CHAPTER

10

Myelin Structure and Biochemistry

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OUTLINE

The morphological distinction between white matter and gray matter is one that is useful for the neurochemist. White matter, so called for its glistening white appearance, is composed of myelinated axons, glial cells, and blood vessels. Gray matter contains, in addition, the nerve cell bodies with their extensive dendritic arborizations. The predominant element of white matter is the myelin sheath, which comprises about 50% of its total dry weight and is responsible for the gross chemical differences between white and gray matter.

THE MYELIN SHEATH

The myelin sheath is a greatly extended and modified plasma membrane that is wrapped around the nerve axon in a spiral fashion. A comprehensive review of the older literature on the structure, biochemistry and other aspects of myelin is available in a book published almost 30 years ago (Morell, 1984), whereas newer developments in the myelin field are covered in detail in a recent two-volume set (Lazzarini, 2004). See also Chapter 31 ("Formation and Maintenance of Myelin") and Chapter 39 ("Diseases Involving Myelin"). The myelin membranes originate from, and are part of, Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS) (see Chapter 1). Each myelin-generating cell furnishes myelin for only one segment of any given axon. The periodic interruptions where short portions of the axon are left uncovered by myelin are the *nodes of Ranvier*, and they are critical to the functioning of the axon and the myelin. The segments of myelinated axons between nodes are called internodes.

Myelin facilitates conduction

Myelin is an electrical insulator, although its function of facilitating conduction in axons has no exact analogy in electrical circuitry (Waxman & Bangalore, 2004). In unmyelinated fibers, impulse conduction is propagated by local circuits of ion current that flow into the active region of the axonal membrane, through the axon, and out through adjacent sections of

the membrane (Figure 10-1). These local circuits depolarize the adjacent piece of membrane in a continuous sequential fashion. In myelinated axons, the excitable axonal membrane is exposed to the extracellular space only at the nodes of Ranvier; this is the location of sodium channels. When the membrane at the node is excited, the local circuit generated cannot flow through the high-resistance sheath and therefore flows out through and depolarizes the membrane at the next node, which might be 1 mm or farther away (see Fig. 10-1). The low capacitance of the sheath means that little energy is required to depolarize the remaining membrane between the nodes, which results in an increased speed of local circuit spreading. Active excitation of the axonal membrane jumps from node to node; this form of impulse propagation is called *saltatory conduction* (Latin *saltare*, "to jump"). Such movement of the wave of depolarization is much more rapid than is the case in unmyelinated fibers. Furthermore, because only the nodes of Ranvier are excited during conduction in myelinated fibers, sodium flux into the nerve is much less than in unmyelinated fibers, where the entire membrane is involved.

Comparison of two different nerve fibers which both conduct at 25 m/sec at 20°C demonstrates the advantage of myelination. The 500 μm-diameter unmyelinated giant axon of the squid requires 5,000 times as much energy and occupies about 1,500 times as much space as a 12 μm-diameter myelinated nerve in a frog. Conduction velocity in myelinated fibers is proportional to the diameter, while in unmyelinated fibers it is proportional to the square root of the diameter. Thus, differences in energy and space requirements between the two types of fibers are exaggerated at higher conduction velocities. If nerves were not myelinated and equivalent conduction velocities were maintained, the human spinal cord would need to be as large as a good-sized tree trunk. Myelin, then, facilitates conduction while conserving space and energy (Waxman & Bangalore, 2004).

Myelin has a characteristic ultrastructure

Myelin, as well as many of its morphological features, such as nodes of Ranvier and Schmidt-Lanterman clefts, can be seen readily in the light microscope (Fig. 10-2). Further insight comes from biophysical studies of structures with parallel axons, sciatic nerve as representative of the PNS and optic nerve or tract as representative of the CNS. Myelin, when examined by polarized light, exhibits both a lipid-dependent and a protein-dependent birefringence. Low-angle X-ray diffraction studies of myelin provide electron density plots of the repeating unit that show three peaks (each corresponding to protein plus lipid polar groups) and two troughs (lipid hydrocarbon chains). The repeat distance varies somewhat depending on the species and whether the sample is from CNS or PNS. Thus, the results from these two techniques are consistent with a protein-lipid-protein-lipid-protein structure, in which the lipid portion is a bimolecular leaflet and adjacent protein layers are different in some way. Figure 10-3 shows data for mammalian optic nerve with a repeat distance of 80 Å. This spacing can accommodate one bimolecular layer of lipid (about 50 Å) and two protein layers (about 15 Å each). The main repeating unit of two such fused unit membranes is twice this, or 160 Å. (See Kirschner & Blaurock, 1992 for discussion and references). Although it is useful to think of myelin in terms of alternating protein and lipid layers, this concept has been modified to be compatible with the "fluid mosaic" model of membrane structure that includes intrinsic transmembrane proteins as well as extrinsic proteins.

Information concerning myelin structure is also available from electron microscope studies, which visualize myelin as a series of alternating dark and less-dark lines (protein layers) separated by unstained zones (the lipid hydrocarbon chains) (Figs. 10-4–10-7). There is asymmetry in the staining of the protein layers. The less dark, intraperiod line represents the closely

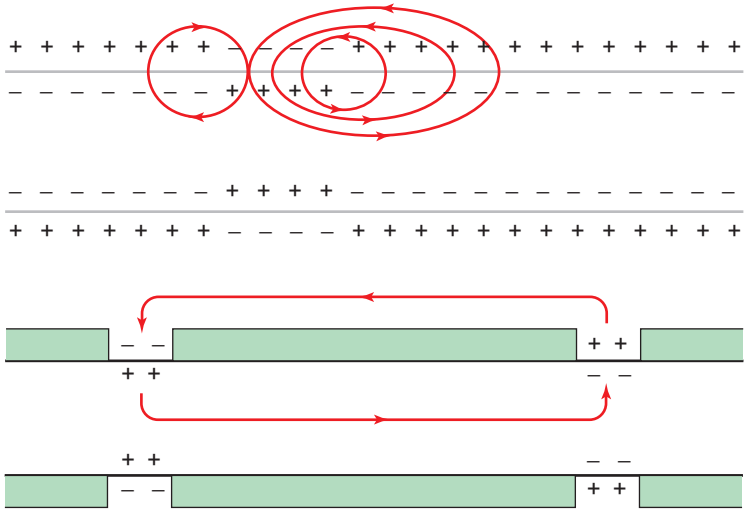


FIGURE 10-1 **Impulse conduction in unmyelinated (top) and myelinated (bottom) fibers.** The arrows show the flow of action currents in local circuits into the active region of the membrane. In unmyelinated fibers the circuits flow through the adjacent piece of membrane, but in myelinated fibers the circuit flow jumps to the next node.

apposed outer protein layers of the original cell membrane; the membranes are not actually fused, as they can be resolved as a double line at high resolution (Figs. 10-6 and 10-7).

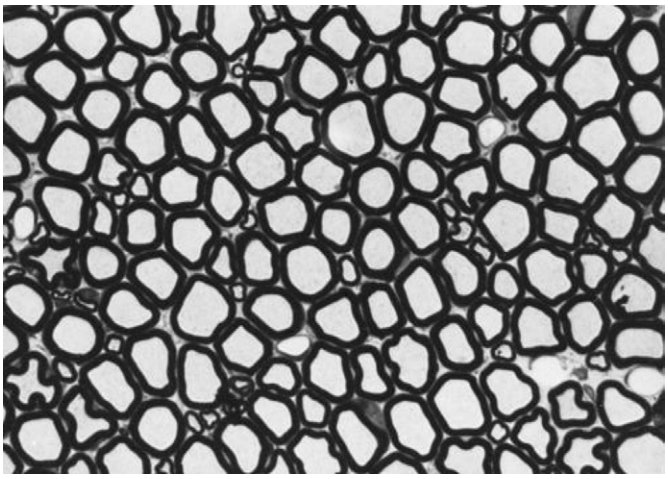


FIGURE 10-2 **Light micrograph of a 1μ Epon section of rabbit peripheral nerve (anterior root), stained with toluidine blue.** The myelin sheath appears as a thick black ring around the pale axon. x 600, before 30% reduction. (Courtesy of Dr. Cedric Raine.)

The dark, or major period, line comprises the fused inner protein layers of the cell membrane. The repeat distances observed by electron microscopy are less than those calculated from the low-angle X-ray diffraction data, a consequence of the considerable shrinkage that takes place after fixation and dehydration. However, the difference in periodicity between PNS myelin and CNS myelin is maintained; peripheral myelin has an average repeat distance of 119 Å and the central myelin of 107 Å.

Nodes of Ranvier

Two adjacent segments of myelin on one axon are separated by a node of Ranvier. In this region the axon is not covered by myelin. Nodes of Ranvier in the PNS and CNS share a common molecular organization and perform the same functions. The main axolemmal proteins responsible for node function are the high densities of voltage-gated Na^+ and K^+ channels that are clustered at these sites. These ion channels are responsible for the transmembrane currents that permit saltatory conduction. Nodes are enriched not only in ion channels but also in a variety of cell adhesion molecules, cytoskeletal scaffolds, and other signaling proteins. For example, the scaffolding protein ankyrinG binds to Na^+ channels and is directly responsible for restricting and stabilizing channels

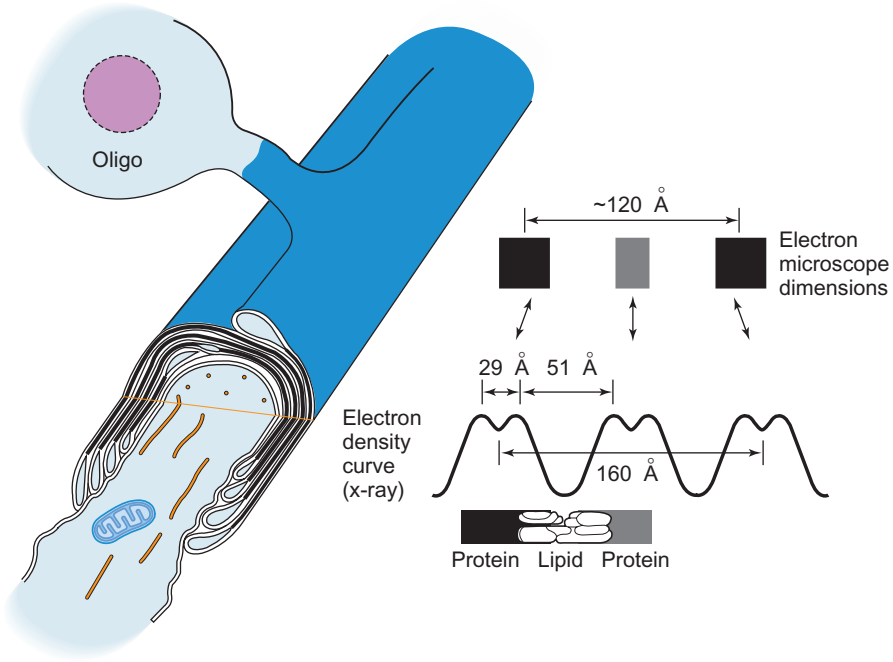


FIGURE 10-3 **A composite diagram summarizing some of the ultrastructural data on CNS myelin.** At the top an oligodendroglial cell is shown connected to the sheath by a process. The cutaway view of the myelin and axon illustrates the relationship of these two structures at the nodal and paranodal regions. (Only a few myelin layers have been drawn for the sake of clarity.) At the internodal region, the cross-section reveals the inner and outer mesaxons and their relationship to the inner cytoplasmic wedges and the outer loop of cytoplasm. Note that in contrast to PNS myelin, there is no full ring of cytoplasm surrounding the outside of the sheath. The lower part of the figure shows roughly the dimensions and appearance of one myelin repeating unit as seen with fixed and embedded preparations in the electron microscope. This is contrasted with the dimensions of the electron density curve of CNS myelin obtained by X-ray diffraction studies in fresh nerve. The components responsible for the peaks and troughs of the curve are sketched below. Adapted from W. T. Norton (1977).

at nodes. AnkyrinG links nodal membrane proteins to the underlying spectrin-actin cytoskeleton.

