyte and Schwann cells and ultimately results in demyelination and cell death (Miyatake and Suzuki, 1972). Children with Krabbe's disease display hyperirritability, fever followed by seizures, and also cognitive decline and developmental arrest (Kolodny et al., 1991;Wenger et al., 1997; Suzuki, 2003).

1.8.2.4 Pelizaeus-Merzbacher's disease (PMD)

PMD is a recessive X-linked dysmyelinating disorder of the CNS. Mutation in the Proteolipid Protein 1 (PLP1) gene, a major structural component of myelin, located on the chromosome Xq22.3 (Mattei et al., 1986) is the causative factor of PMD (Gencic et al., 1989; Hudson et al., 1989). Patients with PMD have reduced white matter because of the inability of OLs to myelinate axons. Involuntary eye movement or nystagmus is noted in early infancy, with poor motor control of the head. A progressive disease, PMD patients exhibit abnormally slow &scanning speech, ataxia and cognitive impairment later in life (Koeppen, 2005; Garbern, 2007).

1.8.2.5 Progressive multifocal leukoencephalopathy (PML)

First described in 1958,Progressive Multifocal leukoencephalopathy (PML) is a demyelinating disorder of the brain caused by a destructive infection of OLs by polyomavirus JC (Astrom et al., 1958; Berger and Major, 1999). It is an opportunistic infection which occurs predominantly in immunosuppressed patients with lymphoid malignancies, HIV infection and individuals with cell transplantation (Richardson, 1988; Major et al., 1992; Berger and Houff, 2009). The JC virus which can replicate only in OLs, presents with progressive demyelinating lesion in white matter, or near grey-white matter junctions. Infected OLs are characterized by enlarged hyperchromatic nuclei. The demyelination in the CNS is secondary to the death of these virus-infected OLs (Hair et al., 1992; Weber et al., 2001). PML patients develop neurological symptoms including dementia, apraxia, visual deficits and motor problems within weeks of infection. Without immunomodulatory therapies, the disease progresses very

fast and can lead to death of the patient within months (Krupp et al., 1985; Berger and Major, 1999).

1.8.3 Other Diseases

The importance of myelination to normal brain function is evident from the pathology of various disorders of myelin as discussed above. However, the significance of myelination has been more apparent recently from the pathology of numerous disorders of the brain, which were previously thought to have no myelin defects. For example, in Down's syndrome and Alzheimer's Disease, the expression levels of CNPase is significantly decreased (Vlkolinsky et al., 2001). Furthermore, patients suffering from schizophrenia show decreased density of OLs (Tkachev et al., 2003; Uranova et al., 2007; Vostrikov et al., 2007), down-regulation of myelin genes (Hakak et al., 2001; Haroutunian et al., 2007) and decreased white matter volume (Sigmundsson et al., 2001). In addition, myelin defects also characterize several disorders of later life, e.g. age related cognitive decline (Hinman and Abraham, 2007), major depression and bipolar disorder (Tkachev et al., 2003; Fields, 2005). Disorders like autism (Hendry et al., 2006; Ke et al., 2009) and cerebral palsy (Ancel et al., 2006; Juliet et al., 2009) are also known to show myelin irregularities.

1.9. Regulation of Oligodendrocytes Differentiation and Myelination

Oligodendrocyte differentiation is highly regulated multistep process. Oligodendrocytes differentiation and myelination is regulated by various extrinsic and intrinsic factors (Figure 1.6).

1.9.1 Extrinsic signaling mechanisms controlling oligodendrocyte differentiation and myelination

Oligodendrocytes differentiation and myelination is highly regulated process controlled by number of mechanisms, include axonal surface ligands, secreted molecules, and axonal activity. Myelination of an individual axon is determined by expression of inhibitory or permissive cues for myelination on the surface of the axon itself. Intriguingly, most of the axonally expressed ligands found to influence myelination to date have been inhibitory, preventing oligodendrocyte differentiation and/or myelination. These factors have included axonal expression of ligands such as Jagged, which signals via Notch in OPCs, Polysialylated form of the neural cell adhesion molecule (PSA-NCAM) and LINGO-1, all of which inhibit either OPC differentiation or myelination (Emery, 2010a). Jagged-1 and Delta-1 are axonally expressed ligands that bind the

Notch1 receptor and increases expression of the Hes1and Hes5 transcription factors that inhibit OPC differentiation. The Leucine rich repeat and immunoglobulin domain containing-1 (LINGO1) mediated inhibition of oligodendrocyte differentiation through the decreased activity of Fyn kinase and subsequent increases in RhoA signaling , a pathway implicated in OPC differentiation (Mi et al., 2005; Liang et al., 2004; Mitew et al., 2014). In contrast to the peripheral nervous system in which axonal expression of neuregulins is the dominant permissive signal for myelination by Schwann cells, neuregulin signaling to oligodendrocytes is largely dispensable for myelination, though CNS overexpression of neuregulins does induce hypermyelination. Signaling via the Wnt/β-catenin pathway has emerged as a key regulator of oligodendrocyte development. Interestingly, the β-catenin effector TCF7L2/TCF4 is highly enriched in early-differentiating oligodendrocytes, but is down regulated in mature oligodendrocytes. Wnt signaling components are present in MS lesions, suggesting that dysregulated Wnt/ β-catenin signaling could contribute to the lack of remyelination often seen in this disease (Emery, 2010a; Emery, 2010b).

The G-protein coupled receptor 17 (GPR17) is an oligodendrocyte specific receptor that is expressed transiently in late stage OPCs/early differentiated oligodendrocytes (Cahoy et al., 2008; Chen et al., 2009; Fumagalli et al., 2011). GPR17 strongly inhibited OPC differentiation and maturation (Chen et al., 2009) by increasing the expression of ID2/ID4 (Inhibitor of Differentiation) expression and nuclear localization, suggesting a mechanism for the GPR17 mediated differentiation block . The relevant endogenous ligands for GPR17 in the context of myelination are yet to be categorically established. Similarly, inhibition of γ-secretase activity within oligodendrocytes during their differentiation in neuronal co-cultures promotes formation of myelin segments (Emery, 2010b; Mitew et al., 2013).

In addition to mitogen withdrawal, OPCs stop dividing and differentiate in the presence of 3,3', 5-triiodothyronine (T_3) The importance of T3 signaling is highlighted by the myelination deficits observed in congenital human hypothyroidism patients (Gupta et al., 1995, Jagannathan et al., 1998) and reduction in myelin gene expression in rodent models of perinatal hypothyroidism (Rodriguez-Pena et al., 1993, Ibarrola and Rodriguez-Pena, 1997, Schoonover et al., 2004). T_3 plays an important role in the timely and efficient production of myelin during development by accelerating OPC differentiation.

Insulin-like growth factor -1(IGF-1), in addition to its role in proliferation and survival of oligodendrocytes, it also promote oligodendrocytes differentiation. Studies have shown that over expressing IGF-1 causes an increase in overall brain growth and myelination, with an increased number of mature oligodendrocytes and myelin gene expression (Carson et al., 1993, Ye et al., 1995, Goddard et al., 1999).

Neuronal activity promotes CNS myelination by modulating surface expression of axonal ligand and cytokines. Adenosine released by active axon may activate purinergic receptors on OPCs and promote their differentiation and myelination. Axonal release ATP induces adjacent astrocytes to release the promyelination cytokine leukemia inhibitory factor (LIF).Direct stimulation of OPCs by glutamate released by synaptic-like structures. OPCs express ionotrophic glutamate receptors and voltage-gated ion channels, and respond to glutamate released from synaptic like structure with a depolarization event not unlike the action potential of a neuron. This synaptic input onto OPCs is also observed in the context of remyelination in the adult CNS and is rapidly lost as the cells differentiate into mature oligodendrocytes, suggesting that it likely has role in regulating OPC behavior. Treatment of OPCs with glutamate in vitro can inhibit both their proliferation and subsequent differentiation via a block in rectifier K^+ channels; this suggests that the role of glutamatergic signaling to OPCs may be to limit, rather than promote, myelination (Emery, 2010b).

