CHAPTER

31

Formation and Maintenance of Myelin

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OUTLINE

INTRODUCTION

Myelination is the production of the myelin membrane that wraps axons in the central and peripheral nervous systems. Myelin structure and function have been described in detail in Chapter 10. This chapter focuses on the formation of myelin, which is the differentiation of two cell types, the Schwann cell in the peripheral nervous system (PNS) and the oligodendrocyte in the central nervous system (CNS). Pathologies associated with demyelinating diseases and conditions are discussed in Chapter 39. Mechanisms of remyelination following demyelination are discussed here. In the

interests of space, numerous aspects of the control of myelination are discussed here very briefly. The reader is referred to Lazzarini, (2004) for greater detail.

Myelination occurs during nervous system development and is essential for normal nervous system function

As the nervous system matures, portions of the PNS myelinate first, then the spinal cord and the brain last (Morell, 1984). In all parts of the nervous system there are many small axons that are never myelinated. In fact there are data indicating that as many as 76% of axons in the subcortical white matter may be unmyelinated, but there are clear regional differences (Partadiredja et al., 2003; Seggie & Berry 1972; LaMantia & Rakic 1990). It is generally true that large neural pathways become myelinated before they are completely functional. A relevant observation is that rats and other nest-building animals are quite helpless at birth, and they myelinate predominantly postnatally. By contrast, grazing animals, such as horses, cows, and sheep, have considerably more CNS myelin at birth and a correspondingly higher level of complex activity immediately postnatally. Most information on myelination has been obtained from rodent models, including many recently developed transgenic mouse systems. These allow directed deletion or overexpression of specific genes for detailed analysis of the regulation of myelination. More recently, zebrafish have become a valued model since they allow detailed analysis of cells *in vivo*, with a series of fluorescently-tagged transgenic fish lines that can be used for live imaging of cell migration and differentiation, or for imaging the induction of specific signaling pathways or other regulatory events in real time (Monk & Talbot 2009).

SCHWANN CELL DEVELOPMENT

Schwann cells are the myelinating cells of the peripheral nervous system

They are derived from the neural crest during embryonic development (Figure 31-1). The neural crest is the source of numerous peripheral neural and non-neural cell types, and the production of Schwann cells involves specification of a subset of these cells to the Schwann cell lineage as Schwann cell progenitor cells. Specification requires expression of the transcription factor Sox10, and Sox10 activates other transcription factors, including Oct6/Scip and Brn2 as this lineage differentiates. These progenitor cells initially develop into immature Schwann cells, which eventually differentiate into myelinating or nonmyelinating Schwann cells. These different cell types are distinguished by specific marker expression. Thus, Schwann cell progenitors express brain fatty acid binding protein (BFABP) and desert hedgehog (DHH) along with low levels of several myelin markers, such as protein zero (P0), myelin proteolipid protein (PLP), and peripheral myelin protein 22 (PMP22). As Schwann cell progenitors differentiate into immature Schwann cells, they increase expression of glial fibrillary acidic protein (GFAP) and S100β, and decrease expression of N-cadherin, cadherin-19 and AP2α, and as they mature further to myelinating Schwann cells, they increase expression of myelin proteins such as P0 or PMP22 (Jessen & MIrsky 2005).

Schwann cell lineage differentiation is regulated by a series of transcription factors

Oct6/Scip and BRN2, in combination with Sox10, direct the differentiation program of Schwann cells through Krox20/Egr2, which is considered the master regulator driving Schwann cell myelination, increasing expression of numerous myelin genes, including P0, PMP22 and myelin basic protein (MBP).

As immature Schwann cells interact with axons during development, they initially encompass a group of clustered axons (Gamble & Breathnach 1965). As both axons and Schwann cells enlarge and mature, the Schwann cell divides and one daughter cell surrounds multiple axons, while the other daughter cell mediates radial sorting of axons, in which it associates with a single large-diameter axon that it will eventually myelinate (Webster, 1984) (Figure 31-2). Over time, groups of unmyelinated axons, called Remak bundles, continue to be surrounded by a non-myelinating Schwann cells, while myelinated axons maintain a one-to-one relationship, with a single Schwann cells myelinating a single axon. The nonmyelinating Schwann cells maintain expression of p75 NGF receptor and GFAP, while myelinating Schwann cells cease p75 NGF receptor and GFAP expression as they increase myelin gene expression.

OLIGODENDROCYTE DEVELOPMENT

Oligodendrocytes are the myelinating cells of the CNS

Oligodendrocytes and their progenitor cells are widely distributed throughout the adult CNS, but in general they arise as progenitor cells from localized ventral neuroepithelial cells during midneurogenesis. They subsequently differentiate through a series of well-characterized developmental stages.

Much early work was possible because of *in vitro* analysis of the oligodendrocyte cell lineage

This field has moved forward in part because of unique characteristics of the oligodendrocyte grown in tissue culture. Much of its developmental program is carried out by purified oligodendrocyte progenitor cells in culture in the absence of neurons, and investigators have studied relatively pure oligodendrocytes in culture since 1980 (McCarthy & de Vellis 1980). As a consequence, the initial stages of differentiation of this lineage have been effectively analyzed and a series of valuable markers for each stage have been identified (Pfeiffer, 1984). Until recently, the cultured cells have allowed investigation of the regulation of oligodendrocyte differentiation more effectively than *in vivo* investigations.

