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CHAPTER

4

Myelination, Dysmyelination, and Demyelination

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*Abbreviations: ARIA, acetylcholine inducing activity; ATP, adenosine triphosphate; bFGF, basic fibroblast growth factor; CNP, 2′-3′-cyclicnucleotide-3′-phosphodiesterase; CNS, central nervous system; CPG, choline phosphoglycerides; EAE, experimental autoimmune encephalomyelitis; E-NCAM, embryonic neural cell adhesion molecule; EPG, ethanolamine phosphoglycerides; FABP, fatty-acid binding protein; Gal-C, galactocerebroside; GAP, growth associated protein; GGF, glial growth factor; HMG-CoA, hydroxymethylglutaryl-Coenzyme A; HNK-1, human natural killer cell–antigen 1; IGF-1, insulin-like growth factor-1; IHN, isonicotimic acid hydrazide; IPG, inositol phosphoglycerides; jp, jimpy mouse; jp-msd, myelin-synthesis deficient mouse; MAG, myelin-associated protein; MBP, myelin basic protein; MOG, myelin/oligodendrocyte glycoprotein; MyTI, myelin transcription factor; N-CAM, neural cell adhesion molecules; NDF, Neu-differentiation factor; NGF, nerve growth factor; NGF-R, nerve growth factor receptor; OL, oligodendroblast; Omgp, oligodendrocyte-myelin glycoprotein; P_0, peripheral myelin protein zero; PDGF, platelet-derived growth factor; PLP, proteolipid protein; PMD, Pelizaeus–Merzbacher disease; PMP-22, peripheral myelin protein, PNS, peripheral nervous system; RER, rough endoplasmic reticulum; rsh, rumpshaker mouse; SCIP, suppressed cAMP–inducible Pou-domain transcription factor; shi, shiverer mouse; shi-mid, myelein-deficient mouse; SPG, serine phosphoglycerides; TET, triethyltin; TGF-β, transforming growth factor-β; Tr, trembler mutant mouse.

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I. Introduction

Normal functioning of the nervous system involves the transmission, processing, and integration of information as nervous impulses. Impulse transmission along axons is greatly facilitated by the presence of myelin, the compact multilamellar extension of the plasma membrane of specialized glial cells that spirals around larger axons. In the central nervous system (CNS), oligodendroglial cells are responsible for the synthesis and maintenance of myelin, whereas Schwann cells subserve this role in the peripheral nervous system (PNS). Schwann cells produce a single segment of myelin (called an *internode*), whereas oligodendroglial cells furnish multiple myelin segments around different axons, although only one segment for a given axon. There are periodic interruptions along the axons between adjacent myelin internodes; termed *nodes of Ranvier,* these short intervals where axons are not enveloped by myelin are vital for normal nervous system function (see later this chapter).

Myelin is an electrical insulator, and the periodic interruptions at the nodes allow for rapid and efficient transmission of nervous impulses. In unmyelinated axons, impulse transmission involves a wave of membrane depolarization that moves down the axon in a continuous sequential manner. However, in myelinated axons, the myelin internodes function as high-resistance insulators, so the excitable axonal membrane, containing a high concentration of voltage-sensitive sodium channels, is exposed only at the nodes of Ranvier. Impulse conduction thus involves excitation at the nodes only, and the impulse jumps from node to node (saltatory conduction) (see Funch and Faber, 1984; Waxman *et al.,* 1989; and Morell *et al.,* 1994, for details). Saltatory conduction is much more rapid and requires much less energy for membrane repolarization than conduction in unmyelinated axons. Myelin thus greatly increases the efficiency of the nervous system, facilitating conduction while conserving metabolic energy and space. It is not difficult to imagine how even minor loss of myelin or perturbations in its structure and/or function could have deleterious effects on normal nervous system function.

II. Morphologic and Structural Aspects of Myelin Formation

Structural aspects of the process of myelination are most easily illustrated in the PNS. Each myelin-forming Schwann cell produces an elaborate specialized extension of its plasma membrane, which is wrapped spirally around a segment of one axon (Fig. 1). Schwann cells, the glial cells of the PNS, are derived from the portion of the neural epithelium that gives rise to the neural crest (Le Douarin, 1982). During development, Schwann cells invade the developing nerves, where they migrate along bundles of axons, proliferate (probably in response to an axonal mitogen; Webster and Favilla, 1984), and segregate the axons individually within invaginations on the surface of Schwann cells. As they cease migrating, they synthesize a basal lamina (Billings-Gagliardi *et al.,* 1974), composed of laminin, merosin, type IV collagen, fibronectin, nidogen/entactin, and heparan sulfate proteoglycan (Sanes and Cheney, 1982; Tohyama and Ide, 1984; Bannerman *et al.,* 1986; Leivo and Engvall, 1989; Sanes *et al.,* 1990). As the axons continue to enlarge, the larger axons become further segregated so that a single Schwann cell envelops a single axon. After the plasma membrane of the Schwann cell has completely enclosed the axon, the external surfaces of the plasma membrane fuse to form a structure known as the *mesaxon.* The mesaxon then elongates and spirals around the axon, eventually resulting in a "jelly-roll" structure consisting of double layers of the Schwann cell plasma membrane. Myelin internodes can be as much as 2 mm long and contain 5 mm of myelin spiral (Friede and Bischhausen, 1980). Myelin compaction occurs as cytoplasm is extruded and the cytoplasmic faces are condensed to produce the dark major period line visible in electron micrographs (Fig. 2). The juncture of what was originally the outer faces of the apposing plasma membranes form the lighter appearing intraperiod line. Mature myelin thus has a characteristic compact multilamellar structure, but cytoplasmic inclusions continu-

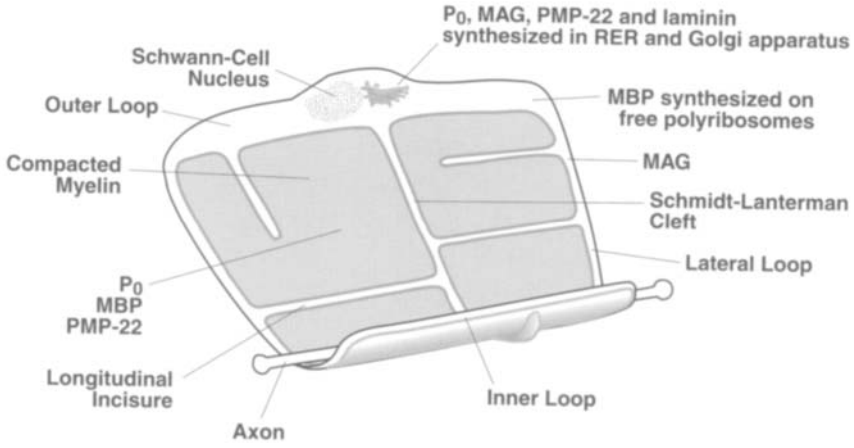

FIGURE 1 Diagram of a single myelinated internode of the PNS, as it would appear if the myelin sheath, with its associated Schwann cell, were unrolled from around the axon. Note the very large myelin membrane surface area, as well as the many cytoplasm-containing structures (lateral, inner, and outer loops; Schmidt–Lanterman clefts; and longitudinal incisures), all continuous with the cytoplasm of the Schwann cell perikarya. This cytoplasmic continuity is presumably necessary for metabolic maintenance of compact myelin. Sites of synthesis, as well as ultrastructural locations, of the major myelin proteins (discussed in a later section) are also shown. In mature compact myelin, the large trapezoidal sheet of myelin membrane is tightly spiraled around the axons, with apposing inner surfaces of the original plasma membrane fusing to give the electron-dense intraperiod lines and the outer surfaces of the original plasma membrane forming the lighter appearing intraperiod line (see also electron micrograph in Fig. 2). Abbreviations: RER, rough endoplasmic reticulum; MBP, myelin basic protein; MAG, myelin-associated glycoprotein; PMP-22, peripheral myelin protein-22.

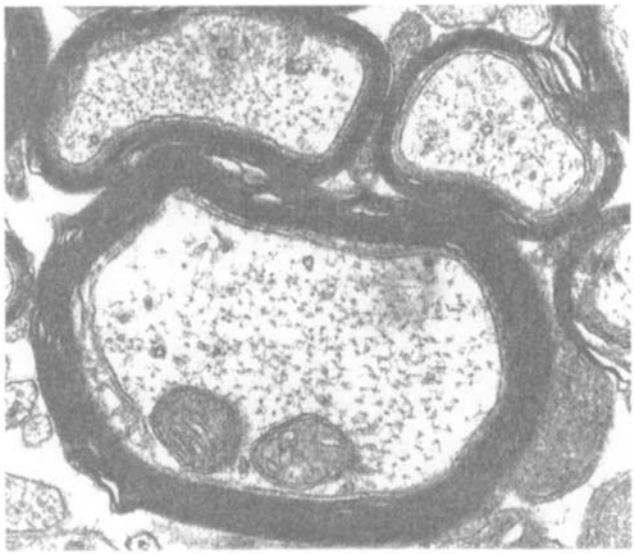

FIGURE 2 Electron micrograph of compact myelin from the mammalian CNS. Although there are minor ultrastructural differences, compact PNS myelin has a similar ultrastructural appearance. Note the alternating pattern of darker major dense lines and paler intraperiod lines, originally formed by fusion of apposing surfaces of the inner and outer leaflets, respectively, of the oligodendroglial plasma membrane. Cytoplasm-containing internal mesaxons can be seen on two of the myelinated axons.

ous with the perikarya cytoplasm of the Schwann cells are also present (Figs. 1 and 2). In addition, the myelin internode contains several ultrastructurally and biochemically distinct membrane domains, including the outer plasma membrane of the myelin-forming cell and the compact myelin itself, as well as the cytoplasm-containing Schmidt–Lanterman incisures, paranodal loops, nodal microvilli, and outer and inner mesaxons. The latter cytoplasm-containing structures provide connections with the perikaryal cytoplasm, and are vital for myelin maintenance.

The process of myelination in the CNS is similar, except that a single oligodendroglial cell extends a number of processes from its cell body; each process then envelopes and myelinates a single segment of a given axon (Fig. 3). Much of the local membrane assembly to give mature, compact myelin occurs within the oligodendroglial cytoplasmic processes (Waxman and Sims, 1984). The size of the fibers and the thickness of the sheaths are very different in the PNS and the CNS, but the overall surface area of myelin generated by an oligodendrocyte around multiple axons may be no larger than that formed by a Schwann cell around a single internode. The term *oligodendrocyte,* meaning "few processes," is actually somewhat of a misnomer,