1.9.2 Intrinsic Control of Oligodendrocyte Differentiation and Myelination

Intrinsic factors regulate the oligodendrocytes and myelination operates through transcriptional, posttranscriptional, and epigenetic mechanisms.

1.9.2.1 Transcriptional regulation

During development, the oligodendrocyte lineage largely arises from SVZ progenitors in the ventral neural tube expressing the transcription factors Nkx6.1, Nkx6.2 and Olig2 in response to Shh (Richardson et al., 2006). Olig2 is essential for the specification of the lineage, with Olig2 null mice displaying a near complete loss of PDGFRα^{+ve} OPCs (Ligon et al., 2006; Lu et al., 2002). Although Nkx6.1 and Nkx6.2 are required for the specification of Olig2+ve cells in the ventral neural tube, they are not absolutely required for the generation of the oligodendrocyte lineage, as some Olig2+ve progenitors are generated from more dorsal regions of the neural tube independently of Nkx6.1 and Nkx6.2 (Cai et al., 2005). Olig1 and Olig2, transcription factors

expressed in the oligodendrocyte lineage throughout the CNS and preceding expression of Pdgfrα (Lu et al., 2000; Zhou et al., 2000).

Once the OPC lineage is specified, number of transcription factors act to maintain them as proliferating OPCs and to limit their differentiation. OPCs express high levels of the transcription factors Sox5, Sox6, Hes5, Id2, and Id4 which inhibit the oligodendrocyte differentiation and myelination. Id2 and Id4 are downstream from the BMPs and the anti differentiation G protein– coupled receptor Gpr17. Id2 and Id4 may inhibit OL differentiation by interacting with Olig1 and Olig2, probably blocking their activity in the nucleus (Samanta and Kessler 2004; Chen et al., 2009). Id2 and Id4 expression is down regulated during differentiation and protein expression also shifts from nuclear to cytoplasm. The Notch signaling pathway limits OPC differentiation through its transcriptional target Hes5. Hes5 binds to and competes with the promyelination factor Sox10, preventing its activity at the myelin basic protein (MBP) promoter. Consistent with this, the brains of Hes5 null mice show accelerated expression of markers of myelinating cells including MBP and proteolipid protein (PLP) (Liu et al., 2006). Sox5 and Sox6 are expressed in neural progenitor cells and OPCs, being down-regulated during differentiation. Sox5 and Sox6 are capable of binding the promoter region of the MBP gene, antagonizing the activity of Sox10 (Stolt et al., 2006).

Transcription factors Nkx2.2, Sox10 and Olig1 are all expressed from relatively early in the oligodendrocyte lineage. These are not required for specification of oligodendrocytes instead they all are required for oligodendrocytes differentiation and myelin genes expression. Olig1 has been shown to be expressed during specification in the pMN domain but appears dispensable for this developmental process, as Olig1 null mice do not seem to affect the formation of pdgfrα+ precursor cells (Lu et al., 2002). Further studies have now shown that Olig1 is necessary for differentiation and maturation of oligodendrocytes (Xin et al., 2005). Sox10 is the earliest transcription factor to be specifically turned on in oligodendrocyte lineage cells immediately after specification(Wegner, 2008). Ablation of Sox10 in mice shows a clear terminal differentiation defect with minimal impact on progenitors (Stolt et al., 2002). This is partly explained by the ability of Sox10 to bind directly to regulatory regions and subsequently induce expression of a number of myelin genes, including Mbp, Plp, connexin-32 (Cx32), and connexin-47 (Cx47) (Stolt et al., 2002; Bondurand et al., 2001; Schlierf et al., 2006). Sox10 has been shown to work synergistically with Olig1 to drive Mbp expression (Li et al.,

2007) and with Olig2 to possibly activate Nkx2.2 expression (Liu et al., 2007) after the completion of oligodendrocyte specification (Zhou et al., 2001; Fu et al., 2002). Nkx2.2-deficient mice demonstrate a role for this transcription factor in oligodendrocyte differentiation but not specification (Qi et al., 2001). However, Nkx2.2 does not appear to activate genes involved in oligodendrocyte differentiation as Sox10 does, but rather acts as a repressor and may serve as one of the earliest switches of oligodendrocyte differentiation by facilitating repression of PDGFR-α signaling (Zhu et al., 2014). A separate set of transcription factors has now been identified that functions after cell cycle exit to mediate terminal differentiation of oligodendrocytes. It is well accepted that proliferation and differentiation are mutually exclusive events in the oligodendrocyte lineage (Casaccia-Bonnefil and Liu, 2003) and that cell cycle exit is an obligatory step associated with dramatic reorganization of the transcriptome and chromatin (Magri et al., 2014a; Magri et al., 2014b). One of the first identified transcription factors modulating this critical transition of oligodendrocyte development is YY1. Conditional ablation of Yy1 in the oligodendrocyte lineage induced a phenotype characterized by defective myelination, ataxia, and tremor. Examination of the mice revealed this to be the result of impaired oligodendrocyte progenitor differentiation due to increased expression of transcriptional inhibitors of myelin gene expression (He et al., 2007). Transcription factor Tcf4 is an effector of the Wnt/β-catenin signaling pathway transiently expressed during oligodendrocyte differentiation at the late OPC/early pre-myelinating phase, required for exit of the cell cycle/onset of oligodendrocyte differentiation. Mice lacking Tcf4 have OPCs but fail to generate mature oligodendrocytes during embryogenesis (Emery, 2010a).

A transcription factor associated with the later stages of myelin formation is myelin gene regulatory factor (MRF), which is required for the generation of mature myelinating oligodendrocytes. Knockdown of MRF in oligodendrocytes did not impair the initial stages of differentiation or the expression of early markers such as GalC but did prevent the expression of most myelin genes. Conversely, over expression of MRF was sufficient to induce myelin gene expression (Emery et al., 2009). MYRF is also required for maintenance of mature oligodendrocytes, as inducible ablation of MRF in adult mice resulted in severe CNS demyelination (Koenning et al., 2012). ChIP-sequencing studies have now shown that MYRF directly binds putative enhancer regions of oligodendrocyte-specific and myelin genes, including Mag, Mog, Mbp, and Plp1. Comparison of available ChIP-sequencing data also revealed

overlapping genomic binding sites for MYRF, Sox10, and Olig2 (Bujalka et al., 2013). Indeed, Sox10 has been shown to directly activate MRF, and once induced; the two transcription factors cooperate synergistically to activate myelin-specific genes (Hornig et al., 2013). A final transcription factor worth noting is ZFP191. Using a forward genetics approach, Howng et al. identified a mouse strain characterized by overt absence of CNS myelin but abundant numbers of late-stage, process-extending oligodendrocytes. Linkage mapping and complementation testing would reveal a single nucleotide insertion in the Zfp191 gene. While the function of ZFP191 in the myelination program remains unknown, it is clear that it is functionally active after YY1 and MYF, as mutant mice showed equivalent levels of CC-1–a marker of oligodendrocyte differentiation to wild-type mice (Howng et al., 2010). Both the Yy1 (He et al., 2007) and MRF (Emery et al., 2009) mutant mice showed reduced levels of CC-1 compared to control mice.

