

# Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System

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**Baumann, Nicole, and Danielle Pham-Dinh.** Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System. *Physiol Rev* 81: 871–927, 2001.—Oligodendrocytes, the myelin-forming cells of the central nervous system (CNS), and astrocytes constitute macroglia. This review deals with the recent progress related to the origin and differentiation of the oligodendrocytes, their relationships to other neural cells, and functional neuroglial interactions under physiological conditions and in demyelinating diseases. One of the problems in studies of the CNS is to find components, i.e., markers, for the identification of the different cells, in intact tissues or cultures. In recent years, specific biochemical, immunological, and molecular markers have been identified. Many components specific to differentiating oligodendrocytes and to myelin are now available to aid their study. Transgenic mice and spontaneous mutants have led to a better understanding of the targets of specific dys- or demyelinating diseases. The best examples are the studies concerning the effects of the mutations affecting the most abundant protein in the central nervous myelin, the proteolipid protein, which lead to dysmyelinating diseases in animals and human (jimpy mutation and Pelizaeus-Merzbacher disease or spastic paraplegia, respectively). Oligodendrocytes, as astrocytes, are able to respond to changes in the cellular and extracellular environment, possibly in relation to a glial network. There is also a remarkable plasticity of the oligodendrocyte lineage, even in the adult with a certain potentiality for myelin repair after experimental demyelination or human diseases.

## I. INTRODUCTION

Glial cells constitute the large majority of cells in the nervous system. Despite their number and their role dur-

ing development, their active participation in the physiology of the brain and the consequences of their dysfunction on the pathology of the nervous system have only been emphasized in the recent years.

Virchow (639) first described that there were cells other than neurons. He thought that it was the connective tissue of the brain, which he called "nervenkitt" (nerve glue), i.e., neuroglia. The name survived, although the original concept radically changed.

The characterization of the major glial cell types was the result of microscopic studies, and especially the techniques of metallic impregnation developed by Ramon y Cajal and Rio Hortega. Using gold impregnation, Ramon y Cajal (495) identified the astrocyte among neuronal cells, as well as a third element which was not impregnated by this technique. A few years later, using silver carbonate impregnation, Rio Hortega found two other cell types, the oligodendrocyte (514), first called interfascicular glia, and another cell type that he distinguished from the two macroglial cells (i.e., macroglia), and that he called microglia (513).

The morphological characteristics of macroglia have been reviewed (453). The progress of morphological techniques and the discovery of cellular markers by immunocytochemical techniques indicate the notion of multiple functional macroglial subclasses. Our understanding of the role of glia in central nervous system (CNS) function has made important progress during recent years. Glial cells are necessary for correct neuronal development and for the functions of mature neurons. The ability of glial cells to respond to changes in the cellular and extracellular environment is essential to the function of the nervous system. Furthermore, there is now growing recognition that glia, possibly through a glial network, may have communication skills that complement those of the neurons themselves (Fig. 1). There are currently expanding discoveries of specialization of both neurons and glial

cells and their persistent interactions that gives also a new insight to the understanding of pathological outcomes. The association of neuronal and glial expression for some neurotransmitters, their transporters, and receptors contributes to the understanding of their functional cooperation. It now seems likely that oligodendrocytes have functions other than those related to myelin formation and maintenance.

As an overview, the number of glial cells increases during evolution; glial cells constitute 25% of total cells in the *Drosophila*, 65% in rodents, and 90% in the human brain (456). The very few morphological studies do not take into account the different glial cell types. Brain white matter tracts are deprived of neuronal cell bodies but contain glial cells; because brain white matter constitutes as big a volume as the cortex gray matter, the overall glia-to-neuron ratio is considerably increased (456).

