Myelin Formation, Structure and Biochemistry

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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 the 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, which 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 20 years ago [1], whereas newer developments in the myelin field are covered in detail in a recent two-volume set [2]. The myelin membranes originate from, and are part of, Schwann cells in the PNS and oligodendrocytes in the CNS (see Ch. 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 [3]. 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

†Pierre Morell tragically passed away early in the preparation of this chapter. A remembrance of his life and work appears on p. xxiii

Basic Neurochemistry: Molecular, Cellular and Medical Aspects
0-12-088397-X

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Published by Elsevier, Inc.
out through adjacent sections of the membrane (Fig. 4-1). 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 (Fig. 4-1). 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 *sallare*, ‘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/s at 20°C demonstrates the advantage of myelination. The 500µm 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 12µm 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 fiber 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 [3].

**myelin has a characteristic ultrastructure.** Myelin, as well as many of its morphological features, such as nodes of Ranvier and Schmidt–Lantermann clefts, can be seen readily in the light microscope (Fig. 4-2). 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

---

**FIGURE 4-1** 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.

**FIGURE 4-2** Light micrograph of a 1 µm 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. (Courtesy of Dr Cedric Raine.)
the lipid portion is a bimolecular leaflet and adjacent protein layers are different in some way. **Figure 4-3** 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 and Blaurock [4] 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 4-4 to 4-7**). There is asymmetry in the staining of the protein layers. The less dark, or intraperiod, line represents the closely apposed outer protein

**FIGURE 4-3** 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 with permission from Norton, W. T. The myelin sheath. In E. S. Goldensohn and S. H. Appel (eds), *Scientific Approaches to Clinical Neurology*. Philadelphia: Lea & Febiger, 1977, pp. 259–298.)

**FIGURE 4-4** 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 (arrowhead) can be seen in lower left. AX, axon. (Courtesy of Dr Cedric Raine.)
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 4-6, 4-7). The dark, or major period, line is 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 the 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. At the paranodal region and the Schmidt–Lantermann clefts (see below), 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 4-8 and 4-9, 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 (Figs 4-3, 4-9).

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.

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

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

**FIGURE 4-7** A typical CNS myelinated fiber from the spinal cord of an adult dog. Contrast this figure with the PNS fiber in Figure 4-4. 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 microtubules and neurofilaments.

**FIGURE 4-8** A diagram showing the appearance of CNS myelin if it were unrolled from the axon. One can visualize this structure arising from Figure 4-3 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 the appearance of this sheet 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 Hirano, A. and Dembitzer, H. M. A structural analysis of the myelin sheath in the central nervous system. J. Cell Biol. 34: 555–567, 1967.)
Schmidt–Lantermann 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. 4-9). 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 (Fig. 4-9).

Myelin is an extension of a glial plasma 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, similarly to the single axon shown in Figure 4-10A. 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 (Fig. 4-10A). 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 (Fig. 4-10). 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 but also points of difference with respect to myelination in the PNS. CNS nerve fibers 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 (Fig. 4-3). Unlike peripheral nerve, where the sheath is surrounded by Schwann cell cytoplasm on the inside and outside (Fig. 4-10), the cytoplasmic tongue in the CNS is restricted to a small
that there are signaling mechanisms from myelin or myelin-forming glia to axons. A common theme, emerging from recent research on transgenic mice deficient for 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 [1]. 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 mass of both CNS and PNS myelin is characterized by a high proportion of lipid (70–85%) and, consequently, a low proportion 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 lipid bilayer with integral membrane 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 (Fig. 4-11). Models of compact myelin are based on data from electron microscopy, immunostaining, X-ray diffraction, surface probes studies, structural abnormalities in mutant mice, correlations between structure and composition in various species, and predictions of protein structure from sequencing information [4].

### Central Nervous System Myelin Is Enriched in Certain Lipids

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Bovine</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrosides</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Myelin Ceramide</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Myelin Sphingomyelin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 4-1 lists the composition of bovine, rat, and human myelin compared to bovine and human white matter, human gray matter, and rat whole brain [1] (see Ch. 3). While there are no absolutely 'myelin-specific' lipids, cerebroside (galactosyl ceramide) is the most typical of myelin. With the exception of 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, which eliminates the obligate terminal step in cerebroside biosynthesis and thereby additionally sulfatide formation [6]. 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