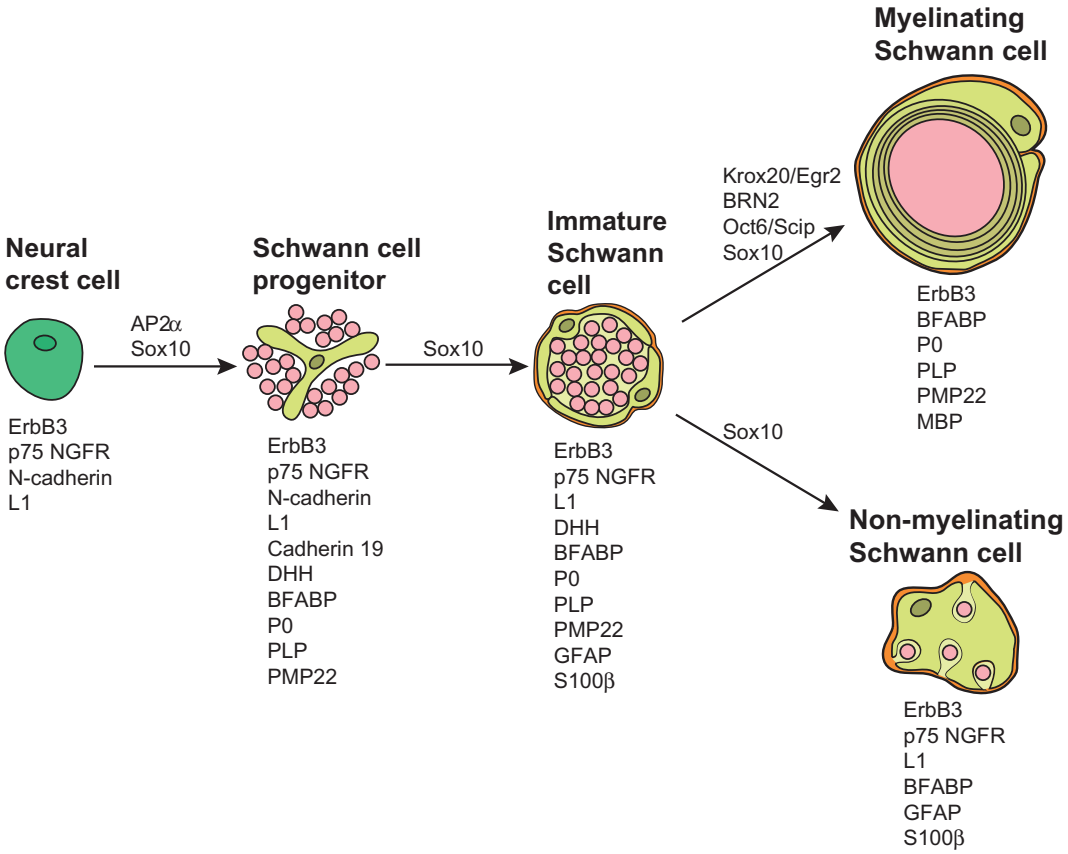

FIGURE 31-1 **Development of the Schwann cell lineage.** The initial neural crest cell expresses ErbB3, the p75 NGF receptor, L1 and N-cadherin. This cell develops into the Schwann cell progenitor when it begins to express AP2α and Sox10. The Schwann cell progenitor cell continues to express several neural crest markers and starts expressing cadherin 19, desert hedgehog (DHH), brain fatty acid–binding protein (BFABP), and low levels of P0, PLP and PMP22. This cell then matures to an immature Schwann cell that has lost expression of N-cadherin and cadherin 19 and increases expression of GFAP and S100β. Increased expression of the transcription factors Krox20/Egr2, BRN2 and Oct6/Scip lead to myelination by the myelinating Schwann cell. These cells have lost GFAP and S100β expression and they increased myelin protein expression. In Schwann cells that become nonmyelinating Schwann cells, myelin gene expression ceases and GFAP and S100 remain. This figure is based on figures and data from multiple studies, in particular (Jessen & Mirsky 2005).

The oligodendrocyte progenitor cell initially plated from embryonic or neonatal rodent brain expresses A2B5, a polysialoganglioside, on cell surfaces. *In vivo*, A2B5 is expressed on numerous cells, but in cultured glial cells, it is relatively specific to the early oligodendrocyte progenitor cell (Figure 31-3). As this cell differentiates, it loses A2B5 expression and begins expression of NG2 proteoglycan and platelet-derived growth factor receptor alpha (PDGFRα). The late oligodendrocyte progenitor cell additionally expresses O4, which is a mixed antigen of sulfatide and other surface antigens on oligodendrocytes and in myelin (Bansal et al.,1989). PDGFRα and NG2 are downregulated as the late progenitor cell becomes an immature oligodendrocyte, but these cells continue to express O4 antigen throughout their further development. They begin to express O1 antigen, which is galactosylcerebroside, a glycolipid that is found in myelin. As they become mature oligodendrocytes, they begin to express high levels of MBP, PLP and other proteins that are abundant in myelin.

The oligodendrocyte progenitor cell is highly responsive to PDGF, which induces proliferation. It also proliferates in response to fibroblast growth factor (FGF), although these cells express several different FGF receptors, which can induce different responses. As they mature, they begin to differentiate in particular in response to thyroid hormone, although other molecules also induce their differentiation, including some of the FGFs or ciliary neurotrophic factor (CNTF). Insulin-like growth factor (IGF) acts both as a survival and mitogenic growth factor for oligodendrocyte progenitor cells and as a differentiation factor.

The discovery of several transcription factors that are expressed at early stages of oligodendrocyte specification and differentiation has helped to identify these cells during development *in vivo*

Olig1, Olig2, Nkx2.2 and Sox10 are early markers *in vivo* the oliogodendrocyte lineage (Woodruff et al., 2001) (Figure 31-3), although some of these early markers are expressed transiently in progenitor populations that will generate neurons. Olig1 and Olig2 are required for oligodendrocyte

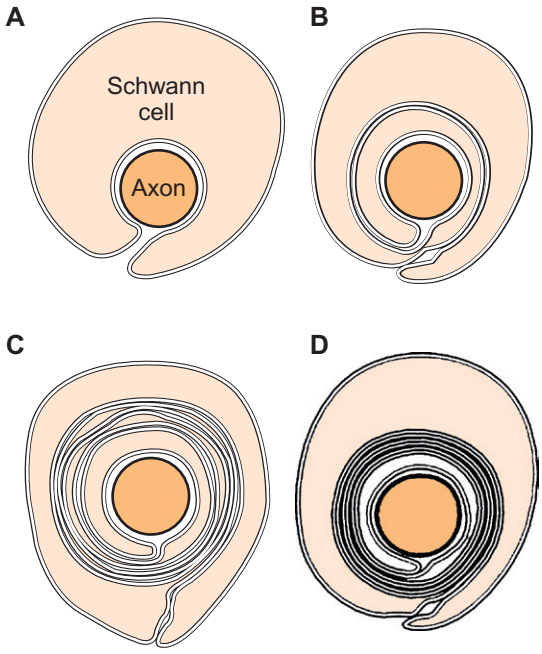

FIGURE 31-2 **Myelin formation in the peripheral nervous system. (A)** The Schwann cell has surrounded the axon but the external surfaces of the plasma membrane have not yet fused in the mesaxon. **(B)** The mesaxon has fused into a five-layered structure and spiraled once around the axon. **(C)** A few layers of myelin have formed but are not completely compacted. Note the cytoplasm trapped in zones where the cytoplasmic membrane surfaces have not yet fused. **(D)** Compact myelin showing only a few layers for the sake of clarity. Note that Schwann cell cytoplasm forms a ring both inside and outside of the sheath. (Adapted with permission from Norton, W. T. The myelin sheath. In E. S. Goldensohn and S. H. Appel (eds), Scientific Approaches to Clinical Neurology. Philadelphia: Lea & Febiger, 1977, pp. 259–298.)

specification. Olig1, Olig2 and Sox10 remain expressed throughout the oligodendrocyte lineage to mature cells. As in culture, as neuroepithelial cells *in vivo* develop into oligodendrocyte progenitor cells, they begin to express PDGFRα and the NG2 proteoglycan. These markers are used to identify the oligodendrocyte progenitor cell throughout development and in the adult. Their expression is reduced as the cells start to differentiate into myelin-producing cells, which then express high levels of myelin markers such as MBP, PLP and 2′,3′ cyclic nucleotide 3′phosphohydrolase (CNP). PLP and CNP are not exclusively markers of mature cells, since low levels of PLP and CNP are also expressed by oligodendrocyte progenitor cells.

