Oligodendrocytes: Myelination and Axonal Support

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Myelinated nerve fibers have evolved to enable fast and efficient transduction of electrical signals in the nervous system. To act as an electric insulator, the myelin sheath is formed as a multilamellar membrane structure by the spiral wrapping and subsequent compaction of the oligodendroglial plasma membrane around central nervous system (CNS) axons. Current evidence indicates that the myelin sheath is more than an inert insulating membrane structure. Oligodendrocytes are metabolically active and functionally connected to the subjacent axon via cytoplasmic-rich myelinic channels for movement of macromolecules to and from the internodal periaxonal space under the myelin sheath. This review summarizes our current understanding of how myelin is generated and also the role of oligodendrocytes in supporting the long-term integrity of myelinated axons.

When Virchow analyzed the fine structure of the brain in the 1850s, he recognized that there were more cells within the "Nervenkitt" than astrocytes, but because of the imperfect staining methods, they remained obscure and were only named the "third element" (reviewed in Somjen 1988; Rosenbluth 1999). It was decades later that Pío del Río-Hortega (1921) applied a staining method involving silver carbonate, thereby shedding new light on the rest of the interstitial cells. These cells were found to contain numerous short processes and were named oligodendroglia and microglia. Defining features of oligodendrocytes were their small cell bodies filled with nuclei containing large amounts of chromatin, and their cellular extensions that lacked fibers but were filled with cy-toplasmic granules (Fig. 1). When the tissue was optimally preserved, the silver impregnation uncovered a tremendous complexity of extensions (Fig. 1). del Río-Hortega was able to distinguish four types of oligodendrocytes (Fig. 1): Type I cells generate many different myelin segments on small diameter axons in diverse orientations; type II cells are similar to type I in size and number, but myelin segments run in parallel to each other; type III oligodendrocytes ensheath fewer axons of larger diameter; and type IV oligodendrocytes have a cell body closely apposed to a single very large axon similar to Schwann cells. From the staining, it became clear that some of the processes run in parallel to axons and appeared to cover the axons with "myelin," a term that was introduced by Virchow already in
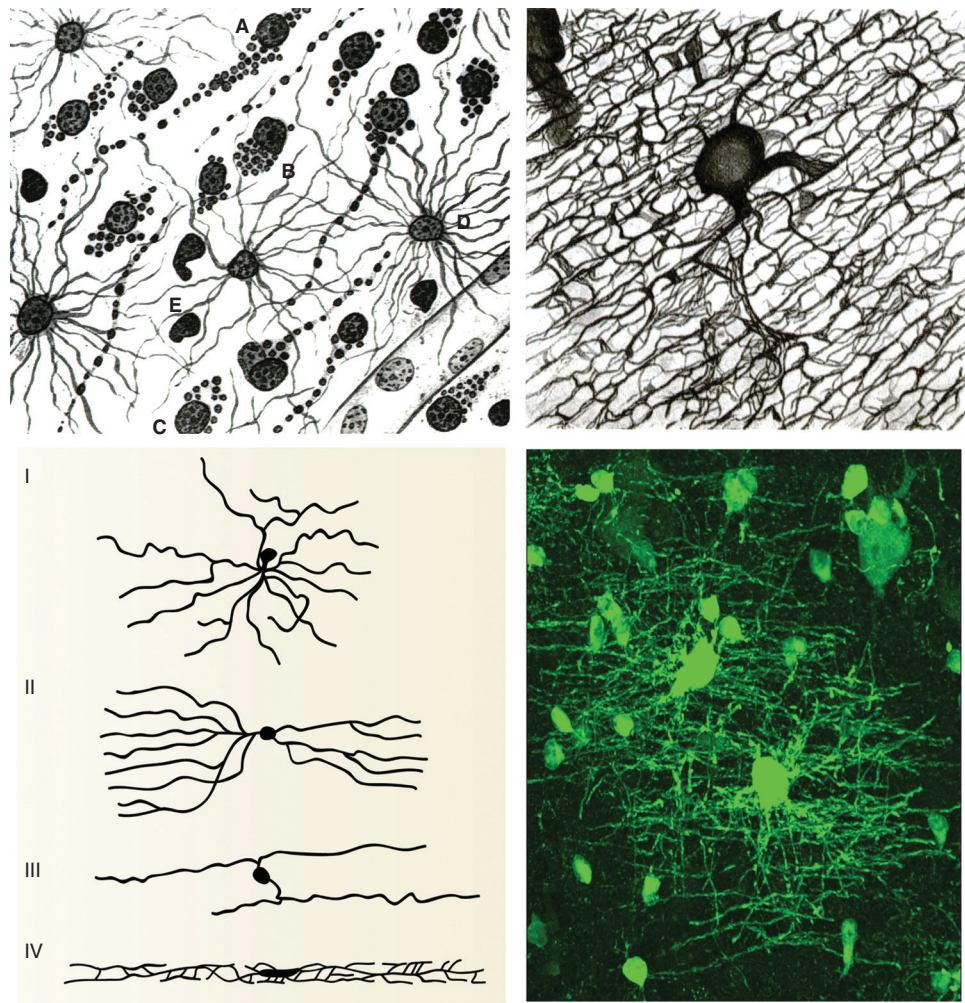
Figure 1. Morphology of oligodendroglia in the cerebellum of a cat. (*Top right*) Cellular processes and branches follow the orientation of the nerve fibers and form complex wraps as shown in del Río-Hortega (1921). (*Top left*) White matter of a newborn human brain: A, Elongated interfascicular cells filled with spherical granules of variable size; B and C, round granular cells; D, astrocytes with long processes; and E, nucleus of a microglia as shown in del Río-Hortega (1921). (*Bottom left*) The four types of oligodendrocytes recognized by del Río-Hortega. (*Bottom right*) Oligodendrocytes expressing proteolipid protein (PLP)−enhanced green fluorescent protein (EGFP) in transgenic mice. Because of its bulky EGFP tag, most of it is found within the cytoplasmic-rich spaces or myelin, including the myelinic channels.