---

**TABLE 4-1** Composition of central nervous system myelin and brain

<table>
<thead>
<tr>
<th>Substance*</th>
<th>Human</th>
<th>Bovine</th>
<th>Rat</th>
<th>Human</th>
<th>Bovine</th>
<th>Gray matter (human)</th>
<th>Whole brain (rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>30.0</td>
<td>24.7</td>
<td>29.5</td>
<td>39.0</td>
<td>39.5</td>
<td>55.3</td>
<td>56.9</td>
</tr>
<tr>
<td>Lipid</td>
<td>70.0</td>
<td>75.3</td>
<td>70.5</td>
<td>54.9</td>
<td>55.0</td>
<td>32.7</td>
<td>37.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>27.7</td>
<td>28.1</td>
<td>27.3</td>
<td>27.5</td>
<td>23.6</td>
<td>22.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>22.7</td>
<td>24.0</td>
<td>23.7</td>
<td>19.8</td>
<td>22.5</td>
<td>5.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Sulfatide</td>
<td>5.8</td>
<td>3.6</td>
<td>7.1</td>
<td>5.4</td>
<td>5.0</td>
<td>1.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Total galactolipid</td>
<td>27.5</td>
<td>29.3</td>
<td>31.5</td>
<td>26.4</td>
<td>28.6</td>
<td>7.3</td>
<td>21.3</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>15.6</td>
<td>17.4</td>
<td>16.7</td>
<td>14.9</td>
<td>13.6</td>
<td>22.7</td>
<td>19.8</td>
</tr>
<tr>
<td>Phospholipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>11.2</td>
<td>10.9</td>
<td>11.3</td>
<td>12.8</td>
<td>12.9</td>
<td>26.7</td>
<td>22.0</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>7.9</td>
<td>7.1</td>
<td>3.2</td>
<td>7.7</td>
<td>6.7</td>
<td>6.9</td>
<td>3.8</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>4.8</td>
<td>6.5</td>
<td>7.0</td>
<td>7.9</td>
<td>11.4</td>
<td>8.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>0.6</td>
<td>0.8</td>
<td>1.2</td>
<td>0.9</td>
<td>0.9</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Plasmalogens</td>
<td>12.3</td>
<td>14.1</td>
<td>14.1</td>
<td>11.2</td>
<td>12.2</td>
<td>8.8</td>
<td>11.6</td>
</tr>
<tr>
<td>Total phospholipid</td>
<td>43.1</td>
<td>43.0</td>
<td>44.0</td>
<td>45.9</td>
<td>46.3</td>
<td>69.5</td>
<td>57.6</td>
</tr>
</tbody>
</table>

*Protein and lipid figures in percentage dry weight; all others in percentage total lipid weight.

†Plasmalogens are primarily ethanolamine phosphatides.
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 [6, 7]. 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 galactocerebrosidase and sulfatide also have important functions in the differentiation of oligodendrocytes, with sulfatide being particularly important [7].

In addition to cerebroside/sulfatide, the major lipids of myelin are cholesterol and phospholipids [1]. 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 4-1). A characteristic phospholipid, and the single most prominent one, is ethanolamine-containing plasmalogen (glycerophospholipid containing an alkyl ether bond—see Ch. 3). 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 4-1 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; for example, myelin of rat has less sphingomyelin than does that of bovine or human (Table 4-1). 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 4-1, there are some other minor lipids, including polyphosphoinositides (see Ch. 3), which account for between 5% and 8% of the total myelin phosphorus; some fatty acid esters of galactocerebrosidase; and two galactosyldiacylglycerides [1]. Myelin from mammals also contains 0.1–0.3% 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: sialosylgalactosylspheramide (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-lactone tetraosylsphamide, 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 60–80% of the total in most species. Many other proteins and glycoproteins are present to a lesser extent. 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 dodecyl sulfate 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 is Mr for relative molecular mass, and another is to state molecular weight in kilodaltons, 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 composition of human and rat brain myelin are illustrated in Figure 4-12, B and D, 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 Fig. 4-11.

**Proteolipid protein.** Myelin PLP, also known as the Folch–Lees protein [8, 9], has the unusual physical property of solubility in organic solvents. The molecular mass of PLP is about 30,000, although it migrates anomalously on sodium dodecyl sulfate (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 Fig. 4-11. An important role for PLP in stabilizing the intraperiod line of CNS myelin has generally been assumed, based largely on the fact that the extracellular loops of this protein are present at this location. Furthermore, the CNS intraperiod line is
ATPase. T, tubulin. 170 kDa GP, 170 kDa glycoprotein. 14 kDa MBP are also called P1 and Pr, respectively, in the terminology for is most apparent in lane the PNS. The 26 kDa MOG is probably the faint band just above PLP that stained band in lane faintly to be seen well on the gels) is just above a discrete Coomassie-blue-respectively. Note that the location shown for MAG (which stains too 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.