In order to understand the *in vivo* stages and markers of oligodendrocyte differentiation and myelination, two major oligodendrocyte developmental systems have been investigated most extensively: oligodendrocyte development in the spinal cord and in the developing telencephalon (Woodruff et al., 2001). In both systems oligodendrocytes are primarily derived from ventral domains, although a relatively small population can arise independently in dorsal domains. Spinal cord development starts with domain organization within the cord, which results from localized responses to gradients of the patterning molecule sonic hedgehog secreted from the notochord and floor plate and bone morphogenetic proteins secreted from the dorsal spinal cord. Oligodendrocytes are specified in the motor neuron precursor (pMN) domain, which is a ventral domain of the developing spinal cord that initially generates motor neuron progenitors that also express Olig1/Olig2. Motor neuron progenitors cease Olig gene expression, and subsequent progenitors within this domain become oligodendrocyte progenitor cells, which migrate throughout the developing spinal cord.

Similarly, in the developing embryonic telencephalon, the cells destined to become oligodendrocytes appear initially in the ventral forebrain, the anterior hypothalamic region and the medial ganglionic eminence. These progenitor cells migrate tangentially up from the ganglionic eminence into the dorsal telencephalon, where this population is initially a progenitor cell for the GABA-ergic interneurons. These cells subsequently become the oligodendrocyte progenitor cell population that proliferates and populates the cortical region. Many differentiate into premyelinating cells and eventually into myelinating oligodendrocytes (Figure 31-4), but PDGFRα/NG2-positive cells remain in the adult central nervous system as oligodendrocyte progenitor cells, and they are the most proliferative cell population in the non-injured adult brain.

A number of transcriptional and epigenetic regulators control oligodendrocyte progenitor cell differentiation into premyelinating and myelinating cells

A number of transcriptional and epigenetic regulators control oligodendrocyte progenitor cell differentiation into premyelinating and myelinating cells (Yu et al., 2010). These include both negative regulators, such as the Notch pathway, the Wnt/β–catenin pathway and the BMP pathway, and positive regulators such as the Olig and some of the Sox family of transcription factors. Early progenitors express Sox 5, Sox 6, Sox9, Hes5, Id2, Id4 and E2A, which maintain these cells as progenitor cells, but these are downregulated as other transcriptional regulators such as Sox 10 and Nkx2.2 increase in expression. Olig1, Olig2 and Sox10 remain important transcriptional regulators throughout this lineage, although Olig1 becomes localized in the cytoplasm as oligodendrocytes mature (Arnett et al., 2004). Tcf4 increases in expression as oligodendrocyte progenitor cells differentiate into premyelinating oligodendrocytes, and along with Sox10, Olig1 and Nkx2.2, it appears essential for that differentiation step. YY1, Myt1, ZFP191 and MRF are transcription factors that play roles as oligodendrocytes mature, and deletion of MRF or ZFP191 in this lineage blocks cells at the premyelinating oligodendrocyte stage *in vivo* (Emery, 2010).

Epigenetic pathways that regulate oligodendrocyte differentiation are being actively investigated. In particular, histone acetylation and deacetylation play important roles, and histone deacetylases (HDACs) are required for early oligodendrocyte differentiation. HDACs act both by directly deacetylating DNA and by acting as co-repressors upon binding to transcriptional regulators. Myelin gene expression inhibitors such as Hes5, a mediator of Notch signaling, or β–catenin, a mediator of Wnt signaling, are downregulated by

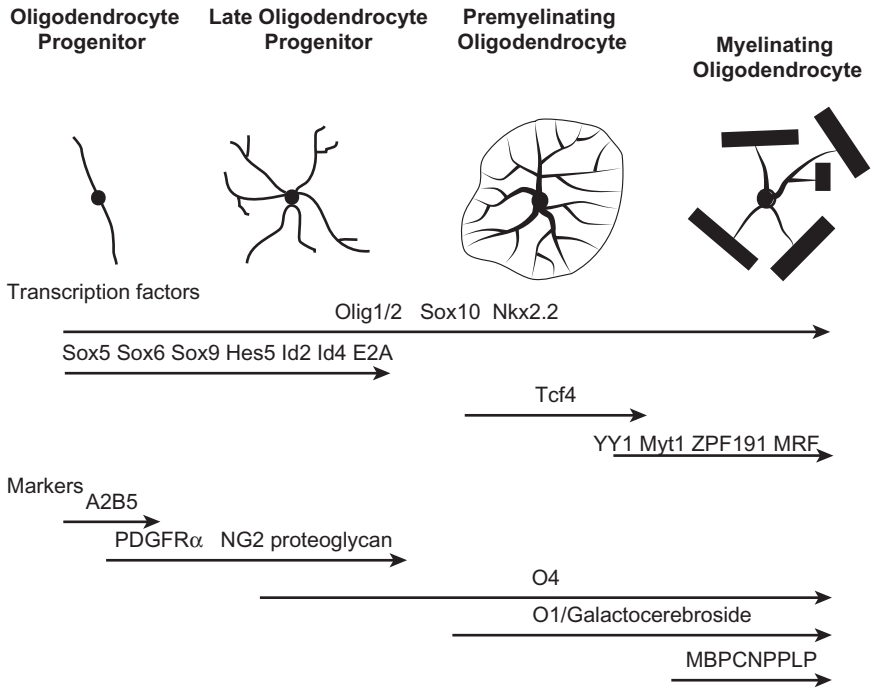

FIGURE 31-3 **Development of the oligodendrocyte lineage.** The bipolar oligodendrocyte progenitor cell expresses the Olig1/2, Sox10 and Nkx2.2 transcription factors indicative of this lineage and early inhibitory transcription factors that maintain its progenitor state as it migrates through the nervous system. It expresses PDGF receptor alpha and NG2 proteoglycan through its early development to the late progenitor stage, which starts to express early myelin antigens such as O4. Inhibitory transcription factors are downregulated as the Tcf4 transcription factor and the myelin antigen O1 start to be expressed. Tcf4 is then downregulated and several other transcription factors are expressed that are essential for the myelinating phenotype. At this stage, high levels of myelin proteins are expressed.

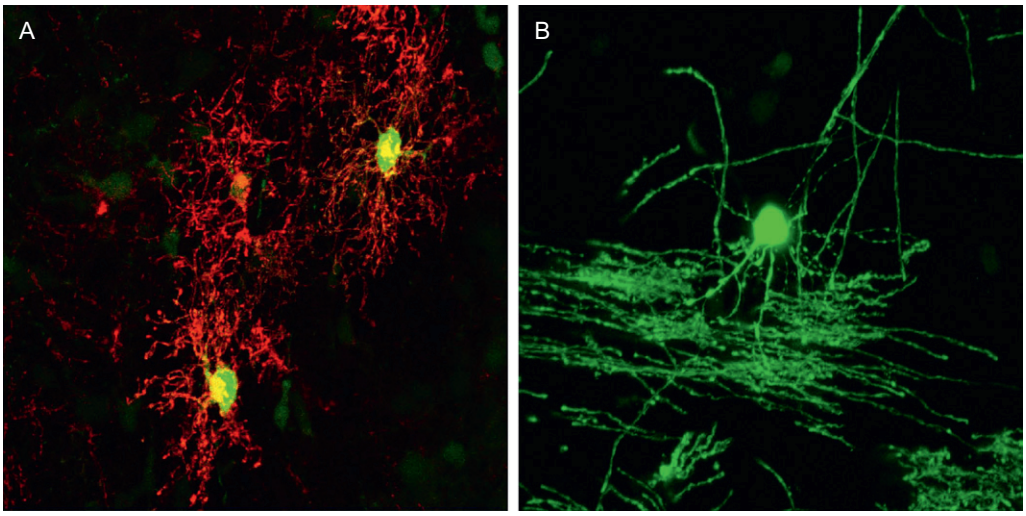

FIGURE 31-4 **Premyelinating and myelinating oligodendrocytes *in vivo*.** Representative images of **(A)** premyelinating oligodendrocytes from P6 mouse cortex, labeled with EGFP (in *Plp*-EGFP mice (Mallon et al., 2002) and PLP (Texas Red) or **(B)** a myelinating oligodendrocyte from mature mouse striatum labeled with EGFP (in *Plp*-EGFP mice). (Figure 4B reproduced from Mallon et al., 2002).