1858, long before its origin and function were known.

Penfield (1924) reinforced the view that the formation of myelin is likely the main role of oligodendrocytes. However, myelination was not considered their only function. Within the gray matter, a fraction of oligodendrocytes were termed "perineuronal" satellite cells, which did not form myelin, but were in close contact with the cell body of neurons, suggesting an interdependent relation. Some oligodendroglia were found in close association with small vessels and were, therefore, subclassified as "perivascular satellites."

The major findings that supported the role of oligodendrocytes in myelin generation were

their high number in white matter tracts, their appearance in development at the time of myelination, and their position close to the myelin sheaths. Curiously, the large number of granules in the cytosol of oligodendrocytes, reminiscent of cells of secretory glands, was also taken as evidence for a function in myelin formation. At that time, myelin was not considered to be an extension of the oligodendroglial plasma membrane, however, but rather a fatty axonal substance secreted into the extracellular space. The importance of the "axon cylinder" for saltatory impulse propagation was well recognized, but it was the seminal work of Betty Ben Geren (1954), using electron microscopy (EM) in the chick peripheral nervous system, which showed that myelin is not axon derived, but rather a continuous membranous extension of Schwann cells. A principally similar relationship, with spiral wrapping of the glial plasma membrane around the axon, was confirmed later for oligodendrocytes in the central nervous system (CNS) (for an excellent early review, see Bunge 1968; Hildebrand et al. 1993). Together, these studies have turned oligodendrocytes and myelin from a putty-amorphous "Nervenkitt" into a fascinating study object of cell biology.

MYELIN STRUCTURE

Myelin became the subject of intense investigations with the refinement of EM, and the application of biochemistry (later, proteomics), when it was found that myelin can be physically purified from most other brain membranes (Norton and Poduslo 1973). Mouse genetics was an early link between these two levels of analysis by providing molecular insight into the myelin architecture. By EM analysis, myelin is a multilayered stack of uniformly thick membranes with a characteristic periodic structure of alternating electron-dense and -light layers, termed the "major dense line" and the "intraperiod line," respectively (Fig. 2). These are adhesion zones and represent the closely apposed cytoplasmic and extracellular myelin membrane bilayers, which appear in repeating patterns of ~12 nm ("periodicity"). Compacted myelin provides the