### FIGURE 4-12
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 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. 4-13). The 18.5 kDa MBP and the 14 kDa MBP are also called P₁ and P₂, 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 migrates 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.

### TABLE 4-2
Some spontaneously occurring animal mutants affecting myelin

<table>
<thead>
<tr>
<th>Names of mutants</th>
<th>Inheritance*</th>
<th>Affected gene</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jimpy mouse, rumpshaker mouse, myelin-deficient (md) rat, shaking dog</td>
<td>X-linked</td>
<td>Proteolipid protein (PLP)</td>
<td>Variable degrees of oligodendrocyte death and CNS myelin deficiency; decreased spacing at intraperiod line of compact CNS myelin; see text</td>
<td>1, 9, 10, 43, 44</td>
</tr>
<tr>
<td>Shiverer mouse, myelin-deficient mouse</td>
<td>AR</td>
<td>Myelin basic protein (MBP)</td>
<td>Deletion or inversion of several MBP exons; very little functional MBP expressed; severe CNS hypomyelination and failure of compaction of major dense line; see text</td>
<td>1, 10, 43</td>
</tr>
<tr>
<td>Trembler mouse (PMP-22)</td>
<td>AD</td>
<td>Peripheral myelin protein-22 (PMP-22)</td>
<td>Hypomyelination specific for the PNS; caused by point mutations in transmembrane domains; see text</td>
<td>1, 45</td>
</tr>
<tr>
<td>Quaking mouse</td>
<td>AR</td>
<td>QKI family of proteins (QKI5, QKI6, QKI7 expressed in oligodendrocytes)</td>
<td>Hypomyelination more severe in CNS than PNS; abnormal expression of RNA-binding proteins likely to interfere with normal splicing or transport of mRNAs for myelin proteins; see text</td>
<td>1, 46–48</td>
</tr>
<tr>
<td>Taiep rat (acronym: trembling, ataxia, immobility, epilepsy, paralysis)</td>
<td>AR</td>
<td>Unknown</td>
<td>Impaired myelin formation followed by demyelination in the CNS; accumulation of microtubules in oligodendrocytes interferes with transport of myelin proteins or mRNAs; see text</td>
<td>49</td>
</tr>
</tbody>
</table>

*AD, autosomal dominant; AR, autosomal recessive; CNS, central nervous system; PNS, peripheral nervous system.
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, CNS myelin devoid of PLP/DM20 cannot sustain normal axonal function. Despite the apparent similarity of the PLP and DM20, DM20 cannot replace PLP in transgenic mice [11] – the same long-term axonal degeneration occurs in mice expressing exclusively DM20 protein. This may be because PLP uniquely interacts both with inositol hexakisphosphate [12], a molecule involved in vesicle transport, and with integrins, modulating interaction with the extracellular matrix [13]. 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. Both human patients (see Ch. 38) and genetically engineered or naturally occurring animal mutants (Table 4-2) with defects in the PLP gene exhibit hypomyelination and often early death. This may result from production of either abnormal protein that cannot fold correctly or simply increased amounts of normal PLP [9], which induce an unfolded protein response and are toxic to oligodendrocytes.

While PLP/DM20 expression is highest in oligodendrocytes in the CNS, PLP/DM20 mRNA is also expressed in myelinating Schwann cells in the PNS [9], 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 [10], again suggesting that this protein has unique functions unrelated to formation of myelin in appreciable amounts. It is also synthesized although not incorporated into myelin in appreciable amounts.

Myelin basic proteins. The MBP of myelin has long been of interest because it was the initial myelin antigen, which, when injected into an animal, elicited a cellular immune response that produced the CNS autoimmune disease called experimental allergic encephalomyelitis (EAE, see Ch. 38). MBP can be extracted from myelin as well as from white matter with either dilute acid or salt solutions; 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 [10, 14, 15]. The classical MBP gene has seven exons, with the full length MBP (21,500 M_\text{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), 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. 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 in Figure 4-13. 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 [16].

The MBPs are extrinsic proteins localized exclusively at the cytoplasmic surface in the major dense line (Fig. 4-11), 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. A severe hypomyelination and failure of compaction of the major dense line in MBP deficient shiverer mutants supports this hypothesis (Table 4-2). 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 methylation of an arginine at residue 106. The rapid turnover of the phosphate groups present on many of the MBP molecules [17] suggests that 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 (gene of the oligodendrocyte lineage), which is more than 100 kb in length [14]. 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. 4-13). 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 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 [10, 14].