Flanking each node at the paranodal region and in the Schmidt-Lanterman clefts, the cytoplasmic surfaces of myelin are not compacted, and Schwann or glial cell cytoplasm is included within the sheath. To visualize these structures one may refer to Figures 10-8 and 10-9, which show that if myelin were unrolled from the axon it would be a flat, spade-shaped sheet surrounded by a tube of cytoplasm. Thus, as shown in electron micrographs of longitudinal sections of axon paranodal regions, the major dense line formed by apposition of the cytoplasmic faces opens up at the edges of the sheet, enclosing cytoplasm within a loop (see Figures 10-3 and 10-9). These loop-shaped terminations of the sheath at the node are called *lateral loops*. The loops form membrane complexes with the axolemma called *transverse bands*, whereas myelin in the internodal region is separated from the axon by an extracellular gap of *periaxonal* space. The transverse bands are helical structures that seal the myelin to the axolemma, but provide, by spaces between them, a tortuous path from the extracellular space to the periaxonal space.

Schmidt-Lanterman clefts are structures where the cytoplasmic surfaces of the myelin sheath have not compacted to form the major dense line and therefore contain Schwann or glial cell cytoplasm (Fig. 10-9). They are common in peripheral myelin, but rare in the CNS. These inclusions of cytoplasm are present in each layer of myelin. The clefts can be visualized in the unrolled myelin sheet as tubes of cytoplasm similar to the tubes making up the lateral loops but in the middle regions of the sheet, rather than at the edges (Figure 10-9).

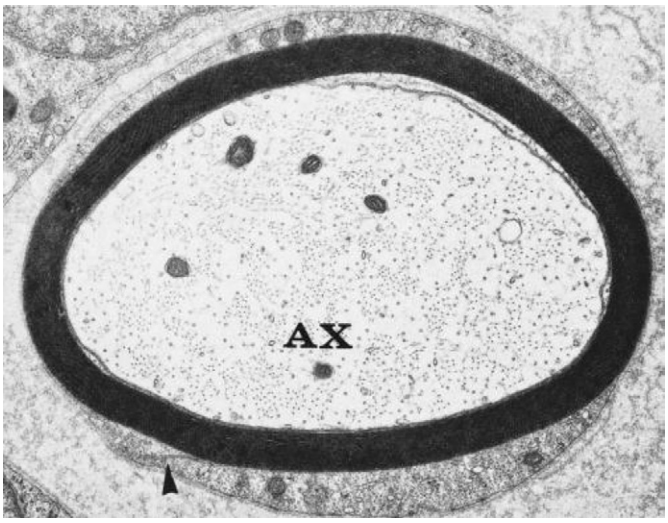


FIGURE 10-4 **Electron micrograph of a single peripheral nerve fiber from rabbit.** Note that the myelin sheath has a lamellated structure and is surrounded by Schwann cell cytoplasm. The outer mesaxon (arrow) can be seen in lower left. (AX) axon ×18,000. (Courtesy of Dr. Cedric Raine.)

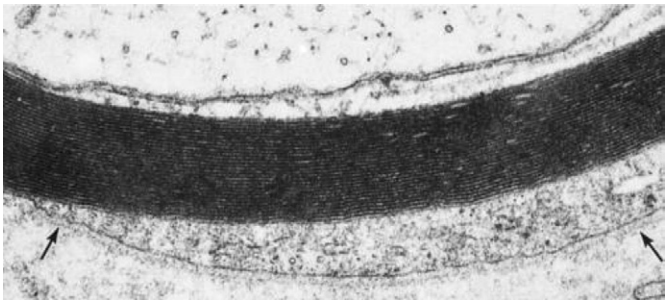


FIGURE 10-5 **Higher magnification of Figure 10-4 to show the Schwann cell cytoplasm covered by basal lamina (arrows). ×50,000.**

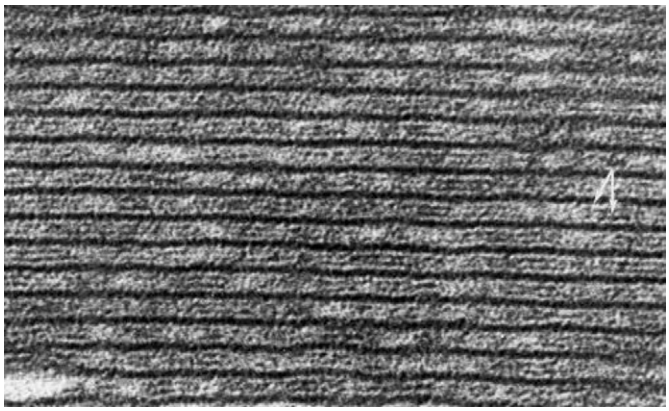


FIGURE 10-6 **Magnification of the myelin sheath of Figure 10-4.** Note that the intraperiod line (arrows) at this high resolution is a double structure. ×350,000. (Courtesy of Dr. Cedric Raine.)

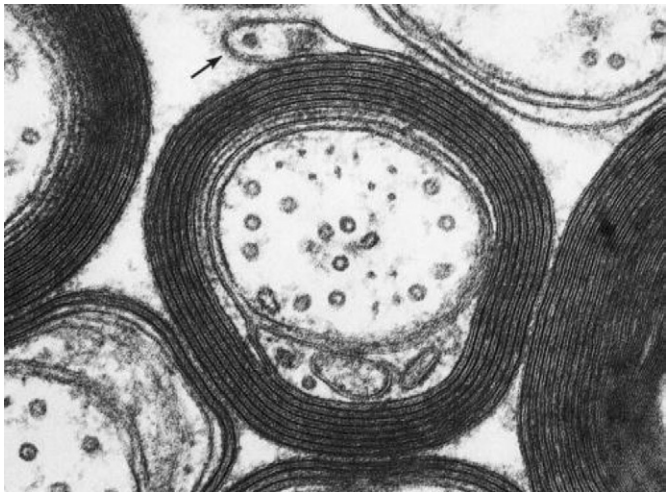


FIGURE 10-7 **A typical CNS myelinated fiber from the spinal cord of an adult dog.** Contrast this figure with the PNS fiber in Figure 10-4. The course of the flattened oligodendrocytic process, beginning at the outer tongue (arrow), can be traced. Note that the fiber lacks investing cell cytoplasm and a basal lamina, as is the case in the PNS. The major dense line and the paler, double intraperiod line of the myelin sheath can be discerned. The axon contains neurotubules and neurofilaments. ×135,000.

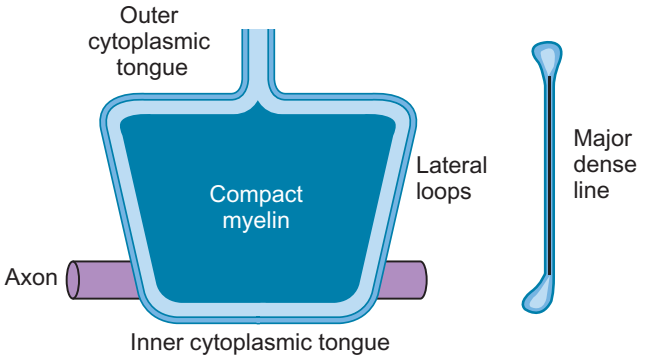


FIGURE 10-8 **A diagram showing the appearance of CNS myelin if it were unrolled from the axon.** One can visualize this structure arising from Figure 10-3, if the glial cell process were pulled straight up and the myelin layers separated at the intermediate period line. The whole myelin internode forms a spade-shaped sheet surrounded by a continuous tube of oligodendroglial cell cytoplasm. This diagram shows that the lateral loops and inner and outer cytoplasmic tongues are parts of the same cytoplasmic tube. The drawing on the right shows how this sheet would appear if it were sectioned along the vertical line, indicating that the compact myelin region is formed of two unit membranes fused at the cytoplasmic surfaces. The drawing is not necessarily to scale. Adapted from A. Hirano and H. M. Dembitzer (1967).

Myelin is an extension of a cell membrane

Myelination in the PNS is preceded by invasion of the nerve bundle by Schwann cells, rapid multiplication of these cells, and segregation of the individual axons by Schwann cell processes. Smaller axons (≤1 μm), which will remain unmyelinated, are segregated; several may be surrounded by one Schwann cell, each within its own pocket, similar to the single axon (see Chapter 31). Large axons (≥1 μm) destined for myelination are enclosed singly, one cell per axon per internode. These cells line up along the axons with intervals between them; the intervals become the nodes of Ranvier.

Before myelination the axon lies in an invagination of the Schwann cell. The plasmalemma of the cell then surrounds the axon and joins to form a double membrane structure that communicates with the cell surface. This structure, called the *mesaxon*, then elongates around the axon in a spiral fashion (see Chapter 31). Thus, formation of myelin topologically resembles rolling up a sleeping bag; the mesaxon winds about the axon, and the cytoplasmic surfaces condense into a compact myelin sheath and form the major dense line. The two external surfaces form the myelin intraperiod line.

In the CNS, myelin is formed by oligodendrocytes. This has many similarities with respect to myelination in the PNS, but also has points of difference therewith. CNS nerve fibers

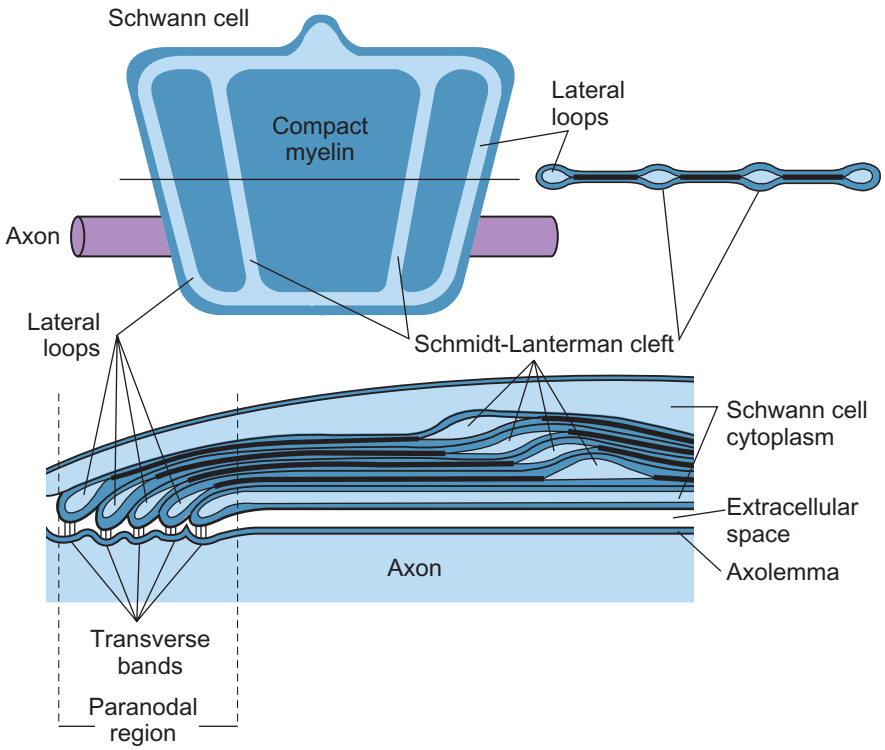


FIGURE 10-9 **A diagram similar to Figure 10-8, but showing one Schwann cell and its myelin sheath unrolled from a peripheral axon.** The sheet of PNS myelin is, like CNS myelin, surrounded by a tube of cytoplasm and has additional tubes of cytoplasm, which make up the Schmidt-Lanterman clefts, running through the internodal regions. The horizontal section (top right) shows that these additional tubes of cytoplasm arise from regions where the cytoplasmic membrane surfaces have not fused. Diagram (bottom) is an enlarged view of a portion of the top left diagram, with the Schwann cell and its membrane wrapped around the axon. The tube forming the lateral loops seals to the axolemma at the paranodal region, and the cytoplasmic tubes in the internodal region form the Schmidt-Lanterman clefts. These drawings are not to scale. Adapted from A. Hirano and H. M. Dembitzer (1967).

are not separated by connective tissue, nor are they surrounded by cell cytoplasm, and specific glial nuclei are not obviously associated with particular myelinated fibers. CNS myelin is a spiral structure similar to PNS myelin; it has an inner mesaxon and an outer mesaxon that ends in a loop, or tongue, of glial cytoplasm (Figure 10-3). Unlike peripheral nerve, where the sheath is surrounded by Schwann cell cytoplasm on the inside and outside, the cytoplasmic tongue in the CNS is restricted to a small portion of the sheath (Figures 10-3 and 10-8). This glial tongue is continuous with the plasma membrane of the oligodendroglial cell through slender processes. One oligodendrocyte can myelinate as many as 40 or more separate axons.