high electrical resistance and low capacitance that is essential for saltatory impulse propagation. At their edges, myelin lamellae are tightly connected by autotypic tight junctions between the membranes of the outer leaflet, consisting of claudin-11 (Gow et al. 1999; Morita et al. 1999). These interlamellar strands run radially through myelin, forming the so-called "radial components." Most of what we know about myelin ultrastructure is based on EM studies performed on glutaraldehyde-fixed and -dehydrated tissue, often associated with shrinkage and collapse of intracellular spaces. A recent technical advance has been (fixation-free) high-pressure freezing EM with an enhanced preservation of tissue architecture, including the cytoplasmic spaces within myelin (Mobius et al. 2008). These noncompacted regions comprise the outer and inner periaxonal "tongues" (or "lips") of myelin membranes, the "paranodal loops," and the "Schmidt–Lanterman incisures" (the latter in the peripheral nervous system). Cytoplasmic channels, reminiscent of Schmidt–Lanterman incisures, can also be found in developing myelin sheaths of the CNS, but largely disappear when myelination is completed (Snaidero et al. 2014). Another method to visualize these cytoplasmic channels within myelin (termed "myelinic channels" in the following) is to inject diffusible dyes into oligodendrocytes or transgenically express fluorescent proteins. Using the fluorescent dye Lucifer Yellow, an extensive network of interconnected cytoplasmic pockets (~1.9 pockets per 10 μm sheath length) was observed in slices of acutely isolated spinal cord (Velumian et al. 2011). During development, myelinic channels are likely to serve as tracks for the delivery of the newly synthesized membrane to the growing tip (see below). In the adult nervous system, they may provide a functional connection between the oligodendroglial soma and the periaxonal space, allowing the distribution of glial metabolites to the axonal compartment. Considering that oligodendrocytes are also interconnected through gap junctions with each other and astrocytes, glial cells may provide a functional "syncytium" within white matter tracts (Abrams and Scherer 2012; Bedner et al. 2012; Nualart-Marti et al. 2013).
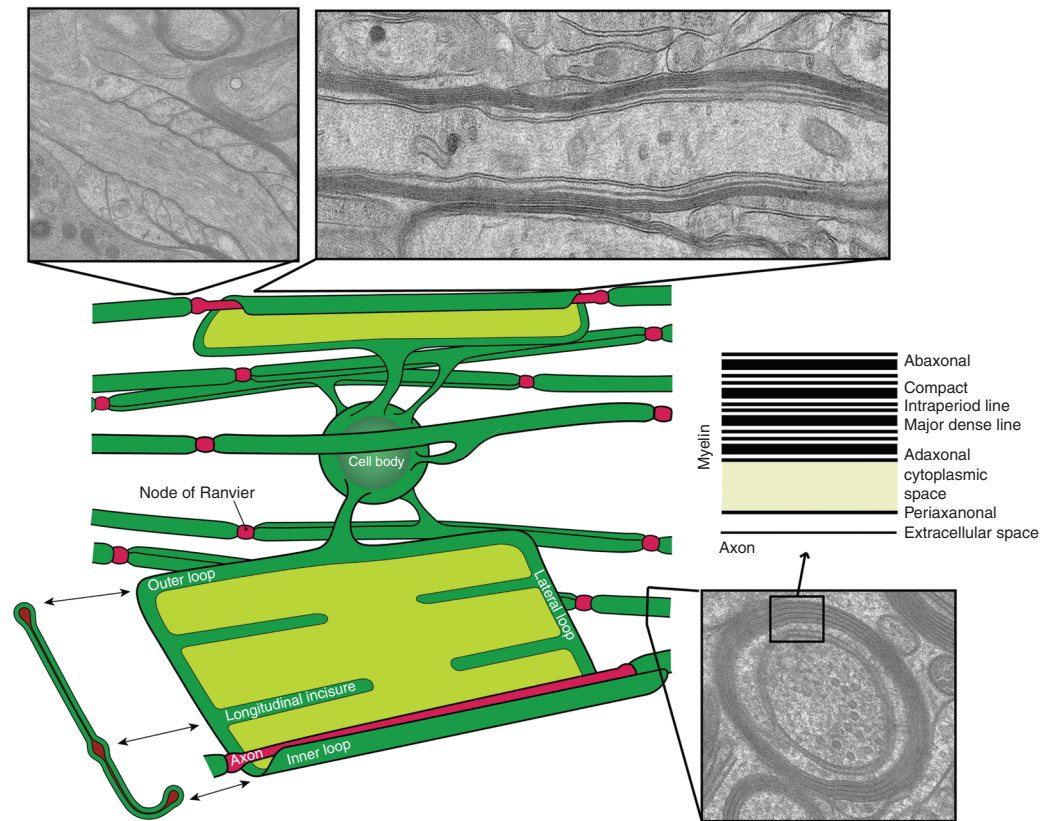
Figure 2. Schematic view of myelinating oligodendrocytes in its wrapped and conceptually "unrolled" state. Electron micrographs show (*left*) paranodal loops, a longitudinal view (*right*), and a cross-section view (*bottom*) of myelin. (From Aggarwal et al. 2011a; modified, with permission, from Elsevier Limited © 2011.)

The myelin sheath is also firmly attached to the axon at each end of an internode in which the myelin membrane never obtains a compacted structure, but terminates in the paranodal loops. These form septate junctions with the axonal surface, held together by the adhesion proteins Caspr and contactin on the axonal side and Neurofascin-155 on the glial surface (Salzer et al. 2008). In the intermodal region (i.e., between the two paranodal domains), the innermost lamellae of the myelin sheath remain noncompacted and form a cytoplasmic compartment, the adaxonal part of the myelinic channel system. It faces the periaxonal space along its length and is ideally positioned to communicate to the neuron.

Apart from its unique ultrastructure, there are several other exceptional features of myelin.

One is certainly its molecular composition. Myelin is a poorly hydrated structure, containing only 40% of water as compared with 80% in the gray matter. Myelin contains 70%–80% lipids (by dry weight) and there is only a small set of proteins that reside within compacted myelin, of which myelin basic protein (MBP) and PLP are most abundant. Even if recent proteome studies have uncovered many more proteins within purified myelin fractions (Werner et al. 2007; Ishii et al. 2009; Dhaunchak et al. 2010; Patzig et al. 2011; Manrique-Hoyos et al. 2012), it still holds true that the proteome of compact myelin is dominated by a few major, predominantly low molecular weight, and mostly myelin-specific proteins.