2′,3′-Cyclic nucleotide 3′-phosphodiesterase: In addition to PLP and MBP, there are many higher-molecular-weight proteins present in myelin (Fig. 4-12). These vary in amount depending on species (rodents generally have more than larger mammals) and age (immature myelin has more). A doublet with Mr 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) [18]. 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 nonphysiological activity, because only the 3′·5′ cyclic nucleotides have been shown to have biological activity. Nevertheless, evolutionary conservation of the catalytic site indicates that its amino acid sequence probably 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 an extra 20 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 regulating cytoskeletal dynamics to promote process outgrowth and
differentiation in oligodendrocytes. Furthermore, 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, so the relevance of this to oligodendrocytes and myelination is also unclear. An interesting possibility combining some of the above information is that CNP could be involved in some specialized aspects of RNA transport and/or processing in oligodendrocytes. Yet 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 leading to axonal degeneration in its absence [18]. Clearly, more research is needed to fully understand the functions of this intriguing myelin/oligodendrocyte-related protein.

Myelin-associated glycoprotein and other glycoproteins of CNS myelin. The myelin-associated glycoprotein (MAG) is a quantitatively minor, 100 kDa glycoprotein in purified CNS and PNS myelin [19, 20] that electrophoreses at the position shown in Figure 4-12. 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 periaxial glial membranes of myelin sheaths. This location next to the axon and its membership in the Ig superfamily (see Ch. 7) 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’ [sialic acid –binding immunoglobulin-like lectins] 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 periaxial 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 result 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 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 26kDa protein called the myelin-oligodendrocyte glycoprotein (MOG) [21]. 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 demyelinging diseases of the CNS and is a leading candidate to be an important antigen in multiple sclerosis (see Ch. 38). 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 [7]. 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) [19, 22]. It was first characterized as a 120kDa 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 nervous system myelin also contains unique proteins.

P0 glycoprotein. Gel electrophoretic analysis (Fig. 4-12A, C) shows that a single 30kDa protein, P0, accounts for more than half of the PNS myelin protein. P0 is a type 1 membrane glycoprotein containing about 220 amino acids after removal of its signal sequence. Rat P0 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 [19, 23]. 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 P0 include phosphorylation and acylation.

The principal difference in the overall protein composition of PNS and CNS myelin is that P0 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 P0 proteins, which are so different in sequence, post-translational 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 P0 in transfected cells results in cell–cell interactions that are due to homophilic binding of its extracellular domains, suggesting that P0 stabilizes the intraperiod line of PNS myelin by similar homophilic binding (Fig. 4-11). The relatively large, glycosylated, extracellular Ig-like domain of P0 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 by Kirschner et al. [23] suggests that homophilic interactions between P0 molecules involve both protein–protein and protein–carbohydrate interactions. Furthermore, investigation of the crystal structure of the P0 extracellular domain suggests that P0 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. P0 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 P0 has profound consequences for myelin structure, in contradistinction to the previously noted, relatively benign CNS consequence of deletion of the PLP gene. P0-null mice exhibit abnormal motor coordination, tremors, occasional convulsions and a severe hypomyelination with thin, noncompacted myelin sheaths.

Expression of the correct amount of P0 is apparently essential for normal myelin formation and maintenance. Young mice heterozygous for the P0-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 P0 exhibit a dose-dependent dysmyelinating neuropathy ranging from transient hypomyelination to severe arrest of myelination and impaired sorting of axons. The critical dosage of P0 required for normal myelin formation is similar to observations 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 P0, 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 P0 is inappropriately located in normally dynamic mesaxonal membranes, causing them to adhere like compact myelin and halting myelination. It is clear that control of P0 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 P0 are expressed in Schwann cells and neural crest cells early in embryonic development well before myelination, which suggests that P0 could have other functions, potentially involving Schwann-cell–axon interactions and signal transduction. The cytoplasmic domain of P0 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 [17].

**Peripheral myelin protein-22.** In addition to the major P0 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. 4-12C) [19, 24]. Similarly to P0, PMP-22 has a single site for N-linked glycosylation. However, unlike P0, 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 nondividing 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 Fig. 4-11, it is not known if its extra-cellular 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 nonmyelinating 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 P0 and this interaction with P0 may be relevant to its function. Also, as is the case with P0 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 (Table 4-2) and some neuropathies in humans (see Ch. 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.