HDACs to reduce their inhibition of oligodendrocyte development (Yu et al., 2010).

Other epigenetic modulators such as microRNAs (miRNAs) also impact oligodendrocyte differentiation. miRNAs are small (~21–24 nucleotides) RNAs that inhibit protein expression after binding to the 3′-untranslated region (3′-UTR) of numerous mRNAs. Hybridization of these miRNAs to mRNA blocks protein expression either by inhibiting protein translation or by increasing turnover of the encoding mRNA. After initial processing in the nucleus, mature miRNAs are generated

from double-stranded RNA precursors in the cytoplasm by the ribonuclease III enzyme, Dicer. When Dicer was conditionally deleted from the oligodendrocyte lineage, cells could not progress beyond the progenitor stage, and upon analysis, the predominant microRNAs lost in these cells were miR219 and miR-338. These two miRNAs, and likely others, appear to repress negative regulators of oligodendrocyte differentiation and/or positive regulators of the progenitor state, and in their absence, cells cannot move beyond the progenitor stage (Dugas et al., 2010; Zhao et al., 2010).

REGULATION OF MYELINATION

Extensive recent research has focused on identifying the axonal signals that regulate myelination

It is well established that axon diameter regulates the thickness of myelin (see Chapter 10), but in addition axonal signaling and electrical activity have been implicated in cell–cell contact and the regulation of myelination. Recent studies suggest interaction of nectin-like (Necl) proteins Necl-1 and Necl-4 on axons and on the axonal membrane of Schwann cells, respectively, mediate the interaction of PNS axons with Schwann cells (Perlin & Talbot 2007). The best-characterized axonal signal regulating myelination is neuregulin (NRG) 1 type III expressed on the surface of PNS axons, which signals through ErbB2/3 receptors on Schwann cells. Neuregulins control survival and proliferation of early Schwann cell progenitors, but NRG1 on axonal surfaces regulates the myelination process. The absence of NRG1 on PNS axons leads to hypomyelination, and overexpression produces hypermyelination. Thus, the thickness of PNS myelin appears regulated by the amount of NRG1 type III on the surface of PNS axons. Additionally, ectopic expression of NRG1 on axons typically associated with nonmyelinating Schwann cells leads to fate switches of the nonmyelinating Schwann cells to myelinating Schwann cells (Taveggia et al., 2005). Thus, NRG1 on the surface of peripheral axons appears to be one of the strongest regulators of Schwann cell fate and myelination identified to date.

Recent studies suggest that the NRG1/ErbB receptor signaling pathway or other surface receptor signaling regulating myelination act though the phosphatidyl inositol 3′ kinase (PI3kinase)/Akt pathways to mTOR (mammalian target of rapamycin). The impact of the PI3Kinase/Akt pathway has been shown in both PNS and CNS myelination, where overexpression of dominant negative PTEN (phosphatase and tensin homolog deleted on chromosome 10, a negative regulator of Akt) (Goebbels et al., 2010) or constitutively active Akt (Flores et al., 2008), respectively, lead to hypermyelination. Akt is itself downregulated by PTEN and, at least in the PNS, it appears that myelin thickness is regulated by differential levels of activated Akt and PTEN. Thus, a novel pathway involving PTEN has been shown to act as the brake for myelination in the PNS. In addition to Akt activation, Dlg1 (mammalian disks large homolog 1) expression in Schwann cells is also mediated by NRG1/ErbB signaling (Figure 31-5). Dlg1 directly interacts with PTEN to stabilize it and reduce its degradation. Thus, NRG1/ErbB signaling acts to increase both Akt signaling and Dlg1 expression. As the Dlg1 increasingly stabilizes PTEN, Akt is increasingly inactivated, reducing mTOR signaling and ending active myelination (Cotter et al., 2010).

NRG1/ErbB signaling increases PNS myelination in a PI3K/Akt independent manner as well. Thus, ErbB2/3 signaling additionally activates phospholipase C, thereby increasing intracellular calcium levels. This activates calcineurin, leading to activation of NFATc3 and NFATc4 (nuclear factor of activated T cells) and nuclear translocation of NFATc4. NFATc4 complexes with SOX10, increasing Krox20/Egr2 transcription, which, as noted above, is considered the master regulator of myelination (Kao et al., 2009).

In addition to ErbB signaling to Akt/mTOR and calcineurin, the NRG1/ErbB pathway also signals to the mitogen-activated protein kinase (MAP kinase) pathway, specifically to extracellular signal-regulated kinase 1 and 2 (Erk1/2). NRG1 has multiple effects on the Schwann cell lineage, and loss of Erk1 and Erk2 in the Schwann cell lineage leads either to loss of Schwann cells or to the inability of Schwann cells to differentiate and myelinate, depending on the timing of Erk2 deletion. At very early developmental stages, the primary impact of NRG1/ErbB signaling is on survival of the lineage. Thus, deletion of Erk1/2 in very early Schwann cell–lineage cells eliminates that survival signaling of NRG1 for early Schwann cell progenitors, leading to significant loss of cells in this lineage. With respect to signaling regulating myelination, deletion of Erk1/2 at the Schwann cell progenitor stage causes severe hypomyelination with apparently normal numbers of Schwann cells, although whether this is related to ERK1/2 signaling driven by NRG1/ErbB/ signaling was not tested (Newbern et al., 2011).

The impact of NRG1 on myelination in the CNS is less clear. There are regional domains that respond to the concentration of NRG1 on axons (Taveggia et al., 2008), but in general CNS myelination appears not to be regulated by NRG1 (Brinkmann et al., 2008). Additionally, while NRG1/ErbB signaling apparently mediates both active myelination and the end of myelination in Schwann cells, no single pathway has been identified yet in oligodendrocytes to control CNS myelination.

While the axonal or environmental signals regulating CNS myelination are not well defined at this point, the intracellular signaling pathways are becoming known. The Akt/PI3Kinase pathway leading to mTOR activation is a major driver of myelination in the CNS (Flores et al., 2008; Narayanan et al., 2009) as it is in the PNS (Goebbels et al., 2010). mTOR activation also regulates differentiation of oligodendrocyte progenitors into an immature oligodendrocyte *in vitro* (Tyler et al., 2009). New data suggest that the Erk2 pathway may regulate the timing of oligodendrocyte differentiation *in vivo* (Fyffe-Maricich et al., 2011), although deletion of both Erk1 and Erk2 primarily impacts oligodendrocyte progenitor cell proliferation, rather than myelination (Newbern et al., 2011). These investigations are ongoing and new pathways will likely be identified as well.