1.9.2.2 Epigenetic regulation

Epigenetic regulation is mediated through mechanisms other than changes in the targeted DNA sequence. Recently, epigenetic and chromatin remodeling events have been shown to be critical for oligodendrocyte differentiation. The several types of epigenetic mechanisms include DNA methylation, histone modification by and histone deacetylases (HDACs) and histone acetyltransferases (HATs), chromatin remodeling mediated by ATP-dependent SWI/SNF complex subunits, and post-translational silencing by non coding RNAs such as small-non coding RNAs (He and Lu, 2013).

OPCs exhibit euchromatic nuclei and transduce extracellular signals to transcription factors, which often recruit large protein complexes containing co-activators or corepressors and histone-modifying enzymes to accessible DNA sequences, allowing for the transcriptional activation or repression of genes regulating lineage determination, proliferation, and migration. The transition from OPC to premyelinating is characterized by progressive heterochromatin formation by down regulation of genes involved in proliferation and inhibition of differentiation (Liu et al., 2016).

HDACs such as HDAC1 and HDAC2 are essential for OPC differentiation as well as myelination both in vitro and in vivo (He and Lu, 2013). It was recently reported that HDACs interact with Tcf4/ Tcf7l2 to promote oligodendrocyte differentiation by competing with β-catenin and are required for differentiation during a critical time window (Huang et al., 2013). At

present, the roles of other HDACs in oligodendrocyte myelination remain to be defined. HDAC11 is reported to regulate oligodendrocyte-specific gene expression in an oligodendroglial cell line (He and Lu, 2013). HDACs interact with other transcriptional regulators to form repressive complexes to inhibit expression of OPC differentiation inhibitors or block the activation of neuronal differentiation genes (He et al., 2007; Ye et al., 2009). HDAC1 can recruit YY1 to the promoter of OL differentiation inhibitors, including Id2, Id4, and Hes5, to repress their transcription (Fig. 3) (Shen et al., 2005; He et al., 2007). Related to histone deacetylation is H3K27 lysine methylation, another epigenetic marker of gene repression that is catalyzed by the enzyme Ezh2, whose expression levels are elevated as neural stem cells (NSCs) differentiate into OPCs and downregulated as NSCs differentiate into neurons or astrocytes (Sher et al., 2008). Specific targets of Ezh2 suggest that this enyzme is likely responsible for the expression of neuronal or astrocytic lineage genes; in Ezh2-silenced oligodendrocytes, these markers are re-expressed and the cells undergo apoptosis (Sher et al., 2008; Sher et al., 2012).Sirt2 is expressed throughout the oligodendrocyte lineage, and is associated with myelin, although its function in myelination is unknown. Loss of Sirt2in Plp-null mice suggests that PLP/DM20 is required for the transport of Sirt2 into the myelin compartment (He and Lu, 2013).

miRNAs function mainly to inhibit gene expression by forming an RNA-induced silencing complex at the perfectly or imperfectly corresponding bases in the 3' untranslated regions of target genes. Micro RNAs have been demonstrated to influence oligodendrocyte differentiation post-transcriptionally by down regulating the differentiation inhibitors. Importantly, inactivation of Dicer1by Cre recombinases in the OL lineage directed by Olig1, Olig2, or CNP promoters causes severe dysmyelination and motor deficits including tremors and seizures. Dicer1deletion leads to an increase of OPC proliferation, and a drastic reduction in myelination (Emery and Lu, 2015). MiR-23 is a negative regulator of lamin B1, over expression of which causes severe myelin loss. MiR-219 and MiR-338 are crucial for oligodendrocyte differentiation, and target oligodendrocyte differentiation inhibitors (e.g. Sox6 and Hes5) and OPC proliferation factors (PDGFRα) or neurogenesis-promoting factors (e.g. FoxJ3 and Zfp238) (He and Lu, 2013).

Smarca4/Brg1 gene encoding the central catalytic ATPase subunit of the SWI/SNF chromatin-remodeling complex is necessary for oligodendrocyte differentiation. Co-immunoprecipitation assays and ChIP-sequencing suggested that Olig2 acts as a pre-patterning factor that directs Brg1

to cis-regulatory enhancers to facilitate the transition from OPCs to immature oligodendrocytes by promoting accessibility of differentiation genes for transcription (Yu et al., 2013).

DNA methylation is an important epigenetic regulatory mechanism during the nervous system development. The majority of DNA methylation occurs on cytosines that precede a guanine nucleotide or CpG sites in the 5' promoter of genes. DNA methylation are regulated by three classes of enzymes, namely, DNA methyltransferases (e.g., Dnmt1 and Dnmt3), the erasers, ten-eleven translocation (TET) family enzymes (TET1, TET2 and TET3) that can oxidize 5-methylcytosine (Kohli and Zhang 2013), and the readers, methyl-CpG-binding domain-containing proteins (MBDs) (Emery and Lu, 2015).

Indeed, TET1, TET2, and TET3 have been shown to be necessary for oligodendrocyte differentiation in vitro (Zhao et al., 2014). Recently, a whole-genome transcriptome and methylome analysis comparing OPCs and oligodendrocytes revealed that DNA methylation is inversely correlated with gene expression during developmental myelination. However, new study show that reduction of DNA methylation via genetic ablation of Dnmt1 in OPCs is not sufficient to induce differentiation, but rather results in severe hypomyelination of the CNS associated with aberrant alternative splicing events and activation of an ER stress response (Moyon et al., 2016). This suggests that DNA methylation acts as a regulator of the OPC state and subsequent transition into differentiating oligodendrocytes.

1.10. Methyl CpG Binding Domain (MBD) Proteins

Methylation of vertebrate genomes are characterized by addition of a methyl group predominantly to the 5 carbon of cytosine (5mC) in CpG dinucleotides (referred to as CpG), with 60–90% of all the CpGs methylated in mammals. DNA methylation is a covalent modification of DNA catalyzed by DNA methyltransferase enzymes (DNMTs) (Bogdanović and Veenstra, 2009). In vertebrates, there are CpG-rich regions referred to as CpG islands, which are predominantly located in regulatory regions. These islands can be found in approximately 60% of all mammalian genes and are mostly found in an unmethylated state (Diaz de Leon -Guerrero et al., 2011).DNA methylation regulates the expression of genes by either inhibiting the binding of transcription factor or recruiting MBD (methyl binding domain) family proteins comprising MBD1, MBD2, MBD3, MeCP2, and MBD4, each of which contains a conserved methyl-CpG

binding domain or MBD (Klose et al., 2005) and a structurally unrelated Kaiso family (Kaiso, ZBTB4, and ZBTB38) (Diaz de Leon -Guerrero et al., 2011). MBD1, MBD2, and MeCP2 are associated with transcriptional repression and chromatin remodeling activities. Mammalian MBD3 does not bind specifically to methylated DNA, and MBD4 is a DNA repair protein although recent evidence suggests that MBD4 may also act as a transcriptional repressor (Klose et al., 2005).

1.11. MeCP2 and Rett Syndrome

Rett syndrome (RTT) is a neurodevelopmental disorder occurring predominantly in females and characterized by normal development up to the first year and a half of age, followed by a subsequent loss of acquired speech and motor skills and begin to develop mental retardation, stereotyped hand movements, ataxia, seizures, microcephaly, autism and respiratory dysfunctions (Zoghbi and Francke, 2001). RTT (Rett Syndrome) has been reported to have an incidence of approximately 1/10,000 female live births (Hagberg et al., 1983). Rett syndrome was first identified by Dr Andreas Rett in 1966 (Rett, 1966). In 1999, it was demonstrated that RTT is associated with a gene mutation of the X linked transcription factor, methyl-CpG binding protein 2 (MeCP2), located on the X chromosome (Xq28) (Amir et al., 1999). This discovery helped to explain the female prevalence of RTT, as MeCP2 is located on the X chromosome (Quaderi et al., 1994), and females that are heterozygous for the mutated MeCP2 allele are able to survive with this debilitating disorder due to X chromosome inactivation (Amir et al., 2000).Males that are hemizygous for MeCP2 mutations have a drastically shortened lifespan of approximately 2 years and typically develop congenital encephalopathy (Ravn et al., 2003; Villard et al., 2000).Genetic analysis has identified 218 mutations linked to RTT (Miltenberger-Miltenyi and Laccone, 2003). Mutations have been found in all MeCP2 domains, indicating that each domain contributes to proper function. These mutations are usually single point mutations in the coding sequences that result in a missense or nonsense mutant, altering a single amino acid or causing a truncated form of the protein. Almost all of the cases reported to date have been sporadic mutations of MECP2, with limited familial exceptions. Eight of these MECP2 mutations have been commonly found in RTT patients and occur in MBD and TRD domains (Adkins and Georgel, 2011).