## II. OLIGODENDROCYTES

### A. Morphology of Oligodendrocytes

The term *oligodendroglia* was introduced by Rio Hortega (513) to describe those neuroglial cells that show few processes in material stained by metallic impregnation techniques. The oligodendrocyte is mainly a myelin-forming cell, but there are also satellite oligodendrocytes (449) that may not be directly connected to the myelin sheath. Satellite oligodendrocytes are perineuronal and may serve to regulate the microenvironment around neurons (349). A number of features consistently distinguish

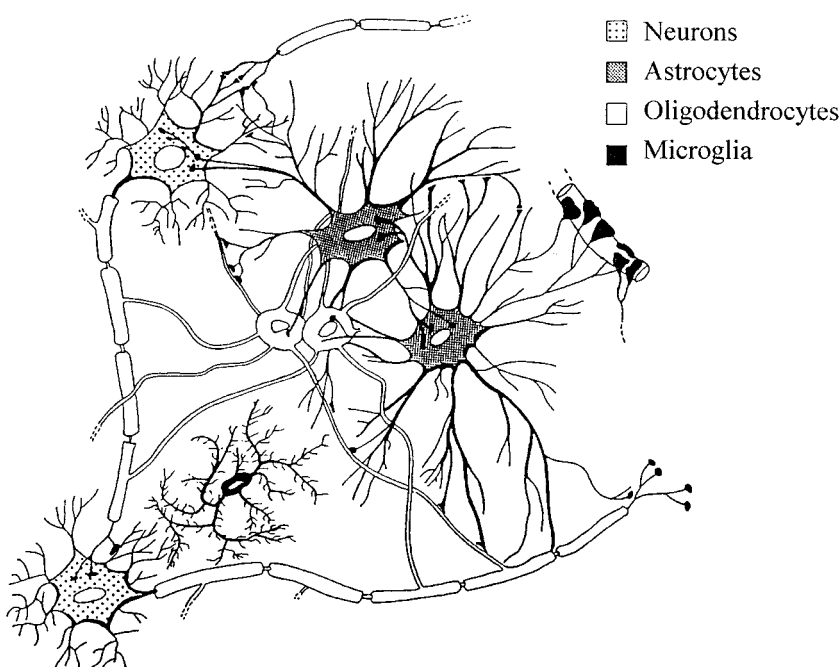


FIG. 1. Schematic representation of the different types of glial cells in the central nervous system (CNS) and their interactions, among themselves and with neurons. Astrocytes are stellate cells with numerous processes contacting several cell types in the CNS: soma, dendrites and axons of neurons, soma and processes of oligodendrocytes, and other astrocytes; astrocytic feet also ensheath endothelial cells around blood capillaries forming the blood-brain barrier and terminate to the pial surface of the brain, forming the glia limitans. Oligodendrocytes are the myelinating cells of the CNS; they are able to myelinate up to 50 axonal segments, depending on the region of the CNS. A number of interactions between glial cells, particularly between astrocytes in the mature CNS, are regulated by gap junctions, forming a glial network. [From Giaume and Venance (218). Copyright 1995 Overseas Publishers Association. Permission granted by Gordon and Breach Publishers.]

oligodendrocytes from astrocytes (reviewed in Ref. 453), in particular their smaller size, the greater density of both the cytoplasm and nucleus (with dense chromatin), the absence of intermediate filaments (fibrils) and of glycogen in the cytoplasm, and the presence of a large number of microtubules (25 nm) in their processes that may be involved in their stability (350). An oligodendrocyte extends many processes, each of which contacts and repeatedly envelopes a stretch of axon with subsequent condensation of this multispiral membrane-forming myelin (82, 84) (Fig. 2). On the same axon, adjacent myelin segments belong to different oligodendrocytes. The number of processes that form myelin sheaths from a single oligodendrocyte varies according to the area of the CNS and possibly the species, from 40 in the optic nerve of the rat (453) to 1 in the spinal cord of the cat (83). Rio Hortega (514) classified oligodendrocytes in four categories, in