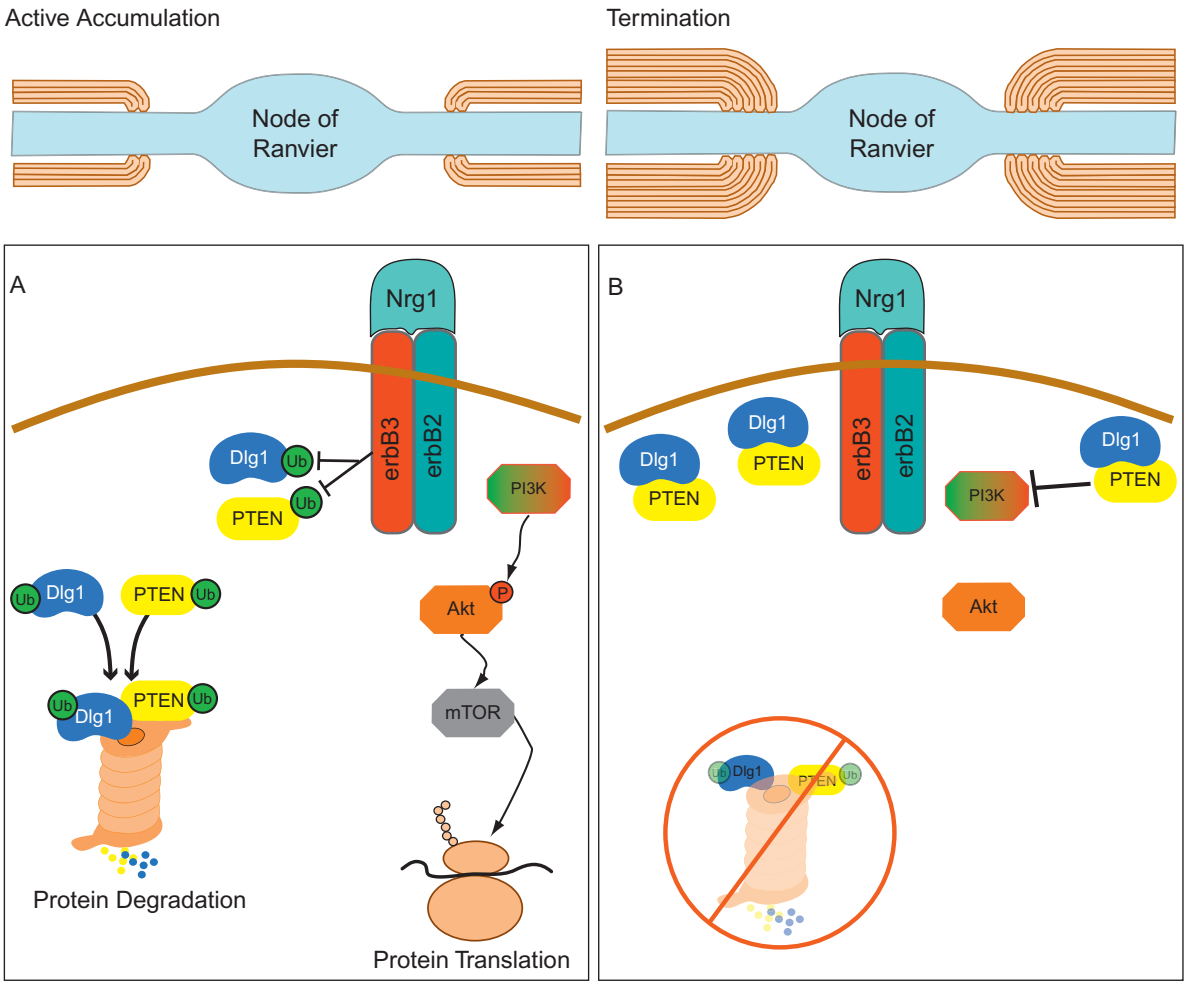

FIGURE 31-5 **NRG/ErbB signaling that regulates both active myelination and termination of myelination in Schwann cells. (A)** During the active phase of myelination of peripheral nerves, axonal expression of neuregulin-1 activates erbB2 and erbB3 receptors on Schwann cells and signals through the PI3K-Akt pathway to initiate myelin production. Neuregulin also induces an increase in Dlg1 levels, presumably by preventing its ubiquitination and subsequent degradation. **(B)** As non-ubiquitinated Dlg1 accumulates, it stabilizes PTEN, reducing its proteosomal degradation, and the number of Dlg1-PTEN complexes increases. This reduces Akt activity and terminates active myelination. (Figure and figure legend from Macklin, 2010.)

DEVELOPMENTAL AND METABOLIC ASPECTS OF MYELIN

Synthesis of myelin components is very rapid during deposition of myelin

Nervous system development is marked by several overlapping periods, each defined by a major event in brain growth and structural maturation (Morell, 1984). In the rat, whose CNS undergoes considerable development postnatally, the maximal rate of cellular proliferation (much of this involving oligodendroglial progenitor cells) occurs at 10 days. The rat cerebrum begins to form myelin postnatally at about 10 to 12 days. The maximal rate of accumulation of myelin in the rat occurs at about 20 days of age, although accumulation continues at a decreasing rate throughout adulthood. A remarkable amount of membrane biogenesis occurs in oligodendrocytes during the period of maximum myelination. Myelin accumulates in 20-day-old rat brain at a rate of about 3.5 mg/day. Rough calculations based on the number of oligodendrocytes and the amount of myelin deposited indicate that, on average, the amount of myelin membrane made by each cell per day is more than three times the weight of its own perikaryon. This very rapid myelin synthesis early in development has been demonstrated biochemically by the very rapid incorporation of radioactive precursors into myelin and substantial increases of enzymes involved in synthesizing myelin components.

Sorting and transport of lipids and proteins takes place during myelin assembly

After myelin components have been synthesized, they must be assembled to form the membranes making up myelin sheaths (Trapp & Kidd 2004). The biogenesis of these sheaths

is an extraordinary process of membrane formation and modeling. In the CNS, this requires the spiraling of numerous oligodendroglial processes around axons and their tight layering to form compact myelin. Furthermore, there is additional modeling of specialized membrane domains with different composition at the inside and outside of the sheaths and in the paranodal glia–axon junctions (Chapter 10). The two major proteins of compact CNS myelin, PLP and MBP, enter the myelin by different routes. PLP is synthesized on membrane-bound polysomes in the perikaryon and transported in membranous vesicles to the myelin being formed at the end of the oligodendroglial processes. By contrast, MBP is synthesized on free polysomes, which are actually located in very close proximity to the newly forming myelin at the end of oligodendroglial processes (Trapp et al., 2004). Its mRNA is transported from the perikaryon to the vicinity of myelin formation in ribonucleoprotein granules by a microtubule-based translocation system. These differences in the route of entry are reflected in different kinetics of incorporation of proteins into myelin membranes in experiments involving labeling with radioactive amino acids after intracranial injection or incubation of brain slices. Radioactive MBP is synthesized and integrated into myelin very rapidly with a lag time of only a few minutes, whereas substantial amounts of radioactive PLP do not appear in myelin until after about 45 minutes (Morell, 1984). Other proteins that are selectively localized in specialized regions of the myelin sheath such as the inner and outer surfaces (e.g., MAG and MOG, respectively) or in the paranodal regions (e.g., neurofascin-155) must be sorted and transported by different mechanisms involving specific sorting signals. It is likely that the sorting mechanisms are related to the apical and basolateral targeting that occurs in simple polarized epithelial cells. Using the model system of transfected Madin-Darby canine kidney (MDCK) epithelial cells, it was shown that MOG sorts exclusively to MDCK basolateral membranes, whereas PLP sorts exclusively to MDCK apical membrane (Kroepfl & Gardinier 2001). However, sorting in myelin-forming cells probably also involves more complicated mechanisms because of the complex variety of membrane domains in myelin sheaths.