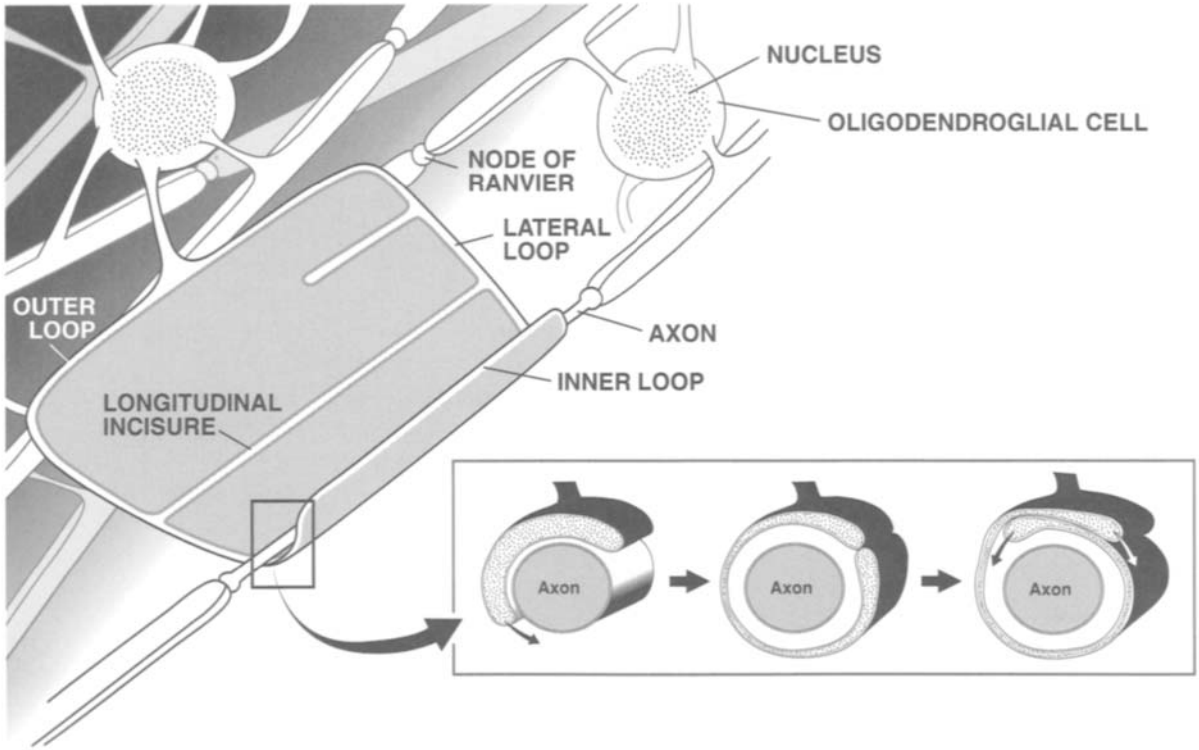

FIGURE 3 Diagram illustrating ultrastructural features of CNS myelin. Two myelin-forming oligodendroglial cells are shown, with each extending numerous processes to form myelin internodes around axons. Myelin from one internode has been unrolled from the axon to illustrate the large membrane surface area as well as various cytoplasm-containing structures (inner, outer, and lateral loops, longitudinal incisures) that are continuous with the perikaryal cytoplasm of the oligodendroglial cell. The nodes of Ranvier, short segments of axon between adjacent myelin internodes, are vital to normal nervous system function (see text). The expanded inset illustrates the process of formation of the initial wrap of newly forming myelin. An oligodendroglial cell process makes contact with the axon and eventually surrounds it. The apposing layers of membrane then fuse to form the mesaxon, which spirals around the axon a number of times. The process of myelination is completed when most cytoplasm is extruded from the uncompacted myelin to give mature, compact myelin (step not shown).

as a given oligodendrocyte may myelinate anywhere from less than five axons up to dozens of axons (Butt and Ransom, 1989; Bjartmar *et al.,* 1994). As in the PNS, the onset of myelination is preceded by proliferation of oligodendroglia. As development continues, both the diameter and length of the axons increase, and this is associated with a corresponding increase in nodal length as well as increases in myelin thickness. Thus, despite its compact highly ordered appearance, myelin continues to expand in all planes during growth and development.

In general, myelination follows the order of phylogenetic development, with the peripheral nerves myelinating first, then the spinal cord, and finally the brainstem, cerebellum, and cerebrum. There is, however, considerable overlap in this progression. In addition, each fiber tract may have its own spatiotemporal pattern of myelination, so that the degree of myelination may differ in different fiber tracts at a given developmental stage. For example, myelination in the spinal cord proceeds in a rostral–caudal gradient, whereas in the optic nerve, myelination progresses with a retinal to chiasmal gradient. Although not necessarily an absolute prerequisite for function, in general, fiber tracts are myelinated before they become fully functional.

Myelination is a major metabolic and structural event that occurs during a relatively brief but precisely defined period in the normal progression of events involved in nervous system development. In both the CNS and PNS, an enormous amount of myelin membrane is formed (some PNS axons may have as many as 100 layers) and this membrane must be maintained at a considerable distance from the supporting glial cell body. The surface area of myelin per adult oligodendrocyte in the rat brain has been calculated to be $1–20 \times 10^5\ \mu m^2$, several orders of magnitude greater than the perikaryal membrane

surface area of about 100 μm^2 (Pfeiffer *et al.,* 1993). Myelinating glial cells are thus maximally stressed in terms of their metabolic and synthetic capacity during this time, with each cell synthesizing myelin equivalent to up to 3 times the weight of its perikarya each day (Morell *et al.,* 1994). Because of these very high levels of synthetic and metabolic activity, these myelinating cells are especially vulnerable to nutritional deficits and/or to toxic insults or injuries during this period.

III. Axon–Glia Interactions during Development and Myelination

The tightly programmed sequence of events eventually resulting in the formation of mature compact myelin and the consequent initiation of impulse transmission is regulated by interactions between axons and glial cells at numerous stages (see Waxman and Black, 1995, for detailed discussion). During the early stages of myelination, the axon is loosely ensheathed by processes arising from immature, relatively undifferentiated glial cells. Loose glial ensheathment of axons in the optic nerve is seen in the rat beginning at postnatal day 6. This is followed by spiral wrapping of the axon by oligodendroglial processes that form compact myelin. In some tracts, the immature myelin sheath is initially close to the oligodendroglial cell body (Remahl and Hildebrand, 1990), but with maturation the sheath is displaced radially and often is connected to the cell body by only a thin cytoplasmic bridge. A single oligodendrocyte can myelinate axons of various diameters in their vicinity and can form myelin sheaths of different thicknesses around axons of differing diameter.

The development, maturation, and maintenance of the myelin sheath is dependent on both the normal functioning of the myelinating glial cells and the integrity of its relationship to the axon it ensheaths. During development, physical features of the myelin sheath, such as thickness and number of lamellae, are not preprogrammed within the myelin-forming cells, but rather depend on local regulation by the axon, with larger axons having thicker myelin sheaths (Waxman and Sims, 1984). Myelin internodal distance is also matched to fiber diameter (Hess and Young, 1952) and the internodal distance : diameter ratio is different for fibers in different tracts. There is some evidence that myelination is initiated when a developing axon reaches a "critical diameter." However, myelination occurs over a range of axonal diameters (Fraher, 1972) and at various times along a single axon (Waxman *et al.,* 1972; Waxman, 1985). The signal for initiation of myelination thus appears to be specific for particular axons, or for specific domains along axons.

The axonal membrane contains molecules that trigger mitogenesis in Schwann cells and oligodendrocytes (Salzer *et al.,* 1980a; 1980b; DeVries *et al.,* 1983; Chen and DeVries, 1989) and regulate the rate and degree of myelin formation (Black *et al.,* 1986; Waxman 1987a,b). Although some Schwann cells go on to myelinate axons, others only ensheath bundles of unmyelinated axons. These nonmyelinated Schwann cells express distinct molecular markers such as the low-affinity nerve growth factor receptor (NGF-R), neural cell adhesion molecules (N-CAM and L1), and growth associated protein-43 (GAP-43), but none of the myelin specific proteins (see following sections, as well as Mirsky and Jessen, 1990; Curtis *et al.,* 1992). Transplantation studies have shown that the axon determines the phenotype of the Schwann cell (Aguayo *et al.,* 1976; Weinberg and Spencer, 1976). Thus, there are a number of potentially distinct physical interactions between Schwann cells and axons as Schwann cells proliferate, migrate, ensheath axons, and form myelin sheaths during development of the PNS.

Although the axon influences and helps direct the formation of myelin, the myelin sheath also significantly defines features of the axon. The premyelinated axon is electrically excitable (Foster *et al.,* 1982; Waxman *et al.,* 1989) and the loss of excitability in the internodal axonal membrane that occurs with myelination involves an active suppression of Na^+ channels by the overlying oligodendrocyte or myelin sheath (Black *et al.,* 1985, 1986). Proliferation of oligodendrocyte precursors in the optic nerve is dependent on axonal electrical activity in that the blockage of optic nerve electrical activity by transection or exposure to tetrodotoxin resulted in a dramatic loss of oligodendrocyte precursor cells (Barres and Raff, 1993). Potassium channels may also participate in myelin formation; the potassium channel blocker, TEA^+ was shown to be effective in eliminating myelination in spinal cord explants while leaving axonal conduction and synapse formation intact.

IV. Myelin-Forming Cells and Their Ontogenic Development

The ontogenic development of the myelinating Schwann cell lineage has been relatively well-characterized, particularly in rodents. Most of our knowledge derives from cell-culture studies, but *in vivo* developmental studies in normal as well as in transgenic and gene knock-out mice have also proved useful. Understanding of the events and stages involved is of potential clinical relevance, not only with respect to various toxic neuropathies and to PNS nerve regeneration, but also because Schwann cell precursors may be attractive

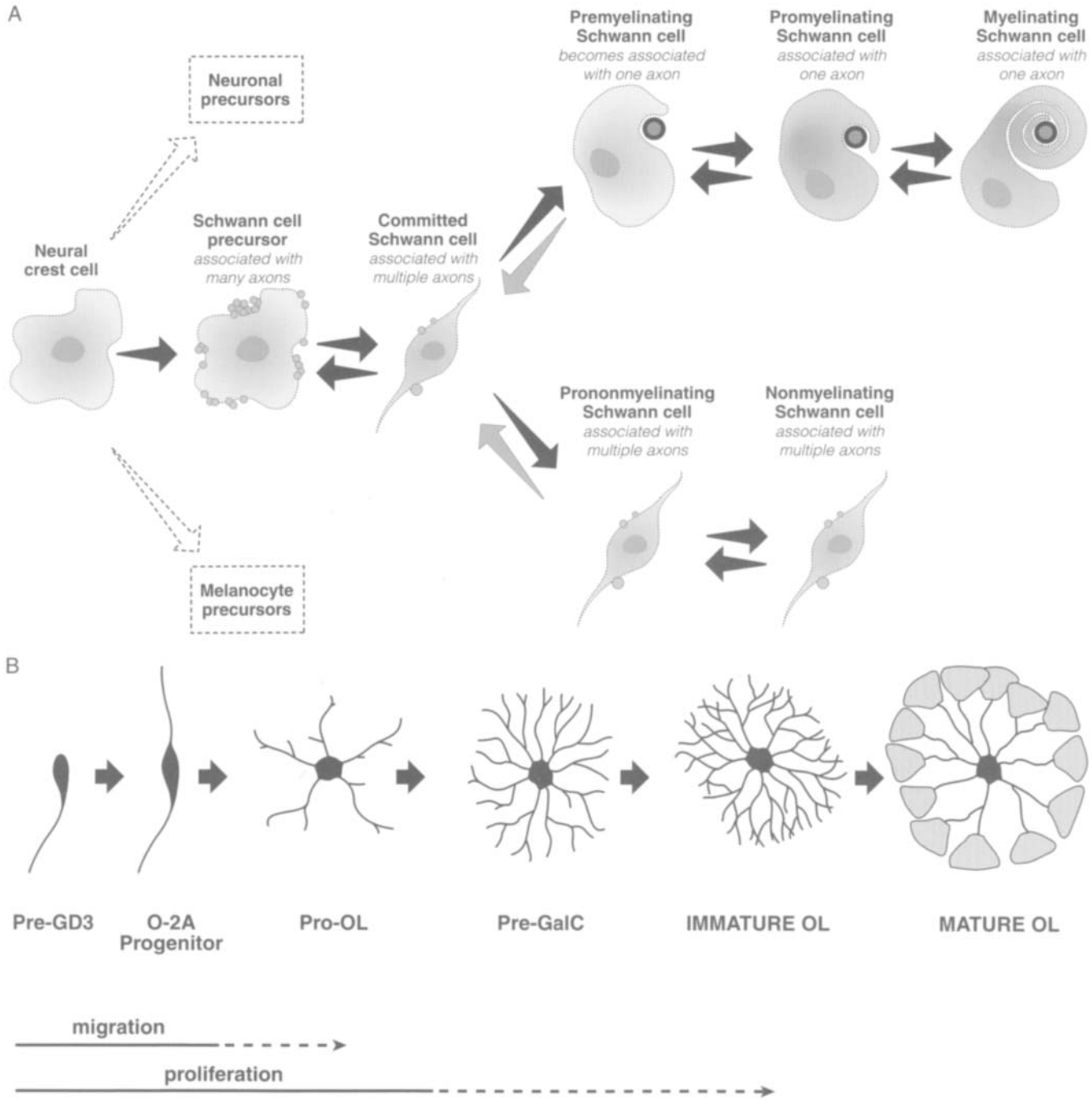

FIGURE 4 Ontogenic development of myelin-forming Schwann cells of the PNS and CNS. (A) Myelinating Schwann cells of the PNS originate from primitive neural crest cells, proliferative multipotential cells also capable of differentiating into neurons or melanocytes. The first step along the Schwann cell lineage gives the Schwann cell precursor, a proliferative cell that becomes associated with many axons and expresses the low-affinity nerve growth factor receptor (NGF-R), growth-associated protein 43 (GAP-43), and the neural cell adhesion molecules N-CAM and L1. The subsequent "committed" Schwann cell becomes associated with progressively fewer axons and expresses, in addition to the previously noted markers, S-100 protein (from this stage onward, all Schwann cells express S-100). Committed Schwann cells develop into either nonmyelinating Schwann cells, which remain associated with several axons and express galactocerebroside (GalC) in addition to the previous markers, or into myelinating Schwann cells. Myelinating Schwann cells progress through a proliferative "premyelinating" stage, characterized by transient expression of suppressed cAMP–inducible Pou-domain transcription factor (SCIP), followed by a "promyelinating" GalC-positive stage, becoming associated with a single axon in the process. The final differentiation into a mature myelinating Schwann cell involves