relation to the number of their processes (also described in Ref. 83). According to their morphology and the size or thickness of the myelin sheath they form, Butt et al. (89) also distinguish four types of myelinating oligodendrocytes, from small cells supporting the short, thin myelin sheaths of 15–30 small diameter axons (type I), through intermediate types (II and III), to the largest cells forming the long, thick myelin sheaths of one 1–3 large diameter axons. Morphological heterogeneity is, in fact, a recurrent theme in the study of interfascicular oligodendrocytes (84). At the electron microscopic level, oligodendrocytes have a spectrum of morphological variations involving their cytoplasmic densities and the clumping of nuclear chromatin. Mori and Leblond (406) distinguish three types of oligodendrocytes: light, medium, and dark. The dark type has the most dense cytoplasm. On the basis of labeling with tritiated thymidine of the corpus callosum of young rats, they suggest that light oligodendrocytes are the most actively dividing cells and that oligodendrocytes become progressively dark as they mature.

Before their final maturation involving myelin formation, oligodendrocytes go through many stages of development. Their characterization is often insufficient by morphological criteria alone both *in vivo* and *in vitro*. A number of distinct phenotypic stages have been identified both *in vivo* and *in vitro* based on the expression of various specific components (antigenic markers) and the mitotic and migratory status of these cells. They are described in section II, *B* and *C*.

## B. Specific Components of Oligodendrocytes

Characterization of a number of specific biochemical markers has increased our knowledge on the stages of oligodendrocyte maturation, both *in vivo* and *in vitro*. These components, although they may be present in other cell types or other tissues, or in specific cells only at certain stages, must be viewed as modular elements that generate, with other elements, complex and unique surface patterns (502). All the biochemical and molecular characteristics of oligodendrocytes are not described here. We limit ourselves to those that have given insights into our understanding of this cell type. Although some markers are very useful in culture to determine the sequences of maturation, and ultimately their mechanism, they may be more difficult to use *in situ*, because some of them are present on other cell types *in vivo*.

Oligodendrocytes originate from migratory and mitotic precursors, then progenitors, and mature progressively into postmitotic myelin-producing cells (see sect. II C5). The sequential expression of developmental markers, identified by a panel of cell specific antibodies, divide the lineage into distinct phenotypic stages (249, 455; reviewed in Ref. 344) characterized by proliferative capac-

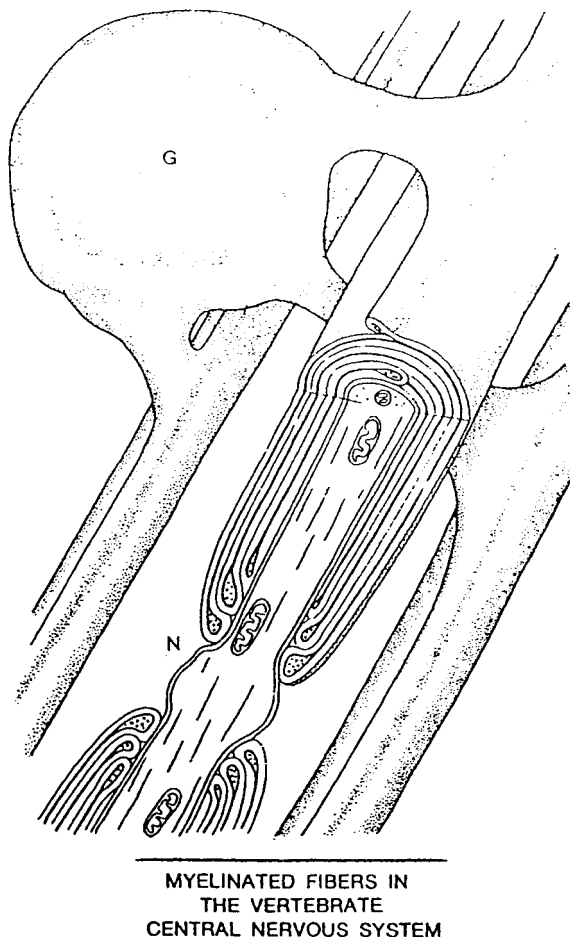


FIG. 2. The oligodendrocyte and the mature myelin sheath. An oligodendrocyte (G) sends a glial process forming compact myelin spiraling around an axon. Cytoplasm is trapped occasionally in the compact myelin. In transverse sections, this cytoplasm is confined to a loop of plasma membrane, but along the internode length, it forms a ridge that is continuous with the glia cell body. In the longitudinal plan, every myelin unit terminates in a separate loop near a node (N). Within these loops, glial cytoplasm is also retained. [Adapted from Bunge (84).]