Myelin deposition in the PNS may result in a single axon having up to 100 myelin layers, and it does not appear that myelin is laid down by a simple rotation of the Schwann cell around the axon. In the CNS, such rotation is precluded by the fact that one glial cell can myelinate several axons. During myelination, there are increases in the length of the internode, the diameter of the axon, and the number of myelin layers. Myelin is therefore expanding in all planes at once. Any mechanism to account for this growth must assume the membrane system is able to expand and contract, and that layers slip over each other.

Myelin affects axonal structure

Traditionally, the role of the myelin membrane has been relegated to its passive properties (increased transverse membrane resistance and decreased membrane capacitance). However, it is now appreciated that the myelin sheath also performs active functions such that the presence of a myelin sheath affects the structure of the axon that it surrounds (Trapp & Kidd, 2004), thereby optimizing its properties for transmission of action potentials by saltatory conduction. Therefore, one major role of the myelin sheath is to actively recruit ion channels and other axonal membrane proteins to specific, polarized locations along the axon. In general, myelinating glia provide factors that interact with axonal cell adhesion molecules to initiate assembly of these polarized domains (Schafer & Rasband, 2006). For example, in the PNS, Schwann cells secrete a protein called gliomedin that is an instructive signal for the assembly of Na^+ channel clusters at nodes of Ranvier. On the extracellular side of the axonal membrane gliomedin binds to, and clusters, the axonal cell adhesion molecule neurofascin-186 (Eshed et al., 2005). Neurofascin-186 in turn functions as an attachment site for ankyrinG, which is found in the axonal cytoplasm. As ankyrinG accumulates at nascent nodes, Na^+ and K^+ channels are recruited to this scaffold and clustered in high density (Dzhashiashvili et al., 2007). Lastly, this entire protein complex is linked to the actin cytoskeleton through ankyrinG binding to βIV spectrin. While all of these same axonal proteins are found at CNS nodes, the glial derived signals that initiate nodal assembly in the CNS remain unknown.

The myelin sheath actively sculpts the membrane protein composition along the entire length of the axon, not just at nodes of Ranvier. Adjacent to the nodes are the paranodes, with their own unique sets of axonal and glial cell adhesion molecules (see below) and cytoskeletal scaffolding proteins. Paranodes themselves can function as a lateral membrane diffusion barrier, restricting proteins to distinct membrane domains. Flanking each paranode is another region called the juxtaparanode. This domain is enriched in a different class of voltage-gated K^+ channels, the Kv1 family of channels. Like the Na^+ channels at nodes of Ranvier, they are recruited to juxtaparanodes through interactions with axonal cell adhesion molecules, cytoskeletal scaffolds, and glial cell adhesion molecules (Poliak et al., 2003). The majority of the length of the myelinated axon is internode. Although this domain is not enriched in voltage-gated ion channels, a unique set of cell adhesion molecules and cytoskeletal proteins is also found in this region and these are organized by the overlying myelin sheath. Thus, myelin regulates the polarized organization of the axonal membrane. Figure 10-10 shows an example of a node of Ranvier labeled with antibodies to illustrate nodal, paranodal, and juxtaparanodal domains of myelinated axons.

Another one of the effects of myelin is to increase axonal diameter by inducing biochemical changes in components of the axonal cytoskeleton such as neurofilaments (see Chapter 6). The effects of myelin on axonal structure imply that there are signaling mechanisms from myelin or myelin-forming glia to axons. A common theme, emerging from recent research on transgenic mice deficient in some of the myelin proteins described later in this chapter, is that, in addition to their roles in the structure of the myelin sheaths, several of them are necessary for the normal formation, maintenance and survival of the axons that are ensheathed.

CHARACTERISTIC COMPOSITION OF MYELIN

The composition of myelin is well characterized because it can be isolated in high yield and purity by subcellular fractionation

If CNS tissue is homogenized in media of low ionic strength, myelin peels off the axons and reforms in vesicles of the size range of nuclei and mitochondria. Because of their high lipid content, these myelin vesicles have the lowest intrinsic density of any membrane fraction of the nervous system. Procedures for isolation of myelin take advantage of both of these properties: large vesicle size and low density (Norton & Poduslo, 1973; Morell, 1984). Peripheral nerve myelin can be isolated by similar techniques, but especially vigorous homogenization conditions are required because of the large amounts of connective tissue and, sometimes, adipose tissue present in the nerve. The slightly lower density of PNS myelin requires some adjustment of gradient composition to prevent loss of myelin.

Myelin *in situ* has a water content of about 40%. The dry masses of both CNS and PNS myelin are characterized by a high percentage of lipid (70–85%) and, consequently, a low percentage of protein (15–30%). By comparison, most biological membranes have a higher ratio of proteins to lipids. The currently accepted view of membrane structure is that of a

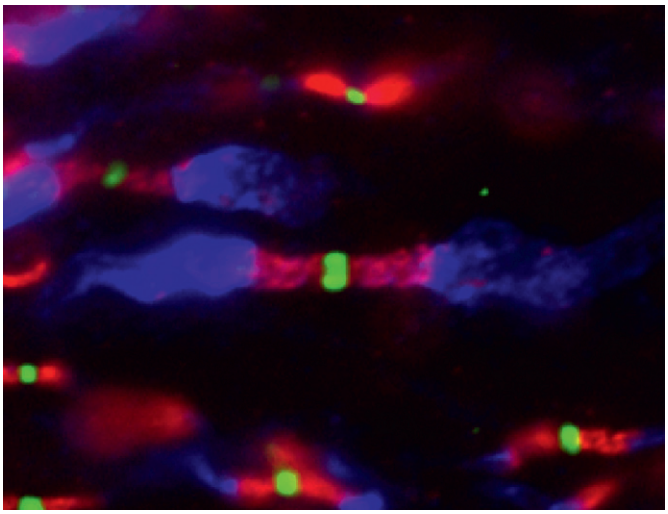


FIGURE 10-10 **A node of Ranvier from optic nerve, triple-labeled using antibodies against Na1 channels (green), Caspr (red), and Kv1.2 K^+ channels (blue) to define nodal, paranodal and juxtaparanodal domains, respectively.**

lipid bilayer with integral membrane proteins embedded in the bilayer and other extrinsic proteins attached to one surface or the other by weaker linkages. Proteins and lipids are asymmetrically distributed in this bilayer, with only partial asymmetry of the lipids. The proposed molecular architecture of the layered membranes of compact myelin fits such a concept (Figure 10-11). Models of compact myelin are based on data from electron microscopy, immunostaining, X-ray diffraction, surface-probe studies, structural abnormalities in mutant mice, correlations between structure and composition in various species, and predictions of protein structure from sequencing information (Kirschner & Blaurock, 1992).

Central nervous system myelin is enriched in certain lipids

Table 10-1 lists the composition of bovine, rat, and human myelin compared to bovine and human white matter, human gray matter, and rat whole brain (Morell, 1984) (see Chapter 5). While there are no absolutely "myelin-specific" lipids, cerebroside (galactosyl ceramide) is the one most typical of myelin. Other than during early development, the concentration of cerebroside in brain is directly proportional to the amount of myelin present. As much as one-fifth of the total galactolipid in myelin is sulfatide, in which the 3-hydroxyl moiety on the galactose of cerebroside is sulfated. Presumably, the glycolipids in myelin, as in other membranes, are preferentially localized on the extracellular membrane face at the intraperiod line. Because of the specificity and quantitative significance of galactocerebroside in oligodendrocytes and myelin, it had long been thought that it would be essential for the formation and maintenance of myelin, but in fact it is not. A UDP–galactose:ceramide galactosyltransferase–null mouse was generated, eliminating the obligate terminal step in cerebroside biosynthesis and thereby additionally sulfatide formation (Marcus & Popko, 2002). Thus, these mice synthesize no cerebroside or sulfatide. Surprisingly, the myelin formed by these mice is relatively normal, although there are subtle structural alterations in the myelin sheaths and neurological abnormalities, both of which become progressively more severe with age. Particularly severe defects occur in the CNS paranodal loops, where glia–axon tight junctions are located. Abnormalities in the PNS of these knockout mice are much less severe. Mice lacking the sulfotransferase that converts cerebroside to sulfatide exhibited similar paranodal disorganization in the CNS, indicating that sulfatide is important for establishing the normal oligodendroglial–axon interactions in the paranodal region (Marcus & Popko, 2002; Taylor et al., 2004). The lack of sulfatide also results in abnormal distribution of Na^+ and K^+ channels in the paranodal and nodal regions of myelinated axons. In addition to their role in myelin itself, experiments with cultured oligodendrocytes have demonstrated that both galactocerebroside and sulfatide also have important functions in the differentiation of oligodendrocytes, with sulfatide being particularly important (Taylor et al., 2004).

In addition to cerebroside/sulfatide, the major lipids of myelin are cholesterol and phospholipids (Morell, 1984). On a molar basis, CNS myelin preparations contain cholesterol, phospholipid and galactolipid in a ratio varying between 4:3:2 and 4:2:2. Thus, myelin contains substantially more molecules of cholesterol than any other single lipid, although on the basis of weight the content of galactolipids is comparable and total phospholipids are most abundant (Table 10-1). A characteristic phospholipid, and the single most prominent one, is ethanolamine-containing plasmalogen (glycerophospholipid containing an alkenyl ether bond—see Chapter 5). Lecithin phosphatidylcholine (PtdCho; 'lecithin') is also a major myelin constituent, and sphingomyelin is a relatively minor one. Cholesterol is enriched on the extracellular face of the myelin membrane, whereas ethanolamine plasmalogen is asymmetrically localized to the cytoplasmic half of the bilayer. Not only is the lipid class composition of myelin highly characteristic of this membrane, the fatty acid composition of many of the individual lipids is distinctive.

The data in Table 10-1 indicate that myelin accounts for much of the total lipid of white matter, and that the lipid composition of gray matter is quite different from that of myelin. The composition of brain myelin from all mammalian species studied is very much the same. There are, however, some species differences; e.g., myelin of rat has less sphingomyelin than does that of bovine or human (Table 10-1). Although not shown in the table, there are also regional variations; for example, myelin isolated from the spinal cord has a higher lipid-to-protein ratio than brain myelin from the same species.