candidates for transplantation to facilitate remyelination and repair in injured CNS. A detailed discussion of this subject is beyond the scope of this chapter, but the reader is referred to several comprehensive reviews on this subject if more details are desired (Jessen and Mirsky, 1991; Gould *et al.,* 1992; Mirsky and Jessen, 1996; Zorick and Lemke, 1996). Schwann cells develop from neural crest cells and most of the key developmental stages in Schwann cell maturation seem to depend on axon-associated signals. Schwann cell precursors give rise to immature Schwann cells, which have a distinct phenotype (Fig. 4A). These cells then develop into either myelin-forming or nonmyelin-forming Schwann cells, under the control of reversible processes regulated by axon-associated signals. Even mature Schwann cells show a high degree of plasticity. Following a demyelinating insult, Schwann cells dedifferentiate into more primitive precursor cells, but generally do not die. When appropriate conditions present themselves (such as the regrowth of axons that occurs following a nerve-crush injury), these cells can proliferate, reestablish contact with axons, redifferentiate to the myelin-forming phenotype, and remyelinate the axons, thereby restoring normal function.

It is possible that primitive neural crest cells enter the Schwann cell lineage only when they first encounter axons in the developing nerves, and that a signal related to axonal contact guides them down the path to mature Schwann cells. Such signals may involve members of the Neu-differentiation factor (NDF) family. Members of the NDF growth factor family, including glial growth factor (GGF), heregulin, acetylcholine-inducing activity (ARIA), and neuregulin, are alternatively spliced products of a single gene, and these molecules are emerging as important regulators of Schwann cell lineage development (Dong *et al.,* 1995; Zorick and Lemke, 1996). Alternatively, it is possible that selected neural crest cells have already entered the Schwann cell lineage before they encounter axons (see Mirsky and Jessen, 1996, for discussion).

Investigation of which transcription factors regulate Schwann cell development is a current area of active study. Among factors likely to play significant roles are the zinc-finger transcription factors Krox-20 (Topilko, 1994), SCIP (see Zorick and Lemke, 1996), Pax3 (Kioussi *et al.,* 1995), and possibly c-jun (Stewart, 1995). A number of signalling molecules also regulate Schwann cell proliferation and myelination, including insulin-like growth factor-1 (IGF-1), which promotes expression of a myelinating phenotype in cell culture, and transforming growth factor βs (TGF-β), which inhibit myelin formation. The latter may be involved in generating the nonmyelinating Schwann cells, which ensheath smaller PNS axons.

The developmental lineage of the oligodendrocyte, the myelin-producing cell of the CNS, is also relatively well characterized, particularly in rodent *in vitro* systems (Fig. 4B). Oligodendrocytes originate as neuroectodermal cells of the subventricular zones and then migrate, proliferate, and further differentiate into mature, postmitotic myelin-forming oligodendroglial cells. Their development is discussed only briefly here, but a more comprehensive review is available (Warrington and Pfeiffer, 1992). Panels of cell- and stage-specific antibodies have proved especially useful in characterizing the sequential expression of various developmental markers, and this has allowed identification of distinct phenotypic stages, each characterized by its proliferative capacity, migratory ability, and distinct morphologies. Primitive precursor cells differentiate into proliferative, migratory bipolar O2A progenitor cells. These cells are bipotential, being capable of differentiating into either astrocytes or oligodendrocytes. The oligodendrocyte lineage progresses through several additional stages, including an immature oligodendrocyte expressing galactocerebroside, sulfatide, and cyclic nucleotide phospho-

downregulation of NGF-R, GAP-43, N-CAM, and L1 expression, with upregulation of expression of GalC and myelin proteins, and *in vivo,* the synthesis and elaboration of myelin. Because of the high degree of plasticity of Schwann cells, most of the developmental steps shown are reversible. (Modified from Mirsky and Jessen [1996] and Zorick and Lemke [1996]). (B) Myelinating oligodendroglial cells of the CNS originate from neuroectodermal cells of the subventricular zones of the developing brain. The earliest precursor cells recognized to date (Pre-GD3 stage) are proliferative, unipolar cells that express the embryonic neural cell adhesion molecule (E-NCAM). These cells develop into GD3 ganglioside-expressing proliferative bipolar cells, termed *O-2A progenitor cells* because they are capable (in culture, at least) of developing into either "type 2" astrocytes or oligodendrocytes. Development continues through a postmigratory but proliferative multipolar pro-oligodendroblast (Pro-OL) and a Pre-GalC stage, characterized by lack of expression of GalC. The onset of terminal oligodendroglial cell differentiation (immature OL stage) is identified by the surface appearance of a subset of "myelin components," consisting of the lipids GalC and sulfatide, as well as the enzyme cyclic nucleotide phosphodiesterase (CNP). Immature OLs then undergo final differentiation into mature oligodendrocytes (mature OL), characterized by regulated expression of myelin components such as MBP and PLP and by the synthesis and elaboration of sheets of myelin membrane.

diesterase (all myelin components), finally arriving several days later at the mature oligodendrocyte stage. Mature oligodendrocytes express all of the myelin-specific proteins and are capable of myelin synthesis *in vitro* in the absence of axons.

It is worth noting that oligodendroglial cells show some developmental plasticity, and this may be of clinical relevance with respect to CNS remyelination. In addition, a small population of oligodendrocyte progenitors persist in the adult rat brain (see Wolswijk and Noble, 1995, for details), and these constitute a potential source of myelin-forming cells for CNS remyelination. Immature, cycling oligodendroglial progenitor cells endogenous to adult white matter are capable of remyelinating CNS axons following lysolecithin-induced demyelination (Gensert and Goldman, 1997). Also, O2-A progenitor cells from mice subjected to coronavirus-induced demyelination show increased phenotypic plasticity and enhanced mitotic potential, properties that may be linked to the efficient remyelination that occurs following the demyelinating phase of this disease (Armstrong *et al.,* 1990). Manipulation of these progenitor cells by various factors (see later this chapter) thus may also be useful in promoting remyelination in a clinical context.

As is the case for Schwann cells in the PNS, development of oligodendrocytes is governed by a number of growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), IGF-1, TGF-β, and nerve growth factor (NGF), as well as several cytokines (see Pfeiffer *et al.,* 1993). Oligodendrocytes differ from Schwann cells in that they can be induced to produce myelin in culture in the absence of axons. This raises the question of the extent to which neurons and their axons influence oligodendrocytes with respect to development and myelination *in vivo.* It is, however, difficult to imagine the lack of significant interaction between these two cells in the developing nervous system, and in fact, many such interactions are known. Neurons produce many of the growth factors involved, and they are known to modulate steady-state levels of myelin components as well as mRNA levels for these components (Singh and Pfeiffer, 1985; Macklin *et al.,* 1986; Kidd *et al.,* 1990; Barres and Raff, 1993,).

As in the PNS, production of myelin by oligodendrocytes requires the coordinated synthesis of massive amounts of myelin components. The marked up-regulation of myelin-specific proteins, as well as of enzymes involved in synthesis of myelin lipids (see later this chapter), reflects corresponding increases in abundance of the respective mRNA transcripts, suggesting that most regulation of the program for myelination occurs at the level of transcription (see Hudson, 1990, for review). A possible candidate for the coordinate control of CNS myelination is myelin transcription factor 1 (MyTI), a zinc-finger DNA-binding protein first identified by its ability to recognize the myelin proteolipid protein (PLP) gene (Kim and Hudson, 1992). MyTI mRNA transcripts are most abundant in oligodendrocyte progenitor cells, suggesting that this factor acts at a very early stage in the regulation of transcription for myelinogenesis (Armstrong *et al.,* 1995).

V. Composition of Myelin

Myelin of both the CNS and PNS has a distinctive composition that differs somewhat from that of most cellular membranes. It is the major component of white matter of the CNS, accounting for about half the dry weight of this tissue, and is responsible for its glistening white appearance. The same is true for larger nerves of the PNS, such as the sciatic nerve. Myelin *in situ* has a water content of about 40%, and is characterized by its high lipid content (70–85% of its dry mass) and its correspondingly low protein content (15–30%). Most biologic membranes have a much higher protein : lipid ratio, usually somewhere around unity. The insulating properties of myelin, vital to its physiological function, are related to this high lipid content. The high lipid content of myelin also results in a buoyant density less than that of other biologic membranes, and advantage can be taken of this to isolate myelin with a high yield and a high degree of purity (Norton and Poduslo, 1973a).

The lipid content of CNS myelin (Table 1) is characterized by high levels of galactolipids (about 32% of lipid dry weight), including galactocerebroside (Gal-C) and its sulfated derivative, sulfatide, and cholesterol (about 26% of total lipid weight), with phospholipids accounting for most of the remainder (Norton and Cammer, 1984; DeWille and Horrocks, 1992; Morell *et al.,* 1994). Plasmalogens, phospholipids having a fatty aldehyde linked to the C1 of glycerol instead of a fatty acid, are especially prominent in myelin. Ethanolamine plasmalogens and phosphatidylcholine (lecithin) are the major phospholipid species. Gangliosides are also present in minor amounts. PNS myelin has a similar composition, although there are quantitative differences (see Smith, 1983). PNS myelin has less cerebroside and sulfatide and more sphingomyelin than CNS myelin. These differences are minor, however, relative to the larger differences in protein composition discussed later.

The protein composition of myelin is relatively simple in that a few major structural proteins account for the bulk of the total protein (Table 2). The PLP and the myelin basic proteins (MBP) together account for about 80% of the protein content of CNS myelin (Nor-

TABLE 1 Lipid Composition of Rat and Human CNS and PNS Myelin and Rat Whole Brain[a]

Component[b]	Human CNS myelin	Rat CNS myelin	Rat whole brain	Human PNS myelin[c]	Rat PNS myelin[c]
			(percent total dry weight)		
Protein	30.0	29.5	56.9	28.7	—
Lipid	70.0	70.5	37.0	71.3	—
			(percent total lipid weight)		
Cholesterol	27.7	27.3	23.0	23.0	27.2
Total galactolipid	27.5	31.5	21.3	22.1	21.5
Cerebroside	22.7	23.7	14.6	—	15.8
Sulfatide	3.8	7.1	4.8	—	5.7
Total phospholipid	43.1	44.0	57.6	54.9	50.6
EPG[d]	15.6	16.7	19.8	19.4	19.2
CPG	11.2	11.3	22.0	8.3	9.6
Sphingomyelin	7.9	3.2	3.8	17.7	7.0
SPG	4.8	7.0	7.2	9.4[f]	11.0
IPG	0.6	1.2	2.4	—[f]	2.5
Plasmalogens[e]	12.3	14.1	11.6	16.1	—

[a]See Norton and Cammer, 1984; Morell *et al.*, 1994; and Morell and Toews, 1996b, for references and additional details.

[b]Other lipids are also present in myelin, including gangliosides, galactosyl diglycerides, and fatty acid esters of cerebroside; although not shown in the table, polyphosphoinositides may account for up to 7% of total myelin lipid phosphorus (see Morell *et al.*, 1994).

[c]Calculated from data in Norton and Cammer (1984), using 800 and 750 as average molecular weights for phosphoglycerides and sphingomyelin, respectively.

[d]Abbreviations: EPG, ethanolamine phosphoglycerides; CPG, choline phosphoglycerides; SPG, serine phosphoglycerides; IPG, inositol phosphoglycerides.

[e]Primarily ethanolamine plasmalogens.