ities, migratory abilities, and dramatic changes in morphology (Fig. 3). Many of these markers have been at first identified in tissue cultures. Some of them are characteristic myelin components (see sect. III C). Myelination requires a number of sequential steps in the maturation of the oligodendroglial cell lineage (249, 455) accompanied by a coordinated change in the expression of cell surface antigens often recognized by monoclonal antibodies. Differentiation involves the loss of certain surface or intracellular antigens and the acquisition of new ones. Some of the surface antigens are lineage markers; discrete phases in these lineages are marked by differential expression of additional antigens or phase markers (502).

### 1. Markers of maturation of the oligodendrocyte lineage

A) NESTIN. Nestin is a protein recognized by the rat-401 monoclonal antibody (257), whose expression specifically

distinguishes neuroepithelial stem cells (from which the name nestin is originated) from other more differentiated cells in the neural tube (327). Nestin defines a distinct sixth class of intermediate filament protein, closely related to neurofilaments. Nestin is also expressed by glial precursors (327), such as radial glia (257), and in the cerebellum by immature Bergmann fibers, and also by adult Bergmann fibers recapitulating developmental stages when placed in the presence of embryonic neurons (573). Using cortical- or CG-4-derived oligodendrocyte lineage cells, culture experiments have shown that high levels of nestin protein are also expressed in proliferating oligodendrocyte progenitors, but the protein is downregulated in differentiated oligodendrocytes (206).

B) PROTEOLIPID PROTEIN. A special note should be made concerning the proteolipid protein (PLP) gene expression, as a marker of developmental maturation of myelinating glial cells, in the CNS as in the peripheral nervous