The production of myelin lipids is a major biosynthetic aspect of myelination. Myelin is an extremely cholesterol-rich membrane, and most of the cholesterol required for myelination is synthesized locally (Jurevics & Morell 1995). Some of the lipids and proteins in myelin forming cells are associated with raft-like domains, which are enriched in cholesterol, glycosphingolipids and glycosylphosphatidylinositol-linked proteins (Taylor et al., 2004). These rafts are likely to play an important role in the trafficking of membrane components and signal transduction mechanisms involved in the assembly of myelin sheaths. Much research designed to elucidate these phenomena and other aspects of myelin assembly is ongoing. However, a more detailed description of this research is beyond the scope of this chapter, and the reader is referred to more comprehensive references (Taylor et al., 2004; Trapp et al., 2004).

The composition of myelin changes during development

The composition of myelin isolated from immature, rapidly developing brain is different from that of the adult (Morell, 1984). As the rat brain matures, the content of galactolipids, MBP and PLP in purified myelin increases, whereas phosphatidylcholine and high molecular weight proteins decrease. These studies on the composition of myelin from immature brains are consistent with the concept that myelin first laid down by oligodendrocytes may represent a transitional form with properties intermediate between those of mature compact myelin and the oligodendroglial plasma membrane. However, interpretation of biochemical studies on purified myelin is complicated by the fact that myelin fractions, isolated by conventional procedures, can be separated into subfractions of different densities. The lighter fractions are enriched in multilamellar myelin, whereas the denser fractions contain a large proportion of single-membrane vesicles that morphologically resemble microsomes or plasma membrane fragments. In general, as one goes from light myelin subfractions to heavier ones, the lipid/protein ratio and the amount of MBP decrease, the amount of PLP decreases or remains relatively constant, and the amounts of MAG, CNP and other high-molecular-weight proteins increase. The interpretation of these findings is that the light subfractions contain primarily compact myelin, while the heavier fractions are enriched in other oligodendroglially derived membranes from the cell processes, inner and outer surfaces of the sheaths, and paranodal loops. Therefore, the differing lipid and protein composition of isolated immature myelin may reflect either transitional forms of developing myelin or a greater content of associated oligodendroglial membranes relative to compact myelin recovered from the thinner immature myelin sheaths, or a combination of these factors. Nevertheless, metabolic studies with radioactive precursors lend support to the view that the heavier fractions isolated from developing brain represent at least in part transitional membranes in the process of conversion to compact myelin. For example, PLP appears first in the heavier fractions and later in lighter fractions in a manner that suggests a precursor-product relationship.

GENETIC DISORDERS OF MYELINATION

Rodent mutants of myelination have been investigated since the 1950s

Rodent mutants of myelination have been investigated since the 1950s. Until recently, these were all spontaneous mutations that were identified in animal colonies. Starting in the 1980s, many of the genes that were mutated in these rodents were cloned and identified with the respective mutants (Table 31-1). Several major myelin proteins were among these spontaneous mutants, including the *shiverer* mouse, which has a deletion in the *Mbp* gene; the mld mouse, which has an inversion in the *Mbp* gene; or mutations in the *Plp* gene in the *jimpy*, msd and *rumpshaker* mice, the MD rat, the shaking pup and other species. Mutations in the less-abundant proteins of myelin have also highlighted the importance of a number of proteins, such as QK1 primarily in the CNS, PMP22 in PNS myelin, or connexins, which encode gap junctional proteins that are found in both CNS and PNS myelin. These proteins are not abundant, but their appropriate expression is essential for generating and maintaining normal

TABLE 31-1 Some Spontaneously Occurring Animal Mutants Affecting Myelin

Names of mutants	Inheritance*	Affected gene	Comments
Jimpy mouse, *rumpshaker* mouse, myelin-deficient (md) rat, *shaking* dog	X-linked	proteolipid protein (PLP)	Variable degrees of oligodendrocyte death and CNS myelin deficiency; decreased spacing at intraperiod line of compact CNS myelin
Shiverer mouse, myelin-deficient mouse	AR	myelin basic protein (MBP)	Deletion or inversion of several MBP exons, very little functional MBP expressed; severe CNS hypomyelination and failure of compaction of major dense line
Trembler mouse (PMP-22)	AD	peripheral myelin protein-22 (PMP-22)	Hypomyelination specific for the PNS caused by point mutations in transmembrane domains
Quaking mouse	AR	QKI family of proteins (QKI5, QKI6, QKI7 expressed in oligodendrocytes)	Hypomyelination more severe in CNS than PNS; abnormal expression of RNA–binding proteins is likely to interfere with normal splicing or transport of mRNAs for myelin proteins
Taiep rat (acronym: trembling, ataxia, immobility, epilepsy, paralysis)	AR	unknown	Impaired myelin formation followed by demyelination in the CNS, accumulation of microtubules in oligodendrocytes interferes with transport of myelin proteins or mRNAs

References: Hudson, 2004; Campagnoni & Skoff, 2001; Baumann & Pham-Dinh, 2001; Nave & Griffiths, 2004; Wrabetz & Feltri, 2004; Hardy, 2004; Song et al., 2003.
*AD, autosomal dominant; AR, autosomal recessive

myelin. Several of these mutants are particularly important because they represent human diseases. For example, the different *Plp* mutants have been informative about the pathology underlying Pelizaeus-Merzbacher disease, a severe form of developmental delay in boys (Chapter 39). Some of these *Plp* mutations are point mutations that lead to misfolded protein, and much of their pathology results from the induction of the unfolded protein response in cells. The oligodendrocyte is particularly vulnerable to the unfolded protein response.

More recently a number of induced mutations have been generated, knocking out specific myelin genes either in totally null animals or in cell-specific deletion studies. These mutants allow investigators to study the function of many different genes. It is of interest that *Plp* knockout mice have a totally different phenotype than point mutations or gene dosage mutants. There is no unfolded protein response in these mice. Rather, myelin is produced that has generally normal appearance, but as discussed below, the myelin generated in this model cannot sustain axonal function and there is eventual axonal degeneration. Thus, how a specific gene is mutated can dramatically impact the phenotype.