[f]Value includes both SPG and IPG.

ton and Cammer, 1984; Morell *et al.*, 1994). In contrast, P_0 protein, a protein not found in the CNS, accounts for more than half the total protein of PNS myelin.

TABLE 2 Protein Composition of CNS and PNS Myelin[a]

Component	CNS	PNS
	(percent total myelin protein)[c]	
PLP/DM20[b]	50	<0.01[d]
P_0	<0.01[d]	>50
MBP	30	18
CNP	4	0.4
MAG	1	0.1
P2	<1	1–15
PMP-22	<0.01[d]	5–10

[a]Only major proteins are shown; see text for discussion of other myelin proteins, and Morell *et al.* (1994) and Newman *et al.* (1995) for additional references and details.

[b]Abbreviations: PLP, proteolipid protein; MBP, myelin basic protein; CNP, cyclic nucleotide phosphodiesterase; MAG, myelin-associated glycoprotein; PMP-22, peripheral myelin protein-22.

[c] Composite values representative of adult mammalian myelin.

[d]Although mRNA for this protein has been detected, the protein itself, if present at all, is present in myelin at only low to undetectable levels.

MBP, P2-protein, and PMP-22 account for most of the remainder of PNS myelin proteins. PLP, the major protein component of CNS myelin, is present in PNS myelin at only very low levels, if at all (see later). In both CNS and PNS myelin, there are a number of other minor but integral protein components, and the list will continue to grow as research continues. These include structural proteins and proteins involved in cell–cell interactions, as well as a large number of enzymes, receptors, and second messenger–related proteins. All of these have vital roles in maintaining the complex structure of myelin and/or in its function. Characteristics of some myelin proteins, including selected aspects of their gene structure and expression, follows, but it is necessarily brief. For a more detailed discussion of individual myelin proteins, see Lemke (1988), Morell *et al.* (1994), Campagnoni (1995), and Newman *et al.* (1995).

It is worth noting at this point that the composition of myelin changes during development, with the first myelin deposited having a somewhat different composition than that present in adults (Norton and Cammer, 1984). In the rat brain, myelin galactolipids increase by about 50%, and phosphatidylcholine decreases by a similar amount. Similar changes have been noted in human myelin as well. Other minor changes in lipids and gangliosides also occur. The protein portion of myelin also changes somewhat during development; both MBP and PLP increase during development, whereas

the amount of higher molecular weight proteins decreases.

A. Myelin Basic Proteins (MBPs)

Myelin basic proteins (MBPs) are highly basic proteins of related isoforms derived from alternative splicing of a single gene. The MBP gene consists of seven exons distributed over about 32 kb of chromosome 18 in the mouse (Roach *et al.,* 1985) and human (Sparkes *et al.,* 1987) and chromosome 1 in the rat (Koizumi *et al.,* 1991); at least six transcripts are expressed via alternative splicing of RNA (Table 3). The MBP gene is actually a "gene within a gene," being part of a much larger (approx. 105 kb in mice and 179 kb in humans) transcriptional unit, called the *Golli-mbp* gene (Campagnoni *et al.,* 1993; Pribyl *et al.,* 1993). Portions of the *Golli-mbp* gene are expressed outside the nervous system, including the immune system, although the exact function of these gene products remains unknown. This may be of relevance to clinical disorders related to autoimmunity against MBP.

Expression of the various MBP protein products is also very complex; in addition to alternative splicing of a number of exons, there is also considerable transcriptional and posttranscriptional control (see Campagnoni, 1995, for details). This complexity of gene expression is augmented by posttranslational protein modifications, including loss of C-terminal arginine, N-acylation, glycosylation, phosphorylation, methylation, deamination, and substitution of some arginine residues with citrulline (Toews and Morell, 1987; Smith, 1992; Morell *et al.,* 1994). MBPs are extrinsic membrane proteins localized to the cytoplasmic membrane surface (major dense line) of myelin of both the CNS and PNS. In the CNS this protein accounts for approximately 30% of the total myelin protein, whereas in the PNS it accounts for only 18% (Greenfield *et al.,* 1980). Myelin lipids can promote MBP self-association, suggesting it may exist as oligomers on the cytoplasmic surface of the myelin membrane (Smith, 1992). It has been suggested that stabilization and maintenance of the myelin structure may be due to specific associations between MBPs and sulfatides and gangliosides (Ong and Yu, 1984; Maggio and Yu, 1989, 1992; Mendz, 1992; Smith, 1992).

B. Proteolipid Protein (PLP) and DM20

Proteolipid protein (PLP) and DM20 integral membrane proteins with several transmembrane domains. PLP is the most abundant protein in CNS myelin (50%), and although mRNA for this protein is present in the PNS, the protein itself is present at only very low levels in PNS myelin (Lemke, 1992) and its function in PNS myelin is unknown (Puckett *et al.,* 1987; Kamholz *et al.,* 1992). A report (Garbern *et al.,* 1997) of a human PLP null mutation phenotype characterized by a demyelinating peripheral neuropathy suggests that PLP/DM20 is necessary for proper myelin formation in the PNS as well as the CNS. This report also demonstrates by immunoelectron microscopy the presence of PLP in compact PNS myelin (however, see also Puckett *et al.,* 1987). Like MBP, PLP is one of the products of alternative splicing of a single gene, having a molecular weight of approximately 25kDa. DM20, a second isoform that migrates as a 20kDa band on SDS gel electrophoresis, is identical to PLP except for the deletion of amino acid residues 116–150 (Macklin, 1992). The PLP–DM20 gene, located on chromosome X in the mouse, rat, and human, is approximately 17 kb in length and consists of seven exons. The alternative splicing of this gene to give PLP and/or DM20 is developmentally regulated, with the DM20 splice product predominating during early myelination. Some mutations of the PLP/DM20

TABLE 3 "Myelin" Mutant Mice

Myelin gene (chromosome)	Mouse mutant	Human disease equivalent	References[a]
MBP	*shiverer*		Roach *et al.,* 1985
(18)	*shiverer*^{mld}		Popko *et al.,* 1988
PLP	*jimpy*	Pelizaeus–Merzbacher Disease	Nave *et al.,* 1986
(X)	*jimpy*^{msd}		Gencic and Hudson, 1990
	rumpshaker		Schneider *et al.,* 1992
P_0	*P_0-deficient*	Charcot–Marie–Tooth disease, type 1B	
(1)			Giese *et al.,* 1992
PMP-22	*trembler*	Charcot–Marie–Tooth disease type 1A	Suter *et al.,* 1992a
(1)	*trembler*^{J}		Suter *et al.,* 1992b
??	*quaking*^{viable}		Ebersole *et al.,* 1992
(17)			

[a]See text for additional references and discussion.

gene (e.g., *jimpy* mice; see later this chapter) result in developmental abnormalities prior to any myelination, suggesting that gene may be involved in other functions besides myelination. In addition, expression is not confined to myelin-producing oligodendrocytes in the CNS. The role of protein products of this gene outside the nervous system remain unknown, however. Missense mutations of the PLP/DM20 gene give rise to a host of CNS pathologies, the most devastating being Pelizaeus–Merzbacher disease (PMD) (see Seitelberger, 1995). In some forms of PMD, there is complete deletion of the PLP/DM20 gene (Raskind *et al.,* 1991). The described single base pair deletion in humans leads to the absence of PLP/DM20 expression; this produces a disease similar to, but less severe than, classic PMD, but also involving a progressive demyelinating peripheral neuropathy (Garbern *et al.,* 1997).

A number of both positive and negative *cis*-acting elements, as well as some *trans*-acting factors, have been identified for this gene (see Campagnoni, 1995, for details). In its orientation in the myelin membrane, the extracellular domains of PLP may be instrumental in stabilizing the intraperiod line of myelin (Morell *et al.,* 1994). In addition, PLP may play an active role as an ion channel (Toews and Morell, 1987; Lees and Bizzozero, 1992). As noted previously, DM20 is generally a relatively minor product of the PLP gene but it shows a pattern of developmental regulation distinct from PLP. It is expressed earlier in development than PLP and is the major PLP gene product in the developing embryo (Ikenaka *et al.,* 1992; Macklin, 1992; Timsit *et al.,* 1992). Its presence in "premyelinating" glial cells and in cells outside the glial cell lineage suggest a possible functional role unrelated to myelination.

C. Myelin-associated Glycoprotein (MAG)

Myelin-associated glycoprotein (MAG) is the principal glycoprotein of central nervous system myelin (for review, see Milner *et al.,* 1990; Quarles *et al.,* 1992). MAG is heavily glycosylated and is specific to myelin sheaths, with an especially high concentration in the periaxonal regions of both CNS and PNS myelin. It is a member of the immunoglobulin gene superfamily (Sutcliffe *et al.,* 1983; Lai *et al.,* 1987; Salzer *et al.,* 1987). Examination of the extracellular, N-terminal domains suggest that MAG is most closely related to the cell adhesion molecules N-CAM, L1, and contactin. In the PNS, MAG immunostaining is seen in glial membranes of the Schmidt–Lantermann incisures, paranodal loops, and mesaxons (Trapp, 1990) and is distinctly absent from the compact myelin sheath. It is thought that MAG plays a major role in membrane–membrane interactions during myelin formation and maintenance (Salzer *et al.,* 1990; Quarles *et al.,* 1992; Morell *et al.,* 1994). It is presumed to be involved in the adhesion of the myelin sheath to the axonal plasmalemma and in membrane spacing (Trapp, 1990), and it has been implicated in various peripheral neuropathies (Mendell *et al.,* 1985; Tatum, 1993). MAG exists as two isoforms (L-MAG and S-MAG) that are derived by alternative splicing from a single gene. L-MAG is produced almost exclusively in the CNS and is the predominant variant during early development and active myelination (Campagnoni, 1988; Trapp, 1990). S-MAG is the major isoform in the adult CNS and in the PNS at all ages. It is thought that the differences in distribution within the CNS and PNS may be associated with either the phosphorylation or other posttranslational modifications (e.g., sulfation of oligosaccharide moieties, acylation of the transmembrane domain) altering interactions with the cytoskeleton (Trapp, 1990; Quarles *et al.,* 1992). Homotypic interaction may be operational in Schmidt–Lantermann incisures, paranodal loops, and mesaxon membranes in PNS myelin, whereas heterotypic interactions with axolemmal constituents may mediate glia–axon adhesion (Salzer *et al.,* 1990; Trapp, 1990; Quarles *et al.,* 1992).

D. 2′-3′-Cyclic Nucleotide-3′-Phosphodiesterase (CNP)

2′-3′-Cyclic nucleotide-′-phosphodiesterase (CNP) is localized within oligodendrocytes in the CNS and within Schwann cells in the PNS. One of the earliest markers for cells of the oligodendroglial lineage, CNP is an enzyme that hydrolyzes 2′,3′-cyclic nucleotides to form 2′-nucleotides exculsively. Because no physiologically relevant substrate molecules have been found in myelin, however, this enzymatic activity may actually be vestigial and unrelated to its function in myelin. The current view of CNP is that it may be a key component of an interactive protein network within oligodendroglial cells, possibly involved in extension of processes (e.g., see Braun *et al.,* 1990). It is isoprenylated, suggesting possible involvement in signal transduction processes (Braun *et al.,* 1991). Furthermore, the presence of potential nucleotide-binding domains on CNP (Sprinkle, 1989) suggest it might exert regulatory influence on various cellular processes such as growth and differentiation by serving as a link between extracellular signals and intracellular effector molecules. Its exact function within myelin and oligodendroglial cells, however, remains unknown.

E. Myelin/Oligodendrocyte Glycoprotein (MOG)

Mylein/Oligodendrocyte glycoprotein (MOG) is localized primarily at the external surfaces of the myelin

sheath and oligodendrocytes. It is developmentally regulated, appearing with the onset of myelination as one of the last myelin protein genes expressed (Scolding *et al.,* 1989). Features of the protein structure suggest it may be a member of the immunoglobulin gene superfamily (Gardinier *et al.,* 1992). Because anti-MOG antibodies can cause demyelination *in vivo* (Schluesener *et al.,* 1987), it has received attention for a potential role in autoimmune-mediated demyelination such as experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (Gunn *et al.,* 1989; Bernard and Kerlero de Rosbo, 1991).