In addition to the lipids of CNS myelin listed in Table 10-1, there are some other minor lipids including polyphosphoinositides (see Chapter 5) that account for between 5 to 8% of the total myelin phosphorus, some fatty acid esters of galactocerebroside, and two galactosyldiglycerides (Morell, 1984). Myelin from mammals also contains 0.1 to 0.3%

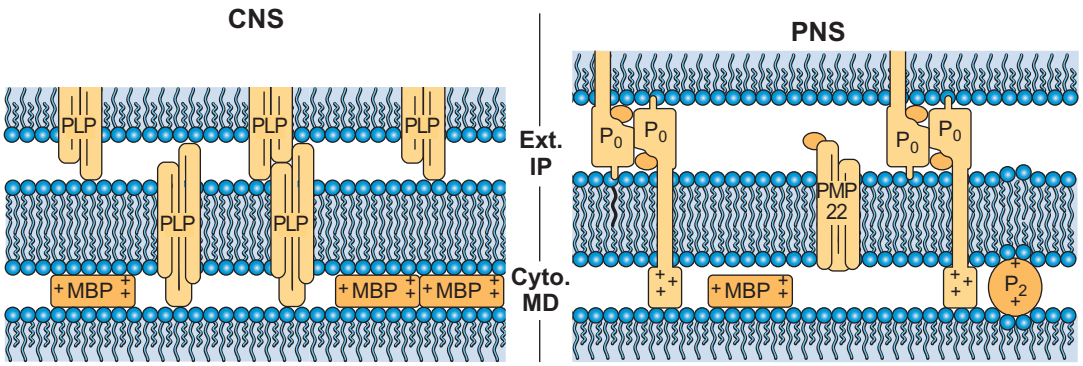


FIGURE 10-11 **Diagrammatic representation of current concepts of the molecular organization of compact CNS and PNS myelin.** The apposition of the extracellular (Ext.) surfaces of the oligodendrocyte or Schwann cell membranes to form the intraperiod (IP) line is shown in the upper part of the figure. The apposition of the cytoplasmic (Cyto.) surfaces of the membranes of the myelin-forming cells to form the major dense (MD) line is shown in the lower part of the figure. The width of the lipid bilayers and the spacing of the intraperiod and major dense lines in this figure are proportional to those determined by X-ray diffraction (Kirschner & Blaurock, 1992). See the text for a detailed description of this model. The dark orange structures on P_0 and PMP represent the single oligosaccharide moieties on each protein. The blip at the apex of P_0 represents the tryptophan residue, which X-ray analysis suggests may interact with the opposing bilayer, but for purposes of simplicity we have not shown the expected tetramerization of P_0. Although PLP molecules may exhibit homophilic interactions as suggested at one position in the figure, there is no strong experimental evidence to support this as in the case of P_0. These diagrams do not include CNP, MAG and other quantitatively minor proteins of isolated myelin, because they probably do not play a major structural role in most of the compact myelin. In fact, many of them are localized selectively in regions of myelin sheaths distinct from the compact myelin.

TABLE 10-1 **Composition of CNS Myelin and Brain**

	Myelin			White matter			
Substance[a]	**Human**	**Bovine**	**Rat**	**Human**	**Bovine**	**Gray matter (human)**	**Whole brain (rat)**
Protein	30.0	24.7	29.5	39.0	39.5	55.3	56.9
Lipid	70.0	75.3	70.5	54.9	55.0	32.7	37.0
Cholesterol	27.7	28.1	27.3	27.5	23.6	22.0	23.0
Galactocerebroside	22.7	24.0	23.7	19.8	22.5	5.4	14.6
Sulfatide	3.8	3.6	7.1	5.4	5.0	1.7	4.8
Total galactolipid	27.5	29.3	31.5	26.4	28.6	7.3	21.3
Ethanolamine phosphatides	15.6	17.4	16.7	14.9	13.6	22.7	19.8
Phosphatidyl choline	11.2	10.9	11.3	12.8	12.9	26.7	22.0
Sphingomyelin	7.9	7.1	3.2	7.7	6.7	6.9	3.8
Phosphatidylserine	4.8	6.5	7.0	7.9	11.4	8.7	7.2
Phosphatidylinositol	0.6	0.8	1.2	0.9	0.9	2.7	2.4
Plasmalogens[b]	12.3	14.1	14.1	11.2	12.2	8.8	11.6
Total phospholipid	43.1	43.0	44.0	45.9	46.3	69.5	57.6

[a]Protein and lipid figures in percent dry weight; all others in percent total lipid weight.
[b]Plasmalogens are primarily ethanolamine phosphatides.

ganglioside (complex sialic acid–containing glycosphingolipids). The major ganglioside in CNS myelin is a monosialoganglioside (GM1), and there are very low amounts of the polysialogangliosides characteristic of neuronal membranes. Myelin from certain species (including human) contains an additional novel ganglioside as a major component: sialosylgalactosylceramide (GM4).

Peripheral and central nervous system myelin lipids are qualitatively similar

However, there are quantitative differences. PNS myelin has less cerebroside and sulfatide and considerably more sphingomyelin than CNS myelin. Of interest is the presence of the LM1 ganglioside, sialosyl-lactoneotetraosylceramide, as

a characteristic component of myelin in the PNS of some species. These differences in lipid composition between CNS and PNS myelin are not, however, as dramatic as the differences in protein composition discussed below.

Central nervous system myelin contains some unique proteins

The protein composition of CNS myelin is simpler than that of other brain membranes, with the myelin basic protein (MBP) and proteolipid protein (PLP) making up 60 to 80% of the total in most species. Many other proteins and glycoproteins are present to lesser extents. With the exception of MBP, myelin proteins are neither easily extractable nor soluble in aqueous media. However, like other membrane proteins, they may be solubilized in sodium dodecylsulfate solutions and, in this condition, can be separated readily by electrophoresis in polyacrylamide gels. This technique separates proteins primarily according to their molecular weight (a common notation convention is Mr for relative molecular mass; another is stating molecular weight in kiloDaltons, abbreviated kDa). The presence of bound carbohydrates or unusual structural features distort somewhat the relationship between electrophoretic migration and molecular weight, so that terminology for location of a protein in such a gel is taken to mean "apparent" molecular weight. The protein compositions of human and rat brain myelin are illustrated in Figure 13B and 13D, respectively. The quantitative predominance of two proteins in human CNS myelin is clear, i.e., MBP and PLP. These two proteins are major constituents of all mammalian CNS myelin membranes, and similar proteins are present in myelin membranes of many lower species. The overall orientation of these two proteins in compact CNS myelin is depicted in Figure 10-11.

Proteolipid protein

Myelin PLP, also known as the Folch-Lees protein (Greer & Lees, 2002; Hudson, 2004), has the unusual physical property of solubility in organic solvents. The molecular mass of PLP is about 30,000, although it migrates anomalously on SDS gels and gives a lower apparent molecular mass. The amino acid sequence, strongly conserved during evolution, contains four membrane-spanning domains, and PLP is described as one of the tetraspan proteins. Both the N- and C-termini are on the cytoplasmic side as shown in Figure 10-11. An important role for PLP in stabilizing the intraperiod line has generally been assumed, based largely on the extracellular loops of this protein being present at this location. Furthermore, the intraperiod line is abnormally condensed both in the PLP knockout mouse (Campagnoni & Skoff, 2001) and in spontaneously occurring PLP mutants (see Chapter 31), confirming a structural role for PLP in determining the membrane spacing at the intraperiod line. PLP has an alternatively spliced isoform, DM20 (Mr = 20,000), which is present in CNS myelin at lower concentration than PLP (Fig. 10-12). DM20 has similar physical properties to PLP and is identical in sequence, except for a deletion of 35 amino acids in the intracellular domain (Greer & Lees, 2002; Hudson, 2004). PLP/DM20 contains about 3 moles of fatty acids (primarily palmitate, oleate or stearate) per mole of protein in ester linkage at several cysteines. There is rapid turnover of the fatty acids independent of the peptide backbone.

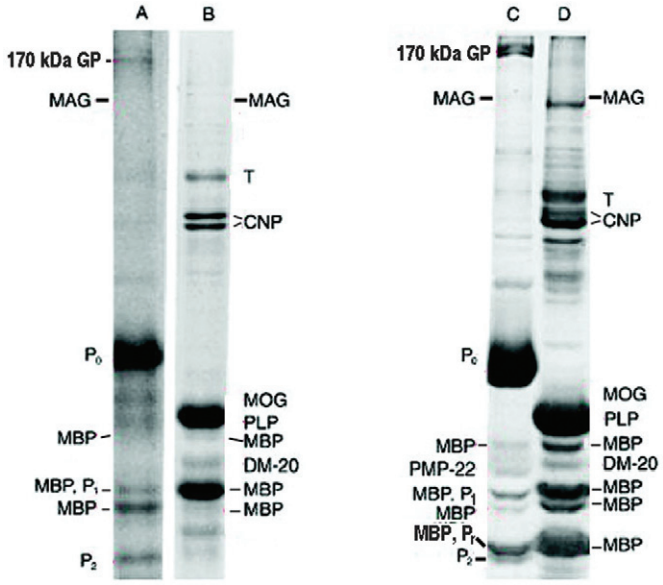


FIGURE 10-12 **Polyacrylamide gel electrophoresis of myelin proteins in the presence of sodium dodecyl sulfate (SDS).** The proteins of human PNS myelin (A), human CNS myelin (B), rat PNS myelin (C) and rat CNS myelin (D) were solubilized with the detergent SDS, electrophoresed and stained with Coomassie Brilliant Blue. The electrophoretic system separates proteins primarily according to their molecular size with the smallest proteins migrating the farthest toward the bottom of the gel. Abbreviations for the proteins are the same as in the text or as defined below. The three MBP bands in lanes A and B are the 17.2, 18.5, and 21.5 kDa isoforms generated by alternative splicing of the mRNA in humans, and the four MBP bands in lanes C and D are the 14.0, 17.0, 18.5, and 21.5 kDa isoforms generated in rats (see Fig. 10-13). The 18.5 kDa MBP and the 14 kDa MBP are also called P_1 and P_t, respectively, in the terminology for the PNS. The 26 kDa MOG is probably the faint band just above PLP that is most apparent in lane D. CNP electrophoresis as a tight doublet, and the lower and upper bands are sometimes referred to as CNP1 and CNP2, respectively. Note that the location shown for MAG (which stains too faintly to be seen well on the gels) is just above a discrete Coomassie blue–stained band in lane D, which is probably the 96 kDa subunit of Na^+, K^+ -ATPase. T, tubulin. 170 kDa GP, 170 kDa glycoprotein.

The PLP gene is expressed very early in development, and in fact DM20 mRNA appears earlier than PLP during development, even before myelin formation in embryos and in premyelinating oligodendrocytes (Hudson, 2004). It is thought that it might have a role in oligodendrocyte migration or differentiation in addition to a structural role in myelin. The PLP/DM20 gene may have evolved from an ancestral gene encoding a pore-forming polypeptide, lending support to the hypothesis that myelin may be involved in ion movement. Although PLP and DM20 serve important functions, they are not essential. Contrary to the general expectation that PLP would be needed for formation of compact, multilamellar myelin, a knockout mouse for PLP/DM20 is initially relatively normal with respect to myelin formation (except for the difference in the intraperiod line spacing), life span, and

motor performance (Hudson, 2004). This suggests that other proteins or lipids of myelin may contribute to adherence of the extracellular faces of the bilayers at the intraperiod line. On the other hand, myelin in the PLP-null mutant is extra sensitive to osmotic shock during fixation, suggesting that PLP does enhance the stability of myelin, possibly by forming a "zipper-like" structure after it is compacted. Furthermore, in older PLP/DM20 knockout mice, there is significant axonal degeneration, suggesting that while myelin can form in the absence of PLP/DM20, myelin devoid of PLP/DM20 cannot sustain normal axonal function. Despite the apparent similarity of the PLP and DM20, DM20 cannot replace PLP in transgenic mice (Stecca et al., 2000), i.e., the same long-term axonal degeneration occurs in mice expressing exclusively DM20 protein. This may be because PLP uniquely interacts both with inositol hexakisphosphate (Yamaguchi et al., 1996), a molecule involved in vesicle transport, and with integrins, modulating interaction with the extracellular matrix (Gudz et al., 2002). Thus, PLP has selective and apparently important functions in the CNS, relative to DM20. While the loss of PLP/DM20 has clear neuropathological consequences in older animals, the loss of these proteins is significantly less serious than expression of mutated or excess PLP/DM20. A variety of naturally occurring rodent and human mutations in PLP (see Chapters 31 and 39), which produce either mutated protein that cannot fold correctly or simply increased amounts of normal PLP (Hudson, 2004), have severe functional consequences, including early death, apparently due to cellular toxicity of misfolded PLP/DM20 or excess amounts of normal PLP/DM20.