MYELIN MAINTENANCE

Maintenance of myelin once it is formed is a poorly understood process

As noted below, there is clearly turnover of myelin proteins and lipids, but how this is regulated has not been extensively investigated. One model that has provided important insight is the Taiep rat (named for its phenotype of tremor/ataxia/immobility/epilepsy/paralysis) (Duncan et al., 1992) (Table 31-1).This animal generates relatively normal myelin, but there is an increasing accumulation of microtubules in oligodendrocytes as the animal ages, and this has been proposed to disrupt transport of myelin proteins or mRNAs to the myelin membrane. The microtubule accumulation appears early and leads to reduced numbers of myelinated axons during the developmental stage and thinner myelin. The myelin defect advances further with age, and at 12 months of age many areas of the nervous system have few myelinated axons. This leads eventually to axonal degeneration. The impact of the cytoskeleton on myelination and on myelin maintenance is an active area of investigation.

Myelin components exhibit great heterogeneity of metabolic turnover

A novel characteristic of myelin is that its overall rate of metabolic turnover is substantially slower than that of other neural membranes (Morell, 1984). This was shown in early biochemical studies that entailed injecting rat brains with a radioactive metabolic precursor and then quantifying loss of radioactivity from individual components as a function of time. Structural lipid components of myelin, notably cholesterol, cerebroside and sulfatide, as well as proteins of compact myelin, are relatively stable, with half-lives on the order of many months. One complication in interpreting such studies is that the metabolic turnover of individual myelin components is multiphasic, consisting of an initial rapid loss of radioactivity followed by a much longer slower loss. For example, initially MBP and PLP exhibit half-lives of two to three weeks, but later, their half-lives are too long to be calculated accurately. A possible interpretation of these data is that some of the newly formed myelin remains in outer layers or near cytoplasmic pockets (incisures and lateral loops) where it is accessible for catabolism—thus accounting for the rapid turnover of the pool. The more stable metabolic pool would consist of deeper layers of myelin less accessible for metabolic turnover.

There are signal transduction systems in myelin sheaths

There are signal transduction systems in myelin sheaths (Taylor et al., 2004; Trapp et al., 2004). Therefore, in contrast to

the relatively slow rate of overall metabolism, some aspects of myelin metabolism involve rapid events with half-lives on the order of minutes. For example, the monoesterified phosphate groups of polyphosphatidylinositol (those at positions 4 and 5) are labeled very quickly even in mature animals, and this presumably is related to the function of phosphoinositides in signal transduction (Chapter 23). Additionally, the phosphate groups on MBP turn over rapidly (Eichberg & Iyer 1996). Although the representation of myelin structure in Figure 31-2 is static, studies that demonstrate relatively rapid metabolism of certain myelin components suggest there may be some dynamic aspect of myelin structure, such as occasional separation of the cytoplasmic faces of the membranes.

The dynamic nature of myelin sheaths likely contributes to the functional state of axons

Numerous enzymes and neurotransmitter receptors are found in myelin (Chapter 10), and glutamate receptors in particular have been of interest, given the impact of excitotoxicity in neurodegenerative conditions. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and N-methyl-d-aspartate (NMDA) receptors are all found in myelin, but their function is unclear. Activation of these receptors in oligodendrocytes in culture can lead to oligodendrocyte cell death. Further, there is evidence that they impact myelin function in ischemia. Thus, ischemic conditions induce axonal damage and glutamate receptor antagonists are quite protective for axonal action potentials and axonal survival. However, whether the glutamate-induced damage results from AMPA or NMDA receptor activities is still under investigation.

Peripheral neuropathies result from loss of myelin in the peripheral nervous system

Peripheral neuropathies result from loss of myelin in the peripheral nervous system, which can result from problems within the Schwann cell itself or from neuronal/axonal problems impacting the maintenance of myelin. Many inherited peripheral neuropathies result from altered gene dosage or point mutations in myelin-specific genes, such as the P0 gene (*Mpz*), the Pmp22 gene (*Pmp22*) or connexin genes (e.g., connexin 32, Gjb1) (Scherer & Wrabetz 2008). These result in both dysmyelinating (developmental defects in myelination) and demyelinating (loss of myelin in the adult) phenotypes, depending on the mutation (see Chapter 39).

A number of environmental toxins impact myelination during development or myelin maintenance in the adult

Some of these, such as hexacarbon neuropathy, may result in demyelination subsequent to axonal injury. Others result from direct effects of toxins on Schwann cell function. Chemotherapy with drugs such as paclitaxel can induce neuronal dying back or Schwann cell damage, leading to peripheral neuropathies. Schwann cells are particularly susceptible to toxicants during active myelination. Environmental compounds such as tellurium directly inhibit cholesterol biosynthesis, which destabilizes the myelin (Morell & Toews 1996). While myelin gene expression is reduced in response to tellurium, markers of nonmyelinating Schwann cells, such as p75 NGF receptor, increase. The damage from neurotoxicants can be reversed upon removal of the toxin, leading to a reduction in p75 NGF receptor expression and re-expression of cholesterol biosynthetic enzymes and myelin genes. Other toxins, such as cuprizone, impact primarily CNS myelin (see Chapter 39).

Leukodystrophies define a number of genetic disorders that impact CNS myelination (dysmyelination) or myelin maintenance once it is formed (demyelination)

Pelizaeus-Merzbacher disease, resulting from mutations in the myelin PLP gene (*Plp1*), is a dysmyelinating disease, and in animal models of the disease, essentially no myelin is made during development. By contrast, adrenoleukodystrophy is a progressive demyelination in the adult CNS, resulting from peripheral accumulation of very long-chain fatty acids that eventually accumulate also in the brain, resulting in loss of myelin. Other leukodystrophies include metachromatic leukodystrophy and Krabbe's leukodystrophy, which result from mutations in lipid enzymes that catabolize myelin lipids. In these patients, myelin is generated but the inability to break down these lipids leads to increasing myelin pathology with age (Chapter 39).

Adrenoleukodystrophy is one of the peroxisomal disorders (see Chapter 39). Peroxisomes function to break down peroxides generated in a number of oxidative reactions in essentially all cells. Peroxisome function is essential for all cells, but peroxisome loss has a particularly devastating effect in the brain, specifically in white matter (Baes & Aubourg 2009). This is likely because their primary function involves intermediary lipid metabolism, a major element in myelin. The damage can range from severe peroxisome defects that result in neurodevelopmental problems to less severe defects that result in degenerative problems in the adult. Recent studies suggest that peroxisome function is particularly needed for myelin function and for the survival of myelinated axons. Thus, when peroxisome function was selectively eliminated in the oligodendrocyte lineage in transgenic mice, myelin production was relatively normal, as was survival of mature oligodendrocytes. However, as the mice matured, there was increasing axonal pathology, along with, but sometimes followed by, myelin loss.

This is reminiscent of some other genetic mouse models, where specific myelin proteins such as PLP or CNP are eliminated from myelin, yet relatively normal myelin is generated. However, the major pathology associated with these mice is an increasing axonal pathology as they age. These different animal models suggest that active interactions between the myelin and its underlying axons are essential for normal axonal function (Nave, 2010). When that interaction is altered, either by loss of specific myelin proteins or of peroxisome function, the myelin may remain, but its trophic effect is lost and the axons begin to degenerate.