Another defining feature of myelin is its extraordinary stability. This was convincingly

shown when 5000-yr-old myelin with an almost intact ultrastructure was dissected from a Tyrolean Iceman (Hess et al. 1998). The underlying molecular basis for the stability of myelin is likely its lipid composition with high levels of saturated, long-chain fatty acids, together with an enrichment of glycosphingolipids (~20% molar percentage of total lipids) and cholesterol (~40% of molar percentage of total lipids) (O'Brien 1965; Coetzee et al. 1996). In addition, myelin comprises a high proportion of plasmalogens (etherlipids) with saturated long-chain fatty acids (Chrast et al. 2011). In fact, ~20% of the fatty acids in myelin have hydrocarbon chains longer than 18 carbon atoms (~1% in gray matter) and only ~6% of the fatty acids are polyunsaturated (~20% in gray matter). Van der Waals dispersion forces, which are generated by the interaction between methylene groups of these long and saturated hydrocarbon chains, provide the major forces that hold these molecules together. In addition, >50% of galactosylceramide and sulfatide are hydroxylated at the 2-C atom by fatty acid 2-hydroxylase (FA2H), thereby providing additional hydroxyl groups for hydrogen bonding and, thus, increasing the packing density of lipids in myelin (Eckhardt et al. 2005; Zoller et al. 2008).

Another peculiarity of the myelin membrane is its metabolic stability. Myelin membrane components have a half-life on the order of several weeks to months. The half-life of cholesterol in myelin was estimated to be >7–8 mo (Smith and Eng 1965). Recently, using in vivo pulse-chase labeling of proteins synthesis with ^{15}N isotopes, followed by mass spectrometry at various ages, myelin proteins together with histones, nucleoporins, and lamins were found to be among the most long-lived proteins in a mouse (Toyama et al. 2013). Thus, myelin is a stable system, which is in contrast to most membranes that are in highly dynamic, far-from-equilibrium steady state (Aggarwal et al. 2011).

However, myelin is compartmentalized into structurally and biochemically distinct domains. It is likely that the noncompacted regions are much more dynamic and metabolically active than the tightly compacted regions, which lack direct access to the membrane-trafficking machinery of oligodendrocytes.

MYELIN ASSEMBLY

Most oligodendrocytes generate between 20 and 60 myelinating processes with intermodal lengths of ~20 μm–200 μm and up to 100 membrane turns (Matthews and Duncan 1971; Hildebrand et al. 1993; Chong et al. 2012). When estimating the "surface" area that one oligodendrocyte would have (in terms of myelin membrane), it sums up to $5–50 \times 10^3$ μm^2, a number not reached by any other cell in our body (Pfeiffer et al. 1993; Baron and Hoekstra 2010). Myelin wrapping of single axonal segments takes only a few hours (at least in the zebrafish), which appears demanding on the machinery of protein and lipid synthesis (Czopka et al. 2013).

Myelination is a complex sequence of events that is best understood when conceptualized as separate steps, including (1) proliferation and migration of oligodendrocyte precursor cells (OPCs) in white matter tracts, (2) recognition of target axons and axon–glia signaling, (3) differentiation of OPCs into myelinating oligodendrocytes, (4) membrane outgrowth and axonal wrapping, (5) trafficking of membrane components, (6) myelin compaction, and (7) node formation. We will discuss here some aspects, but refer to comprehensive reviews for a more detailed description (Barres and Raff 1999; Baumann and Pham-Dinh 2001; Miller 2002; Emery 2010; Simons et al. 2012; Bakhti et al. 2013a; Freeman and Rowitch 2013).

First, OPCs migrate away from the neuroepithelium of the ventricular/subventricular zone of the brain into the developing white matter, in which they proliferate and form an evenly spaced network of processing-bearing cells. In vivo time-lapse imaging in transgenic zebrafish and mice show that OPCs continuously extend and retract numerous processes as they migrate and settle into their final positions (Kirby et al. 2006; Hughes et al. 2013). These dynamic and explorative processes are self-repulsive toward other OPCs and, thereby, define the uniform density and spacing of these cells. After OPCs have moved into their defined territories, their

further paths can be very different. Although some OPCs remain in a precursor state, others will differentiate into myelin-forming oligodendrocytes. To ensure that the number of oligodendrocytes matches the number of axons to be myelinated, an excess of cells are produced, which are later eliminated by apoptosis (Raff et al. 1993; Barres and Raff 1994; Trapp et al. 1997). One mechanism that determines the final number of oligodendrocytes is the competition for limiting amounts of target-derived growth and survival factors, such as platelet-derived growth factor (PDGF)-A, fibroblast growth factor (FGF)-2, insulin-like growth factor (IGF)-1, neurotrophin (NT)-3, and ciliary neurotrophic factor (CNTF) (Barres and Raff 1994; Miller 2002). This mechanism could be confirmed in mice overexpressing PDGF-A, which caused a dramatic increase in the number of OPCs in the embryonic mouse spinal cord, followed by production of ectopic oligodendrocytes (Calver et al. 1998). This excess of oligodendrocytes is later counterbalanced by an increase of apoptosis at an immature stage of differentiation, resulting in a normal number of myelin-forming oligodendrocytes.

