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The Translational Biology of Remyelination: Past, Present, and Future

Robin J. M. Franklin¹ and Vittorio Gallo²

Amongst neurological diseases, multiple sclerosis (MS) presents an attractive target for regenerative medicine. This is because the primary pathology, the loss of myelin-forming oligodendrocytes, can be followed by a spontaneous and efficient regenerative process called remyelination. While cell transplantation approaches have been explored as a means of replacing lost oligodendrocytes, more recently therapeutic approaches that target the endogenous regenerative process have been favored. This is in large part due to our increasing understanding of (1) the cell types within the adult brain that are able to generate new oligodendrocytes, (2) the mechanisms and pathways by which this is achieved, and (3) an emerging awareness of the reasons why remyelination efficiency eventually fails. Here we review some of these advances and also highlight areas where questions remain to be answered in both the biology and translational potential of this important regenerative process.

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Key words: progenitor, remyelination, multiple sclerosis

Introduction

Demyelination, the loss of myelin from an otherwise intact axon, is an unusual pathological event in the CNS in that it is often followed by remyelination, a spontaneous regenerative process involving the generation of new oligodendrocytes and the formation of new myelin sheaths (Franklin and ffrench-Constant, 2008; Gallo and Armstrong, 2008). With the restoration of myelin comes the return of myelin functions lost during demyelination, namely, salutatory conduction, the maintenance of axonal integrity and the recovery from functional deficits (Duncan et al., 2009; Franklin et al., 2012). Primary demyelination is a feature of several neurological conditions. In those where demyelination features as a single acute event, such as in traumatic injury, the likelihood is that these lesions will, eventually, undergo spontaneous repair (e.g., Lasiene et al., 2008). However, the situation is somewhat different in the most prevalent demyelinating disease of the adult central nervous system (CNS), multiple sclerosis (MS). MS is a chronic demyelinating disease, often of many decades duration, in which autoimmune-mediated damage to myelin (and in the acute inflammatory phase, to axons) continues to occur episodically. Like all regenerative processes,

the efficiency of remyelination declines progressively throughout adulthood (Goldschmidt et al., 2009): the basis of this decline is discussed later in this review. The effects of aging on remyelination are likely to have a profound bearing on the natural history of the disease even though the rate at which remyelination occurs in humans is difficult to assess. The observation that extensive remyelination can be found in older individuals with MS (Patrikios et al., 2006) can not be taken as evidence that remyelination is unaffected by aging (which would make remyelination unique amongst regenerative processes), since in this study there was no way of knowing when a particular lesion occurred or how long it took to remyelinate. The consequence of failing remyelination is that axons remain chronically demyelinated and vulnerable to the irreversible degeneration that underpins the currently untreatable secondary progressive phase of MS. By implication, reversing the declining efficiency of remyelination by its therapeutic enhancement is likely to prove an effective means of addressing the unmet clinical need of providing neuroprotection in the secondary progressive MS (Franklin et al., 2012). Although remyelination enhancement could theoretically be achieved by transplantation of myelinogenic cells (despite the

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many practical hurdles of cell identity and source, delivery, and histocompatibility), current evidence suggest that this will not be necessary since the proof-of-principle studies in mice indicate that the effects of ageing on the spontaneous regenerative process are reversible and that strategies targeting the endogenous remyelination will prove effective throughout the disease duration (Huang et al., 2011; Ruckh et al., 2012). While cell transplantation remains (and is likely to remain) the approach of choice for the demyelinating conditions of genetic origin (Goldman et al., 2012; Gupta et al., 2012), where the oligodendrocyte lineage is inherently defective, promotion of endogenous remyelination is the current favored strategy for remyelination in MS and will therefore form the focus of this review.

Endogenous Adult Progenitor Cells with the Potential to Remyelinate

Substantial OL death occurs in MS and demyelinating disorders (Barnett and Prineas, 2004; Cannella et al., 2007), and in all animal models of demyelination (Aguirre et al., 2007; Mason et al., 2004; Mi et al., 2011; Woodruff and Franklin, 1999; Zhou et al., 2012). Induction of apoptosis in OLs by lentivirus-mediated caspase 9 expression under the MBP promoter results in rapid focal demyelination (Caprariello et al., 2012), demonstrating that demyelination is initiated by OL death itself. Oligodendrocytes (OLs) and Schwann cells are the only cells in the central nervous system that are able to repair demyelinated axons. Schwann cell remyelination of the CNS, which has been reported in MS, spinal cord injury and various experimental models (Itoyama et al., 1983; Zawadzka et al., 2011), occurs under particular circumstances such as when demyelination is associated with astrocyte loss. Here, we will focus on oligodendrocyte remyelination. Although cellular interactions between OLs and other cell types of the CNS regulate the complex process of remyelination, OLs themselves are ultimately responsible for myelin repair. Therefore, recognizing and understanding the intrinsic OL potential to remyelinate under different pathological conditions, and the cellular and molecular signals that regulate this process are crucially important to design future therapies aimed at promoting remyelination.