1.12. MeCP2 Structure and Function

MeCP2 produces two isoforms (MeCP2_E1 and MeCP2_E2) by alternating splicing of mRNA, differing in only 21 amino acids at the N terminus. MeCP2_E1 isoform is expressed 10 times more in brain with respect to MeCP2_E2 (Mnatzakanian et al., 2004). The longer splice variant MeCP2_E1 (also called MeCP2B) utilizes the translational site in exon 1, splices out exon 2 and generates a 498 kDa protein from exon 1, 3 and 4. Another splice variant MeCP2_E2 (also called MeCP2A) utilizes the ATG signal on exon 2, utilizes all the four exons to generate a 486 kDa protein (Kerr et al., 2011). MeCP2_E1 is highly expressed in primary neurons, as compared to primary astrocytes (Kaddoum et al., 2013; Zachariah et al., 2012). Further, physiological significance of these two isoforms were elucidated and it was found that elevated expression of MeCP2_E2 isoform promotes the neuronal death by suppressing the expression of FoxG1. FoxG1 is a protein that promotes neuronal survival and mutations of which are also linked to Rett syndrome (Dastidar et al., 2012). MeCP2_E2 is dispensable for Rett Syndrome phenotypes but essential for embryo viability and placenta development. Mutation of MeCP2_E1 alone is sufficient to cause Rett syndrome which suggests its critical role in Rett syndrome phenotype (Itoh et al., 2012). DNA methylation pattern at the regulatory elements of MeCP2 may regulate the differential expression of its two isoforms in specific brain regions (Olson et al., 2014).MeCP2 protein has five structural domains comprising N-terminal domain (NTD), the Methyl binding domain (MBD), the intervening domain (ID), the transcription repression domain (TRD) and the C-terminal domain (CTD). Among these, MBD domain of MeCP2 is essential for its binding to Methyl CpG dinucleotide which is linked with most of the disease causing mutations. TRD is required for its association with co-repressor proteins for the function of transcription repression. Pathogenic mutation in ID and CTD domains suggest their importance in MeCP2 dysfunction (Figure1.7) (Bedogni et al., 2014; Hansen et al., 2010; Yusufzai and Wolffe, 2000; Lewis, 1992).

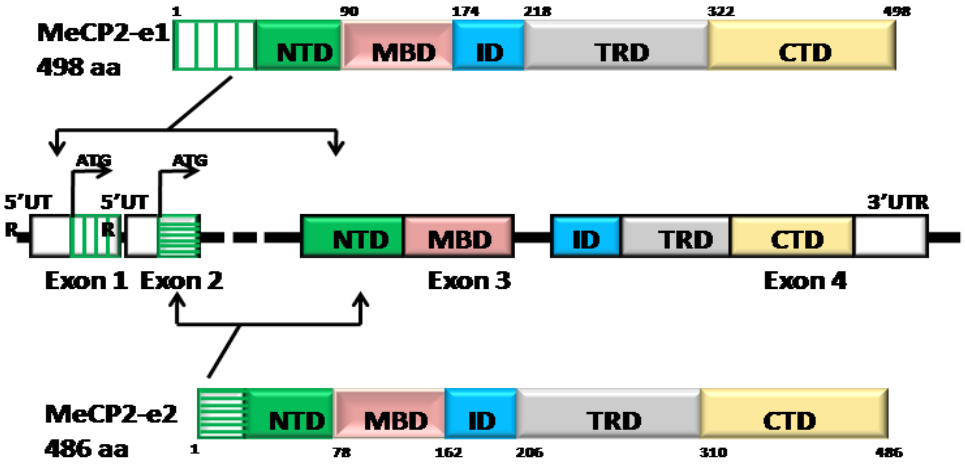
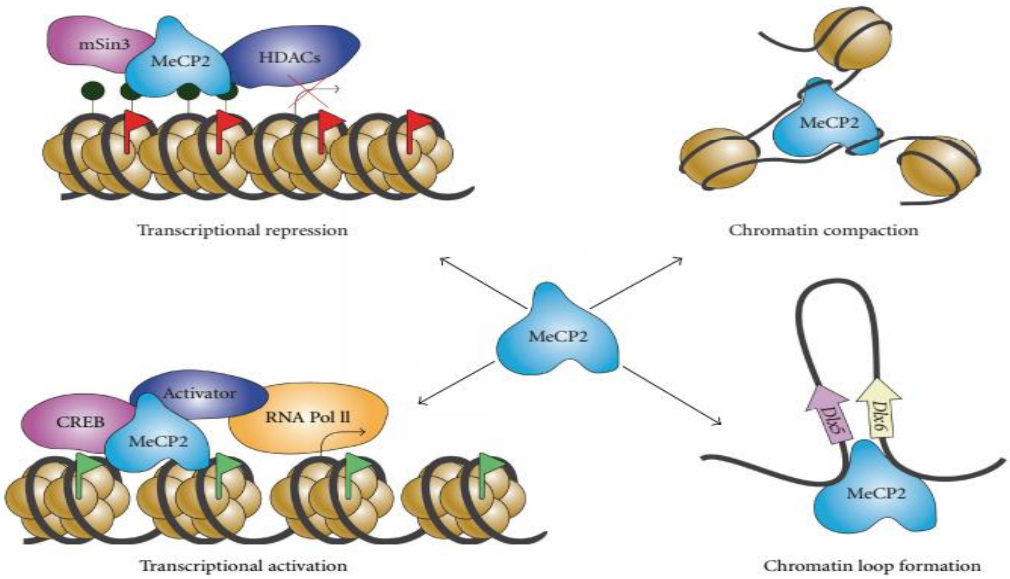


Figure 1.7: Structure and Splicing of MeCP2. MeCP2 upon alternative splicing form two different isoforms (MeCP2-e1 and MeCP2-e2). MeCP2-e1 is 498 amino acids long and has 21 unique N-terminal residues (vertical green stripes). MeCP2-e2 is 486 amino acids long and has 9 unique residues (horizontal green stripes). The remaining protein sequence is common to both isoforms and can be divided in 5 domains : the NTD (N-terminal domain), the MBD (methyl-CpG binding domain), the ID (intervening domain), the TRD (transcription repression domain), the CTD (C-terminal domain).