Maturating OPCs pass through different stages of morphological differentiation and express a number of cell-specific marker proteins and lipids (Baumann and Pham-Dinh 2001). Based on morphology and molecular phenotype, cells in this lineage can be sequentially grouped into OPCs, immature/premyelinating oligodendrocytes, and mature/myelinating oligodendrocytes. The speed of oligodendrocyte differentiation is highly variable, however. In the hindbrain, oligodendrocytes are already produced a few days after the appearance of OPCs, whereas a similar transition takes several weeks in the cerebral cortex. Although it has become clear that OPCs can persist for a long time in their immature stage, it appears that once the decision has been made to trigger differentiation, it occurs at a very rapid pace. Recent time-lapse imaging in zebrafish shows that, at least in this species, there is only a brief window for an oligodendrocyte to complete myelination (Watkins et al. 2008; Czopka et al. 2013). When myelination has started, it takes that oli-

godendrocyte only 5 h to generate all myelin sheaths.

Because the differentiation of oligodendrocytes has to be adjusted to the developmental program of neurons, the general assumption is that neuronal signals control the transformation of OPCs into myelin-forming oligodendrocytes. However, unlike in the peripheral nervous system (PNS), in which neuregulin-1 on the axonal membrane controls myelination by Schwann cells, an equivalent single-key regulator that triggers myelination by oligodendrocytes in the CNS has not been identified. Moreover, it appears that also the relief from inhibitory neuronal signals is critical for OPCs to exit a "repressed" state (Emery 2010). These inhibitory axonal signals (e.g., Jagged, PSA-NCAM, and LINGO-1), in turn, activate various transcriptional regulators, such as Sox 5/6, Hes5, and Id2/4, which actively prevent OPCs from entering terminal differentiation (Piaton et al. 2010; Taveggia et al. 2010). Many of these inhibitory transcription factors are also controlled by microRNAs (miRNAs), histone deacetylases, and signaling pathways, of which the Wnt/β-catenin pathway has recently emerged as a key regulator of myelin gene expression and myelination (Shin et al. 2005; Fancy et al. 2009; Budde et al. 2010; Dugas et al. 2010; Kim et al. 2010; Zhao et al. 2010; Tawk et al. 2011).

From the wealth and complexity of the signals that ultimately prevent OPCs from entering terminal differentiation, it becomes clear that the timing of oligodendrocyte differentiation is a critical step in the life of an oligodendrocyte. Electrically active neurons appear to stimulate OPC proliferation and selectively induce myelination of an active neural circuit (Gibson et al. 2014). Once the decision has been made to initiate myelination, oligodendrocytes must be properly positioned because mature oligodendrocytes appear to have very little capacity to migrate into new positions or to delay myelin synthesis. A number of "feedforward signals" have been identified that help to speed up the morphological differentiation of OPCs once the starting signal(s) has been launched. For example, microRNAs, in particular, miRNA-219 and miRNA-338, actively repress the activity of crit-

ical transcription factors and signaling pathways, such as Sox5/6, Hes5, and PDGFRα (Dugas et al 2010; Zhao et al. 2010; Hudish et al. 2013). At the same time that the activity of other transcription factors, including repressors of repressors and activators of transcription, like myogenic regulatory factors (MRFs), Nxk2.2, Olig1, Sox10, YY1, and Zfp191, are turned on (He et al. 2007; Wegner 2008; Emery et al. 2009; Howng et al. 2010; Meijer et al. 2012; Bujalka et al. 2013). Once myelination has started, the PtdIns(3,4,5)P3/Akt/mTor and ERK1/2-dependent signaling pathways are essential in driving myelin formation forward until fully matured sheaths with optimal g-ratios have been generated (Flores et al. 2008; Tyler et al. 2009; Goebbels et al. 2010; Ishii et al. 2013).

Although much has been learned about the oligodendrocyte lineage progression, we know little about how oligodendrocytes decide which axon to select for myelination (Simons and Lyons 2013). One would assume that specific adhesion molecules must be expressed on the surface of axons, which are recognized by oligodendrocytes. However, evidence has been provided that target size alone can determine the initiation of myelination (Lee et al. 2012a). When OPCs were cultured in the presence of inert polystyrene fibers to model axons, they were able to ensheath such "axons," suggesting that oligodendrocytes (unlike Schwann cells) do not require axonal adhesion molecules and growth factors to initiate the formation of myelin. The caliber size appeared to be critical as fibers <400 nm were rarely ensheathed. It is possible that, because of physical constraints, the ensheathing processes of oligodendrocytes are unable to navigate around axons below a specific threshold caliber. Although larger axons are preferentially myelinated in vivo, axons as small as 200–300 nm can be myelinated in some regions of the CNS. It is likely that oligodendrocytes receive specific signals from small-caliber axons to initiate myelination. The nonreceptor tyrosine kinase Fyn could be one of these signals as loss of Fyn causes a severe hypomyelination of such small-caliber axons without affecting myelination of larger axons (Umemori et al. 1994).