It is well established that remyelination primarily occurs from endogenous neural progenitor cells (NPCs) and OPCs surviving demyelinating insults and generating new, mature OLs. Analysis of the spatio-temporal dynamics of cellular recovery in demyelinated lesions demonstrated that cells with an OPC phenotype first appear before new myelinating oligodendrocytes are generated (Aguirre et al., 2007; Fancy et al., 2004; Levine and Reynolds, 1999; Watanabe et al., 2002). Genetic fate-mapping studies using a variety of reporter proteins expressed under the control of OPC gene promoters

directly demonstrated enhanced oligodendrogenesis from OPCs after demyelination (Aguirre et al., 2007; Zawadzka et al., 2010; Guo et al., 2011; Nakatani et al., 2013). Importantly, this pool of OPCs is renewable between demyelinating episodes (Penderis et al., 2003). A more recent study used a retroviral reporter system to selectively, genetically and permanently label endogenous dividing NG2-expressing progenitors after spinal cord injury confirmed that this cell population is actively involved in myelin repair (Powers et al., 2013). These findings are consistent with reports demonstrating that grafted rodent and human glial progenitors, as well as iPSC-derived OPCs, can extensively repopulate and remyelinate demyelinated areas (Groves et al., 1993; Najim et al., 2013; Windrem et al., 2004). These findings indicate that endogenous adult OPCs and NPCs represent the major cellular targets to promote remyelination in the CNS.

A therapeutic approach of exploiting endogenous progenitor cells for remyelination therefore calls for clearer understanding of many intrinsic properties of adult OPCs, namely: (1) What are the characteristic features of activated, remyelinating OPCs? (2) What is their potential to migrate to different brain regions to repopulate demyelinated areas (3) How efficient are these progenitor populations in expanding to regenerate the necessary OL numbers, and how effectively do they differentiate into mature, myelinating OLs? (4) Which and where are the major NPC/OPC populations capable of regenerating oligodendrocytes and myelin after demyelination? (5) Are there injury-induced OPCs derived from other cell lineages? These issues have been studied for many years, and some significant progress has recently been made to further uncover the molecular and developmental properties of these progenitors, and identify specific cellular phenotypes and their regenerative potential.

The endogenous population of adult OPCs is characterized by expression of the PDGFR α (Kang et al., 2010; Zhu et al., 2008). NG2 is highly responsive to injury (e.g., Rhodes et al., 2006), and recently its role during remyelination was demonstrated, not only in OPC proliferation, but also as a component of demyelination-induced inflammatory damage (Kucharova et al., 2011). Fate mapping studies and use of different transgenic mouse strains demonstrated that these cells are capable of efficiently regenerating myelinating OLs after demyelination (Aguirre et al., 2007; Zawadzka et al., 2010). Like perinatal OPCs, adult OPCs express the transcription factors Olig1/2 (Aguirre et al., 2007; Cassiani-Ingoni et al., 2006; Fancy et al., 2004; Ligon et al., 2006; Lu et al., 2002; Sim et al., 2002; Zhou et al., 2001), Ascl1 (Nakatani et al., 2013; Parras et al., 2004) and Nkx2.2 (Fancy et al., 2004; Soula et al., 2001; Zhou et al., 2001) under physiological and pathological conditions. Importantly,