PERIVENTRICULAR LEUKOMALACIA

Wendy B. Macklin

Cerebral white matter damage results in periventricular leukomalacia (PVL), which is a common form of brain injury in preterm infants. PVL is a serious health issue, leading to some form of neurologic and/or cognitive deficit in as many as 90% of surviving preterm infants with a birth weight less than 1500 g (Khwaja et al., 2008). PVL is primarily a white matter disorder, with essentially complete loss of cells in necrotic areas of white matter and loss of premyelinating oligodendrocytes with accompanying astrogliosis and microglial activation in other areas. There is often some neuronal loss, particularly of subplate neurons, which are important for early organization of the developing cortex. PVL primarily results from hypoxia/ischemia in preterm infants, although infection and inflammation also contribute to the damage.

The particular vulnerability of the preterm infant brain to PVL likely results from several developmental features of the fetal brain. The preterm infant brain has significantly lower cerebral blood flow compared to term infants or adults, and this reduced blood flow makes this stage of brain development particularly vulnerable to ischemic damage. Slightly reduced blood flow that might go unnoticed in term infants could have serious consequences for preterm infants. The specific damage induced by cerebral ischemia in the preterm brain likely also results from the presence of two cell types that are abundant in developing white matter at this stage. Microglial cells are found in brain at very early stages of development, but they are particularly abundant in white matter during the last trimester of gestation, the period of greatest risk of PVL. Additionally, in the third trimester of development, human fetuses are beginning to myelinate axons in the forebrain, and during this early stage of myelination, the oligodendrocyte progenitor cell differentiates into the late oligodendrocyte progenitor/premyelinating cell. The premyelinating cell is most abundant in white matter, and it remains in human parietal white matter for as long as three months at the end of gestation before it starts to myelinate axons (Back et al., 2001).

Within the oligodendrocyte lineage, the premyelinating cell is particularly vulnerable to oxidative damage and other insults. In tissue culture studies of rodent cells, late oligodendrocyte progenitor cells are far more vulnerable to oxidative damage than mature oligodendrocyte, in part because they are more sensitive to loss of glutathione. Increased reactive oxygen species can be produced by the oligodendrocyte progenitor cells themselves, leading to their death (Back et al., 1998). In PVL tissue, the premyelinating cell expresses high levels of protein nitration and lipid peroxidation, hallmarks of oxidative damage (Haynes et al., 2003). Thus, ischemic damage during the last trimester of gestation appears to have its greatest impact on premyelinating cells, leading to their death.

As noted above, the abundance of microglia in white matter during the third trimester also contributes to the damage. Microglia are likely to be the major source of the reactive oxygen species, and proinflammatory cytokines released by activated microglia also contribute to the death of premyelinating oligodendrocytes. Microglia would be particularly activated in tissue that is also exposed to infection or other sources of inflammation, which, as noted above, are contributing factors for PVL.

Other sources of damage result from excitotoxicity during ischemia/reperfusion. Premyelinating oligodendrocytes express Ca^{2+}-permeable glutamate receptors and the major brain glutamate transporter EAAT2. During ischemia, extracellular glutamate accumulation, both from neurons and from reversal of the glutamate transporters upon energy depletion, could induce Ca^{2+}-mediated premyelinating cell death.

Therapeutic approaches to reducing the incidence of PVL are coming from careful monitoring of changes in brain blood flow to reduce the likelihood of ischemia and from reducing potential oxidative damage or excitotoxicity, but PVL remains a serious concern for preterm infants.

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REMYELINATION

Much work has focused on the regulation of myelination, which relates clinically to developmental disorders of brain development, but another clinically important research area is remyelination. Much of the data obtained on the regulation of myelination appears to be comparable during remyelination, although unique aspects of remyelination have been identified.

Peripheral nerve regeneration has been studied extensively

In the PNS, remyelination occurs quickly following segmental demyelination, which is the direct loss of myelin but the retention of axons. This contrasts with Wallerian degeneration, which is the loss of myelin secondary to loss of axons. In Wallerian degeneration, recovery of the axons is

required prior to remyelination. In response to either type of injury, Schwann cells in the PNS de-differentiate and turn over myelin proteins. Myelin gene expression is downregulated and gene expression for the p75 NGF receptor and the transcription factor Oct6/Scip rapidly increases. Schwann cells proliferate extensively and begin remyelination once intact axons are accessible. If the demyelination resulted from crush/transection of the nerve, this may take some time, but if it is a toxin-induced demyelination, remyelination will start as soon as the toxin is removed.

Demyelination in the CNS has far more extensive long-term consequences than in the PNS, since a single oligodendrocyte can myelinate 40 or more axons

Demyelination in the CNS can also result from toxin exposure, but the most common human CNS demyelinating disease is multiple sclerosis, which is immune-mediated demyelination and axonal degeneration (Chapter 39). Significant research is focused on identifying remyelination approaches to repair CNS damage in multiple sclerosis and other CNS demyelinating diseases. Two general approaches are under study: repairing damage with transplanted cells or by stimulation of repair by endogenous oligodendrocyte progenitor cells, which are known to remain in and around multiple sclerosis lesions.

A number of exogenous sources of potential remyelinating cells are being studied. Schwann cells from the PNS are known to enter damaged areas of the CNS in spinal cord injury and remyelinate axons. Olfactory ensheathing cells are of significant interest, since they might be obtainable from patients and they are capable of ensheathing CNS axons. Stem cells are under investigation, including cells derived from embryonic stem cells, adult stem cells or induced pluripotent stem cells (iPS cells). Many different experimental conditions are being studied to assess how best to differentiate cells to enhance their ability to remyelinate, but at the same time reduce their tendency to become other cell types.

A further issue complicating exogenous repair is how best to deliver these cells to the lesioned areas. Lesioned tissue likely provides chemotactic signals for migrating cells, but fully differentiated oligodendrocytes do not migrate. Thus, using progenitor cells is optimal for transplantation, in order to enhance migration to appropriate locations, but the cells must receive appropriate signals at the lesion in order to differentiate and myelinate.

The alternative to exogenous sources of remyelinating cells is the stimulation of endogenous oligodendrocyte progenitor cells to remyelinate. Oligodendrocyte progenitor cells remain within multiple sclerosis lesions (Chang et al., 2000) and in animal models of demyelination they are abundantly available. When there is remyelination in the adult, the myelin is significantly thinner than the original myelin sheath. Given the fact that there are progenitor cells in the lesion and far more progenitor cells in the tissue around the lesion, it is important to understand why the cells in the lesions do not remyelinate. Several possibilities exist. They may not be receiving appropriate differentiation signals from axons, or they may be receiving overriding inhibitory signals from the lesion. Alternatively, they may be insufficient in number to be effective at remyelination and the progenitor cells outside the lesion may not respond to migration cues to move to the region of demyelination. These possibilities are under investigation. This is a promising area for drug development, since demyelinating diseases are devastating and, with the abundance of adult oligodendrocyte progenitor cells throughout the brain, repair by these progenitors may be approachable. Without such repair, axons are nonfunctional. They eventually degenerate, leading to the multitude of clinical symptoms in multiple sclerosis and other neurologic diseases.

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