Once a particular axon has been engaged by a myelinating oligodendrocyte process, dramatic changes in plasma membrane architecture are induced. How the processes are converted into flat sheets that spread and wind along the axons to generate a multilayered stack of membranes has been difficult to address. By light microscopy, a coil of an average periodicity of 5.7–7 μm along the internodal dimension has been observed (Butt and Berry 2000; Pedraza et al. 2009; Sobottka et al. 2011; Ioannidou et al. 2012). This has led to the suggestion that either myelin extends as a coil twisting around the axon in a corkscrew motion (Pedraza et al. 2009) or myelin thickening is achieved by forming new layers on top of the inner ones in a "croissant-like" manner (Sobottka et al. 2011). Recently, by using EM of high-pressure frozen samples and three-dimensional reconstructions based on serial block-face EM, snapshots of the myelin ultrastructure have been obtained during development of the optic nerve (Snaidero et al. 2014). This analysis revealed that the innermost layers of myelin have the shortest lateral extensions, whereas the layers toward the top of the myelin sheath build up in size with each consecutive wrap. Together, the data suggest that myelin grows by two distinct but coordinated motions: the wrapping of the leading edge at the inner tongue around the axon, that is, underneath the previously deposited membrane, and the lateral extension of myelin membrane layers toward the nodal regions (Fig. 3). Thus, the lateral cytoplasmic-rich edges of each myelin layer always stay in close contact with the axonal surface and move in a continuous helical manner toward the future node in which they align and form the paranodal loops. This model implies that newly synthesized membrane material has to be transported all the way through the developing myelin sheath toward the innermost layer facing the axon. In fact, as already discussed above, there is an elaborated system of cytoplasmic channels within compacted myelin that provide a helical path for the transport of vesicles to the growth zone at the leading edge.

The different myelin components are synthesized in oligodendrocytes at several subcellular localizations and are transported by various
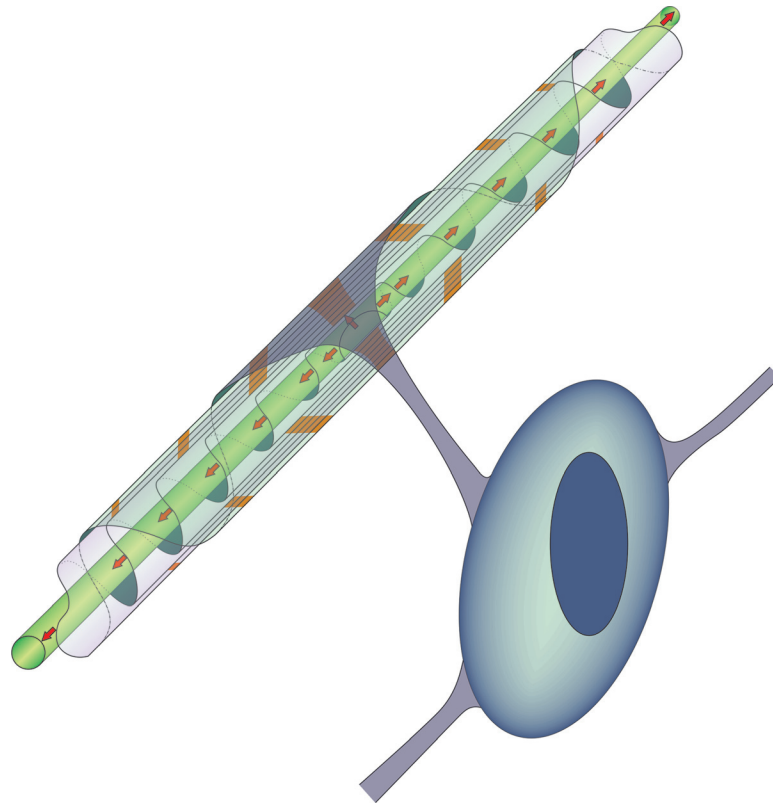
Figure 3. Model showing the direction of how myelin wraps around the axon. There are two motions: the wrapping of the leading edge at the inner tongue around the axon underneath the previously deposited membrane and the lateral extension of myelin membrane layers toward the nodal regions (for details, see Snaidero et al. 2014).

mechanisms to the growing myelin sheath. Early pulse-labeling experiment had suggested that (at least in Schwann cells) newly synthesized lipids and integral myelin proteins first reach the outer myelin membranes and then diffuse laterally, at least after the initial wraps have been made (Gould and Dawson 1976; Gould 1977). In addition to membrane transport via the biosynthetic secretory pathway, the membrane-associated protein MBP needs to be delivered to myelin, which in development occurs by the transport of its messenger RNA (mRNA) within cytoplasmic granules (Colman et al. 1982; Ainger et al. 1993). Subsequently, the local translation of MBP is initiated at the tip of the glial process, which is in contact with the axon in vivo (or in the outermost edge of the oligodendrocyte in vitro) (White et al. 2008; Laursen et al.