**P2 protein.** PNS myelin contains a positively charged protein different from MBP that is referred to as P2 (M, =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 [25]. The amount of P2 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. P2 protein is generally considered a PNS myelin protein but it is expressed in small amounts in CNS myelin sheaths of some species. P2 is an antigen for experimental allergic neuritis, the PNS counterpart of EAE (see Chs 36 and 38). P2 appears to be present in the major dense line of myelin sheaths, where it may play a structural role similar to MBP (Fig. 4-11). Interestingly, the larger amounts of P2 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 P2 in large sheaths than small ones [4]. 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 peripheral and central 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% [1]. 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 P1 in the CNS nomenclature. The 18.5 kDa component is present and is often referred to as the P2 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. For example, the shiverer mutant mouse, which expresses no MBP (Table 4-2), has a greatly reduced amount of CNS myelin, with no compaction of the major dense line. By contrast, shiverer PNS has essentially normal myelin, both in amount and structure, despite the absence of MBP. This CNS/PNS difference in the role of MBP is probably because the cytoplasmic domain of P2 has an important role in stabilizing the major dense line of PNS myelin. Animals doubly deficient for P0 and MBP have a more severe defect in compaction of the PNS major dense line than P0-null mice, which indicates that both proteins contribute to compaction of the cytoplasmic surfaces in PNS myelin [23].

**Myelin-associated glycoprotein.** As in 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 [19,20]. 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 Ch. 30). 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 [26]. 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, which 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 the most important function of MAG in the PNS is transmitting 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 that 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. 4-12), are described here briefly.

**Tetraspan proteins.** Intriguingly, there are numerous tetraspan proteins (containing four transmembrane spanning domains) in myelin and related glial membranes [27], including PLP/DM20, PMP-22, myelin and lymphocyte protein (MAL/MVP17/VIP17) and plasmolin 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 (Figs 4-3, 4-9), 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 17 kDa myelin and lymphocyte tetraspan protein (MAL). This protein was initially identified in compact myelin as MVP17, a novel myelin membrane protein [28], 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 glycosphinolipid-enriched protein/lipid rafts and may function in their sorting and transport to myelin [29, 30]. MAL has been established to be part of an extended gene family, which includes plasmolin, another myelin tetraspan protein [31]. Plasmolin is also associated with glycosphinolipid-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 are a family of tight junction proteins found in many tissues, which 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 [32]. 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 was quickly established to be also a member of the claudin family, i.e., claudin-11 [33]. 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 [33] 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 β integrin [33].

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 [34]. 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 connexin-32 and connexin-29 are found in myelin [35], 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 probably also exist in CNS myelin. Connexin-32 mutations are associated with the peripheral neuropathy Charcot–Marie– Tooth disease (see Ch. 38) but, interestingly, have little CNS pathology.

**Paranodal proteins other than tetraspan proteins.** The overall structure of the axonal membrane at the nodes of Ranvier themselves is essential for normal axonal firing, and this appears to be regulated in trans by the proteins and lipids in the paranodal myelin membranes. The axonal and glial membranes in this region of the fiber demonstrate an exquisite division into highly specialized domains, whose biochemical structures are currently a very active subject of research. The correct positioning of sodium channels exclusively at the nodes and not in the internodes is necessary for generating action potentials. Similarly, the potassium channels are localized very specifically to the juxtaparanodal axonal membrane and, as noted above, cerebroside- and sulfatide-null mice have disorganized paranodes and consequently altered nodal membranes, for example altered sodium channel organization. A variety of membrane proteins, including several members of the immunoglobulin superfamily, are selectively localized in these nodal and paranodal domains and must play important roles in the formation and stabilization of these complex structures. For example, an important trans interaction at the paranode occurs between neurofascin-155 on the glial membrane and contactin/Caspr (contactin-associated protein) multimers on the axonal membrane to form septate-like junctions. A detailed description of the proteins and lipids in these structures is beyond the scope of this chapter, and the reader is referred to excellent recent reviews of this area that are available elsewhere [32, 36].

**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 [37]. 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 with respect not only to maintenance of its own structure but also to 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 and not contaminants. These include cAMP-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:cereamide galactosyl-transferase 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 phosphatidylcholine can also be synthesized within myelin. Perhaps even more elemental building blocks can be assembled
into lipids by myelin enzymes, since acyl-coenzyme A (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 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 [9].

Neurotransmitter receptors associated with myelin. Neurotransmitter receptors have been identified on oligodendrocytes and oligodendrocyte progenitor cells as well as in compact myelin [37, 38]. Their functional role has not been established but some intriguing hypotheses have been put forth. A wide variety of these receptors has been found, including muscarinic acetylcholine (mACH) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, N-methyl-d-aspartate (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 [13]. 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 8–12 kDa isoforms and appears to function in controlling axonal diameter and the arrangement of the radial component [39]. 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 1-periaxin-dystrophin-related protein 2-dystroglycan (PDG) complex associated with the Schwann plasma membrane [36, 40]. 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 (Fig. 4-12B, D, CNS myelin). The 48 kDa myelin/oligodendrocyte-specific protein (MOSP) is a component found only in CNS myelin and oligodendroglial membranes, which appears to associate with tubulin [41].