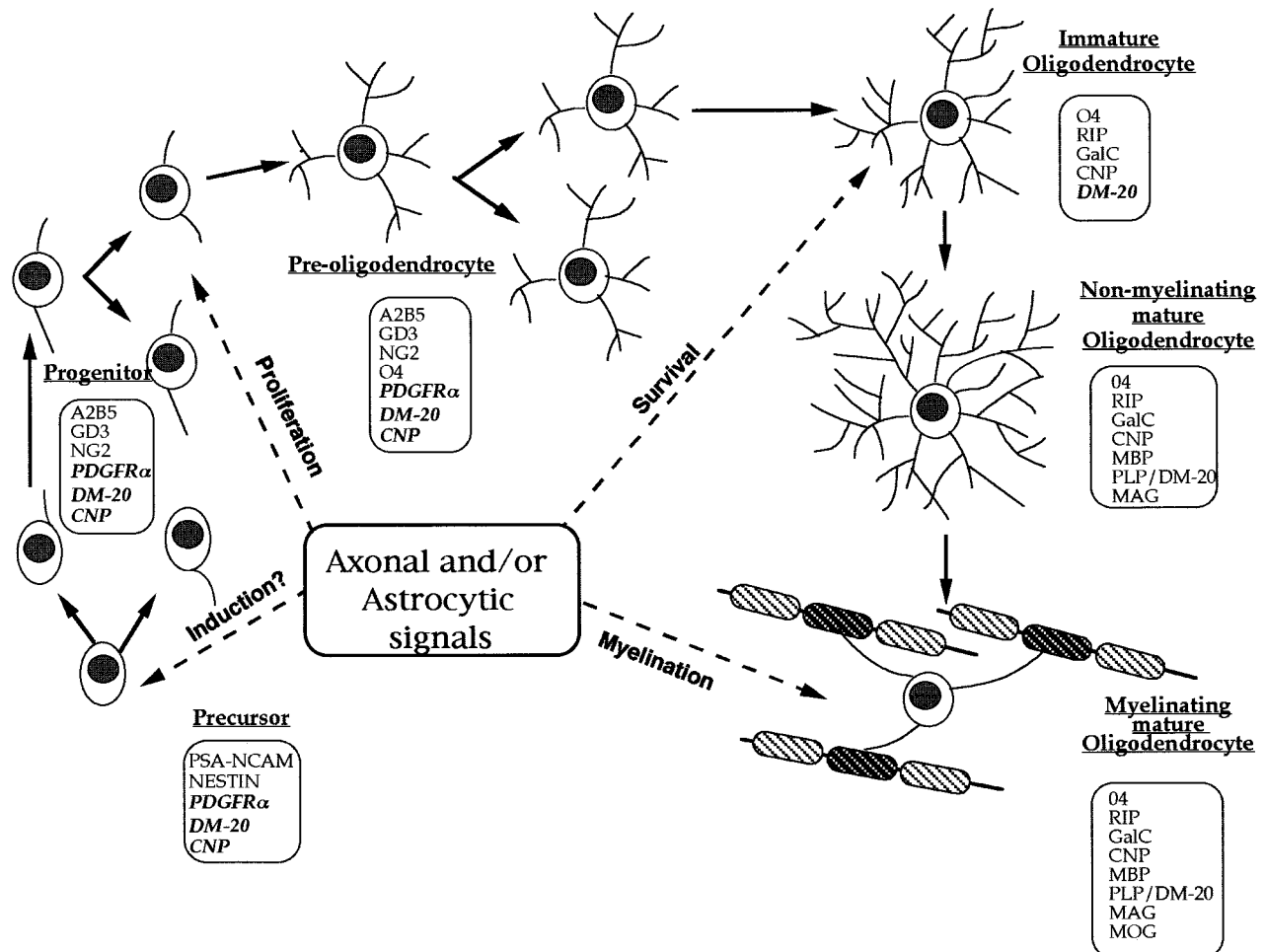


FIG. 3. Schematic representation of the developmental stages of cells of the oligodendrocyte lineage. Schematic drawing of the morphological and antigenic progression from precursor cells to myelinating mature oligodendrocytes, through progenitors, preoligodendrocytes, and immature nonmyelinating oligodendrocytes. Timing of neuronal and astrocytic signaling is indicated. Stage-specific markers are boxed. RNAs are in italics. [From Lubetzki et al. (344). See also Hardy and Reynolds (249) and Pfeiffer et al. (455).]



system (PNS). By RT-PCR and in situ hybridization, the mRNA of DM-20, coding for an isoform of PLP, can be detected in the developing CNS at a very early stage of development, before the onset of myelination (272, 454, 610, 611). With the use of PLP-Lac Z transgenic mice, expression of the splicing variant DM-20 of the PLP gene is detected in the mouse embryo within very discrete regions of the CNS and rather extensively throughout the PNS (576, 666). The DM-20 protein is detected precociously in different regions of the nervous system, in oligodendrocyte precursors from the spinal cord (144), or in ensheathing cells from the olfactory bulb (145).

C) PLATELET-DERIVED GROWTH FACTOR  $\alpha$ -RECEPTOR. The platelet-derived growth factor  $\alpha$ -receptor (PDGFR- $\alpha$ ) transcripts are also detected at very early stages of the developmental maturation of myelinating glial cells. Although PDGF R- $\alpha$  and PLP/DM-20 cells are usually found in a close vicinity, either in the same or in adjacent territories, they are rarely coexpressed by the same cells, raising the question of single or multiple oligodendrocyte lineage (reviewed in Refs. 510, 577).

D) PSA-NCAM. The embryonic polysialylated form of neural cell adhesion molecule (NCAM), PSA-NCAM, defines in the absence of expression of GD3, the precursor stage (239, 248), from which oligodendrocyte progenitors arise.