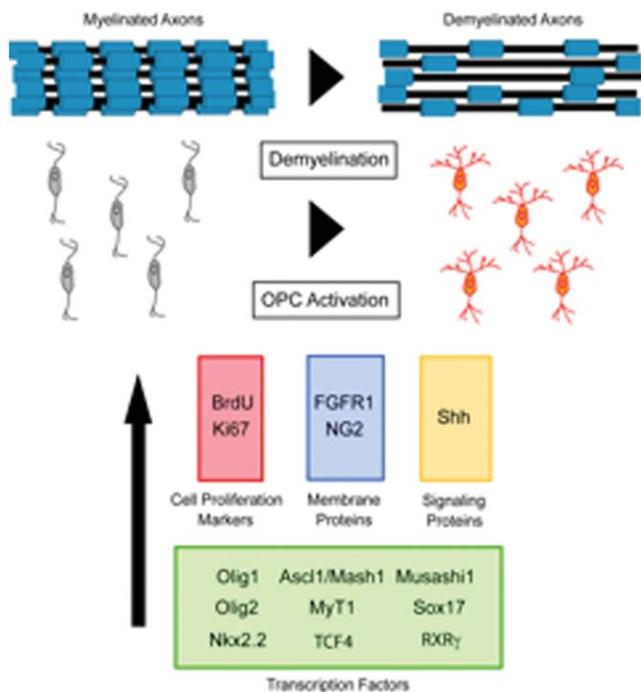


FIGURE 1: Phenotype of endogenous adult progenitor cells with the potential to remyelinate. The figure shows cellular proteins that are transiently upregulated (large arrow on the left) in endogenous adult OPCs after demyelination and can be used to identify “activated OPCs”, i.e. cells that respond to the demyelination insult. These proteins include cell proliferation markers, membrane and signaling proteins, and transcription factors.

expression of the marker proteins that characterize OPCs in the normal and pathological rodent brain has also been revealed in MS tissue (Arnett et al., 2004; Chang et al., 2002; Nait-Oumesmar et al., 2007). The identification and characterization of OPCs in the pathological brain raises the important question of whether there is indeed a phenotype corresponding to an “activated” OPC (Fig. 1).

Both BrdU pulse-chase and fate mapping studies demonstrated that dividing OPCs expressing proliferation markers are actively involved in remyelination (Aguirre et al., 2007; Carroll et al., 1998; Gensert et al., 1997). Intrinsic regulators of OPC proliferation also play an important role in remyelination. In response to CNS demyelination, loss of Cdk2 alters OPC renewal, cell cycle exit, and differentiation (Caillava et al., 2011). Furthermore, Cdk2 deletion accelerates remyelination of demyelinated CNS axons from parenchymal OPCs (Caillava et al., 2011). Interestingly, Cdk4, not Cdk2, may play a crucial role in PNS remyelination after nerve injury (Atanasoski et al., 2008), indicating that different molecular mechanisms regulate OPC and Schwann cell precursor expansion and recruitment during CNS versus PNS remyelination. Finally, developmental regulation of cell cycle activity (Belachew et al., 2002), together with impaired recruitment and differentiation (Shen et al., 2008; Sim et al.,

2002), likely underlies the reduction in the OPC regenerative potential observed in adult compared with the developing brain (Sim et al., 2002).

Other cellular features, besides proliferation markers, appear to be typical of “activated” OPCs. Fibroblast growth factor receptor 1 (FGFR1) expression is upregulated in OPCs after cuprizone-induced demyelination and its deletion during chronic demyelination reduces the number of OPCs, but promotes their differentiation to oligodendrocytes (Zhou et al., 2012). Both Shh and a member of the SoxF transcription factor family, Sox 17, are expressed at high levels in OPCs after demyelination, but are either undetectable or expressed at much lower levels in OPCs under normal physiological conditions (Feret et al., 2013; Ming et al., 2013; Moll et al., 2013). Shh and Sox17 have both been identified as positive regulators of oligodendrogenesis and remyelination (Feret et al., 2013; Ming et al., 2013), and Shh transcripts are detected only in OPCs engaged in remyelination (Feret et al., 2013). Musashi1 (Msi1), Myt1, Tcf4 (Tcf7l2) and Nkx2.2 are expressed in OPCs in white matter regions during development, and their expression is upregulated in OPCs after demyelination (Dobson et al., 2008; Fancy et al., 2004, 2009; Sim et al., 2002; Vana et al., 2007; Watanabe et al., 2004). Furthermore, although Olig2-expressing progenitors appear to be a major cell population involved in remyelination (Aguirre et al., 2007; Fancy et al., 2004; Menn et al., 2006), maintenance of nuclear Olig2 expression is required for oligodendrocyte regeneration, as translocation of Olig2 from the nucleus to the cytoplasm is associated with induction of GFAP expression and astrogliogenesis (Cassiani-Ingoni et al., 2006). Finally, Olig1, which relocates to the nucleus in “activated” OPCs, and Ascl1/Mash1 are also required for remyelination (Arnett et al., 2004; Nakatani et al., 2013). Loss of Olig1 in transplanted NPCs results in astrocyte generation, rather than oligodendrocytes (Whitman et al., 2012), and selective deletion of Ascl1/Mash1 in OPCs prevents remyelination (Nakatani et al., 2013). In conclusion, it appears that expression of proliferation markers, as well as high levels of FGFR1, Shh, Sox17, Msi1, Myt1, Nkx2.2, Tcf4, and Ascl1/Mash1, together with nuclear Olig1/2 expression, may characterize “activated” OPCs engaged in the remyelination process.