F. Oligodendrocyte–Myelin Glycoprotein (Omgp)

Oligodendrocyte–myelin glycoprotein (Omgp) is one of the minor protein components of myelin that appears in the CNS during the period of myelination. It is highly glycosylated and appears to be specific to oligodendrocytes and myelin membranes in the CNS (Mikol and Stefansson, 1988). The protein is anchored to the membrane through a glycosylphosphatidylinositol intermediate. A subpopulation of Omgp molecules contain the human natural killer cell antigen–1 (HNK-1) carbohydrate (Mikol *et al.,* 1990b). The presence of a tandem leucine repeat domain in the predicted polypeptide sequence and the apparent presence of Omgp at the paranodal regions of the myelin sheath have lead to speculation that its major role is as an adhesion molecule that mediates axon–glial cell interactions (Mikol *et al.,* 1990a).

G. P_0 Protein (Peripheral Myelin Protein Zero)

P_0 protein (peripheral myelin protein zero) accounts for more than 50% of the protein in peripheral nerve myelin (Ishaque *et al.,* 1980). This has lead to the suggestion that P_0 is the PNS equivalent of PLP in the CNS, although the properties of these two proteins are very different. P_0 is a transmembrane protein with a glycosylated extracellular domain, a single membrane spanning region, and a highly basic intracellular domain (Lemke,(Lemke and Axel, 1985). It is thought to play an important role in the compaction of myelin through the homotypic interaction of molecules on adjacent myelin lamellae (Lemke, 1988). Like MAG, it is a member of the immunoglobulin superfamily. Unlike many of the other myelin protein genes, expression of the P0 gene is highly restricted to Schwann cells. The P_0 gene contains six exons distributed over about 7 kb in both rats and mice (Lemke, 1988; You *et al.,* 1991). Based on transgenic experiments, elements regulating its expression appear to reside in first 1.1 kb of the 5′-flanking region (Messing *et al.,* 1992). Mutation of the P_0 gene in humans is associated with Charcot–Marie–Tooth disease, type 1B, an inherited peripheral neuropathy (Hayasaka *et al.,* 1993; Kulkens *et al.,* 1993).

H. Peripheral Nerve Protein P2

Peripheral nerve protein P2 is a basic protein distinct from MBP. Interest in P2 arose from its ability to induce experimental allergic neuritis, a demyelinating disease of the PNS (Kadlubowski and Hughes, 1980). It has sequence homology to cellular retinol and retinoic acid–binding proteins (Crabb and Saari,1981; Eriksson *et al.,* 1981) and fatty acid–binding proteins (FABPs) (Veerkamp *et al.,* 1991), and has high affinity for oleic acid, retinoic acid, and retinol (Uyemura *et al.,* 1984). The P2 protein gene belongs to an ancient family of FABPs that diverged into two major subfamilies (Medzihradszky *et al.,* 1992). P2 mRNA levels parallel myelination during development as well as the levels of microsomal enzymes involved in fatty acid elongation. This suggests the P2 protein may be involved in fatty acid elongation or in the transport of very long-chain fatty acids to myelin (Narayanan *et al.,* 1988).

I. Peripheral Myelin Protein-22 (PMP-22)

Peripheral myelin protein-22 (PMP-22) is a glycoprotein with an apparent molecular weight of about 22 kDa (Kitamura *et al.,* 1976; Smith and Perret, 1986). The rat and human genes have been cloned (Spreyer *et al.,* 1991; Welcher *et al.,* 1991; Hayasaka *et al.,* 1992); although these have a high homology to the growth arrest–specific mRNAs for gas 3 and PASII-glycoprotein, the function of PMP-22 in PNS myelin remains unknown. The PMP-22 gene maps to mouse chromosome 11 and a point mutation in this gene is apparently responsible for the autosomal dominant mutation in the Trembler (Tr) mutant mouse (Suter *et al.,* 1992a,b). In humans, the gene maps to chromosome 17; this gene is duplicated in patients with Charcot–Marie–Tooth disease, type 1A, and this alteration is presumably related to the pathology (Patel *et al.,* 1992; Valentijn *et al.,* 1992; see Campagnoni, 1995, and Newman *et al.,* 1995 for additional references and discussion). The rat PMP-22 gene, the expression of which is largely confined to the PNS, is developmentally regulated in Schwann cells, where its expression coincides with myelination (Spreyer *et al.,* 1991; Snipes *et al.,* 1992). mRNA expression is coordinately down-regulated along with other myelin proteins during tellurium-induced primary demyelination and during degeneration induced by nerve transection or crush; message levels are consequently up-regulated

along with other myelin genes during remyelination if it occurs (Spreyer *et al.,* 1991; Toews *et al.,* 1997).

J. Enzymes Associated with Myelin

Although myelin initially was believed to be metabolically inert (due largely to the very slow metabolic turnover of some of its components) and to function exclusively as an electrical insulator, it is now known that the picture is considerably more complex and interesting. All protein and lipid components of myelin turn over with measurable turnover rates (see Benjamins and Smith, 1984; Morell *et al.,* 1994). Although some structural components of myelin are indeed relatively stable metabolically, with half-lives of several months, there is also a very rapid turnover of some myelin components. The phosphate groups modifying myelin basic protein in compact CNS myelin turn over with half-lives of minutes or less (DesJardins and Morell, 1983), and the phosphate groups on myelin polyphosphoinositides also show a very rapid turnover rate. At least 40 different enzyme activities have been documented in CNS myelin (Newman *et al.,* 1995). In addition to CNP described previously, these include enzymes related to second messenger signalling, as well as associated receptors and G-proteins (Larocca *et al.,* 1990). A number of enzymes involved in myelin lipid metabolism are also present, including those for phospholipid synthesis and catabolism. Noteworthy among the latter are phospholipase C activities for polyphosphoinositides, and these may have important roles in signal transduction mechanisms in myelin (Ledeen, 1992). Also of note are a cholesterol ester hydrolase, and UDP-galactose: ceramide galactosyltransferase, the terminal enzyme in biosynthesis of galactocerebroside, the most "myelin-specific" lipid. Various proteases, protein kinases and phosphatases, and transport-related enzymes are also present; of particular interest with respect to the transport-related enzymes are carbonic anhydrase (Cammer *et al.,* 1976) and Na^+,K^+-ATPase (Zimmerman and Cammer, 1982). These enzymes may be involved in controlling K^+ levels at nodes of Ranvier (Lees and Sapirstein, 1983) and/or in removal of carbonic acid from metabolically active axons.

K. Cytoskeletal Proteins in Myelin and Myelinating Glia

The cell surface area of myelin-forming cells is so large as to suggest the need for specialized structures and mechanisms for transporting components between the perikaryon and the remote extensions. There is indeed a great deal of protein and lipid transport and targeting within the myelinating glial cell, and cytoskeletal elements play important roles in these processes. P_0, MAG, and laminin (a secreted extracellular matrix component; Cornbrooks *et al.,* 1983) are synthesized and modified in the rough endoplasmic reticulum (RER) and Golgi membranes of the perinuclear cytoplasm and then sorted into different carrier vesicles upon exit from the *trans* Golgi network (Trapp *et al.,* 1993). These proteins must be transported over millimeter distances prior to insertion into their proper surface membrane locations. Various proteins enriched in compact myelin reach their proper destinations via different mechanisms. For example, P_0 reaches compact myelin by vesicular transport, whereas it is the mRNA for MBP that is translocated, with synthesis of MBP occurring close to the site of its insertion into the forming myelin (Colman *et al.,* 1982; Trapp *et al.,* 1987; Griffiths *et al.,* 1989).

As noted previously, the cytoskeleton plays a major role in transport and assembly of myelin. In myelinating Schwann cells, microfilaments are enriched beneath the membranes of the Schmidt–Lanterman incisures, the outer and inner mesaxons, and portions of the outermost compact myelin lamellae and Schwann cell plasma membrane (Trapp *et al.,* 1989; Zimmerman and Vogt, 1989; Kordeli *et al.,* 1990). It is thought that interactions between MAG and microfilaments play a role in membrane motility during myelination (Trapp and Quarles, 1982; Trapp *et al.,* 1984; Martini and Schachner, 1986; Salzer *et al.,* 1987). MAG colocalizes with microfilaments at membranes that move during internodal growth (Trapp *et al.,* 1989). A role for MAG in myelin wrapping and spacing is supported by studies showing precocious spiral wrapping by myelinating Schwann cells transfected with additional copies of MAG (Owens *et al.,* 1990), and impaired or prevented wrapping when MAG expression is reduced or eliminated in Schwann cells (Owens and Bunge, 1991; Mendell *et al.,* 1985). Microfilaments also help to define and maintain organelle-rich channel regions and organelle-free non-channel regions of the myelin internode. The channel regions are important to formation and maintenance of the myelin internode and to intracellular transport of myelin components. They include the external cytoplasmic channels, Schmidt–Lanterman incisures, para nodal loops, and periaxonal cytoplasm. Microfilaments associated with the abaxonal plasma membrane and the adaxonal periaxonal membrane may have multiple functions in dealing with the external environment, including endocytosis (Blok *et al.,* 1982), pinocytosis (Phaire-Washington *et al.,* 1980), and exocytosis (John *et al.,* 1983; Koffer *et al.,* 1990), as well as stress resistance.

Just as they do in axons, microtubules may also subserve a role in the directional movement of organelles within glial cells. Post-Golgi vesicles transported in this

way could be involved in the growth, turnover, or modification of compact myelin at distant sites. Microtubules are the largest of the cytoskeletal filaments and provide a dynamic substrate for organelle trafficking and structural organization (for review see Dustin, 1984; Kirschner and Mitchison, 1986; Schroer and Sheetz, 1991). They are present in all major cytoplasmic compartments of the myelin internode, but are excluded from compact myelin (Peters *et al.,* 1991). In myelinating Schwann cells, microtubules are crucial to the transport of myelin proteins and organelles (Trapp *et al.,* 1995). This function is determined by the orientation and organization of microtubules, which in turn are influenced by axons (Kidd *et al.,* 1994). During the formation of the myelin sheath, contact with a myelin-inducing axon results in a more complex microtubule organization (Kidd *et al.,* 1994). As in axons, microtubules are inherently unstable and oscillate between phases of elongation and collapse (Dustin, 1984; Kirschner and Mitchison, 1986). The extent of depolymerization and repolymerization is determined by complex assembly/disassembly kinetics and can be influenced by modifications such as binding of MAPs (Sloboda *et al.,* 1976; Pryer *et al.,* 1992). Microtubule disassembly causes marked accumulation of P_0, MAG, and laminin in the perinuclear cytoplasm of myelinating Schwann cells (Trapp *et al.,* 1995). Because of this, chemically induced neurotoxicity involving microtubules may lead to alterations not only in axons, but also in myelin and myelinating cells as well.

In myelinating Schwann cells, the major intermediate filament is vimentin (Dahl *et al.,* 1982; Schachner *et al.,* 1984; Kobayashi and Suzuki, 1990). In most cells, intermediate filaments are considered to have a structural role in mechanically maintaining cell shape against external forces (Klymkowsky *et al.,* 1989; Skalli and Goldman, 1991) It has been proposed that vimentin intermediate filaments interact with microfilament-associated molecules and with microtubules in resisting stress (Wang *et al.,* 1993). It is thought that intermediate filaments play a role in the process of myelination in the PNS because myelinating Schwann cells contain abundant intermediate filaments and the content of these filaments between myelinating and nonmyelinating Schwann cells can vary substantially.