**DEVELOPMENTAL AND METABOLIC ASPECTS OF MYELIN**

The developmental progress of myelination varies between regions and species. As the nervous system matures, portions of the PNS myelinate first, then the spinal cord, and the brain last [1]. In all parts of the nervous system there are many small axons that are never myelinated. It is generally true that large neural pathways become myelinated before they are completely functional. A relevant observation is that rats and other nest-building animals are quite helpless at birth and myelinate predominantly postnatally. By contrast, grazing animals such as horses, cows and sheep have considerably more CNS myelin at birth and a correspondingly higher level of complex activity immediately postnatally.

Synthesis of myelin components is very rapid during deposition of myelin. Nervous system development is marked by several overlapping periods, each defined by a major event in brain growth and structural maturation [1]. In the rat, whose CNS undergoes considerable development postnatally, the maximal rate of cellular proliferation (much of this involving oligodendroglial precursor cells) occurs at 10 days. The rat brain begins to form myelin postnatally at about 10–12 days. The maximal rate of accumulation of myelin in the rat occurs at about 20 days of age, although accumulation continues at a decreasing rate throughout adulthood. A remarkable amount of membrane biogenesis occurs in oligodendrocytes during the period of maximum myelination. Myelin accumulates in a 20-day-old rat brain at a rate of about 3.5 mg/day. Rough calculations based on the number of oligodendrocytes and the amount of myelin deposited indicate that on average the amount of myelin membrane made by each cell per day is more than three times the weight of its own perikaryon. This very rapid myelin synthesis early in development has been demonstrated biochemically by the
very rapid incorporation of radioactive precursors into myelin and substantial increases of enzymes involved in synthesizing myelin components.

**Sorting and transport of lipids and proteins takes place during myelin assembly.** After myelin components have been synthesized, they must be assembled to form the membranes making up myelin sheaths [16]. The biogenesis of these sheaths is an extraordinary process of membrane formation and modeling. In the CNS, this requires the spiraling of numerous oligodendroglial processes around axons and their tight layering to form compact myelin. Furthermore, there is additional modeling of specialized membrane domains with different composition at the inside and outside of the sheaths and in the paranodal glia–axon junctions. The two major proteins of compact CNS myelin, PLP and MBP, enter the myelin by different routes. PLP is synthesized on membrane-bound polysomes in the perikaryon and transported in membranous vesicles to the myelin being formed at the end of the oligodendroglial processes. By contrast, MBP is synthesized on free polysomes, which are actually located in very close proximity to the newly forming myelin at the end of oligodendroglial processes [16]. Its mRNA is transported from the perikaryon to the vicinity of myelin formation in ribonucleoprotein granules by a microtubule-based translocation system. These differences in the route of entry are reflected in different kinetics of incorporation of proteins into myelin membranes in experiments involving labeling with radioactive amino acids after intracranial injection or incubation of brain slices. Radioactive MBP is synthesized and integrated into myelin very rapidly with a lag time of only a few minutes, whereas substantial amounts of radioactive PLP do not appear in myelin until after about 45 minutes [1]. Other proteins that are selectively localized in specialized regions of the myelin sheath such as the inner and outer surfaces (e.g. MAG and MOG, respectively) or in the paranodal regions (e.g. neurofascin-155) must be sorted and transported by different mechanisms involving specific sorting signals. It is likely that the sorting mechanisms are related to the apical and basolateral targeting that occurs in simple polarized epithelial cells. Using the model system of transfected Madin–Darby canine kidney (MDCK) epithelial cells, it was shown that MOG sorts exclusively to MDCK basolateral membranes, whereas PLP sorts exclusively to MDCK apical membrane [42]. However, sorting in myelin-forming cells probably also involves more complicated mechanisms because of the complex variety of membrane domains in myelin sheaths. Furthermore, some of the lipids and proteins in myelin forming cells are associated with raft-like domains, which are enriched in cholesterol, glycosphingolipids and glycosylphosphatidylinositol-linked proteins [7]. These rafts are likely to play an important role in the trafficking of membrane components and signal transduction mechanisms involved in the assembly of myelin sheaths. Much research designed to elucidate these phenomena and other aspects of myelin assembly is ongoing. However, a more detailed description of this research is beyond the scope of this chapter, and the reader is referred to more comprehensive references [7, 16].