Aging strongly affects the potential of endogenous OPCs to remyelinate (Hampton et al., 2012; Ruckh et al., 2012; Sim et al., 2002) and epigenetic mechanisms modulating OPC differentiation program are also regulated by age (Shen et al., 2008). Histone deacetylases (HDACs) are involved in downregulation of inhibitors of oligodendrocyte differentiation during development (Marin-Husstege et al., 2002; Ye et al., 2009). The efficiency of this process is affected by aging, and remyelination is impaired by *in vivo* administration of HDAC inhibitors acting on OPCs (Shen

et al., 2008). Therefore, it appears that HDACs play an important role in remyelination efficiency and their activity is required to suppress inhibitors of OPC differentiation. In addition to HDACs, Na⁺-dependent deacetylases also play a role in oligodendrocyte regeneration and remyelination efficiency. A recent study demonstrated that the nuclear sirtuin SIRT1 acts as an inhibitor of remyelination, because inactivation of this protein leads to expansion of the OPC pool, enhanced remyelination and delayed paralysis in animal models of demyelination (Rafalski et al., 2013). In summary, epigenetic reactivation of developmental mechanisms in OPCs is likely to be an essential component of oligodendrogenesis and remyelination. It will have to be established whether activation of epigenetic mechanisms in distinct pools of OPCs determines recruitment of subpopulations of these cells during myelin regeneration processes of the adult brain.

Two major sources of OPCs are present in the adult brain: the subventricular zone (SVZ) and the parenchyma, which includes the white matter itself (Aguirre et al., 2004, 2007; Levison and Goldman, 1993; Menn et al., 2006; Nait-Oumesmar et al., 1999). The SVZ progenitors involved in remyelination of white matter regions express NG2 and Olig2, as well as markers of Type-C cells, including EGFR (Aguirre et al., 2004, 2007; Menn et al., 2006; Nait-Oumesmar et al., 1999). Differently from Type-B cells, SVZ OPCs actively divide and their proliferation rate is enhanced after demyelination of the corpus callosum (Aguirre et al., 2007; Menn et al., 2006; Nait-Oumesmar et al., 1999). Both parenchymal and SVZ progenitor pools participate in remyelination, although their relative contributions to oligodendrocyte regeneration and remyelination of demyelinated lesions has not yet been quantitatively determined. Furthermore, although many of the molecular pathways involved in proliferation, migration and differentiation of SVZ and parenchymal OPCs after demyelination have been identified, it is still unclear whether these distinct pools of endogenous progenitors are functionally distinct and whether intrinsic molecular mechanisms distinguish their proliferative responses to demyelination. Future studies should be aimed at revealing these differences, by, for example, selectively inhibiting recruitment of either SVZ or parenchymal OPCs in order to measure their relative involvement in remyelination.

A question of great interest in oligodendrocyte regeneration and remyelination is whether specific neural cell lineages display plasticity in postnatal and adult brain under pathological conditions, i.e., can neuronal or glial progenitors “committed to” either a neuronal or an astrocytic fate become diverted to generate different progenies? The potential for instructing immature neurons to generate glia would enable larger pools of endogenous neural progenitors to be targeted and avoid some of the problems associated with heterologous

transplantation as a therapeutic approach, such as rejection of heterologous cells, and efficiency and invasiveness of the grafting process. Studies have described changes in cell fate potential after genetic manipulation of specific neuronal progenitor cell populations under normal physiological conditions and in culture. For example, adult hippocampal NPCs change their fate from neurons to OPCs and oligodendrocytes after retroviral-mediated overexpression of bHLH transcription factor *Ascl1* (*Mash1*) (Jessberger et al., 2008). This process is regulated by cell-autonomous and niche-dependent mechanisms (Jessberger and Gage, 2009). Cell lineage plasticity also occurs in *GAD65*- and *Dcx*-expressing neuroblasts of the adult SVZ, which generate oligodendrocytes in corpus callosum after focal demyelination (Jablonska et al., 2010). This process occurs together with induced expression of *Mash1* and *Olig2* in these progenitors, strongly suggesting that bHLH transcription factors act coordinately as essential regulators of oligodendrogenesis from *GAD65*- and *Dcx*-expressing neuroblasts. The BMP antagonist chordin was identified as a SVZ signal that promotes oligodendrogenesis from these progenitors, indicating that niche-dependent mechanisms also regulate lineage plasticity of neuroblasts in the SVZ (Jablonska et al., 2010).