(Zachariah and Rastegar, 2012)

Figure 1.8: MeCP2 function in gene regulation and chromatin organization

MeCP2 has highly disorganized structure which acquires local secondary structure upon binding to other macromolecule and thereby inviting multiple protein-protein interactions. Further post translation modification of MeCP2 also regulates its activity and interaction with other proteins (Bedogni et al., 2014). MeCP2 phosphorylation in response to neuronal activity lead to regulation of activity regulated genes. Neuronal activity phosphorylates MeCP2 at serine 421 (S421) which leads to transcriptional induction of BDNF gene (Zhou et al., 2006). It has been identified that dephosphorylation and phosphorylation of MeCP2 at serine 80 (S80) and serine 421 (S421) respectively, in response to neuronal activity, regulates transcription of target genes. S80 is major phosphorylation site in neurons under resting condition and its dephosphorylation upon neuronal activity attenuates its chromatin association (Tao et al., 2009). However, MeCP2 S421A mutation in mice shows defective synaptic development and abnormal behavior. Interestingly, ChIP(chromatin immunoprecipitation) sequencing shows that MeCP2 S421 phosphorylation occurs on MeCP2 bound across genome and it does not regulate the expression of specific genes, rather it functions as a histone like factor and facilitates genome wide response of chromatin to neuronal activity (Cohen et al., 2011). Recent studies have shown

that MeCP2 phosphorylation at three sites (S86, S274, and T308) are induced differentially by neuronal activity, brain derived neurotrophic factor (BDNF), and other agents which increase the intracellular cAMP levels. Phosphorylation at T308 site inhibits its interaction with NCoR co-repressor complex and thus suppresses the MeCP2 mediated transcriptional repression. These findings suggest that loss of MeCP2 and NCoR /SMRT co-repressor interaction lead to RTT (Ebert et al., 2013; Lyst et al., 2013). MeCP2 regulation of target genes expression is mediated through its interaction to other partner proteins, including co-repressors mSIN3a (Nan et al., 1998) and cSki (Kokura et al., 2001), transcription factor YY1 involved in activation as well as repression (Forlani et al., 2010),YB1 (RNA interacting protein) (Young et al., 2005), Swi/Snf family ATPase Atrx (α-thalassemia/mental retardation syndrome X linked)(Nan et al., 2007), NcoR (nuclear receptor co-repressor) and Dnmt1 (DNA methyl transferases) (Kokura et al., 2001). In addition, MeCP2 also binds to co-repressor CoREST (corepressor of RE1 silencing transcription factor) (Ballas et al., 2005), the transcriptional activator CREB (cAMP response element binding protein)(Chahrour et al., 2008) and the tri-thorax-related protein Brahma (Harikrishnan et al., 2005).It is believed that MeCP2 disorganized structure and its post-translational modification contributes to its multifunctional property.

Although being a ubiquitous protein in nature, MeCP2 is most predominantly expressed in the brain and is specific to neuronal cells compared to other cell types. Its expression level correlates with the maturation of neurons which suggests the noteworthiness of MeCP2 in neuronal maturation and maintenance including synapse formation and dendritic arborization (Kishi and Macklis 2004; Shahbazian et al., 2002). This was supported by the fact that the brain size of RTT patients is small because of smaller, immature neurons rather than fewer cells (Nagai et al., 2005; Miyake and Nagai, 2007). Moreover, reduced spine density, smaller somas and substantially decreased dendritic arborization was seen in many different parts of RTT brain (Zhou et al., 2006; Chapleau et al., 2009). MeCP2 density in neuronal nuclei was found to be as abundant as histone octamer. Neuronal loss of MeCP2 results in change in chromatin organization mediated by increase in histone acetylation and doubling of histone H1 (Skene et al., 2010). Further, it was also found to regulate the nuclear size and RNA synthesis in neurons during their maturation (Yazdani et al., 2012). This implicates that MeCP2 act as global regulator of chromatin states instead of gene specific regulator. In dorsal horn neurons, post inflammation, phosphorylation of MeCP2 occurs which leads to increased expression of target

genes such as the serum- and glucocorticoid- regulated kinase (SGK1), FK 506 binding protein 5 (FKBP5), a glucocorticoid receptor-regulating co-chaperone of hsp-90, and the sulfotransferase family 1A, phenol-preferring, member 1(SULT1A1). SGK1 supports the induction of ankle joint inflammation. Moreover, MeCP2, DNMT (DNA methyl transferase) and HDAC (Histone deacetylase) levels are modulated within the superficial dorsal horn during the maintenance phase of persistent pain states, indicating the prominent role of MeCP2 in chromatin organization during maintenance phase of chronic pain state (Géranton et al., 2007; Tochiki et al., 2012).

MeCP2 duplication or over expression seeds the condition of MECP2 duplication syndrome which is specific in males but can also be found in females. This syndrome may resemble RTT in the beginning, but the disorder turns out to be clinically more severe and different from classic RTT on the long term (Ramocki et al., 2010). Methyl binding domain (MBD) and transcriptional repressor domain (TRD) are responsible for toxicity in MeCP2 duplication syndrome (Heckman et al., 2014). Furthermore, the over expression of MeCP2 impairs the chick embryo neural tube formation by provoking premature differentiation of proliferating progenitors (Petazzi et al., 2014). Severity of neurological symptom in Rett patients also depends on X-chromosome inactivation (XCI) patterns (Braunschweig et al., 2004). Most of the RTT female patients are heterozygous for MeCP2 mutation due to random XCI. Deficiency of MeCP2 in the MeCP2 mosaic mouse model may not affect primary XCI in early development, but does affect the proportion of neurons expressing the wild-type *Mecp2* allele in region- and age-dependent manner (Smrt et al., 2011).

The classical function of MeCP2 is to silence the gene expression through the recruitment of co-repressors comprising HDAC and mSIN3 (Figure1.8) (Nan et al., 1998). BDNF (Brain derived neurotrophic factor) is one of the widely investigated target gene, whose expression is modulated by MeCP2, and have been shown to be involved in the neuronal survival, differentiation and synaptic plasticity. Previous works have shown that MeCP2 represses the expression of BDNF and is de-repressed after neuronal membrane depolarization. Neuronal membrane depolarization phosphorylates MeCP2 causing its dissociation from BDNF promoter (Chen et al., 2003; Martinowich et al., 2003; Zhou et al., 2006). Further, it was demonstrated that BDNF protein level decrease in the brain of *Mecp2* knockout mice (Chang et al., 2006; Li et al., 2012) and increase in mice over expressing *Mecp2* (Chahrour et al., 2008). These observations

signify the importance of altered BDNF level in RTT disease progression. In contrast to earlier studies, BDNF protein level was seen to be enhanced in both MeCP2 knock out and MeCP2 over expressing cultured hippocampal neurons (Larimore et al., 2009). These findings suggest that the control of BDNF expression by MeCP2 can dynamically switch between repression and activation. Epigenetic evaluations has established that MeCP2 act as transcriptional repressor, but a recent report by Zoghbi and colleagues explains that MeCP2 activates expression of target genes in the hypothalamus and cerebellum in mice over-expressing MeCP2 (Figure 1.8). MeCP2 is associated with transcriptional activator CREB1(cAMP responsive element binding protein 1) at the promoter region of an activated targets but not at repressed targets (Ben-Shachar et al., 2009; Chahrour et al., 2008).

1.13. Glia and MeCP2

Glial cells in brain consist of astrocytes, oligodendrocytes and microglia. Astrocytes maintain and regulate the level of ions and neurotransmitters at synapse, provide nutrients and structural support around synapses, and contribute to the integrity of the blood brain barrier (BBB). On the other hand, oligodendrocyte produces myelin sheath around the axons, allows the rapid conduction of nerve impulses and also provides trophic support to axons. In addition to providing insulation and trophic support to neurons, myelinating glia are active participants in the proper functioning of the nervous system, sculpting the structural and electrical properties of axons by controlling their diameter, as well as the spacing and clustering of ion channels at nodes and paranodes. Lastly, microglia are resident inflammatory cells of brain which are involved in regulation of synaptic function (Baumann and Pham-Dinh, 2001; Tremblay et al., 2011; Wang and Bordey, 2008). Earlier reports suggested that MeCP2 is expressed only in neuron and its expression correlate with neuronal maturation while it was shown to be absent in other glial cells (Jung et al., 2003; Kishi and Macklis 2004; Shahbazian et al., 2002). However, in recent times, several reports and studies from other laboratories and our own laboratory have demonstrated the MeCP2 expression in all the glial cells including astrocytes, oligodendrocytes, and microglia (Ballas et al., 2009; Maezawa and Jin, 2010; Tochiki et al., 2012; Vora et al., 2010, Sharma et al., 2015). Rett syndrome has been primarily associated with functional loss of MeCP2 solely in neuronal cells but several recent studies supports that glial cells like neurons

are integral components of the Rett syndrome pathophysiology. Hence, glial MeCP2 studies also need to be given more attention.