VI. Molecular Aspects of Myelin Synthesis and Assembly

Because the structure and composition of myelin is unique, its formation involves activation of a set of unique genes (see Lemke, 1988, for details). These genes include those related to induction of myelination (e.g., glial-specific receptors for differentiation signals), those involved in controlling and directing the initial deposition of myelin (e.g., axon–glial cell-adhesion molecules), and those involved in actual production of compact myelin (e.g., structural proteins of myelin). Genes for enzymes involved in synthesis of lipids enriched in myelin are also preferentially activated as well. The process of myelination is a highly regulated event that begins postnatally during the first few weeks of life in the rodent brain and within the third fetal trimester in the human spinal cord. In the CNS of rodents, maximum levels of synthesis of myelin components and actual accumulation of myelin occurs at about 3 weeks of age (Norton and Poduslo, 1973b; Norton and Cammer, 1984; Morell *et al.,* 1994), and although myelin accumulation continues for an extended time, the rate of synthesis declines considerably by about 6 weeks of age. This time course is similar to the profile of expression of myelin protein genes (see Campagnoni and Macklin, 1988). In the rodent PNS, myelination begins at about birth, peaks at about 2 weeks, and then decreases to a low basal level by the end of the first month (Webster, 1971). As is the case in the CNS, the pattern of myelin synthesis and accumulation is closely paralleled by expression of mRNA for PNS myelin protein components (Lemke and Axel, 1985; Stahl *et al.,* 1990) and for enzymes involved in synthesis of major myelin lipids (hydroxymethylglutaryl-Coenzyme A [HMG-CoA] reductase, the rate-limiting enzyme in cholesterol biosynthesis; and ceramide galactosyltransferase, the rate-limiting enzyme in cerebroside biosynthesis) (Lemke and Axel, 1985). Regulation of the expression of myelin genes occurs at a number of different levels including promotor choice, transcription, mRNA splicing and stability, translation, and posttranslational processing (for reviews see Campagnoni, 1988; Campagnoni and Macklin, 1988; Lemke, 1988; Nave and Milner, 1989; Ikenaka *et al.,* 1991; Mikoshiba *et al.,* 1991; Campagnoni, 1995).

The synthesis and assembly of myelin has been examined by measuring incorporation of radioactive precursors into myelin components both *in vivo* and in tissue slices, by measuring the *in vitro* activities of enzymes involved in synthesis of myelin components, by examining levels of expression of mRNA species for myelin-related genes, and by actual isolation and analysis of myelin (for reviews see Benjamins and Smith, 1984; DeWille and Horrocks, 1992; Morell *et al.,* 1994). After individual myelin components have been synthesized, they must be assembled to form mature compact myelin. Some components, such as PLP, which is synthesized on bound polyribosomes in the oligodendroglial perikaryon, show a time lag of about 45 min between their synthesis and their appearance in myelin, reflecting the

time required for transport from their site of synthesis to the forming myelin. Other components, such as MBP, show only a short lag, in keeping with their synthesis of free polyribosomes in oligodendroglial processes, near the actual site of myelin assembly. In keeping with this difference, studies have shown that myelinating cells spatially segregate mRNA species for myelin-specific proteins (see Trapp *et al.,* 1987). mRNA for MBP is transported to near the sites of myelin assembly before the protein is synthesized (Trapp *et al.,* 1988; Gillespie *et al.,* 1990), whereas PLP mRNA is present in a perinuclear location. Individual lipids also show different kinetics of entry into myelin following synthesis, and some of this may be due to synthesis in, or movement through, different intracellular pools (Benjamins *et al.,* 1976; Benjamins and Iwata, 1979; for review, see Benjamins and Smith, 1984).

The mature myelin internode contains several ultrastructurally and biochemically distinct membrane domains that include the outer plasma membrane of the myelin-forming cell and its attached compact myelin, as well as the Schmidt–Lanterman incisures, paranodal loops, nodal microvilli, the periaxonal membrane, and the membranes of the outer and inner mesaxons (see Figs. 1 and 3). Some of these membrane domains are compositionally distinct, containing different structural proteins and differing in lipid composition as well. With respect to the PNS, P_0, MBP, and PMP-22 are enriched in compact PNS myelin (Trapp *et al.,* 1981; Omlin *et al.,* 1982), whereas PLP and MBP predominate in compact CNS myelin. The exact mechanisms by which individual components are targeted to their respective membrane domains and other molecular aspects of actual myelin membrane assembly are not well understood, but this continues to be an active area of investigation. It seems clear that cytoskeletal elements are closely involved in the intracellular sorting and transport of myelin components, as discussed in a previous section, and they presumably also function in the actual process of myelin assembly.

VII. Mutant Dysmyelinating Mouse Models

In the mouse, terminal differentiation of myelin-forming cells occurs mostly after birth, following establishment of the basic wiring of the nervous system. Many neurologic mutations result in dysmyelination, the inability of myelin-forming glial cells to assemble qualitatively and/or quantitatively normal myelin (see Quarles *et al.,* 1994; Nave, 1995). Because myelination is a postnatal event in rodents, this inability to assembe normal myelin is not immediately lethal, and the animals usually survive for at least several weeks. Mutations that affect myelin are characterized behaviorally by abnormalities such as shivering, ataxia, and frequently including seizures; these signs of abnormal nervous system function begin at about the time when myelin accumulation becomes significant, probably an indication of the importance of myelin for motor control and normal brain function. In general, myelin-deficient mice possess a mutant gene for some structural myelin protein (see Lemke, 1988). The major known "myelin-deficient" mutations in mice are described as follows, as are some related transgenic and "knockout" mouse models.

A. *Shiverer Mice*

The shiverer mouse (shi, mouse chromosome 18) was one of the first neurologic mouse mutants examined at the molecular–genetic level (Roach *et al.,* 1983). Affected homozygotes lack any detectable MBP and fail to make normal CNS myelin. The behavioral phenotype of this mouse is first observed within the second postnatal week, when a general body tremor, which becomes more pronounced with intentional movements, develops (Biddle *et al.,* 1973; Chernoff, 1981). The shivering behavior derives from a loss of spinal motor and reflex control and increases with age, often progressing to include seizures. The life span of the shiverer mouse is limited to approximately 6 months of age.

Histologic examination shows severe dysmyelination throughout the entire CNS, but with normal-appearing PNS myelin (Privat *et al.,* 1979; Kirschner and Ganser, 1980; Rosenbluth, 1980). At the ultrastructural level, the pattern of dysmyelination is dominated by a severe lack of myelin. The myelin-like structures that are occasionally present are loosely wrapped around the axon and the intracellular adhesion zone of the extended cell process that normally forms the "major dense line" of myelin cannot be discerned (Readhead and Hood, 1990). The lack of proper myelin sheath formation may be the result of a defect in myelin compaction or a related process, because oligodendroglia appear to be normally differentiated. Histologic evidence of dysmyelination is supported biochemically by a dramatic reduction of all major myelin proteins, and more specifically by the complete lack of MBP. This is the result of a large (20 kb) genomic deletion encompassing exons 3–7 of the MBP gene (or exons 7–11 of the larger golli-MBP gene) resulting in no coding capacity for any of the MBP isoforms (Roach *et al.,* 1985; Molineaux *et al.,* 1986).

The tremoring phenotype can be cured by a number of manipulations associated with restoration of MBP expression, indicating that the amount of MBP available to the oligodendrocytes is a rate-limiting step in the assembly of CNS myelin. Successful approaches include

reintroduction of the entire wild-type MBP gene into the germ line of the shiverer mouse (Readhead *et al.,* 1987), increasing the transgene copy number (Popko *et al.,* 1987; Shine *et al.,* 1990), and the reintroduction of a MBP minigene encoding only the smallest (14 kDa) MBP isoform (Kimura *et al.,* 1989).

A shiverer-like phenotype can also be generated in normal mice by specifically down-regulating the amount of MBP mRNA available for protein synthesis via transgenic expression of the MBP gene in "anti-sense" orientation under the control of its cognate MBP promotor (Katsuki *et al.,* 1988). Similarly, the formation of antisense MBP mRNA is the presumed primary defect of the *myelin-deficient* (shi-mld) mouse mutant, an allele of the shiverer mutation on chromosome 18 (Doolittle and Schweikart, 1977; Popko *et al.,* 1988). The presence of this antisense RNA is thought to reduce and dysregulate the amount of normal MBP mRNA functionally available, thereby resulting in a level insufficient for normal myelin formation (Fremeau and Popko, 1990; Tosic *et al.,* 1990). The dysmyelination is less severe in the myelin deficient mouse when compared to the shiverer mouse. Isolated white matter tracts in CNS have a 3–4-fold increase in sodium channel density and it has been suggested that some myelin-associated molecule absent from the Shiverer white matter tracts could cause a down-regulation of either the synthesis or accumulation of sodium channels in myelinating axons (Noebels *et al.,* 1991). The lack of dysmyelination in the PNS is thought to be due to the substitution of P_0, the major integral protein of PNS myelin, for the structural function of MBP (Lemke and Axel, 1985).

B. Jimpy Mice—Dysmyelination and Glial Cell Death

The mammalian PLP gene is linked to the X chromosome and defects in this gene are associated with neurologic abnormalities in the mouse and with Pelizaeus–Merzbacher disease in humans. In the mouse, three mutations have been characterized: the jimpy (jp), myelin synthesis–deficient (jp msd), and rumpshaker (rsh). Each derives from a point mutation that alters the structure of the encoded protein. In the jimpy mouse, the mutation is a single nucleotide change in the PLP gene that inactivates the splice-acceptor site of intron 4. The last α-helical transmembrane domain is replaced by an aberrant carboxy terminus, and the resulting abnormally folded protein is degraded in the endoplasmic reticulum shortly after synthesis, failing to reach the Golgi apparatus for further processing and transport (Roussel *et al.,* 1987). The CNS is nearly completely devoid of myelin, with less than 1% of the axons ensheathed; PNS myelin, however, is ultrastructurally intact (Sidman 1964; Herschkowitz *et al.,* 1971). There are only a few layers of abnormally thin myelin around CNS myelin, consisting of either uncompacted membrane whorls or compacted myelin with abnormal ultrastructure (Duncan *et al.,* 1989). The major cause of the dysmyelination in the jimpy mouse seems to be a lack of differentiated oligodendrocytes. The proliferation rate of oligodendrocyte precursor cells is increased but an abnormally high rate of apoptotic cell death eliminates most of these maturating oligodendrocytes (Skoff, 1982; Knapp *et al.,* 1986; Barres *et al.,* 1992). Islands of myelinated fibers are formed by the few oligodendrocytes that escape degeneration and developmental arrest. The behavioral phenotype is evident at 2 weeks of age and consists of general body tremor and ataxia; the animals die with seizures and convulsions by about 4 weeks of age. Heterozygous jimpy females, which are mosaics with respect to the X-linked PLP gene, display normal behavior.

In the allelic mouse mutant, jimpy msd, there are similar ultrastructural alterations in myelin as seen in the jimpy mouse, but about twice as many glial cells escape premature degeneration (Billings-Gagliardi *et al.,* 1980). The rumpshaker mutant is the result of a novel mutation of the PLP gene (Schneider *et al.,* 1992) and displays a phenotype very different from the jimpy and the jimpy msd. Rumpshaker mice have more myelin than other dysmyelinated mutants and the degree of dysmyelination varies among CNS regions, with early myelinated regions appearing normal whereas late myelinating regions are severely hypomyelinated. The oligodendrocytes appear differentiated and most escape apoptotic cell death resulting in a normal complement of mature oligodendrocytes (Griffiths *et al.,* 1990). The rumpshaker mutation appears to allow the oligodendrocyte to survive but somehow interferes with its ability to normally deposit PLP in the myelin membrane (Schneider *et al.,* 1992). Although sparse, some myelin sheaths subsist in the rumpshaker mutants and these show selective immunostaining for DM20 (Schneider *et al.,* 1992). These findings suggest that DM20 may serve a critical purpose in glial cell development that is distinct from any function in myelin formation and maintenance.

C. P_0-Deficient Mice

P_0-deficient mice have been generated by homologous recombination of the P_0 gene in mouse embryonic stem cells with the cloned gene and subsequent generation of germline chimeric mice (Giese *et al.,* 1992). Animals lacking one functional copy of the P_0 gene are phenotypically normal, but the homozygotes develop a behavioral phenotype by the third week of life. These mice show a body tremor and dragging movements of the hindlimbs. There is no evidence of paralysis or sei-

zures and the mutants have a normal life span. Histologically, the deficit is characterized by the inability of the Schwann cell to assemble a compacted multilamellar PNS myelin sheath. The high degree of variability in the pathology is thought to be due to the intervening actions of other proteins such as cell adhesion molecules (MAG; N-CAM), and perhaps other myelin proteins. Using promoter and regulatory regions of the P_0 gene in a fusion gene construct, Schwann cells were destroyed when they began to express P_0, after associating in a 1 : 1 ratio with axons (Messing *et al.,* 1992). The behavioral phenotype of the Schwann cell–ablated mice was similar to the phenotype displayed in the homogygous P_0 mutants. A proliferation of nonmyelin-forming Schwann cells was induced along with skeletal muscle atrophy.