While PLP/DM20 expression is highest in oligodendrocytes in the CNS, PLP/DM20 mRNA is also expressed in myelinating Schwann cells in the PNS (Hudson, 2004), where small amounts of protein are synthesized although not incorporated into myelin in appreciable amounts. It is also expressed in nonmyelinating Schwann cells of the PNS. The levels of PLP and DM20 mRNA are differentially regulated in myelinating and nonmyelinating Schwann cells, with DM20 mRNA being expressed more in nonmyelinating Schwann cells and PLP mRNA being expressed more in myelinating Schwann cells. In addition to expression in the CNS and PNS, low levels of DM20 expression have been found in thymus and heart (Campagnoni & Skoff, 2001), again suggesting that this protein has unique functions unrelated to formation and maintenance of compact myelin. Furthermore, a novel alternatively spliced form of the protein that is soluble has recently been identified in neurons and oligodendrocytes (Campagnoni & Skoff, 2001). This protein may have yet other functions.

Myelin basic proteins

The major basic protein (MBP) of myelin has long been of interest because it was the initial myelin antigen that, when injected into an animal, elicited a cellular immune response that produced the CNS autoimmune disease called *experimental allergic encephalomyelitis* (EAE, see Chapter 39). MBP can be extracted with either dilute acid or salt solutions from myelin as well as from white matter; once extracted, it is very soluble in water. The MBP genes from a number of species are highly conserved, and as with the PLP gene, the MBP gene is alternatively spliced (Aruga et al., 1991; Campagnoni & Skoff, 2001; Campagnoni & Campagnoni, 2004). The classical MBP gene has seven exons, with the full length MBP (21,500 M_r) containing all seven exons, although this protein is one of the minor MBP proteins in myelin. Exons 2, 5B and 6 are present or absent in four other MBP proteins found in myelin. The most abundant MBP in human myelin contains exons 1B, 3, 4, 6 and 7 (18.5 kDa MBP, in box), whereas in rodent myelin both the 18.5 kDa MBP and a 14 kDa MBP containing exons 1B, 3, 4, 5 and 7 are the most abundant (in box). Two different minor MBPs of approximately 17 kDa exist, which are encoded by exons 1B, 2, 3, 4, 5B and 7 or 1B, 3, 4, 6 and 7, respectively. A diagrammatic representation of some of these alternative splicing schemes is presented (Fig. 10-13). The ratio of the MBPs changes with development, with more 14 kDa MBP found in mature rodent tissue. In immature oligodendrocytes, the MBP mRNA is localized in the cell body. However, as the cell matures, the MBP mRNA is localized in the myelin processes, far from the cell body, presumably because newly translated MBP associates rapidly with membranes at its site of synthesis (Trapp et al., 2004).

The MBPs are extrinsic proteins localized exclusively at the cytoplasmic surface in the major dense line (Fig. 10-11), a conclusion based on their amino acid sequence, inaccessibility to surface probes, and direct localization at the electron microscope level by immunocytochemistry. There is evidence to suggest that MBP forms dimers, and it is believed to be the principal protein stabilizing the major dense line of CNS myelin, possibly by interacting with negatively charged lipids. Failure of compaction of the major dense line in MBP-deficient *shiverer* mutants supports this hypothesis (see Chapter 31).

The MBPs are highly unfolded in solution, with essentially no tertiary structure. They show microheterogeneity upon electrophoresis in alkaline conditions. This is due to a combination of phosphorylation, loss of the C-terminal arginine, and deamidation. There is also heterogeneity in the degree of deimination of arginine to citrulline and of methylation of an arginine at residue 106. The rapid turnover of the phosphate groups present on many of the MBP molecules (Eichberg & Iyer, 1996) suggests this post-translational modification might influence the close apposition of the cytoplasmic faces of the membrane. (Whether phosphorylation modifies this process in a dynamic manner is a topic of speculation.) The physiological significance of the heterogeneity of MBPs, which results from alternative splicing and from unique post-translational modifications, is an open question.

Intriguingly, the classical MBP gene is actually part of a larger gene, golli (**g**ene of the **oli**godendrocyte **li**neage), which is greater than 100 kb in length (Campagnoni & Campagnoni, 2004). This gene has three transcription start sites, two of which are used to transcribe the MBP mRNAs, while the most 5′ transcription start site generates golli mRNAs (Fig. 10-13). Transcripts from this upstream promoter are expressed more ubiquitously than MBP mRNAs. Thus, they are expressed in neurons and oligodendrocytes in the nervous system and in T cells in the immune system. Most interestingly from an evolutionary perspective, the golli proteins contain a 133 amino acid domain that contains both unique golli sequences and

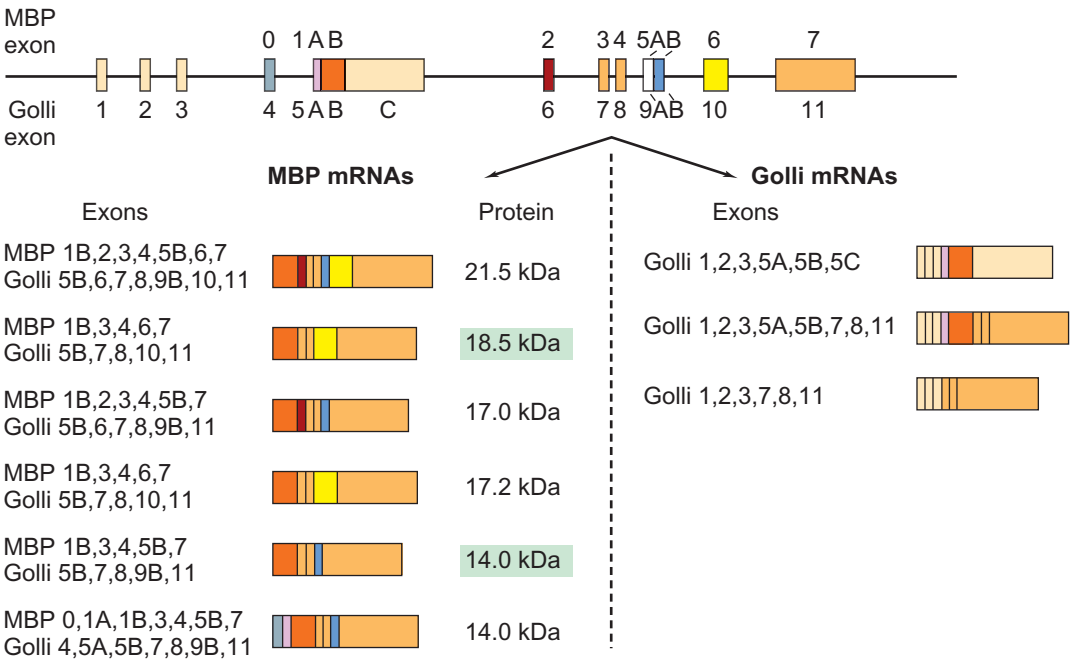


FIGURE 10-13 **The amino acid sequences corresponding to the various mouse MBPs are encoded in a gene containing at least 11 exons (separated by introns—DNA regions whose base sequence does not code directly for proteins).** This gene is depicted here, but the sizes of the exons and introns are not accurately represented. The exons are depicted in boxes with the original MBP exon numbering above, and the golli/MBP exon numbering below. Some of the introns are over 100,000 bases in length, and could not be shown accurately here. This gene can be spliced into two sets of mRNAs: the MBP mRNAs and the golli mRNAs. The MBP exons can be spliced to give an mRNA containing the original 7 MBP exons, which are exons 5B, 6,7,8,9B, 10 and 11 of the golli/MBP gene; this mRNA encodes the 21.5 kDa MBP. Alternative MBP mRNA splicings result primarily in mRNA species with deletions of MBP exons 2 (red) and/or 6 (yellow) (golli exons 6 and/or ,10), which encode the other MBPs, although in humans, elimination of MBP exons 2 and 5B (blue) (golli exons 6 and 9B)can generate a 17.2 kDa MBP. A unique MBP mRNA (M41) encoding a 14 kDa MBP (bottom) was identified in which a novel MBP transcription site was used (exon 0/4, black), and MBP exons 1A and 1B (golli exons 5A and 5B). Additionally, a unique MBP sequence upstream of the classical MBP exon 5 was identified (exon 5A/9A diagonal lines), which may be spliced into some MBP mRNAs, although the full sequence of these mRNAs has not been determined (Aruga et al., 1991). The exons forming the various MBP mRNA species and proteins are indicated. There are three well-characterized golli mRNAs (BG21, J37 and TP8) (Campagnoni & Campagnoni, 2004), which are transcribed from golli exon 1, and which may or may not contain exons from the MBP mRNAs (horizontal lines). This figure is adapted from one published in Campagnoni & Campagnoni (2004).

classic MBP sequences. The golli proteins are expressed during embryonic development and in postnatal tissue, and the proteins are found in multiple subcellular localizations, including nuclei, cytoplasm and cellular processes. Their function is not yet understood, although there is the suggestion that they may be involved in process extension in neural cells (Campagnoni & Skoff, 2001; Campagnoni & Campagnoni, 2004).

2′:3′-cyclic nucleotide 3′-phosphodiesterase

In addition to PLP and MBP, there are many higher–molecular weight proteins present in myelin (Fig. 10-12). These vary in amount depending on species (rodents generally have more than larger mammals) and age (immature myelin has more than mature). A doublet with M_r ~46 kDa and 48 kDa is present in CNS myelin, which comprises several percent of total myelin protein and has the enzyme activity, 2′:3′-cyclic nucleotide 3′ phosphodiesterase (CNP) (Braun et al., 2004). Although there are low levels of CNP associated with other cell types, it is greatly enriched in CNS myelin and oligodendrocytes, for which it is a commonly used biochemical marker. It is expressed at a much lower concentration in Schwann cells at the onset of myelination, and does not increase during development with the accumulation of myelin as in the CNS. The enzyme is extremely active with the substrate 2′, 3′-cAMP, as well as cGMP, cCMP and cUMP analogs, which are all hydrolyzed to the corresponding 2′-isomer. This may be a non-physiological activity, because only the 3′:5′ cyclic nucleotides have been shown to have biological activity. Evolutionary conservation of the catalytic site indicates that its amino acid sequence likely has an important function, although the precise role of CNP has remained elusive over the many years since it was discovered. Two CNP polypeptides are generated by alternative splicing of the mRNA, with the larger polypeptide having 20 extra amino acids at the N-terminus. Immunocytochemistry demonstrates that CNP is not a major component of compact myelin, but is concentrated in specific regions of the myelin sheaths associated with cytoplasm, such as the oligodendroglial processes, inner and outer tongue processes, and lateral loops. The protein is in the cytoplasm, but much of it associates with membranes, because both isoforms are isoprenylated at the C-terminus

and acylated. Some clues about its function have come from reports that it binds to cytoskeletal elements such as F-actin and tubulin and that overexpression in cultured non-neural cells promotes outgrowth of processes. Such findings suggest that its function may be in regulation of cytoskeletal dynamics to promote process outgrowth and differentiation in oligodendrocytes. Aberrant myelination occurring *in vivo* in transgenic mice overexpressing CNP similarly suggests that it could be an early regulator of cellular events that culminate in CNS myelination. However, it is also important to note that the amino acid sequence of CNP puts it in a superfamily of RNA processing enzymes whose physiological roles are unclear, and the relevance of this to oligodendrocytes and myelination is unclear. An interesting possibility combining some of the above information is that it could be involved in some specialized aspects of RNA transport and/or processing in oligodendrocytes. Most puzzling of all is the phenotype displayed by the recently generated CNP-null mice, which appear to myelinate entirely normally, but as adults exhibit axonal swelling, neurodegeneration and premature death. It has been speculated that CNP is a multifunctional protein with an initial role in oligodendroglial differentiation that can be compensated for by another protein, and a second function essential for the normal interaction of oligodendrocytes with axons, thus leading to axonal degeneration in its absence (Braun et al., 2004). Clearly, more research is needed to fully understand the functions of this intriguing myelin/oligodendrocyte–related protein.