E) GANGLIOSIDE GD3. The ganglioside GD3 has been identified by the use of two monoclonal antibodies: R24 (483) and LB1 (127). In fact, R24 recognizes also minor gangliosides. In vitro, there is a high expression of GD3 on oligodendrocyte progenitors in culture (248); thus it is a good marker for oligodendrocyte cell culture; GD3 expression disappears as the cell matures. Nevertheless, in vivo it is also expressed in other glial cell types such as immature neuroectodermal cells, subpopulations of neurons and astrocytes during development, resting ameboid microglia (174, 671), reactive microglia, and astrocytes (223). Thus particular caution should be used when extrapolating from in vitro investigations to the CNS in situ (223).

F) THE MONOCLONAL ANTIBODY A2B5. The monoclonal antibody A2B5 (172) recognizes several gangliosides (198) that remain as yet uncharacterized. It is expressed both on neurons and glial cells in vivo; it is used essentially in oligodendrocyte cultures to follow the maturation of oligodendrocyte progenitors. In culture, ganglioside GT3 and its O-acetylated derivative are the principal A2B5-reactive gangliosides (153, 181). Both antigens are down-regulated as the cell differentiates into the mature oligodendrocyte. This corresponds to the disappearance of cell surface immunostaining by A2B5. Like GD3 monoclonal antibodies, A2B5 binding in vivo is not cell specific.

G) THE RAT NG2 PROTEOGLYCAN. The rat NG2 proteoglycan is an integral membrane chondroitin sulfate proteoglycan with a core protein of 260 kDa. In the mature CNS, cells express this antigen together with PDGFR- $\alpha$  (426, 427,

504). These cells have extensive arborizations of their cell processes and are found ubiquitously long after oligodendrocytes are generated. They do not express antigens specific to mature oligodendrocytes, astrocytes, microglia, and neurons, suggesting that they are a novel population of glial cells which undergoes proliferation with morphological changes in response to stimuli such as inflammation or demyelination (reviewed in Ref. 136a, 426).

H) THE MONOCLONAL ANTIBODY O4. The monoclonal antibody O4 (571) marks a specific preoligodendrocyte stage of oligodendrocyte maturation. It reacts also with sulfatides and still unidentified glycolipids (23).

## 2. Oligodendrocyte/myelin markers

A) GLYCOLIPIDS. There are specific glycolipids in oligodendrocytes and myelin, such as galactosylceramides (GalC) (galactocerebrosides) and sulfogalactosylceramides (sulfatides). Galactosylceramides and sulfogalactosylceramides are early markers that remain present on the surface of mature oligodendrocytes in culture (455, 488) and in vivo (693). It appears often difficult to characterize them on the cell surface of the oligodendrocyte during development with precision, because many of the reagents, i.e., the monoclonal antibodies used, are less specific than thought at first. The main antibody used to identify some of these developmental stages is O1 (571), which recognizes galactocerebrosides, but also monogalactosyldiglycerides and an unidentified antigen present during oligodendrocyte development. This is also the case for the R-MAb (497), which has been mainly used for galactocerebroside identification, but recognizes also sulfatides, monogalactosyldiglycerides, seminolipids, and another unidentified antigen (23). Some polyclonal IgG antibodies to galactocerebrosides alter the organization of oligodendroglial membrane sheets (164).

B) RIP ANTIGEN. RIP antigen has been identified through the use of a monoclonal antibody that was generated against oligodendroglia from rat olfactory bulb (199). It recognizes an unknown cytosolic epitope on oligodendrocytes and labels both oligodendrocyte processes and the myelin sheath (44, 199). Two bands of 23 and 160 kDa have been found by Western Blot, the nature of which have not been elucidated. Interestingly, this marker may serve to determine biochemical subtypes of oligodendrocytes together with carbonic anhydrase II (89).