One can propose that cell fate plasticity is a major factor supporting endogenous brain repair. Glial progenitor cell lineage plasticity is not entirely surprising, since hippocampal and SVZ astroglial stem cells display multipotency both *in vitro* and after grafting *in vivo* (Doetsch et al., 1999; Eriksson et al., 2003; Ganat et al., 2006; Laywell et al., 2000; Yagani-sawa et al., 2005), however the host environment is critical in determining their survival and differentiation. Various growth factors, neurotrophins, cytokines, and proinflammatory molecules are present in the cellular environment of a demyelinated lesion, where it is likely that they promote or restrict cell fate plasticity in activated neuronal or glial progenitors during regeneration. Therefore, a major aim of future studies will be defining endogenous signaling pathways activated under pathological conditions, and demonstrating their effects on neural progenitor cell plasticity.

The experimental literature discussed above points to a major role of endogenous NG2/PDGFR α -expressing progenitors in remyelination. These cells represent an important target to promote myelin repair, as they have the potential to generate both oligodendrocytes and Schwann cells under pathological conditions (Zawadzka et al., 2010). However, there are still major issues that will have to be resolved and that directly affect the outcome of endogenous OPC-mediated remyelination. It is still undefined to what extent OPC/NPC death occurs in MS lesions (Cui et al., 2013), with subsequent reduction of the endogenous pools of these cells. This is an important and unresolved question, as the

size of this pool directly impacts the efficiency of the repair process (see Franklin and ffrench-Constant, 2008 for review). Furthermore, the influence of the pathological cellular environment, in particular inflammation, can influence the response of NG2 cells to demyelination (Rhodes et al., 2006). These factors will have to be carefully taken into account in designing future strategies aimed at promoting OPC-mediated oligodendrocyte regeneration and remyelination.

Functional Role of Neurons in Remyelination

Death of oligodendrocytes and loss of myelin represents a major component of the degenerative processes associated with myelin pathology and demyelination. However, it has become clear that neuronal loss and axonal damage and degeneration also play a crucial role in myelin diseases, including MS (Ganter et al., 1999; Trapp et al., 1998). Evidence obtained in animal models of demyelination, as well as in human MS tissue, supports the idea that compromised axonal function and neuronal loss both cause long-term impairment observed in the chronic progressive phase of MS (Dubois-Dalcq et al., 2005). Therefore, MS and other demyelinating diseases are now regarded as a combination of inflammatory damage to oligodendrocytes and neurodegeneration.

Similar to the process that occurs during development, repair of a demyelinated lesion depends on appropriate matching between oligodendrocytes and axons, i.e. on a coordinated regenerative process that involves both glia and neurons. The number of oligodendrocytes necessary to repair a lesion will ultimately depend on the size of the lesion, in particular the number of demyelinated axons and the length of demyelinated segments. However, the cellular and molecular mechanisms that regulate appropriate myelin thickness and intermodal length during remyelination are still unknown. Are the same axonal signals that act on oligodendrocytes to modulate developmental myelination also effective during remyelination, and what types of communication occur between axons and oligodendrocytes during this process? Some of these crucial cellular interactions have been identified, including electrical activity-mediated communication, release of soluble factors and cell-to-cell contact, and are still being actively investigated.

Electrical activity and proper axonal function are required for developmental myelination (Demerens et al., 1996; Wake et al., 2011). In fact, it is likely that synchronization between oligodendrocyte developmental programs and specific patterns of axonal activity is crucial for timing of oligodendrocyte differentiation and initiation of myelination. Furthermore, imaging studies have demonstrated that neuronal activity and learning cause structural changes in white matter regions of the brain; although the underlying cellular

and molecular events are still largely unknown (see Zatorre et al., 2012 for review). Recent behavioral studies identified a critical developmental period for social experience-dependent oligodendrocyte maturation and myelination (Makinodan et al., 2012), as well as impaired myelination in the adult brain caused by social isolation (Liu et al., 2012). These studies strongly suggest that experience-dependent neuronal activity results in white matter plasticity both in the developing and adult brain.