1.13.1. Astrocytes and MeCP2

Several glial genes expression including α B-crystallin, Glial fibrillary acidic protein (GFAP), glial excitatory amino acid transporter 1 (EAAT1) and S100 A13 were found to be increased in RTT brain (Colantuoni et al., 2001). Apart from the neuronal MeCP2, several studies have shown a non-cell autonomous influence of astrocytic MeCP2 on neuronal cells and its contribution to RTT pathogenesis. In fact, astrocytes from RTT mouse model fail to support the normal neuronal growth and cause dendritic abnormalities. Moreover, the condition media from *Mecp2*- null astrocytes lead to neuronal damage which suggests the aberrant secretion of toxic factors by mutant astrocytes (Ballas et al., 2009). In the similar line of studies, MeCP2 deficiency in astrocytes has also been correlated with abnormal growth and the regulation of the BDNF level. MeCP2 also regulate the astrocytes immune response by altering the expression of pro-inflammatory cytokines (TNF –alpha, IL-1β, and IL-6) and kinase (p38 MAPK) (Maezawa et al., 2009).MeCP2 deficient astrocytes cause abnormal dendritic arborization of wild neuron in co-culture which is a prominent neurological feature of RTT brain. Mutant astrocytes differentiated from isogenic induced pluripotent stem cell (iPSC) lines from human RTT patients also adversely affect the morphology and function of wild type neuron from mouse and human and such non-cell autonomous effects are partially mediated by secreted factor (Williams et al., 2014).

MeCP2 expression in GFP-labeled wild type astrocytes was found to be reduced when co-cultured with *MeCP2*$^{-/+}$astrocytes in a time-dependent manner. It was found that this non cell autonomous effect of MeCP2 deficient astrocytes on MeCP2 expression in wild type astrocytes is mediated via gap junction (GJ), specifically Cx-43-containing GJs. These observations suggest that the MeCP2 deficiency state spreads among *MeCP2*$^{-/+}$ tissue through non cell autonomous transfer of negative regulator of MeCP2 protein level via GJ over time. The negative regulators may include Ca^{2+}, inositol trisphosphate, glutamate, or small regulatory miRNAs, such as miR132 that post transcriptionally down regulates MeCP2 (Maezawa et al., 2009; Klein et al., 2007).

MeCP2 re-expression preferentially in astrocytes in MeCP2 deficient mice restores the RTT phenotype like locomotion and anxiety levels, respiratory abnormalities to a normal pattern and prolonged lifespan, indicate that not only neuron but glia are also integral component in neuropathology of RTT (Lioy et al., 2011). Abnormal glutamate metabolism has been found in RTT brain. MeCP2 involvement in the glutamate clearance in astrocytes culture through regulation of glutamate transporters and glutamine synthetase may influence the progression of RTT (Okabe et al., 2012).

Astrocytes from MeCP2 deficient mice shows reduced expression of stathmin-like 2 (STMN2) protein results in altered microtubule assembly and dynamics. STMN2 in astrocytes promotes the microtubule disassembly and its down regulation in MeCP2 deficient astrocytes leads to increased microtubule growth. Since the astrocytes are also involved in dendritic outgrowth and synaptogenesis, it was thought that STMN2 down regulation in MeCP2 deficient astrocytes may explain the dendritic abnormalities observed in RTT brain (Nectoux and Florian, 2012; Slezak and Pfrieger, 2003). Recently, it was shown that microtubules dynamics and vesicular transport is altered in astrocytes derived from $MeCP2^{308/y}$ mice and human astrocytes derived from iPSC from an RTT patient with a *MECP2*p.Arg294*mutation. Impaired microtubule dependent dynamics and vesicular transport in MeCP2 deficient astrocytes have been found to be restored upon treatment of microtubule stabilizing agent EpothiloneD (EpoD). Thus, microtubules can be potential target for therapeutic in Rett syndrome (Delépine et al., 2016). MeCP2 deficiency is also found to be associated with reduced sensitivity of medullary astrocytes to changes in PCO2/ [H+] (Turovsky et al., 2015). MeCP2 deficiency alone in astrocytes significantly depresses hypercapnic ventilatory response in mice, these results shows the role of astrocytes in regulation of respiratory response to CO_2 and further suggesting the role of astrocytic MeCP2 respiratory abnormalities observed in Rett syndrome (Garg et al., 2015).

In a study, where human embryonal carcinoma cell line, NTera-2, were induced to differentiate into astrocytes, it was observed that MeCP2 in association with Sin3A inhibits the differentiation of NTera-2 into astrocytes like lineage by suppressing GFAP (Glial Fibrillary Acidic Protein) expression. Upon differentiation, the promoter undergoes a conformational change triggered by STAT3 binding, which causes release of Sin3A/MeCP2 complex and GFAP gene activation. Moreover, MeCP2 deficiency in RTT- hiPSC line (human induced pluripotent cells) derived

neural stem cells resulted in an increase in the number of astrocytes differentiated and dysregulation of GFAP expression (Andoh-Noda et al., 2015). Above studies suggest MeCP2 role in cellular differentiation and lineage specific gene expression (Cheng et al., 2011).

A combined approach of gene expression microarray and MeCP2 ChIP-seq in astrocytes found a set of potential MeCP2 target genes whose products are involved in normal astrocytes signaling, cell division and neuronal support functions, and the loss of which may contribute to the Rett syndrome phenotype. Validation of selected target genes transcript by qRT PCR revealed that MeCP2 deficiency in astrocytes consistently affect the expression of *Apoc2, Cdon, Csrp* and *Nrep*. Apoc2 (apolipoprotein C-II), *Cdon (*cell adhesion molecule-related/down regulated by oncogenesis) *and Csrp* (cysteine and glycine-rich protein 1) have elevated expression in MeCP2 deficient mice while the *Nrep* (neuronal regeneration-related protein) has reduced expression. Defects in Apoc2 and Cdon expression in astrocytes may associate to cardiac defect and brain structure abnormalities observed in Rett syndrome. Csrp1, which is upregulated in astrocytes in absence of MeCP2, appears to have a role in neuronal regeneration. Nrep is an important factor involved in glial mobility and neoplasia (Yasui et al., 2013). Recently (Delépine et al., 2015) gene expression profiles of wild-type and mutant astrocytes from *Mecp2* $^{308/y}$ mice demonstrated two interesting genes encoding secreted proteins, CHGB (Chromogranin B) and LCN2 (Lipocalin 2) were dysregulated in *Mecp2*-deficient astrocytes and exert negative non-cell autonomous effects on neuronal properties. LCN2 secreted by reactive astrocytes is involved in binding and transport of lipids and other hydrophobic molecules. Interestingly, LCN2 play important role in regulation of neuronal excitability and spine morphology (Ferreira et al., 2013). Chromogranin B, a component of exocytose vesicles was found in secretory granule cargoes involved in BDNF secretion (Sadakata et al., 2004). Another important molecule NR2F2 (nuclear receptor subfamily 2, group F, member 2) was found to be dysregulated in MeCP2 deficient astrocytes which may be involved down- and up-regulation of several target genes in astrocytes such as Ccl2 (Chemokine (C–C motif) ligand 2), Lcn2 and Chgb, further suggesting the NR2F2 involvement in *Mecp2*-deficient astrocyte could give better understanding of Rett syndrome pathophysiology (Delépine et al., 2015).