C) CARBONIC ANHYDRASE II. Among the seven isozymes that are products of different genes, carbonic anhydrase II (CAII) is the only one that is located in the nervous system, in oligodendrocytes. CAII covers all stages of the lineage and is also a marker of adult oligodendrocytes (217); it is a more diffuse marker of glial cells in the developing animal (95). In the anterior medullary velum of the rat, RIP<sup>+</sup> CAII<sup>+</sup> oligodendrocytes support numer-

ous myelin sheaths for small diameter axons, whereas  $\text{RIP}^+ \text{CAII}^-$  oligodendrocytes support fewer myelin sheaths for large-diameter axons (89). This immunocytochemical classification overlaps the morphological classification of Bunge (84). However, CAII is also reported to be expressed by microglia (671).

D) NI-35/250 PROTEINS. NI-35/250 proteins are transmembrane proteins enriched in mammalian myelin CNS and oligodendrocytes (105). NI-35/250 have been found to be potent inhibitors of axonal regrowth in pathological conditions (see sect. III G3c). The bovine NI-220 cDNA has been recently characterized (578). These proteins are recognized by the monoclonal antibody IN-1. The gene has recently been cloned (114). There are three isoforms of the proteins; one of them, NOGO A, is the one with the inhibitory properties and which is mainly expressed on CNS oligodendrocytes and myelin (see sect. III G3) (reviewed in Refs. 20, 222, 232).

E) SPECIFIC MYELIN PROTEINS. Genes encoding the specific myelin proteins are expressed at different stages of the oligodendrocyte differentiation and maturation. 2',3'-Cyclic nucleotide-3'-phosphohydrolase (CNP), myelin basic protein (MBP), PLP/DM-20, myelin-associated glycoprotein (MAG), and myelin/oligodendrocyte glycoprotein (MOG) genes as well as other minor myelin proteins are described in section III C2.

### 3. Other oligodendrocyte protein markers

A) TRANSFERRIN. Transferrin, the iron mobilization protein, is expressed in oligodendrocytes (62) and choroid plexus epithelial cells. Before the establishment of the blood-brain barrier, neural cells are dependent on serum transferrin biosynthesized in the liver. As the blood-brain barrier gets established during development, neural cells become dependent on transferrin produced by oligodendrocytes and choroid plexus epithelial cells. In addition, transferrin acts as a trophic and survival factor for neurons and astrocytes, suggesting an important function for oligodendrocytes besides myelination (176).

B) S100 PROTEINS. S100 proteins are  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  binding proteins; they are at high concentration in the mammalian brain. It is now known that the S100 family of calcium-binding proteins contains ~16 members, each of which exhibits a unique pattern of tissue/cell type specific expression. Whereas the most abundant isoform, S100  $\beta$ -protein, is expressed predominantly by astrocytes, it is also present in adult rat brain in a minor subpopulation of oligodendrocytes (511).

C) GLUTAMINE SYNTHETASE. Glutamine synthetase catalyzes the conversion of glutamate to glutamine in the presence of ATP and ammonia. By immunocytochemical methods, its presence has been demonstrated in the cytoplasm of astrocytes. It is also found in a discrete population of oligodendrocytes (129).

## C. Origin and Differentiation of Oligodendrocytes

For many years, one has mainly inferred lineage relationships by examining patterns of antigen expression and morphological changes in developing glia. Lineage can now more securely be traced with gene transfer techniques using retroviral vectors (109, 352, 476) and transgenic constructs (191, 224, 666). In the former kind of analysis, a replication-deficient retrovirus bearing a reporter gene such as LacZ coding for the bacterial  $\beta$ -galactosidase, is introduced into a dividing cell population. After the virus enters a cell, DNA copies of viral RNA are synthesized, and a proportion of cells undergoing DNA replication will integrate viral DNA into genomic DNA. From that point on, viral genes will be passed to all the progeny (633). Moreover, the promoter sequences of genes specifically activated in glial precursors induce the expression of the LacZ reporter gene in the glial lineages. In the transgenic mice bearing this type of transgene, LacZ expression characterizes the subpopulation that expresses the transgene during its differentiation.