Earlier studies in the CNS demonstrated that blockage of action potentials in axons results in defective myelination, whereas increase in electrical activity enhances myelination (Demerens et al., 1996). The mechanisms by which electrical activity in axons influence myelination are still incompletely defined, although axonal signals, e.g., glutamate and adenosine, that are released by specific patterns of electrical stimulation in axons and regulate myelination have been identified (Stevens et al., 2002; Wake et al., 2011). Adenosine is thought to act on purinergic receptors expressed by OPCs (Stevens et al., 2002), whereas glutamate is believed to act on receptors of the AMPA, kainate and NMDA subtype (Gallo et al., 1996; Káradóttir et al., 2005; Kukley et al., 2007; Patneau et al., 1994; Ziskin et al., 2007). Importantly, astrocytes are also involved in activity-dependent regulation of myelination and cooperate with other neural cell types to modulate this process. ATP released by axons through specific patterns of stimulation induces release of cytokine leukemia inhibitory factor (LIF) by astrocytes, which in turn promotes myelination (Ishibashi et al., 2006). More recently, electrical activity was found to promote myelination in a frequency-dependent fashion, and neuronal cAMP was identified as a major mediator of this effect (Malone et al., 2013). However, direct evidence that electrical activity *per se* modulates remyelination in the CNS *in vivo* is still lacking.

Compromised axonal function is likely to further reduce remyelination potential, as distribution of metabolites, organization of ionic channels and patterns of electrical activity are permanently altered (see Franklin and ffrench-Constant, 2008, for review). Therefore, it is important to understand how demyelination impacts functional organization of axons and ultimately their physiological properties. First, mitochondrial functions are severely affected in degenerating axons (Dutta et al., 2006), as axonal ATP is depleted and Na⁺-K⁺ ATPase failure occurs as a consequence of inflammation (Smith, 2007). This causes a reduced ability of axons to prevent pathological Na⁺ influx and to extrude Na⁺ ions. Higher intracellular Na⁺ concentrations in axons trigger a cascade of events, including enhanced Na⁺-Ca²⁺ exchange channel activity, increased Ca²⁺ influx, activation of proteases, and disruption of neurofilament integrity and axonal transport (Dutta et al., 2006; Stys et al., 1992). Consistent with this hypothesis, in MS tissue and

animal models, the number of mitochondria in axons significantly changes as a result of demyelination and during remyelination, as demonstrated by immunolabeling with antibodies against porin, a voltage-gated anion channel selectively expressed in the mitochondrial membrane (Zambonin et al., 2011). In particular, mitochondrial content significantly increases in chronically or acutely demyelinated axons, and decreases during remyelination, although the overall number of mitochondria in remyelinated axons is still higher than in myelinated axons (Zambonin et al., 2011). These results indicate that demyelinated axons display a large increase in energy demand, which is partially attenuated by remyelination.

Myelin plays an essential role in organization of axonal domains, because the organization of nodal, paranodal, and juxtaparanodal proteins, including ion channels, is severely compromised in demyelinated axons (see Waxman, 2006 for a review). In demyelinated axons, voltage-gated Na^+ channel (NaV) expression and nodal distribution are significantly altered—in particular the small conductance channel NaV 1.2 is upregulated and more evenly redistributed on the intermodal axolemma (Craner et al., 2003). Furthermore, the larger conductance NaV 1.6 channels, which are normally segregated at the nodes of Ranvier after myelination, are also more evenly distributed on axons (Craner et al., 2004). Similar neuronal abnormalities were also detected in MS tissue (Craner et al., 2004). Therefore, it is likely that a large upregulation of Na^+ conductance will cause a drastic increase in intra-axonal Na^+ ions, with subsequent protease activation and axonal loss.

Expression and distribution of paranodal (paranodin/Caspr) and juxtaparanodal (Kv channels and Caspr) proteins is also altered in demyelinated axons found in MS tissue, as they are all diffusely distributed, rather than clustered in specific domains (Coman et al., 2006). Conversely, in remyelinated lesions (shadow plaques and partially remyelinated plaques), aggregates of nodal and perinodal constituents were detected (Coman et al., 2006), further indicating that myelin contributes to maintaining normal distribution of axonal proteins. Thus, demyelination causes major changes in fundamental structural and physiological properties of axons, which prevent normal saltatory conduction and disrupt normal axo-glial interactions. These abnormalities of intrinsic axonal properties contribute to inhibition of myelin repair, which in turn further compromises axonal viability leading to degeneration.