**The composition of myelin changes during development.** The composition of myelin isolated from immature, rapidly developing brain is different from that of the adult [1]. As rat brain matures, the content of galactolipids, MBP and PLP in purified myelin increases, whereas phosphatidylcholine and high-molecular-weight proteins decrease. These studies on the composition of myelin from immature brains are consistent with the concept that myelin first laid down by oligodendrocytes may represent a transitional form with properties intermediate between those of mature compact myelin and the oligodendroglial plasma membrane. However, interpretation of biochemical studies on purified myelin is complicated by the fact that myelin fractions isolated by conventional procedures can be separated into subfractions of different densities. The lighter fractions are enriched in multilamellar myelin, whereas the denser fractions contain a large proportion of single membrane vesicles that morphologically resemble microsomes or plasma membrane fragments. Generally speaking, as one goes from light myelin subfractions to heavier ones, the lipid:protein ratio and the amount of MBP decrease, the amount of PLP decreases or remains relatively constant, and the amounts of MAG, CNP and other high-molecular-weight proteins increase. The interpretation of these findings is that the light subfractions contain primarily compact myelin while the heavier fractions are enriched in other oligodendroglial-derived membranes from the cell processes, the inner and outer surfaces of the sheaths, and the paranodal loops. Therefore, the differing lipid and protein composition of isolated immature myelin may reflect either transitional forms of developing myelin or a greater content of associated oligodendroglial membranes relative to compact myelin recovered from the thinner immature myelin sheaths, or a combination of these factors. Nevertheless, metabolic studies with radioactive precursors lend support to the view that the heavier fractions isolated from developing brain represent at least in part transitional membranes in the process of conversion to compact myelin. For example, PLP appears first in the heavier fractions and later in lighter fractions in a manner that suggests a precursor–product relationship.

**Spontaneous mutations in experimental animals provide insights about the structure and assembly of myelin.** The myelin mutants often have names relating to their characteristic tremor due to the myelin deficit, e.g. **shiverer**, **jimpy**, **quaking** and **trembler** mice (Table 4-2). Although some of the mutants have been studied for many years [1], it is only recently that recombinant DNA techniques have led to identification of the primary genetic defects in most of them. Some of them are good
models for inherited human diseases affecting myelin, as described in Ch. 38. Furthermore, the naturally occurring mouse mutants, which may express abnormal myelin proteins, sometimes have phenotypes that differ from null mutants produced experimentally by homologous recombination and are thereby instructive with regard to the mechanisms of myelin formation and loss.

Mutation of either of the major structural proteins of CNS myelin can lead to a severe hypomyelination; MBP in shiverer or myelin-deficient (mld) mice and PLP in jimpy mice and several other animal mutants [10, 43, 44] (Table 4-2). Ultrastructural abnormalities in the small amount of myelin that is formed by these mutants have been informative with regard to the structural roles of these proteins in compact CNS myelin. Thus the cytoplasmic surfaces of the spiraled oligodendrocyte membranes do not compact to form a major dense line in the absence of MBP in shiverer mutants, and the intraperiod line is more condensed than normal in jimpy mice, which express very little PLP. The ultrastructure of PNS myelin is normal in both of these mutants since PLP is virtually absent from peripheral myelin and P0 appears to be capable of stabilizing both the intraperiod and major dense lines of myelin of the PNS in the absence of MBP. The shiverer phenotype can be corrected by introducing normal MBP into transgenic mutant mice, resulting in almost complete correction of the shivering, early death and failure of CNS myelin compaction.

The severity of the myelin deficiency in PLP mutants varies. Most PLP mutations are quite severe, but the PLP mutation in rumpshaker mice causes a relatively mild hindlimb shaking phenotype. By contrast, there is severe reduction and abnormal structure of CNS myelin in jimpy mice, along with a profound loss of oligodendroglycans. Furthermore, attempts to correct the jimpy phenotype by introducing the normal gene have not been successful. Thus the jimpy PLP mutation appears to be dominant, causing a misfolded protein response that leads eventually to oligodendrocyte death with morphological features of apoptosis. These oligodendroglial abnormalities appear to be the primary cause of the severe hypomyelination in jimpy mice rather than the absence of PLP in the myelin, since, as noted above, the PLP-null mouse has relatively normal myelin. The PLP mutants demonstrate the fact that protein mutations may be far more severe than simple loss of the protein. It must be noted that mutation of the minor isoforms of these proteins expressed in glia, neurons and other cell types may also impact these phenotypes [10].