1.13.2 Oligodendrocytes and MeCP2

Brain magnetic resonance study in *Mecp2-/y* mice detected a significant reduction in the thickness of the corpus callosum (Saywell et al., 2006). Diffusion tensor imaging (DTI) study in Rett syndrome patients also found significant reduction in fractional anisotropy (FA) in corpus callosum (Mahmood et al., 2010), which suggests white matter impairment in Rett syndrome patients. Myelin-associated oligodendrocytic basic protein (MOBP) is one of the myelin proteins which may be involved in the compaction or stabilization of myelin but exact function of MOBP is still unclear (Montague et al., 2006). MOBP was found to be up regulated in *Mecp2*- null mice and directly regulated by MeCP2 binding through its promoter (Urdinguio et al., 2008). Myelin genes such as MBP (myelin basic protein) and MAG (myelin associated glycoprotein) have been found to be increased in the corpus callosum of *Mecp2* mouse brain (Vora et al., 2010). CNS major myelin proteins comprising MBP, PLP (Proteolipid protein) and MAG plays an important role in myelin sheath integrity and formation. PLP and MBP are distributed within the myelin sheaths and are essential for its compaction. MAG is located in the periaxonal regions and may serve to facilitate cell–cell interactions between myelin and axonal membranes during myelination (Baumann and Pham-Dinh 2001; Fulton et al., 2009). F2-Dihomo-isoprostanes (F2-Dihomo-IsoP), peroxidation products from adrenic acid, a known component of myelin were found to be increased by about two orders of magnitude in RTT patients in stage I of Rett syndrome and decreased in later stages of disease progression. This increased level of F2-Dihomo-isoprostanes in patients suggests the early brain white matter damage in RTT. Some neurological sign in RTT patients overlap with X- linked adrenoleukodystrophy and supports the white matter damage in RTT (Durand et al., 2013). Recently, Nurit Ballas group has shown a significant contribution of oligodendrocytes MeCP2 expression in Rett syndrome pathology (Nguyen et al., 2013). Mice lacking MeCP2 only in oligodendrocytes lineage developed severe hind limb clasping phenotype and restoration of MeCP2 solely in oligodendrocytes resulted in significant improvement of some RTT phenotype including significant improvement of hind limb clasping phenotype and motor activity deficits. Moreover, the loss of MeCP2 only in the oligodendrocyte lineage does not affect the expression pattern of proteins (MBP and PLP) involved in myelination. However, myelin genes MBP and PLP were found to be impaired in *Mecp2*$^{Stop/y}$ mice brain. MBP expression was reduced while the PLP protein level was increased in *Mecp2*$^{Stop/y}$ mice brain. MBP level was only partially restored upon expression of MeCP2 in

oligodendrocytes while level of PLP remain unchanged, suggesting that MeCP2 expression in oligodendrocytes has little effect on the expression of myelin-related proteins and there is possibility of non cell autonomous effect of other cell on expression of myelin proteins (Nguyen et al., 2013). Recently, studies carried out in our laboratory have shown that MeCP2 knock down in cultured rat oligodendrocytes leads to upregulation of several myelin genes expression includes myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin associated oligodendrocytic basic protein (MOBP), neurotrophin (BDNF) and transcriptional regulator YY1(Yin Yang 1) which indicate that MeCP2 act as negative regulator of the myelin gene expression in oligodendrocytes (Sharma et al., 2015).

1.13.3 Microglia and MeCP2

Microglia is known as resident immune cells in brain and primarily functions in brain injury and disease. Despite its main role in immunity, microglia recently have been shown to be involved in synaptic functioning, interaction with neuronal and other non neuronal cells and release of soluble factors in mature and aging brain (Tremblay et al., 2011). *Mecp2* null microglia conditioned media shows potent neurotoxic activity due to release of excess glutamate. Wild type neurons treated with *Mecp2* null microglia condition media shows abnormal dendritic morphology, signs of microtubule disruption and damage of postsynaptic glutamatergic components (Maezawa and Jin, 2010). Previous studies on astrocytes demonstrated that MeCP2-deficient astrocytes detrimentally influence the neuronal dendrite formation (Ballas et al., 2009; Maezawa et al., 2009). Ballas group suggested that *Mecp2* null astrocytes release a soluble neurotoxic factor which shows slow activity and requires long time incubation of at least 3 day in culture but the factor have not been identified. Further, study by Maezawa and Jin found that wild type neuron treated with conditioned media derived from primary mixed glial culture from *Mecp2 null* mice show dendritic damage while conditioned media from highly pure culture of *Mecp2 null* astrocytes show no dendritic abnormalities. This is because of microglia present in mixed glial culture. When wild type neurons treated with conditioned media from *Mecp2* null microglia show robust dendritic abnormalities and this is due to an abnormally high level of glutamate released by *Mecp2* null microglia, causing excitotoxicity that may contribute to dendritic and synaptic abnormalities in RTT. Further, it was identified that increased levels of glutaminase and connexin 32 in *Mecp2-null* microglia are responsible for increased glutamate production and release, respectively. This explain that microglia, and not the astrocytes, are the

major source of soluble neurotoxic factor (Maezawa and Jin, 2010). Further in same line, recent report show that MeCP2 deficiency leads to mitochondrial dysfunction and neurotoxicity in microglia by regulating the glutamine homeostasis. MeCP2 act as repressor of major glutamine transporter SNAT1 in microglia. MeCP2 down regulation or SNAT1 over expression in microglia resulted in mitochondrial dysfunction and neurotoxcity due to overproduction of glutamate (Jin et al., 2015).

It was observed that restoration of MeCP2 in microglia attenuates the symptoms of RTT in mouse model. Thus, via multiple approaches, wild type Mecp2-expressing microglia within the context of Mecp2-null male mouse arrested numerous facets of disease pathology; lifespan was increased; breathing patterns were normalized; apneas were reduced; body weight was increased to near wild type, and locomotor activity was improved. $Mecp2^{+/-}$ females also showed significant improvements as a result of wild type microglial engraftment. More recently, it has been observed that in Mecp2 deficiency condition, microglia become activated and are lost with disease progression. Peripheral macrophage and monocytes population were also found to be depleted in *Mecp2* null mice. Postnatal re-expression of *Mecp2* using $Cx3cr1^{creER}$ extends the lifespan of *Mecp2* null mice. MeCP2 also regulate glucocorticoid- and hypoxia-induced transcripts in Mecp2 deficient microglia and macrophages. Furthermore, MeCP2 modulate the inflammatory genes transcript in response to TNF stimulation to microglia (Derecki et al., 2012; Cronk et al., 2015).These observations suggest a major role of microglia in Rett syndrome pathophysiology.

1.14. MeCP2 and Cancer

As well as to its role in neurological disorders, MeCP2 has also been shown to play a role in many cancers such as breast, colorectal, lung, liver, and prostate cancer. MeCP2 role in cancer is related to the epigenetic regulation of cancer-related genes that involve hypermethylation of gene promoters. MeCP2 promote the growth of prostate cancer cells by regulating the cell proliferation and apoptosis (Parry et al., 2011; Pulukuri et al., 2007; Pampalakis et al., 2009; Pancione et al., 2010; Lin et al., 2007; Shin et al., 2011). Valproic acid, an HDAC inhibitor has been recently shown to regulate expression of the MeCP2 and HDAC1to HDAC3 genes in C6 glioma cells (Kim et al., 2008). In gastric carcinoma cells, micro RNA miR-212 was shown to suppress translation of MECP2 transcripts, which in turn resulted in reduced depth of cellular

invasion (Wada et al., 2010). In addition, MeCP2 has been linked to other cancers, such as myeloma (Wang et al., 2006), hematological malignancies (Meklat et al.,2007), ductal carcinomas (Xu et al., 2012), and cervical cancers (Wang et al., 2013).