D. Trembler Mice

The trembler mouse (Tr; mouse chromosome 11) contains a mutation of the PMP-22 gene, which results in PNS dysmyelination. The PMP-22 gene encodes an integral membrane protein specific to Schwann cells (22 kDa peripheral myelin protein) believed to be important for normal Schwann cell development (Spreyer *et al.,* 1991; Welcher *et al.,* 1991). Histologically, the majority of large caliber axons in the sciatic nerve are devoid of a myelin sheath and, if present at all, these are abnormally thin and relatively uncompacted (Henry and Sidman, 1988). The total number of Schwann cells is dramatically increased at the time of segregation of axons, and myelin deposition is arrested. In the absence of PNS myelin, the mice display a behavioral phenotype characterized by a coarse-action tremor that begins at the end of the second postnatal week and results in moderate quadriparesis and a waddling gate. Under controlled conditions, these animals can experience a normal life span.

E. Quaking Mice

The quaking mouse (qk; mouse chromosome 17) is the result of an autosomal recessive mutation (Sidman *et al.,* 1964). Homozygous mice carrying the viable quaking allele (qkv/qkv) show the typical motor coordination signs of dysmyelination in the absence of seizures and have a normal life span. The myelin deficiency, characterized by fewer than normal myelin lamellae, is predominately in the brain and spinal cord, although a lack of normal compaction and enlarged intraperiod lines of some myelinated fibers has been noted in the PNS as well (Trapp, 1988). Interestingly, the distribution of MAG is shifted from the innermost myelin layer facing the axon to throughout the compact myelin sheath.

F. Myelin Mutants in Other Species

Myelin mutants in a number of other species besides humans and mice have also been described (see Duncan, 1995, for detailed discussion). These include X-linked mutations in the dog (shaking pup, Griffiths *et al.,* 1981), pig (type A III hypomyelinogenesis congenita, Blakemore *et al.,* 1974), rat (myelin-deficient; Dentinger *et al.,* 1982; Jackson and Duncan, 1988), and rabbit (paralytic tremor, Taraszewska, 1988), as well as the autosomal recessive *taiep* mutant rat (Duncan *et al.,* 1992).

VIII. Other "Disorders" of Myelination

As noted previously, myelination is a critical process in the maturation of the nervous system. It involves the synthesis of an enormous amount of specialized membrane within a relatively short period of time. Much of the myelin in both the CNS and the PNS is formed during a relatively short "developmental window" (first few years in humans; first 30 days of age in rodents), and this period is preceded by a burst of myelinating-cell proliferation. During these time periods, a large portion of the nervous system's metabolic capacity is devoted to myelinogenesis. During these "vulnerable periods," the process of myelination is especially susceptible to perturbations such as toxic insults, nutritional deficiencies, genetic disorders of metabolism, viral infections, substances of abuse, and other environmental factors (for review, see Wiggins, 1986). Insults occurring during the period of proliferation of myelinating cells may be especially disruptive, as this may lead to an irreversible deficit of myelin-forming cells and consequent permanent hypomyelination. Perturbation of myelination at a later stage may result in a myelin deficit that can be reversed. Depending on the timing of the insult, a myelin deficiency can result from alterations related to several different developmental events, including failure of myelin-forming cells to proliferate, reduction of axonal development resulting in fewer and/or smaller axons to myelinate, and decreased formation of myelin at time of maximal synthesis.

The morphologic term *myelinopathy* describes damage to white matter or myelin, and disorders of myelin can be classified by a number of different factors. Such factors include the preferential effects on either the CNS or on the PNS or an involvement of both systems. In addition, effects on myelin can sometimes be delineated as the result of a primary effect on myelin itself or the myelinating glial cell. Myelin loss due to a primary insult to myelin or the myelinating cell are termed *primary demyelination.* There are a number of factors relevant to selective targeting of various toxic or metabolic in-

sults to myelin (for discussion, see Morell *et al.,* 1994; Morell and Toews, 1996a). An intact axon is a prerequisite for maintenance of normal myelin; alterations in myelin due to an effect on the neuron or the underlying axon is termed *secondary demyelination.* Secondary demyelination is an inevitable consequence of serious damage to neurons supporting myelinating axons or to axonal transcetion or crush (Wallerian degeneration). However, the distinction between primary and secondary demyelination is often somewhat vague; the basis for this distinction usually involves morphologic evidence of the initial target site. The term *hypomyelination* is used to describe developmental alterations of myelination in which an insufficient amount of myelin accumulates. Hypomyleination can be the result of disease processes, undernutrition, or toxic insult. The term *dysmyelination,* when used in its strictest sense, refers to certain inborn errors of metabolism in which a block in the breakdown of a myelin lipid causes accumulation of myelin of an abnormal composition (which eventually leads to a collapse and degeneration of myelin), but it is also in wide use as a general descriptor of situations characterized by any abnormalities in myelin.

Some specific myelinopathies that are preferential to developing organisms are discussed later in this chapter. Additional toxicants have been demonstrated to disturb myelin in the adult animal and the morphologic descriptions and mechanistic processes involved have been previously discussed (Morell, 1994; Morell and Toews, 1997). These include tellurium, diphtheria toxin, 2′3′-dideoxycytidine, vigabatrin, carbon monoxide, triethyltin, lead, hexachlorophene, cuprizone, and isoniazid.

A. *Undernutrition*

In the human infant, several studies have provided evidence supporting the concept of a critical period from birth to about 2 years of age, during which time the nervous system is most vulnerable to malnutrition. The production of neurons is virtually completed by about the midpoint of gestation, but glial cell production continues through the end of gestation into the second postnatal year. The vulnerability of the developing nervous system to various factors is determined by the developmental stage of the cellular activities targeted by a specific insult. The effects of an agent or condition may vary depending on the agent, the time of insult during development, and the species under study (Dobbing, *et al.,* 1971). General factors such as undernutrition can have maximal effects on processes that are most active during what has been called the "brain growth spurt" (Dobbing and Sands, 1979). Depending on the developmental process ongoing at the time of exposure, alterations can be produced in either the number of neurons and extent of axonal arborizations, the number of glial cells, or the degree of myelination.

Myelination in both the CNS and PNS is sensitive to nutritional factors (see Wiggins, 1986; Blass, 1994). Brains of rats undernourished from birth contain a lower amount (20% deficit) of total lipids, cholesterol, and phospholipids and a 50% deficit in cerebrosides (Benton *et al.,* 1966). Following severe nutritional deprivation during lactation and post-weaning, total brain galactolipids, cholesterol, and lipid phosphorus showed a slower rate of accumulation (Krigman and Hogan, 1976). Lipid phosphorus and cholesterol levels recovered by adulthood, whereas galactolipids remained at a 60% decreased level. Myelin recovered from undernourished rats was normal in lipid composition but siginificantly reduced in total amount (Fishman *et al.,* 1971). A reduced proportion of basic and proteolipid protein was seen in myelin isolated from undernourished rats at postnatal days 15 and 20, but the composition was similar to normals by postnatal day 30. At all time points examined, myelin yield was 25% less than normal levels (Wiggins *et al.,* 1976). These studies suggested that undernutrition produced a delay in myelin maturation. Morphologic examination of animals undernourished from birth showed a decreased number of mature oligodendroglia and poorly stained myelin (Bass *et al.,* 1970; Krigman and Hogan, 1976). The number of myelin lamellae per axon and the number of myelin lamellae for a given axon diameter were both lower (Krigman and Hogan, 1976).

Some studies suggest that in some cases, myelination is able to catch-up and achieve normal levels once unrestricted feeding is initiated. Rats deprived by an increased litter size rapidly gained body and brain weight and normal brain lipid composition within 3 weeks after weaning to an unrestricted diet (Benton *et al.,* 1966). However, nutritional deprivation during the first 21 days of life resulted in reduced levels of total brain lipids, cerebrosides, cholesterol, and PLP, and this deficit persisted through 120 days of age (Bass *et al.,* 1970). Similar persistent myelin deficits were found in brains of rats subjected to either moderate or severe food deprivation during the first 30 days of life (Toews *et al.,* 1983). Although metabolic studies showed that after 6 days of free feeding following 20 days of postnatal starvation, incorporation of labeled precursors into myelin proteins was higher than in animals starved for the entire 26 days, and it was still depressed relative to controls (Wiggins *et al.,* 1976). Severe underfeeding in rats from 1 to 14 days of age resulted in a lasting significant deficit in myelin, even with rehabilitation (Wiggins and Fuller, 1978). Overall, these studies point to the possibility of irreversible deficits in myelin resulting from nutritional deficiencies during development. Additional studies suggest

the most vulnerable period for myelin may be the time of oligodendroglial proliferation. Animals deprived during this period are left with a permanent deficit of myelin-forming cells, resulting in irreversible hypomyelination (Wiggins and Fuller, 1978). Apparently, once normal numbers of oligodendroglia have been formed, the process of myelin formation itself is somewhat more capable of nutritional rehabilitation.

Similar effects can be found with a specific nutritional manipulation of depleting protein in the diet. In rats subjected to a protein and calorie deficiency during gestation and lactation, glial numbers were greatly reduced, and by postnatal day 19 the majority of cells in the corpus callosum appeared to be glioblasts rather than differentiated oligodendroglia (Robain and Ponsot, 1978). An early postnatal protein deficiency resulted in reduced levels of brain myelin and an altered myelin composition in rats (Nakhasi *et al.*, 1975). In myelin from offspring of rats maintained on a 4% protein diet during lactation, an excess of high molecular weight proteins and a deficiency of PLP in heavy myelin was found at postnatal day 17, with normal protein composition seen at 53 days (Figlewicz *et al.*, 1978). The MAG persisted in its higher molecular weight form longer than normal, suggesting that protein deficiency results in a delay in development and maturation of the myelination process (Druse and Krett, 1979).

Animals raised on a fat-deficient diet are able to synthesize all fatty acids except the essential fatty acids (linoleic and linolenic acid families). Essential fatty acid deficiency induced prenatally in the mothers and postnatally in the offspring resulted in lower brain weights (White *et al.*, 1971), and a low level of galactolipid and PLP (McKenna and Campagnoni, 1979). In the optic nerve of essential fatty acid–deficient rats, vacuolation, intramyelinic splitting, and Wallerian degeneration were present (Trapp and Bernsohn, 1978). Several studies have demonstrated hypomyelination in the offspring of copper-deficient mothers (DiPaolo *et al.*, 1974; Prohaska and Wells, 1974). In the third generation of mice maintained on a copper-deficient diet, the offspring showed approximately 60% decrease in myelin yield with the major glycoprotein shifted to a higher molecular weight (Zimmerman *et al.*, 1976).

B. Thyroid Deficiency

Thyroid hormones influence the temporal onset of myelination and its compositional maturation. Neonatal thyroidectomy in rats results in a lasting reduction of total cerebroside in the brain and a 30% reduction in myelin yield (Balazs *et al.*, 1969). It is thought, however, that hypothyroidism does not exert a specific effect on myelin but rather delays myelin development and maturation (Dalal *et al.*, 1971; Walters and Morell, 1981). Whereas hypothyroidism resulted in a 1–2 day delay of myelinogenesis with prolonged immature myelin formation, it eventually attained a normal composition, although the myelin deficit persisted.

C. Inorganic Lead

A classic example of differential susceptibility of the developing organism to the effects of an environmental chemical is that of inorganic lead exposure. Children are more vulnerable to lead in terms of external exposure sources, internal levels of lead, and timing of exposures during development. At high exposure levels, lead induces encephalopathy in children and can be life threatening. Experimental animal studies have allowed examination of various specific target sites and processes of development susceptible to lead toxicity (Krigman *et al.*, 1980). During development, the process of CNS myelination shows an increased vulnerability to lead exposure. The amount of lead that accumulates in the brain of the developing animal during lactation can be as much as 4 times higher than brain levels in the lactating dam receiving lead in the drinking water. Under these conditions, myelin was significantly reduced; however, the relationship between the axon diameter and myelin lamellae remained normal, suggesting that the hypomyelination was the result of altered axonal growth (Krigman *et al.*, 1974). Direct administration of lead via to pups intubation from 2 to 30 days of age resulted in a reduction of myelin accumulation in the forebrain and optic nerve. These effects were not due to undernutrition, as the accumulation of brain myelin was decreased significantly relative to controls undergoing a similar degree of malnourishment (Toews *et al.*, 1980). In developing rats, there is a synergistic interaction between lead exposure and mild malnutrution induced by milk deprivation with respect to decreasing the normal developmental accumulation of myelin (Harry *et al.*, 1985). This interaction appeared to be more prevalent in females as compared to males. The decrement in myelin induced by development exposure to inorganic lead is a long-lasting effect that persists into adulthood (Toews *et al.*, 1980, 1983). Myelination is not necessarily the most sensitive target for lead as low doses sufficient to produce some microscopically discernible hemorrhagic encephalopathy in the cerebellum of young rats did not depress myelination (Sundstrom and Karlsson, 1987); this hemorrhagic encephalopathy may be related to concentration of lead in brain capillaries (Toews *et al.*, 1978).