Myelin-associated glycoprotein (MAG) and other glycoproteins of CNS myelin

The myelin-associated glycoprotein (MAG) is a quantitatively minor 100 kDa glycoprotein in purified CNS and PNS myelin (Quarles, 2002; Georgiou et al., 2004) that electrophoreses at the position shown in Figure 10-12. However, because of its small amount (<1% of total protein) and weak staining by Coomassie blue, it does not correspond to one of the discrete protein bands visible in the figure. MAG has a single transmembrane domain that separates a heavily glycosylated extracellular part of the molecule, composed of five Ig-like domains and eight or nine sites for N-linked glycosylation, from an intracellular carboxy-terminal domain. Its overall structure is similar to that of neural-cell adhesion molecule (N-CAM). MAG in rodents occurs in two developmentally regulated isoforms, which differ in their cytoplasmic domains and are generated by alternative splicing of its mRNA. The isoform with a longer C-terminal tail (L-MAG) predominates early in development during active myelination of the CNS, whereas the isoform with a shorter cytoplasmic tail (S-MAG) increases during development to become prominent in adult rodents.

MAG is not present in compact, multilamellar myelin, but is located in the periaxonal glial membranes of myelin sheaths. This location next to the axon and its membership in the Ig superfamily (see Chapter 9) suggest that it functions in adhesion and signaling between myelin-forming cells and the axolemma. Indeed, substantial evidence has now accumulated that MAG is involved in signaling in both directions between glia and axons, although its most important functions appear to be different in the CNS and the PNS. MAG is in the "siglec" [**s**ialic acid–binding **i**mmuno**g**lobulin-like **lec**tins] subgroup of the Ig superfamily and binds to glycoproteins and gangliosides with terminal α2-3 linked sialic acid moieties. Thus, some of the axolemmal binding partner(s) for MAG are likely to be sialoglycoconjugates. A relationship of MAG to other adhesion proteins also is demonstrated by the presence in most species of a sulfate-containing epitope in its oligosaccharide moieties that reacts with the HNK-1 monoclonal antibody. The carbohydrate HNK-1 epitope is expressed on many neural adhesion proteins, including N-CAM and MAG, and has been shown to function in cell–cell interactions.

MAG had long been thought to function in important signaling mechanisms from axons to oligodendrocytes during myelination. However, it is now known that MAG is not essential for myelin formation because MAG-null mice myelinate relatively normally. Nevertheless, in the CNS, these knockouts exhibit a significant delay of myelination, periaxonal and paranodal structural abnormalities, redundant myelin loops, and supernumerary myelin sheaths. In addition, there is degeneration of periaxonal oligodendroglial processes in aging MAG-null mice, suggesting the occurrence of a "dying-back oligodendrogliopathy." Therefore, the absence of MAG causes oligodendrocytes to form myelin less efficiently during development and become dystrophic with aging. Furthermore, although the neurological deficit in MAG-null mice is mild, double knockouts in which the absence of MAG is combined with the genetic ablation of other proteins results in more severe CNS phenotypes than either knockout alone. These *in vivo* findings suggest that MAG-mediated signaling from axons to oligodendrocytes is needed for efficient myelination and maintenance of healthy mature oligodendroglia. As with other proteins in the Ig superfamily, it is likely that the interaction of MAG with its ligand(s) on the axolemma mediates cell-cell signaling by mechanisms involving phosphorylation. The cytoplasmic domains of MAG are phosphorylated on serine and theonine residues by protein kinase C, and L-MAG is also phosphorylated on tyrosine-620. Furthermore, the cytoplasmic domain of L-MAG has been shown to interact with fyn tyrosine kinase, phospholipase Cγ and other oligodendroglial proteins. The L-MAG isoform appears to be particularly important for CNS myelination, because genetically engineered mice lacking only the L-isoform exhibit the same CNS abnormalities as total knockouts, but not the PNS pathology of the total knockouts described below.

There are a large number of other glycoproteins associated with white matter and myelin, and a few in addition to MAG that have been cloned and characterized. One of these is a minor 26 kDa protein called the myelin-oligodendrocyte glycoprotein (MOG) (Pham-Dinh et al., 2004). MOG is also a transmembrane glycoprotein, contains a single Ig-like domain and one site for N-linked glycosylation, and expresses the adhesion-related HNK-1 epitope. Unlike MAG, which is sequestered at the interior of CNS myelin sheaths, MOG is localized on the outside surface of myelin sheaths and oligodendrocytes, apparently directed by a basolateral membrane-targeting signal in its cytoplasmic domain. Consistent with

its surface localization, MOG has been implicated as a target antigen in autoimmune aspects of demyelinating diseases of the CNS and is a leading candidate to be an important antigen in multiple sclerosis (see Chapter 39). Its surface location also suggests that it may function in signal transduction, transmitting information from the extracellular matrix or adjacent myelin sheaths to oligodendrocytes. This role is further suggested by changes in cultured oligodendrocytes when MOG is cross-linked on the cell surface with anti-MOG antibodies (Taylor et al., 2004). However, its physiological function remains obscure, because the recent generation of MOG-null mice yielded an apparently normal phenotype.

Another glycoprotein with a similar name to MOG is the oligodendrocyte-myelin glycoprotein (OMgp) (Mikol et al., 1993; Quarles, 2002). It was first characterized as a 120 kDa phosphatidylinositol-linked glycoprotein in human white matter and subsequently cloned. It is not a member of the Ig superfamily, but is characterized by a cysteine-rich motif at the N-terminus, a series of tandem leucine-rich repeats, and the HNK-1 epitope. These properties suggest that it may function in cell–cell interactions. However, unlike MAG and MOG, it is not specific to myelin-forming cells and is also expressed in neurons. It has attracted substantial interest in recent years because it is one of the myelin-associated inhibitors of axonal regeneration (see below), but its function with regard to myelination is unclear at this time.

Peripheral myelin also contains unique proteins

P_0 glycoprotein

Gel electrophoretic analysis (Figure 12 A, C) shows that a single 30 kDa protein, P_0, accounts for more than half of the PNS myelin protein. P_0 is a type 1 membrane glycoprotein containing about 220 amino acids after removal of its signal sequence. Rat P_0 contains a single extracellular Ig-like domain of 124 amino acids, a hydrophobic transmembrane domain of 26 amino acids and an intracellular domain of 69 amino acids (Quarles, 2002; Kirschner et al., 2004). The amino-terminal extracellular domain has a single site for N-linked glycosylation, and the glycans at that site are very heterogeneous, with many containing sialic acid and sulfate. In addition to glycosylation, other posttranslational modifications of P_0 include phosphorylation and acylation.

The principal difference in the overall protein composition of PNS and CNS myelin is that P_0 replaces PLP as the major protein, although myelin-forming Schwann cells do express very low levels of PLP. It is interesting to note that PLP and P_0 proteins, which are so different in sequence, posttranslational modifications, and membrane topology, may have similar roles in the formation of structures as closely related as myelin of the CNS and PNS, respectively. Expression of P_0 in transfected cells results in cell–cell interactions due to homophilic binding of its extracellular domains, suggesting that P_0 stabilizes the intraperiod line of PNS myelin by similar homophilic binding (Fig. 10-12). The relatively large, glycosylated, extracellular Ig-like domain of P_0 probably accounts for the greater separation of extracellular surfaces in PNS myelin relative to CNS myelin where closer apposition of these surfaces is possible in the presence of the smaller extracellular domains of PLP. Evidence reviewed suggests that homophilic interactions between P_0 molecules involve both protein-protein and protein-carbohydrate interactions (Kirschner et al., 2004). Furthermore, investigation of the crystal structure of the P_0 extracellular domain suggests that P_0 molecules cluster on each membrane surface as tetramers. The crystal structure also suggested that a tryptophan residue at the apex of the extracellular domain could interact directly with the lipid bilayer of the opposing membrane. P_0 protein also has a relatively large positively charged domain on the cytoplasmic side of the membrane that contributes significantly to stabilization of the major dense line in the PNS. The complete knockout of P_0 has profound consequences for myelin structure, in contradistinction to the previously noted, relatively benign CNS consequence of deletion of the PLP gene. P_0-null mice exhibit abnormal motor coordination, tremors, occasional convulsions, and severe hypomyelination with thin, non-compacted myelin sheaths.

Expression of the correct amount of P_0 is apparently essential for normal myelin formation and maintenance. Young mice heterozygous for the P_0-null mutation appear normal, but develop progressive demyelination with age, which resembles chronic inflammatory demyelinating neuropathy and may involve inflammatory mechanisms. Furthermore, transgenic mice overexpressing P_0 exhibit a dose-dependent dysmyelinating neuropathy ranging from transient hypomyelination to severe arrest of myelination and impaired sorting of axons. The critical dosage of P_0 required for normal myelin formation is similar to that observed with other myelin proteins and may reflect the necessity for appropriate amounts of myelin proteins to form stoichiometric complexes in compact myelin. However, in the case of P_0, the pathology that occurs with overexpression may also reflect a mistargeting of the protein and an interesting misuse of its obligate homophilic adhesive properties. Some of the extra P_0 is inappropriately located in normally dynamic mesaxonal membranes, causing them to adhere like compact myelin and halting myelination. It is clear that control of P_0 expression is complex, involving interactions with the axon and basal lamina, rate of cell division, inhibitory and stimulatory growth factors, cAMP levels and transcription factors. It also should be noted that low basal levels of P_0 are expressed in Schwann cells and neural crest cells early in embryonic development (well before myelination), which suggests that P_0 could have other functions, potentially involving Schwann cell–axon interactions and signal transduction. The cytoplasmic domain of P_0 is phosphorylated on serine and tyrosine residues, and this might be indicative of signaling mechanisms within Schwann cells during early development as well as later during myelination (Eichberg & Iyer, 1996).

Peripheral myelin protein-22

In addition to the major P_0 glycoprotein, compact PNS myelin contains a 22 kDa protein called peripheral myelin protein-22 (PMP-22) that accounts for less than 5% of the total protein (Fig. 10-12C) (Quarles, 2002; Suter, 2004). Similarly to P_0, PMP-22 has a single site for N-linked glycosylation.

However, unlike P_0, which is nerve specific, PMP-22 is expressed in many other tissues. It has four hydrophobic potential transmembrane domains and is a tetraspan protein like the major PLP of CNS myelin, but there is no sequence homology to PLP. It is in a highly homologous family of small hydrophobic tetraspan proteins that also include epithelial membrane proteins (EMP-1, -2 and -3). It is referred to as a "growth arrest protein" because its cDNA was first cloned from non-dividing fibroblasts, and the synthesis of PMP-22 and other myelin proteins ceases when Schwann cells begin to proliferate following nerve transection. Although the tetraspan PMP-22 is localized primarily in compact PNS myelin as shown in Figure 10-11, it is not known if its extracellular or cytoplasmic domains play an important structural role for myelin. The relatively small amount of PMP-22 and the fact that it is present in the plasma membranes of both myelinating and non-myelinating Schwann cells suggest that it may have a dynamic function in myelin assembly or maintenance rather than a major structural role. Its tetraspan structure, similar to that of PLP, suggests that one of its roles might be similar to one of the functions of PLP in CNS myelin. PMP-22 has been shown to form complexes with P_0, and this interaction with P_0 may be relevant to its function. Also, as is the case with P_0 and PLP, any significant deviation in gene dosage for PMP-22 or disruption caused by point mutations has severe functional consequences. Mutations of the PMP-22 gene cause the dysmyelinating phenotypes in *trembler* mice and several neuropathies in humans (see Chapter 38). In addition, the association of PMP-22 with growth arrest in Schwann cells and other cell types suggests that it may have an unknown role in regulation of growth or differentiation.