1.15. Glioma

Gliomas are the most common adult primary malignant brain tumor, representing 70% of adult primary brain tumors (Wen and Kesari, 2008; Ohgaki and Kleihues, 2005).Gliomas are thought to arise from glial cells or their precursors and occur in the central nervous system (CNS)(Zong et al., 2012).Gliomas are classified as World Health Organization Grades I to IV based on histopathological appearance, including predominant cell type, nuclear aytpia, mitotic figures, necrosis and microvascular proliferation (Louis et al., 2007). Low-grade gliomas (LGG) consist of grade I tumors (such as pilocytic astrocytoma), which are considered non-malignant, and grade II tumors (diffuse astrocytoma, oligodendroglioma). High-grade gliomas (HGG) consist of grade III tumors (anaplastic astrocytomas, anaplastic oligodendrogliomas) and grade IV tumors (glioblastoma (multiforme) -GBMs). Approximately 55% of malignant gliomas are GBMs (Ostrom et al., 2014). Grade I tumors are benign and generally have a favorable prognosis after surgical resection (Maher et al., 2001).High grade gliomas are diffusive and infiltrate into normal brain parenchyma, rendering surgical resection alone insufficient; thus standard therapy in these cases include adjuvant chemoradiotherapy, which can prolong median survival (Stupp et al., 2005). Extra cranial GBM metastasis occurs in about 0.4% - 0.5% cases (Fonkem et al., 2011). Even though GBMs rarely metastasizes, it remains one of the most lethal cancers, with a median survival of 12-15 months (Wen and Kesari, 2008;Noushmehr et al., 2010).Comprehensive genomic and epigenomic profiling of gliomas indicate that heterogeneities within tumor cells play a dominant role in the progression, development of resistance to therapy and recurrence. Despite the increasing knowledge of molecular aberrations involved in gliomas, the standard therapy remains to be primarily surgical resection, radiotherapy and chemotherapy. Recent genome-wide genomic and epigenomic analyses have revealed that mutations in epigenetic modifiers occur frequently in gliomas and that dysregulation of epigenetic mechanisms is closely associated with glioma formation. Given that epigenetic changes are reversible, understanding the epigenetic abnormalities that arise in gliomagenesis might be key to developing more effective treatment strategies for glioma. Given that inhibitors of histone deacetylases are

currently in clinical trial for the treatment of GBM, it is important to understand the involvement of histone modifications during gliomagenesis. (Galanis et al.,2009;Friday et al., 2012). However, knowledge about the role of dysregulation of epigenetic mechanisms in GBM is still very limited, especially how the epigenome is altered specifically at certain loci and how this affects the phenotypes of GBM. Following the discovery of GSC, therefore, it is important to elucidate the epigenetic mechanisms by which environmental cues control the differentiation of GSC into the diverse array of cell types that form GBM (Kondo et al., 2014).

1.16. C6 Glioma as Model System for Glioblastoma

Rat C6 glioma cell line was originally produced in random-bred Wistar-Furth rats by exposure to N,N'-nitroso-methylurea , and it show similar morphology to glioblastoma multiforme (GBM) when injected into the brain of neonatal rats (Grobben et al., 2002). Glioblastoma (GBM) is the most frequent and aggressive malignant primary brain tumor with only about 12% of patients surviving beyond 36 months (long-term survivors) (Li et al., 2016).C6 derived tumors grown from cells transplanted in Wistar rats have characteristics closer to natural glioblastomas than tumors grown in other rat strains. In addition different brain implantation techniques of the cells (intracerebral, intraventricular, intraparenchymal, use of agar, etc.) can be used for the specific study of tumor growth, parenchymal invasion, dispersion in the CSF, etc.(Grobben et al., 2002). These cells have been reported to have a mutant *p16/Cdkn2a/ Ink4α* locus (Schlegel et al., 2000) with no expression of *p16* and *p19ARF*mRNAs, and a wild type p53 (Asai et al., 1994). Recent molecular characterization study, where change in gene expression between the C6 glioma and rat stem cell-derived astrocytes were compared, revealed that the changes in gene expression observed in the C6 cell line were the most similar to those reported in human brain tumors (Sibenaller et al., 2005).Compared to astrocytes, they also had increased expression of the PDGFβ, IGF-1, EGFR and Erb3/Her3genes, which are frequently over expressed in human gliomas. The C6 rat glioma model has been widely used in experimental neuro-oncology to evaluate the therapeutic efficacy of a variety of modalities, including chemotherapy, anti angiogenic therapy, proteosome inhibitors, treatment with toxins, radiation therapy, photodynamic therapy, oncolytic viral therapy and gene therapy. Since this tumor arose in an outbred Wistar rat, however, there is no syngeneic host in which it can be propagated. This is a very serious limitation that diminishes its usefulness for survival studies since the tumor is

immunogenic, even in Wistar rats. Despite this limitation, the C6 glioma model continues to be used for a variety of studies related to brain tumor biology which includes tumor growth, invasion, migration, BBB disruption, neovascularization, growth factor regulation and production, and biochemical studies. Finally, single-cell clonal study has revealed that C6 cells also have cancer stem cell-like characteristics, including self-renewal, thepotential for multi-lineage differentiation in vitro and tumor formation in vivo (Barth and Kaur, 2009).C6 glioma retain some characteristic of glial precursor and also express some oligodendrocyte and astrocytes genes including PLP, MAG and GFAP (Salvati et al., 2004; Zhu et al., 1994; Ye et al., 1992; Leisewitz et al., 2008).

1.17. Rationale of the Present Study

MeCP2 is a methyl binding protein, mutation of which causes neurodevelopmental disorder Rett syndrome. Most of the research related to MeCP2 was primarily focused on the role of MeCP2 in neuronal cells and its contribution to Rett syndrome. However, neuronal maturation is dependent on signals derived from the glial cells that make up 90% of the CNS cellular mass. Recently, studies have shown MeCP2 expression in glial cells and it also regulates glial gene expression. Oligodendrocytes, the myelinating glial cells of CNS required for normal functioning of neurons, also show expression of MeCP2. To date, there has been not much research focused on MeCP2 role in oligodendrocytes. Moreover, MeCP2 act as global transcriptional modulator and hence the present study hypothesized that:

a. MeCP2 regulates myelin-related gene expression in cultured rat oligodendrocytes

b. MeCP2 directly binds to myelin-related gene promoters

Besides, MeCP2 role in neurodevelopmental disorders, its importance in many cancers such as breast, colorectal, lung, liver, and prostate cancer is well studied (Kim et al. 2008; Parry et al. 2011). However, far too little attention has been paid to MeCP2 role in regulation of glioma malignant behaviour. C6 glioma retains some characteristics of glial precursor and also expresses some oligodendrocyte and astrocytes genes including PLP, MAG and GFAP. Based on these information's, following hypothesis were made:

a. *MeCP2 differentially regulate myelin gene expression in oligodendrocytes and C6 glioma*
b. *MeCP2 regulates the C6 glioma malignant behavior*.

1.18. Objectives

On the basis of above hypotheses following objectives were defined for present study:

1. ***Evaluation of MeCP2 mediated regulation of myelin related gene expression and oligodendrocyte cell behavior***
2. ***Identification of genes involved in myelination for which MeCP2 act as transcriptional repressor or activator in the developing central nervous system***
3. ***Evaluation of MeCP2 function in the regulation of glioma growth and gene expression***