D. Triethyltin (TET)

The basic CNS change induced by exposure to TET is a massive cerebral edema, restricted primarily to the

white matter (Magee *et al.,* 1957; Torak *et al.,* 1970), with the formation of intramyelinic vacuoles (Jacobs *et al.,* 1977). The pathologic effect varies with the age of the animal (Suzuki, 1971). Young rats exposed to TET develop severe spongious white matter similar to that seen in the adult, but with the absence of major clinical signs seen in the adult (Suzuki, 1971; Blaker *et al.,* 1981). It is thought that the severe paralysis seen in the adult animal is due in part to the intracranial pressure developed during severe edema, whereas the open cranial sutures in the young rat may allow for edema in the absence of increased pressure. When newborn rats are exposed to TET, brains became swollen and petechial hemorrhages are observed, particularly in the cerebellum. Necrotic cells were found diffusely throughout the brain (Watanabe, 1977). When older (postnatal day 8) animals were exposed, both the hemorrhagic and necrotic changes occurred, but damage was also seen in the myelinated fibers of the brain stem and cerebellum. Although the morphologic alterations in myelin dissipate with time, biochemical evidence suggests that the amount of myelin produced is decreased and that this myelin deficit persists through adulthood (Blaker *et al.,* 1981; Toews *et al.,* 1983). Chronic exposure to TET from 2 to 30 days after birth decreases myelin yield and cerebroside content (55%) and 2′,3′-cyclic nucleotide 3′-phosphohydrolase activity (20%) (Blaker *et al.,* 1981). In studies using radioactive tracer, Smith (1973) demonstrated that it is the newly forming CNS myelin that is preferentially susceptible to degradation by TET. Interestingly, administration of TET to quaking mice did not produce intramyelinic edema (Nagara *et al.,* 1981).

E. Trialkyllead

When young animals are exposed to trialkyllead, the process of myelination is inhibited (Konat and Clausen, 1976). Unlike triethyltin, this impairment in myelinogenesis is not accompanied by edema of white matter. The impairment appears to be primarily in the deposition of myelin rather than in the program for myelination, as the protein composition of forebrain myelin isolated from triethyllead-intoxicated young rats was normal (Konat and Clausen, 1978). *In vitro* studies suggest that the alteration involves posttranslational processing and transport of integral membrane proteins, processes particularly important for myelin proteins during development (Konat and Clausen, 1980; Konat and Offner, 1982).

F. Hexachlorophene

Hexachlorophene (2,2′-methylene*bis*-3,4,6-trichlorophenol) is an antimicrobial agent that has been used previously in soaps and detergents, as well as in the bathing of newborn babies to prevent bacterial infections (Herter, 1959; Powell *et al.,* 1973; for review, see Towfighi, 1980). Both CNS and PNS myelin show a severe white matter edema following exposure, and young rats are more vulnerable than adults (Towfighi *et al.,* 1974). In young rats, edema of the myelin sheath becomes evident after postnatal day 15, probably because the myelin membrane provides a hydrophobic reservoir for accumulation of this toxic compound and thereby becomes a significant site for fluid accumulation (Nieminen *et al.,* 1973). Developmental exposure results in a decrease of the normal accumulation of myelin during development (Matthieu *et al.,* 1974). In 22-day-old rats nursed by mothers fed hexachlorophene, there was a decrease in myelin yield, yet the myelin composition remained normal. Abnormal "dissociated" myelin accounted for about 10% of the total myelin and contained the typical myelin constituents with the exception of MAG, which was absent (Matthieu *et al.,* 1974).

G. 6-Aminonicotinamide

Degeneration caused by this antimetabolite involves myelin, neurons, astrocytes, and oligodendroglia. In young animals injected with 6-aminonicotinamide, the PNS shows a selective swelling of Schwann cell cytoplasm at the inner surface of the myelin sheath. The nerve is compressed by the swelling and results in an overgrowth of the myelin sheath (Friede and Bischhausen, 1978).

H. Isonicotinic Acid Hydrazide (IHN)

Young ducklings fed a diet containing IHN developed a wobbling gait and head tremor after 2 weeks, progressing to ataxia and inability to stand (Lampert and Schochet, 1968). Examination of the CNS showed spongy degeneration of the myelin-containing white matter.

I. Cuprizone

Cuprizone (*bis*-cyclohexanoneoxalyhydrazone) is a copper chelator that results in CNS demyelination following dietary exposure to weanling mice. The loss of myelin can reach as much as 70% in white matter regions of the cerebrum (Carey and Freeman, 1983). Deficits in adenosine triphosphate (ATP) production secondary to reduced activity of cytochrome oxidase (a copper-requiring enzyme) may lead to alterations in energy-requiring ion transport mechanisms, but the underlying reason for targeting of this compound to CNS myelin is not clear. Interestingly, cuprizone inhibits carbonic

anhydrase, an enzyme present in myelin, and this inhibition takes place well before any demyelination is observed (Komoly *et al.,* 1987). In experimental studies of cuprizone neurotoxicity, mRNA for MAG, a protein located at the myelin–axonal interface, is down-regulated during demyelination and returns to normal levels following cessation of exposure (Fujita *et al.,* 1990). The mRNA for this glycoprotein exists in two major splice variants that are both severely down-regulated. On recovery, one splice variant returns to normal levels whereas the other shows an accumulation above control levels. Prolonged exposure to cuprizone (9 weeks or longer in mice) results in irreversible demyelination (Tansey *et al.,* 1996), possibly due to death of oligodendrocytes and/or oligodendrogilal precursor cells.

J. Tellurium

Exposure of weanling rats to a diet containing tellurium (element 52) leads to a highly synchronous demyelinating peripheral neuropathy (Lampert and Garrett, 1971; Duckett *et al.,* 1979; Said and Duckett, 1981; Takahashi, 1981; Bouldin *et al.,* 1988). When tellurium exposure is discontinued, there is rapid and synchronous remyelination. Although tellurium toxicity in humans is rare, this model is of considerable interest as a system for studying the manner in which a specific metabolic insult can lead to demyelination. Because there is little or no associated axonal degeneration, it has also proved useful for examining events and processes related to PNS remyelination, independently of processes related to axonal regeneration.

Inclusion of 1–1.5% elemental tellurium in the diet of weanling rats leads to a primary segmental demyelination of about 20–25% of myelinating internodes in the sciatic nerve, but with sparing of axons (Bouldin *et al.,* 1988; Harry *et al.,* 1989). This demyelination results in a peripheral neuropathy characterized by hindlimb paresis and paralysis. Older rats are much more resistant to tellurium, and the CNS is generally not affected, although some pathologic alterations can be induced with prolonged exposure periods. The nature of the underlying metabolic insult has been delineated. Tellurium blocks cholesterol synthesis, specifically by inhibiting the enzyme squalene epoxidase, an obligate step in the cholesterol biosynthesis pathway (Harry *et al.,* 1989; Wagner-Recio *et al.,* 1991; Wagner *et al.,* 1995). Tellurite, a water-soluble oxidized metabolite of the administered insoluble element, is the active species *in vitro,* effective at micromolar concentrations in a cell-free system (Wagner *et al.,* 1995). The organotellurium compound dimethyltelluronium dichloride, $(CH_3)_2TeCl_2$, is also effective in inhibiting squalene epoxidase in cultured Schwann cells and in inducing demyelination when administered intraperitoneally (Goodrum, 1997). Presumably, the resulting cholesterol deficit in Schwann cells eventually leads to an inability to maintain preexisting myelin and to assemble new myelin; this in turn leads to the observed demyelination. Although the tellurium-induced inhibition of cholesterol biosynthesis is systemic, deleterious effects are confined largely to the sciatic nerve. In the liver, which supplies cholesterol for most body tissues, the resulting intracellular cholesterol deficit results in a marked upregulation of the cholesterol biosynthetic pathway (Toews *et al.,* 1991b; Wagner-Recio *et al.,* 1991; Wagner *et al.,* 1995), presumably via well-characterized feedback mechanisms (see Goldstein and Brown, 1990, for review). This allows normal levels of cholesterol synthesis in this tissue despite considerable inhibition of one of the steps in the synthesis pathway, and normal levels of lipoprotein-associated circulating cholesterol are maintained. However, unlike many other tissues, the sciatic nerve cannot use circulating cholesterol; all cholesterol required for myelin in the sciatic nerve must be synthesized locally (Jurevics and Morell, 1994). This fact, coupled with the great demand for cholesterol in the rapidly myelinating PNS at the time of tellurium exposure, may account for the specificity of toxicity observed.

Expression of mRNA for myelin proteins is markedly down-regulated during the demyelinating phase of tellurium neuropathy, as is gene expression for enzymes involved in synthesis of lipids enriched in myelin (Toews *et al.,* 1990, 1991a,b, 1997). The latter include HMG–CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Although this enzyme is markedly up-regulated in the liver (as expected from the tellurium-induced intracellular sterol deficit), it is down-regulated in the sciatic nerve in concert with other myelin-related genes (Toews *et al.,* 1991b). Failure to up-regulate the cholesterol biosynthesis pathway in the sciatic nerve is, in fact, probably the major underlying reason for the preferential susceptibility of this tissue. The co-ordinate down-regulation for myelin-related genes seen following exposure to tellurium suggests that gene expression of all proteins involved in myelin synthesis and assembly may be under the co-ordinate control of the overall program for myelination (see Morell and Toews, 1996a; Toews *et al.,* 1997, for discussion). The coordinate down-regulation of myelin gene expression takes place in all myelinating Schwann cells and not just in those undergoing demyelination (Toews *et al.,* 1992). Thus, this down-regulation is not just a secondary response to injury but rather reflects the co-ordinate control of myelin gene expression. When tellurium exposure is discontinued, there is co-ordinate up-regulation of these messages during the remyelinating period.

Thus, tellurium toxicity specifically leads to PNS demyelination because (1) synthesis of cholesterol, a major myelin lipid, is severely inhibited; (2) unlike other tissues, peripheral nerve cannot up-regulate the synthesis of cholesterol in response to the tellurium-induced cholesterol deficit; (3) because the PNS is isolated from the circulation by barriers, it cannot use circulating cholesterol derived from the diet or from synthesis in the liver; and (4) there is a particularly high demand for cholesterol in the myelinating PNS at the time of tellurium exposure.

IX. Concluding Remarks

The process of myelination by oligodendroglia in the CNS and by Schwann cells in the PNS represents a complex series of metabolic and cell–biologic events involving intercellular recognition and interaction, adhesion, synthesis, sorting and assembly of specialized myelin membranes, compaction of myelin lamellae, and axonal (and possibly glial) ion channel reorganization. This entire process must be completed during an intense burst of metabolic activity at a specific predetermined interval during development, and failure to complete this program of myelination within the proper "developmental window" may have permanent deleterious effects. Because myelin-forming cells are operating at near their metabolic capacity, they are especially sensitive to toxic or other types of insults during this "vulnerable period" of nervous system development, and deficiencies in myelin, either qualitative or quantitative, may result. These myelin deficiencies can result from underlying alterations of various developmental events, including failure of myelin-forming cells to proliferate in normal numbers, reduction of axonal development, and/or decreased or altered formation of myelin at its normal time of maximal synthesis. The timing of any toxicant exposure or other insult can also differentially effect one or more of these events necessary for normal myelination. Although these processes are best examined in the developing nervous system, a clearer understanding of the biochemistry, molecular biology, and cell biology of these events is also of particular relevance with regards to remyelination in injured adult nervous tissue. Further delineation of the underlying nature of insults that result from toxic, genetic, nutritional, or other perturbations will also be useful in better understanding these vital processes.

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