P_2 protein

PNS myelin contains a positively charged protein different from MBP that is referred to as P_2 (Mr: ~15,000). It is unrelated in sequence to MBP and is a member of a family of cytoplasmic fatty acid binding proteins (FABP) that are present in a variety of cell types (Martenson & Uyemura, 1992). The amount of P_2 protein is variable among species, accounting for about 15% of total protein in bovine PNS myelin, 5% in humans, and less than 1% in rodents. P_2 protein is generally considered a PNS myelin protein, but it is expressed in small amounts in CNS myelin sheaths of some species. P_2 is an antigen for experimental allergic neuritis, the PNS counterpart of EAE (see Chapters 38 and 39). P_2 appears to be present in the major dense line of myelin sheaths where it may play a structural role similar to MBP (Fig. 11). Interestingly, the larger amounts of P_2 protein that are in myelin of some species correlate with increased widths of the major dense lines as determined by X-ray diffraction, and there appears to be substantially more P_2 in large sheaths than small ones (Kirschner & Blaurock, 1992). The large variation in the amount and distribution of the protein from species to species and sheath to sheath raises so far unanswered questions about its function. Its similarities to cytoplasmic proteins in other cells, whose functions appear to involve solubilization and transport of fatty acids and retinoids, suggest that it might function similarly in myelin assembly or turnover, but there is currently no direct experimental evidence to support this hypothesis.

Some classically defined myelin proteins are common to both CNS and PNS myelin

Myelin basic protein

In PNS myelin, MBP varies from approximately 5 to 18% of total protein, in contrast to the CNS where it is close to 30% (Morell, 1984). In rodents, the same four 21, 18.5, 17 and 14 kDa MBPs found in the CNS are present in the PNS. In adult rodents, the 14 kDa MBP is the most prominent component and is termed P_r in the PNS nomenclature. The 18.5 kDa component is present and is often referred to as the P_1 protein in the nomenclature of peripheral myelin proteins. Another species-specific variation in human PNS is that the major basic protein is not the 18.5 kDa isoform that is most prominent in the CNS, but rather a form of about 17 kDa. It appears that MBP does not play as critical a role in myelin structure in the PNS as it does in the CNS. This is probably because the cytoplasmic domain of P_0 has an important role in stabilizing the major dense line of PNS myelin. This difference is illustrated in the *shiverer* mutant mouse, which expresses very little MBP (see Chapter 31), and a greatly reduced amount of CNS myelin, with no compaction of the major dense line. This contrasts with *shiverer* PNS, which has essentially normal myelin, both in amount and structure, despite the absence of MBP. On the other hand, animals doubly deficient for P_0 and MBP have a more severe defect in compaction of the major dense line than P_0-null mice, which indicates that both proteins contribute to compaction of the cytoplasmic surfaces in PNS myelin (Kirschner et al., 2004).

Myelin-associated glycoprotein

Similarly to the CNS, MAG is present in the periaxonal membranes of myelin-forming Schwann cells, but it is also present in the Schwann cell membranes of the Schmidt-Lanterman incisures, paranodal loops and the outer mesaxon (Quarles, 2002; Georgiou et al., 2004). Therefore, in addition to a role in Schwann cell–axon interactions, MAG may also function in interactions between adjacent Schwann cell membranes at these other locations in the PNS. Both isoforms of MAG are present in the rodent PNS, although S-MAG is the predominant isoform at all ages. PNS myelination in MAG-null mice is initially more normal than CNS myelination. However, as the mice age they develop a peripheral neuropathy characterized by degeneration of myelinated axons, which is the most severe phenotypic abnormality displayed by the knockout mice. The pathology is associated with decreased axonal caliber, increased neurofilament density, reduced expression and phosphorylation of neurofilaments and eventually axonal degeneration. These findings demonstrate an essential role for MAG in signaling from Schwann cells to axons that is necessary for the maintenance of normal myelinated axons in the PNS. Thus, MAG is another example of a myelin-related, glial protein whose absence has profound effects on the ensheathed axon.

In this regard, it is noteworthy that MAG is one of several neural proteins (also including Nogo and OMgp) that inhibit neurite outgrowth in tissue culture and axonal regeneration *in vivo* (see Chapter 32). This inhibitory activity has been

studied intensively in recent years, since it is extremely important for understanding factors that prevent axonal regeneration following neural injury (Filbin, 2003). This area of research has led to remarkable progress in identifying neuronal MAG receptors, and to the identification of a MAG-mediated signaling mechanism that affects neurons and also could be important for the normal maintenance of myelinated axons. Thus, a physiologically important signal promoting the stability of mature myelinated axons could be interpreted inappropriately by a plastic developing neurite *in vitro* or a regenerating neurite *in vivo*, thereby inhibiting its growth. The MAG receptor on neurites that transmits this inhibitory signal appears to be a complex localized in raft-like signaling domains that consists of gangliosides, the glycosylphosphatidylinositol-anchored Nogo receptor and the p75 neurotrophin receptor. This neuronal receptor complex involved in MAG's effects on neurite outgrowth is also likely to function within myelinated axons to promote axonal stability, but this remains to be established.

It is noteworthy that the axonal degeneration that occurs in the PNS of MAG-null mice is not observed in the CNS, possibly because other CNS myelin proteins enhance axonal stability. These could include PLP and/or CNP, both of which are needed for axonal stability in the CNS, where they are present in much higher concentration. In summary, it appears that MAG's most important function in the PNS is to transmit a signal from Schwann cells to axons that is needed for the stability of myelinated axons, whereas its principal function in the CNS is to transmit a signal in the reverse direction that promotes efficient myelination and oligodendrocyte vitality.

Myelin sheaths contain other proteins, some of which have only recently been established as myelin related

The proteins described above represent most of the well-established myelin proteins, which are myelin-specific or have been studied primarily in the context of myelin and demyelinating diseases. However, myelin sheaths contain numerous other proteins in smaller amounts that are also in many other cells and/or have only been identified relatively recently. Some of these are in compact myelin, but others are enriched in specialized structures within myelin sheaths that are distinct from compact myelin. Some of these proteins, which may be among the many minor bands seen on myelin protein gels (Fig. 10-12), are described here briefly.

Tetraspan proteins

Intriguingly, there are numerous tetraspan proteins (proteins containing four transmembrane spanning domains) in myelin and related glial membranes (Bronstein, 2000), including PLP/DM20; PMP-22; myelin and lymphocyte protein (MAL/MVP17/VIP17) and plasmolipin in compact myelin; and oligodendrocyte-specific protein (OSP)/claudin-11, CD9 and connexins in the specialized associated structures of the myelin sheaths, such as the tight junctions or the paranodal loops. The presence or absence of these proteins can be essential to the specialized structure and function of myelin. The paranodal loops, which form the tight junctions between glial processes and axons in the paranodal regions of the sheaths (see Figures 10-3 and 10-9), are crucial for normal firing of myelinated axons. Rapid saltatory conduction of nerve impulses in myelinated fibers is, thus, dependent on the structural integrity of nodes of Ranvier and of the tight junctions at this location, which prevent ion leaking into the internodes.

Other than PLP/DM20 and PMP22, one of the earliest myelin tetraspan proteins characterized was the 17kDa myelin and lymphocyte tetraspan protein (MAL). This protein was initially identified in compact myelin as MVP17, a novel myelin membrane protein (Kim et al., 1995), which was quickly demonstrated to be identical to MAL and VIP17. MAL (MVP17/VIP17) is part of the apical sorting machinery in non-neural polarized cells, and it has been proposed to be involved in protein sorting in myelin membrane domains. It associates with glycosphingolipid-enriched protein/lipid rafts and may function in their sorting and transport to myelin (Frank, 2000; Erne et al., 2002). MAL has been established to be part of an extended gene family, which includes plasmolipin, another myelin tetraspan protein (Magyar et al., 1997). Plasmolipin is also associated with glycosphingolipid-enriched membrane domains from myelin. Thus, this family of proteins, two of which are found in compact myelin, may be involved in sorting of proteins or in signal transduction through lipid rafts in myelin.

Many of the other myelin tetraspan proteins are localized in specialized myelin structures. Claudins, found in many tissues, comprise a family of tight junction proteins that form barriers to the diffusion of solutes between adjacent cells. Tight junctions in the paranodal regions of the PNS that act as barriers for diffusion of small ions involve the tetraspan claudin-5 (Scherer et al., 2004). OSP is found in the radial component of myelin; it was initially identified by differential screening as a novel tetraspan protein found in oligodendrocytes, but it was quickly established also to be a member of the claudin family, i.e., claudin-11 (Gow, 2004). The radial component is a specialized ultrastructural feature in CNS myelin, but not PNS myelin, which appears as lines of tight junctions with reduced spacing between extracellular leaflets. These lines of tight junctions extend in spiraled fashion across the whole thickness of CNS myelin sheaths from one paranodal region to the other. The CNS myelin tight junctions between adjacent layers of spiraled membranes probably contribute stability and, most importantly, act as a barrier to the diffusion of ions that is essential for the normal electrophysiological function of myelinated axons. The principal protein component of these tight junctions is OSP/claudin-11 (Gow, 2004), and in OSP/claudin-11 null mice, these tight junctions are missing from CNS myelin. Thus, OSP/claudin-11 is essential for formation of the radial component in CNS myelin. In addition to its role in formation of tight junctions, OSP/claudin-11 is also involved in oligodendrocyte migration, possibly through its interactions with OSP/claudin-11 associated protein (OAP)1 and beta1 integrin (Gow, 2004).

CD9 is a well-characterized hematopoietic tetraspan protein that has been shown to be present in CNS and PNS myelin, although it is present at higher levels in PNS myelin. In other cells, it is involved in integrin signaling and cell adhesion and motility. It is expressed at late stages of myelination and in the CNS is primarily found in paranodal junctions

(Ishibashi et al., 2004). While compact CNS myelin is apparently normal in CD9-null animals, the paranodal loops are often disconnected from axonal membranes, and the transverse bands of the paranodal loops are lost. In the PNS, in addition to altered paranodes, hypermyelination occurs. Thus, this tetraspan protein appears to act primarily at paranodes, where it is crucial for normal paranodal junctions.

Another type of membrane contact in the paranodal regions is gap junctions, which provide a radial pathway for diffusion of small molecules across the lateral loops. In particular the tetraspan proteins connexin32 and connexin29 are found in myelin (Scherer & Paul, 2004), predominantly in noncompact domains of myelin, including paranodes and Schmidt-Lanterman incisures. While gap junctions typically form between adjacent cells, in myelin they form between adjacent layers of membrane. In Schwann cells, functional gap junctions provide a radial pathway of interconnection throughout the myelin. It has been proposed that this radial pathway through the myelin mediates spatial buffering of extracellular potassium during action potential activity as well as communication from the adaxonal domain of myelin to the cell body. Similar radial pathways containing gap junctions likely also exist in CNS myelin. Connexin32 mutations are associated with the peripheral neuropathy Charcot-Marie-Tooth disease (see Chapter 38), but, interestingly, have little CNS pathology.