A different type of axonal-oligodendroglial communication has been recently characterized, based on the pioneering work of Bergles and colleagues demonstrating the presence of synapses on NG2-expressing OPCs (Bergles et al., 2000). Although these neuron-NG2 cell synapses were originally identified in gray matter, they are also found in NG2 cells present in white matter, both under normal physiological

conditions (Kukley et al., 2007; Ziskin et al., 2007) and after demyelination (Exteberria et al., 2010). Axon collaterals in corpus callosum make direct synaptic contacts with OPCs, which receive both glutamatergic and GABAergic synapses (Lin and Bergles, 2003). At early postnatal developmental stages both neurotransmitter amino acids depolarize the NG2 cell membrane. Recent evidence indicates that glutamate regulates NG2 cell proliferation and differentiation *in vivo* (Mangin et al., 2012), as previously observed in OPCs in different culture systems (Gallo et al., 1996; Yuan et al., 1998). The direct demonstration of synapses being present on actively dividing NG2 cells (Ge et al., 2009; Kukley et al., 2008) is consistent with a developmental function, in particular on cell proliferation. Furthermore, downregulation of neuron-NG2 cell synapses occurs as OPCs mature to premyelinating and myelinating OLs (De Biase et al., 2010), indicating a specific role for these synapses at earlier developmental stages that precede myelination (Gallo et al., 2008).

Synaptic communication between neurons and OPCs is likely to regulate not only OPC development, but also oligodendrocyte regeneration and remyelination. NG2 cells in the SVZ do not display synaptic contacts with neurons; however, they receive glutamatergic synaptic inputs from axonal collaterals once they migrate from the SVZ into the corpus callosum within a few days after toxin-induced focal demyelination (Exteberria et al., 2010). Similar to the process that occurs during development, these synaptic inputs are also downregulated at more mature stages during remyelination (Exteberria et al., 2010). Therefore, axons still establish and maintain contacts with NG2 cells during early phases of remyelination, suggesting that glutamate-induced depolarization contributes to NG2 cell cycle exit and initiation of differentiation also under pathological conditions.

In addition to soluble factors and neurotransmitters, membrane-bound molecules present in axons regulate myelination, and likely remyelination. The immunoglobulin superfamily member's neural cell adhesion molecules (NCAMs) have been implicated in cell-cell adhesion and migration, and their expression is developmentally regulated, indicating specific roles during critical phases of CNS maturation (Coman et al., 2005). PSA-NCAM expression in axons is drastically downregulated during myelination and its loss from the axonal surface coincides with initiation of myelination (Charles et al., 2000). This suggests that PSA-NCAM is an inhibitor of myelination, and downregulation of this adhesion molecule is a necessary step to convert axons to a myelination permissive state. Studies in postmortem MS tissue are consistent with this hypothesis. In fact, PSA-NCAM is normally absent from the adult brain, but is re-expressed on demyelinated axons present in MS plaques, and not in shadow plaques,

strongly suggesting that it might be one of the endogenous inhibitors of remyelination in MS (Charles et al., 2002).

Semaphorins are known as developmental signals for both neurons and glia in the CNS (Armendariz et al., 2012; Pasterkamp, 2012). Semaphorins have emerged as important regulators of both OPC migration and differentiation following demyelination. Developmental studies first identified semaphorins 3A and 3F as repulsive and attractive guidance cues for OPCs, respectively. It has subsequently been shown that adult OPCs express class 3 semaphorin receptors, neuropilins and plexins and that neuropilin expression by OPCs increases after demyelination. Gain and loss of function experiments have shown that semaphorin 3A impairs OPC recruitment to the demyelinated area, while semaphorin 3F overexpression accelerates not only OPC recruitment, but also remyelination rate (Piaton et al., 2011). Semaphorin 3A, which is expressed at high levels in active MS lesions (Syed et al., 2011), also reversibly inhibits OPC differentiation in culture and prevents remyelination when infused in demyelinated lesions of the CNS (Syed et al., 2011), indicating that this protein might contribute to maintaining OPCs in an undifferentiated state and limit their maturation under pathological conditions.