Two allelic trembler mutations, which affect only the PNS, result from different point mutations in transmembrane domains of peripheral myelin protein-22 (PMP-22) [1, 45] (Table 4-2). The trembler phenotypes are characterized by hypomyelination, continued Schwann cell proliferation and partial paralysis of the limbs. These murine mutants are animal models for some of the inherited human neuropathies caused by abnormalities of the PMP-22 gene (Ch. 38). Because the function of PMP-22 is uncertain, the mechanisms by which the point mutations in this protein cause the trembler phenotype are unclear. However, as with the PLP mutations, the pathology may result from an unfolded protein response to abnormal protein that is retained in the endoplasmic reticulum.

A number of spontaneous animal mutants are not direct mutations of compact myelin protein genes but rather appear to cause defects in cellular processes needed for myelin assembly or maintenance. One example is the quaking mouse, with hypomyelination in both the CNS and PNS but a greater deficiency of CNS myelin [1, 46] (Table 4-2). Unlike the shiverer and jimpy mice, which form almost no myelin and die early, quaking mice generate more myelin and live to adulthood. However, the myelin sheaths are thin and poorly compacted, especially in the CNS. The myelin isolated from the CNS of this mutant has all the known myelin proteins but its overall composition resembles that of very immature normal mice. Although the quaking mouse was one of the first dysmyelinating mutants to be described and has been extensively studied, the genetic lesion was identified only recently. The quaking gene encodes a family of RNA-binding proteins generated by mRNA splicing. These QKI proteins appear to regulate the alternative splicing and/or transport of oligodendroglial mRNAs for myelin proteins such as MBP and MAG [46–48].

The more recently identified ‘taiep’ rat mutant (Table 4-2) has impaired accumulation of CNS myelin for up to 2 months followed by a period of demyelination [49]. Adult taiep rats have only 10–25% of the normal amount of CNS myelin. The primary genetic lesion has not yet been identified but the mutant oligodendrocytes exhibit an abnormal accumulation of microtubules during development, suggesting that the mutation may involve a microtubule-associated protein. Biochemical and immunocytochemical studies indicate that excessive microtubule accumulation interferes with transport of myelin proteins and/or their mRNAs, eventually leading to a failure of myelin maintenance [49].

Myelin components exhibit great heterogeneity of metabolic turnover. One of the novel characteristics of myelin demonstrated in early biochemical studies was that its overall rate of metabolic turnover is substantially slower than that of other neural membranes [1]. A standard type of experiment was to evaluate lipid or protein turnover by injecting rat brains with a radioactive metabolic precursor and then follow loss of radioactivity from individual components as a function of time. Structural lipid components of myelin, notably cholesterol, cerebroside and sulfatide, as well as proteins of compact myelin, are relatively stable, with half-lives of the order of many months. One complication in interpreting these studies is that the metabolic turnover of individual myelin components is multiphasic—consisting of an initial rapid loss of radioactivity followed by a much longer slower loss.
For example, initially MBP and PLP exhibit half-lives of 2–3 weeks, but later their half-lives are too long to be calculated accurately. A possible interpretation of these data is that some of the newly formed myelin remains in outer layers or near cytoplasmic pockets (incisures and lateral loops) where it is accessible for catabolism – thus accounting for the rapidly turning-over pool. The more stable metabolic pool would consist of deeper layers of myelin less accessible for metabolic turnover.

By contrast to the relatively slow rate of overall metabolism, the presence of signal transduction systems in myelin sheaths [7, 16] indicates that some aspects of myelin metabolism probably involve rapid events with half-lives on the order of minutes. For example, the monoesterified phosphate groups of polyphosphatidylinositol (those at positions 4 and 5) are labeled very quickly even in mature animals, and this is presumably related to the function of phosphoinositides in signal transduction (see Ch. 20). Additionally, the phosphate groups on MBP turn over rapidly [17]. Although the representation of myelin structure in Figure 4-11 is static, studies that demonstrate relatively rapid metabolism of certain myelin components suggest that there may be some dynamic aspect of myelin structure, such as occasional separation of the cytoplasmic faces of the membranes. The dynamic nature of myelin sheaths probably contributes to the maintenance and functional state of axons. Clearly recent studies on the pathology of multiple sclerosis demonstrate the crucial nature of myelin both for nerve conduction and axonal survival (see Ch. 38). A more complete understanding of the formation and maintenance of myelin sheaths awaits conceptual and analytical advances.

ACKNOWLEDGMENTS

We thank Dr Cedric Raine for the elegant photomicrographs in this chapter and Jeffrey Hammer for help in preparing Figure 4-11 showing the molecular organization of compact myelin.

REFERENCES