Nodal, paranodal, and juxtaparanodal proteins

As described above, the axonal and glial membranes along a myelinated axon demonstrate an exquisite division into highly specialized domains, whose biochemical structures are currently a very active subject of research. It is clear that the correct positioning of proteins in the axonal membrane is critical for proper function. For example, sodium channel clustering at the nodes and not in the internodes is necessary for generating action potentials. In the PNS, glia-derived nodal proteins include gliomedin and NrCAM, both of which are produced by Schwann cells, cleaved from the cell surface, and incorporated into the nodal extracellular matrix (Eshed et al., 2007). These proteins interact with axonal neurofascin-186 to initiate Na^+ channel clustering. In the CNS, an analogous nodal matrix also exists and comprises the chondroitin sulfate proteoglycans brevican and versican (Hedstrom et al., 2007). Whether these extracellular matrix molecules function like gliomedin in the PNS remains unknown.

At paranodes, an important *trans* interaction occurs between neurofascin-155 on the glial membrane and contactin/Caspr (**c**ontactin-**as**sociated **pr**otein) multimers on the axonal membrane. This complex is thought to comprise the transverse bands and form septate-like junctions. The paranodes also function as a kind of barrier, restricting nodal and juxtaparanodal membrane proteins to their distinct domains. For example, mice lacking Caspr, contactin, cerebroside or sulfatide have disorganized paranodal junctions. This results in the broadening of nodal Na^+ channel clusters and the redistribution of juxtaparanodal K^+ channels into the paranodal region (Bhat et al., 2001; Rios et al., 2003). K^+ channels are normally excluded from paranodes and are clustered at juxtaparanodes through interactions with the cell adhesion molecules Caspr2 on the axon and TAG-1 on the glial cell (Poliak et al., 2003). Together these observations emphasize the fact that nodal and juxtaparanodal ion channels can be clustered in axons by at least two different types of glial cell–dependent mechanisms: (1) exclusion from paranodal regions and (2) interactions between axonal and glial cell adhesion molecules.

A detailed description of the proteins and lipids in these structures is beyond the scope of this chapter, and the interested reader is referred to excellent recent reviews of this area that are available elsewhere (Scherer & Arroyo, 2002; Scherer et al., 2004).

Enzymes associated with myelin

Several decades ago it was generally believed that myelin was an inert membrane that did not carry out any biochemical functions. More recently, however, a large number of enzymes have been discovered in myelin (Ledeen, 1992). These findings imply that myelin is metabolically active in synthesis, processing and metabolic turnover of some of its own components. Additionally, it may play an active role in ion transport not only with respect to maintenance of its own structure but also through participation in ion buffering near the axon.

A few enzymes, such as the previously mentioned CNP, are believed to be fairly specific for myelin/oligodendrocytes. There is much more in the CNS than in peripheral nerve, suggesting some function more specialized to the CNS. In addition, a unique pH 7.2 cholesterol ester hydrolase is also enriched in myelin. On the other hand, there are many enzymes that are not myelin-specific, but appear to be intrinsic to myelin rather than contaminants. These include cyclic AMP–stimulated kinase, calcium/calmodulin dependent kinase, protein kinase C, a neutral protease activity and phosphoprotein phosphatases. The protein kinase C and phosphatase activities are presumed to be responsible for the rapid turnover of MBP phosphate groups, and the PLP acylation enzyme activity is also intrinsic to myelin.

Myelin enzymes involved in structural lipid metabolism consist of a number of steroid modifying enzymes and cholesterol esterifying enzymes; UDP-galactose:ceramide galactosyltransferase; and many enzymes of glycerophospholipid metabolism, including all the enzymes necessary for phosphatidyl ethanolamine synthesis from diradyl-sn-glycerol and ethanolamine. It is likely that phosphatidycholine can also be synthesized within myelin. Perhaps even more elemental building blocks can be assembled into lipids by myelin enzymes, since acyl-CoA synthetase is present in myelin, suggesting the capacity to integrate free fatty acids into myelin lipids. The extent of the contribution of these enzymes in myelin (relative to enzymes within the oligodendroglial perikaryon) to metabolism of myelin lipids is not known.

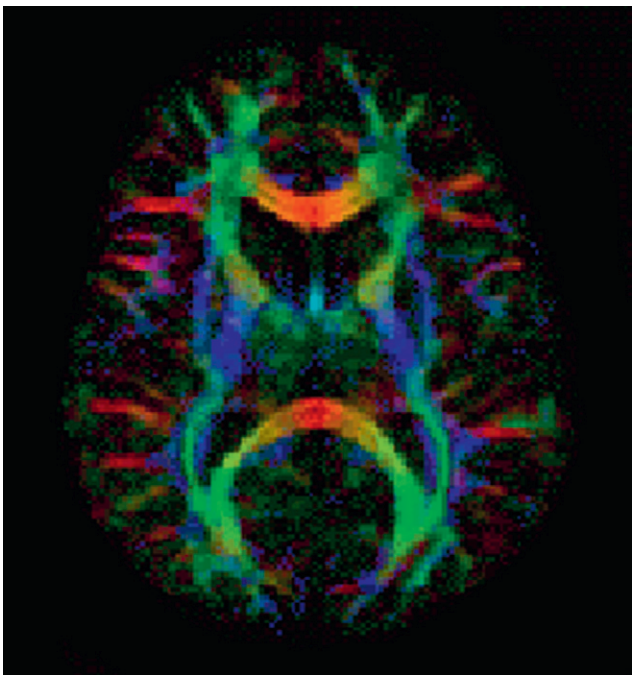
Other enzymes present in myelin include those involved in phosphoinositide metabolism: phosphatidylinositol kinase, diphosphoinositide kinase, the corresponding phosphatases and diglyceride kinases. These are of interest because of the high concentration of polyphosphoinositides of myelin and the rapid turnover of their phosphate groups. This area of research has expanded towards characterization of signal

EXAMINATION OF PATHOLOGIC CHANGES IN WHITE MATTER BY DIFFUSION TENSOR IMAGING

Joyce A. Benjamins

Diffusion tensor imaging (DTI) is an important tool used to examine changes in white matter structural integrity in both development and disease (Mori et al., 2006; Wozniak & Lim, 2006; Jito et al., 2008; Beardsley et al., 2005). The method is a recently developed technique based on magnetic resonance imaging (MRI), and is proving useful in human studies and animal models, both *in vivo* and in fixed tissue. MRI signals are derived from the motion of hydrogen (proton) nuclei in a magnetic field; in tissue, these signals arise primarily from the protons in water. DTI extends the resolution and contrast of conventional MRI by acquiring a series of images with multiple imaging parameters. If the diffusion of water molecules is restricted in one direction, this is referred to as anisotropic diffusion, and differences in diffusion compared to random diffusion are termed fractional anisotropy. This property has been applied to examine the organization of white matter in brain, based on the premise that water is expected to move more easily along myelinated axons rather than in a direction perpendicular to them. Thus, DTI detects the presence of oriented (as opposed to random) structures, with the more highly oriented, well-organized myelinated axons having higher fractional anisotropy (FA) values. While myelination or levels of myelin contribute to these measurements, differences may also indicate changes in water content, axonal packing, astrocytic hypertrophy or abnormalities in tract organization (Spadoni et al., 2007; Harsan et al., 2007).

MRI and DTI studies in humans have shown anomalies in white matter that may in part reflect abnormal myelination. For example, DTI showed lower fractional anisotropy and decreased white matter density in the lateral splenium of the corpus callosum in individuals with fetal alcohol syndrome compared to controls, consistent with disorganized fiber tracts in the region of the optic radiation and crossing visual association fibers, and with a significantly correlated reduction in visual–motor integration (Sowell et al., 2008). DTI is widely used to diagnose and follow the course of multiple sclerosis, and is an important parameter included in assessment of efficacy of therapies to slow disease progression, i.e., demyelination and axon degeneration (Fox, 2008). The application of DTI to follow changes in Alzheimer's and other neurodegenerative diseases is under investigation (Stebbins & Murphy, 2009), and the method has proved useful in assessing changes following stroke or brain injury in both humans and animal models (Jiang et al., 2010).



The white-matter connectivity of the brain can be imaged by using color-coded orientation maps representing the principal directions of the diffusion tensor in various brain regions (i.e., tractography). The image represents an axial section of an adult human brain near the mid-point of the corpus callosum and basal ganglia (contributed by L. Hermoye to Wikipedia).

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EXAMINATION OF PATHOLOGIC CHANGES IN WHITE MATTER BY DIFFUSION TENSOR IMAGING (*cont'd*)

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Wozniak, J. R., & Lim, K. O. (2006). Advances in white matter imaging: A review of *in vivo* magnetic resonance methodologies and their applicability to the study of development and aging. *Neuroscience Biobehavioral Reviews*, *30*(6), 762–774.

transduction system(s), with evidence of G proteins and phospholipases C and D in myelin.

Certain enzymes shown to be present in myelin could be involved in ion transport. Carbonic anhydrase has generally been considered a soluble enzyme and a glial marker, but myelin accounts for a large part of the membrane-bound form in brain. This enzyme may play a role in removal of carbonic acid from metabolically active axons. The enzymes 5′-nucleotidase and Na^+, K^+-ATPase have long been considered specific markers for plasma membranes and are found in myelin at low levels. The 5′-nucleotidase activity may be related to a transport mechanism for adenosine, and Na^+, K^+-ATPase could well be involved in transport of monovalent cations. The presence of these enzymes suggests that myelin may have an active role in ion transport in and out of the axon. In connection with this hypothesis, it is of interest that the PLP gene family may have evolved from a pore-forming polypeptide (Hudson, 2004).

Neurotransmitter receptors associated with myelin

Neurotransmitter receptors have been identified on oligodendrocytes and oligodendrocyte progenitor cells as well as in compact myelin (Ledeen, 1992; Belachew et al., 1999). Their functional role has not been established, but some intriguing hypotheses have been put forth. A wide variety of these receptors have been found, including mACh receptors, AMPA receptors, NMDA receptors and kainate receptors. It has been proposed that muscarinic receptors may be involved in phosphatidylinositol signaling in myelin or oligodendrocytes. There is recent evidence that muscarinic receptor activation alters integrin function in oligodendrocytes by modulating binding to extracellular matrix molecules (Gudz et al., 2002). With regard to adult pathologies, there is an increasing literature indicating that AMPA receptors may mediate glutamate cytotoxicity in oligodendrocyte progenitor cells.

Other myelin-related proteins

Another protein in compact CNS myelin is the myelin-associated oligodendrocytic basic protein, which is localized in the major dense line in several 8 to 12 kDa isoforms, and appears to function in controlling axonal diameter and the arrangement of the radial component (Yoshikawa, 2001). PNS myelin sheaths have long been known to contain a 170 kDa glycoprotein (see earlier editions of this chapter) that accounts for about 5% of the total myelin protein and may be related to the recently characterized L-periaxin-dystrophin-related **p**rotein 2-**d**ystro**g**lycan (PDG) complex associated with the Schwann plasma membrane (Scherer & Arroyo, 2002; Sherman & Brophy, 2004). This PDG complex is essential for stable axon-glia interactions, and mutations of the periaxin gene lead to profound disruptions of axonal ensheathment and segmental demyelination.

Small amounts of proteins characteristic of cells and membranes in general can also be found in myelin. There is evidence that tubulin is an authentic myelin-related component (Figure 10-12 B, D, CNS myelin). The 48 kDa **m**yelin/**o**ligodendrocyte-**s**pecific **p**rotein (MOSP) is a component found only in CNS myelin and oligodendroglial membranes that appears to associate with tubulin (Dyer & Matthieu, 1994).

Modern proteomic methods including mass spectrometry have dramatically expanded our 'catalog' of myelin proteins. More than 300 proteins have been found to be common to both human and mouse CNS myelin (Ishii et al., 2009). However, relatively few of these proteins' functions have been elucidated, emphasizing the tremendous amount of work that remains to be done to understand myelin formation, maintenance, and its many functions.

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