Axonal Protection and Remyelination

Myelin integrity is vital to axonal function and survival, as demonstrated in earlier studies in transgenic mice in which the myelin-specific genes *Cnp* or *Plp* were selectively deleted (Griffiths et al., 1998; Lappe-Siefke et al., 2003). In these mouse mutants, myelin sheaths displayed only minor structural abnormalities, but axons progressively degenerated. The absence of PLP protein directly affected axoplasmic transport and further analysis identified the myelin-associated NAD-dependent deacetylase sirtuin 2 as a molecular mediator of this effect (Werner et al., 2007). Studies in human pathological tissues are also consistent with the notion that myelin maintains axonal stability, as demonstrated in Pelizaeus-Merzbacher disease (PMD) and in MS. PMD is caused by *PLP* gene mutations and causes axon loss (Garbern et al., 2002), indicating that the phenotype observed in animal models reproduces the human disease. In MS tissue, white matter regions in which at least partial remyelination occurred display enhanced preservation of axons (Kornek et al., 2000), consistent with findings in animal models demonstrating that remyelination is protective to axons (Irvine and Blakemore, 2008). The cellular and molecular mechanisms causally involved in the effects of myelin on axonal survival are being elucidated, and IGF1, BDNF and GDNF have been identified as candidates for oligodendrocyte-derived trophic factors that promote axonal growth and survival (Smith et al., 2013; Wilkins et al., 2003). An intriguing recent study has revealed activity-dependent axonal release of the neurotransmitter glutamate triggering oligodendroglial

secretion of exosomes carrying cargoes of specific proteins and RNA that are taken up by neurons and improve neuronal viability (Frühbeis et al., 2013).

Oligodendrocytes and myelin also play a critical role in metabolic support of axons under normal physiological conditions (Nave, 2010). The role of oligodendrocytes in maintaining long-term axonal integrity has been recognized for a number of years, but only recently have some of the fundamental molecular mechanisms been defined. In particular, both glucose and lactate have been identified as main regulators of oligodendrocyte development and myelination (Rinholm et al., 2011). Furthermore, metabolic coupling between axons and oligodendrocytes has been demonstrated, with oligodendrocytes providing aerobic glycolysis products to axons under conditions that cause energy deprivation (Funfschilling et al., 2012). Finally, a crucial role for the lactate transporter MCT1, which is highly enriched in oligodendrocytes, has been recently revealed, as disruption of MCT1 causes axon damage and neuronal loss (Lee et al., 2012). A role for oligodendroglial MCT1 has also been suggested in the pathogenesis of ALS (Lee et al., 2012).

These recent studies have further elucidated how oligodendrocyte loss and demyelination could impact short- and long-term axonal integrity, and ultimately neuronal survival. Conversely, oligodendrocyte regeneration and prompt remyelination would prevent axons from undergoing a sequence of physiological changes that leads to irreversible damage. Remyelination not only restores normal axonal conduction and physiological patterns of electrical activity, but also protects axons from further inflammatory-induced degeneration (Black et al., 2006).

Conclusion and Perspectives

The studies discussed above demonstrate that neurons and oligodendrocytes form a unique, mutually beneficial cellular partnership that can be severely affected by the compromised viability of either cell type. To elucidate the functional role of neurons and their activity on remyelination, future efforts should be focused on developing experimental approaches that will allow simultaneous monitoring of electrical activity in axons and their myelination status *in vivo*. These approaches should be combined with integrated analysis in animal models and in human brain. Field potential recordings (Crawford et al., 2009) combined with imaging techniques (Zatorre et al., 2012) will allow a more accurate evaluation of action potential propagation along axons and functional integrity of white matter regions.

Diffusion tensor imaging studies and the use of more sophisticated multimodal imaging techniques will help define real-time microstructural changes occurring in white matter during remyelination. In neurons, these changes relate to

axon number and diameter, axon density, axon branching and directionality, and extent of remyelination. In non-neuronal cells, changes in macroglia and microglia number and size, as well as angiogenesis will affect imaging results. A relatively new MR technique, serial magnetization transfer (MT), that appears to be particularly sensitive to detecting myelination (Laule et al., 2007), has been used to evaluate demyelination, axonal loss and nerve degeneration (Horsfield, 2005). Furthermore, MT was also used to investigate natural variation of white matter microstructure and composition in healthy subjects (Kang et al., 2011). This experimental approach is also useful to better determine myelin content and distribution in disease. Finally, positron emission tomography can be used to image vital stains for myelin (Stankoff et al., 2011) and maps of multi-exponential T2 relaxation times can provide myelin measures (Laule et al., 2007).

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