2011; Wake et al. 2011). Although MBP is, thus, synthesized in the distal process, that is, closest to the axon, myelin compaction is mediated by MBP progresses from the outside to the inside of the sheath, namely, proximal to distal (Snaidero et al. 2014). It is obvious that if the sites of MBP translation and compaction are spatially separated, there must be a mechanism in place that prevents membrane compaction at the site of MBP synthesis. The myelin protein CNP1 appears to be such a factor that delays myelin compaction. In mice lacking CNP1, myelin compaction proceeds faster and extends to the innermost layers of the myelin sheath, whereas transgenic overexpression of CNP1 results in areas of myelin that lack compaction (Gravel et al. 1996; Snaidero et al. 2014). Thus, the equilibrium of CNP1 and MBP levels seems to reg-

ulate the speed of compaction in early development.

Once MBP is bound to two adjacent cytoplasmic membrane surfaces, it appears to "polymerize" by lateral interactions with previously deposited MBP "monomers," thereby driving membrane zippering at the cytoplasmic surfaces of the myelin bilayer (Aggarwal et al. 2013). The self-association of high-order aggregates is triggered by membrane binding of MBP, which results in charge neutralization and major conformational changes of the protein (Harauz et al. 2009; Aggarwal et al. 2013; Bakhti et al. 2013). One major consequence of the formation of MBP polymers is the depletion of most peripheral and membrane-associated proteins from compacted myelin. This occurs by MBP-mediated protein extrusion and generation of a diffusion barrier, which limits the entry of proteins with larger cytoplasmic domains (Aggarwal et al. 2011).

Compact myelin becomes a protein-poor membrane, lacking major glycoproteins at the extracellular leaflet, which is likely to uncover weak (generic) forces that promote the association of two bilayers at their extracellular surface (Bakhti et al. 2013). In addition, with the maturation of oligodendrocytes, the plasma membrane undergoes major transformations of its structure. Whereas OPCs are covered by a dense layer of large and negatively charged self-repulsive oligosaccharides, the compacted myelin of fully matured oligodendrocytes lacks most of these glycoproteins and complex glycolipids. Such a conversion may contribute to the transformation of oligodendrocyte lineage cells from the self-avoiding and "repulsive" OPCs that tile the entire CNS to "sticky" oligodendrocytes, which ensheath axons with a multilayered stack of self-associating membranes.

METABOLIC SUPPORT OF AXON FUNCTION

Once myelination is completed, the developmentally important cytosolic channels decrease in size, but remain as a significant compartment of (noncompacted) myelin, which can be visualized, for example, by dye injections, the expression of fluorescent proteins (Fig. 1), or high-pressure freezing EM. This cytosolic space connects the mature oligodendroglial soma to the innermost tip of the oligodendrocyte process (Fig. 2). As a "myelinic channel," this tube-like cytosolic compartment (also termed the "inner lip") runs in parallel to the thin extracellular periaxonal space in which it faces the axonal internode along its length (Nave 2010). It is filled with a plethora of soluble and membrane-associated myelin proteins and a molecular complexity that outreaches, by far, the proteome of compacted myelin (Roth et al. 2006; Werner et al. 2007; Ishii et al. 2009; Jahn et al. 2009). Moreover, the detection of microtubules and multivesicular bodies within the myelinic channel by EM (mostly in the paranodal loops and the Schmitt–Lanterman incisures of myelin in the peripheral nervous system) suggests that motor-driven transport processes in this compartment are maintained throughout adult life. Apparently, the fine structure of noncompacted myelin preserves functional axonal integrity as compact myelin not only insulates axons electrically, but also shields the axonal compartment and limits rapid access to metabolites in the extracellular space.

Loss of the normal fine structure of myelin can cause late-onset axonal degeneration and even premature death, as shown in mouse mutants that lack certain myelin-specific proteins (Griffiths et al. 1998). For example, absence of the membrane-anchored protein CNP1 (which normally prevents premature myelin compaction during development; see above) does not alter myelination per se, but is associated with secondary transport problems in the axonal compartment after myelination has been completed. One contributing mechanism could be the abnormal closure of myelinic channels by abnormal "compaction," which might directly or indirectly interfere with efficient metabolic coupling of oligodendrocytes and axons. The resulting pathology is indicative of axonal energy deficits and is followed by widespread axonal loss and premature death in mice (Lappe-Siefke et al. 2003; Edgar and Nave 2009). Even 50% reduced expression of CNP1 (in heterozygous mouse mutants) interferes with long-term

maintenance of white matter tracts. In this case, CNP1 deficiency leads to axonal loss and significant neuroinflammation only at an older age when mice also become behaviorally abnormal (Hagemeyer et al. 2012).

Indeed, recent genetic evidence suggests that oligodendrocytes and axons are metabolically coupled (Fig. 4). Mice, in which oligodendrocytes lack mitochondrial respiration (Fünfschilling et al. 2012), showed that with myelination the energy metabolism of developing oligodendrocytes switches. Although oligodendrocytes in development import glucose and lactate (Rinholm et al. 2011) to allow rapid myelination, building on the synthesis of large amounts of lipids, after myelination the same oligodendrocytes can survive by glycolysis only, without signs of demyelination or degeneration. In fact, oligodendroglia-derived lactate can be readily metabolized within myelinated tracts, in which axons were shown to efficiently take up lactate for mitochondrial ATP production (Fünfschil-

ling et al. 2012). This mechanism of oligodendroglial support requires, in addition to glucose import through glial glucose transporters (GLUT1), the subsequent release of lactate (and/or pyruvate) into the periaxonal space, which is a function of the monocarboxylate transporter MCT1 (Lee et al. 2012). Interestingly, even 50% reduced expression of MCT1 (in heterozygous mouse mutants) interferes with long-term maintenance of myelinated axons (Lee et al. 2012), similar in phenotype to axonal degeneration caused by oligodendrocyte-specific defects (Griffiths et al. 1998; Lappe-Siefke et al. 2003). Thus, it is plausible that either the delivery of metabolites by diffusion through myelinic channels and MCTs or the deposition and turnover of MCTs themselves can be impaired if myelin is structurally perturbed or becomes secondarily disrupted, such as in demyelinating diseases. On the other hand, even a severe developmental dysmyelination, such as in "shiverer" (MBP-deficient) mice, is compat-
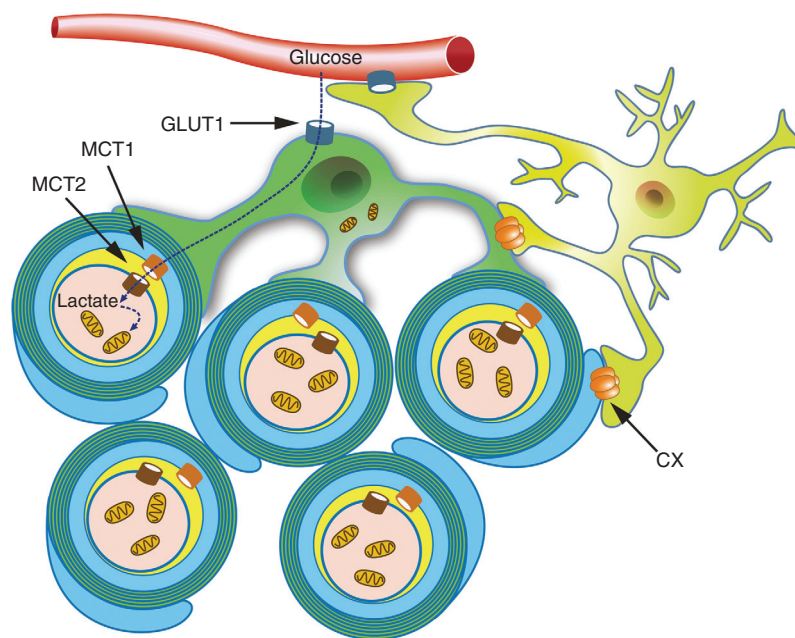
Figure 4. Schematic depiction of an oligodendrocyte that takes up blood-derived glucose and delivers glycolysis products (pyruvate/lactate) via monocarboxylate transporters (MCT1 and MCT2) to myelinated axons. Oligodendrocytes and myelin membranes are also coupled by gap junctions to astrocytes and, thus, indirectly to the blood–brain barrier. CX, Connexin. (From Saab et al. 2013; modified, with permission, from Elsevier Limited © 2013.)

ible with axon function and survival, presumably as long as critical metabolic interactions remain intact (Griffiths et al. 1998). In fact, comparing different myelin disease models, it appears that for the maintenance of axon function, no myelin is better than defective myelin when associated with a loss of axon–glia metabolic coupling. Axonal energy metabolism is important, but unlikely the only aspect of intermediate metabolism in the axonal compartment dependent on associated glial cells. This is also suggested by very recent observations in mice with LKB1-deficient Schwann cells showing late-onset axonal degeneration even in the presence of elevated lactate (Beirowski et al. 2014).

CONCLUDING REMARKS

The formation of myelin is one of the most complex transformations of a plasma membrane that comprises timely coordinated cell–cell interactions with reciprocal intercellular signaling events, the wrapping and extension of the glial plasma membrane around the axon, and the extrusion of cytoplasm from newly generated multilamellar membrane stacks. Each of these events represents a fascinating area of cellular neuroscience with relevance not only for our understanding of normal physiology, but also for various white matter diseases. Whereas the myelin sheath has been regarded for a long time as an inert insulating structure, it has now become clear that myelin is "alive" and metabolically active with cytoplasmic-rich pathways, myelinic channels, for movement of macromolecules into the periaxonal space. The myelin sheath and its subjacent axon need to be regarded as one functional unit that is not only morphological, but also metabolically coupled. In the future, we need to also understand whether macromolecules are exchanged between neurons and glia. Over the past, it has become clear that myelin can undergo significant structural changes in adulthood (Young et al. 2013). Myelin seems to be dynamically regulated by experience both during development and in adult life, thus, the extent of myelin sheath formation may also serve as a form of plasticity to adapt brain function to environmental stimuli (Fields

2008; Liu et al. 2012; Makinodan et al. 2012; Mangin et al. 2012; Young et al. 2013). Clearly, identifying the molecular mechanisms that regulate myelin formation and metabolic pathways within myelin are likely to be key areas for further study.

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