Mammalian species acquire their full complement of cortical neurons during the first half of gestation. Radial glia are established early in development at the time of neurogenesis, during the early gestational period, and support neurons during their migration to the cortex (reviewed in Ref. 493). The subventricular zone (SVZ), which is present in late gestational and early postnatal mammalian brain, is a major source of astrocytes and oligodendrocytes, although astrocytes may also develop from radial glia. Maturation of oligodendrocytes and astrocytes takes place in the mammalian CNS largely in early postnatal life.

### 1. Common precursors for neurons and glia: pluripotentiality of stem cells

In the *Drosophila* CNS, during neurogenesis, there is a binary genetic switch between neuronal and glial determination (261, 285). The mutation of a gene encoding a nuclear protein, glial cells missing (*gcm*), causes presumptive glial cells to differentiate into neurons, whereas its ectopic expression using transgenic constructs forces virtually all CNS cells to become glial cells. Thus, in the presence of *gcm* protein, presumptive neurons become glia, whereas in its absence, presumptive glia become neurons. However, a gene with an identical function has not as yet been identified in the vertebrate nervous system.

In the mammalian cortex, both neurons and glia arise from the proliferating neuroepithelial cells of the telencephalic ventricular and SVZ (149). The SVZ is a mosaic of multipotential and lineage restricted precursors (329), where environmental cues influence both fate, choice, and all surviving cells (279). A number of trophic factors

(see sects. II C8 and III F) influence the actual developmental fate of a progenitor or a multipotent stem cell from the SVZ, which can differ from its developmental potential (91, 241, 281, 484, 682). As a consequence of this growth factor dependency, neurons are not formed postnatally because of the lack of trophic factors necessary for their survival, resulting in their death; this has been shown by a recent clonal analysis of the progeny of single rat SVZ cells using replication deficient retroviral vectors (329).

Because environmental conditions can be provided *in vitro* when adding specific trophic factors to the culture medium (18, 281, 415, 484, 503), glial cells differentiating in cultures may exhibit a considerable plasticity in the extent of lineage switching, not observed *in vivo* (565) (see sect. II C6).

On the other hand, it has been hypothesized that even after development is completed, the Notch pathway may help maintain some mammalian stem cells and precursor cells in adult tissues by preventing them from differentiating, thus allowing for the possibility of new cell generation in renewing or damaged tissues (14).

The identity of neural stem cells in the adult CNS is presently debated and could be either ependymal cells (280) or SVZ astrocytes (149, 323a). For a critical review of these data, see Reference 27.

## 2. Oligodendrocyte precursors in the ventricular zone

Oligodendrocyte precursors originate from neuroepithelial cells of the ventricular zones, at very early stages during embryonic life. This was first suggested by following the expression of specific markers of oligodendrocytes (127, 248, 249, 328, 455), some of which are transcripts of future protein components of myelin (CNP, MBP, PLP) (272, 454, 478, 610, 611, 690).

In spinal cord, oligodendrocytes initially arise in ventral regions of the neural tube. Miller and co-workers (652) have shown, using a special dye (DiI) associated with specific markers of the oligodendrocyte lineage, that oligodendrocyte precursors arise in the ventral spinal cord and then migrate dorsally during development. During subsequent development, the dorsal regions acquire the capacity for oligodendrogenesis, probably both intrinsically (94) and through the ventral to dorsal migration of oligodendrocyte precursors. This has been confirmed using cultures of the thoraco-lumbar area of the rat spinal cord, which showed that the ability to give rise to oligodendrocytes is restricted to the ventral region of this area of the spinal cord until E14 (652). Signals from the notochord/floor plate, involving the morphogenetic protein sonic hedgehog, are necessary to induce the development of ventrally derived oligodendroglia (392, 441, 442, 467, 479).

*In situ* hybridization for mRNAs of proteins involved in oligodendrocyte maturation has been used to charac-

terize early oligodendrocyte precursors. Ventral ventricular cells express mRNA for PDGFR- $\alpha$  (478). Similarly, a discrete population of cells expressing mRNA for the myelin protein CNP (690) and DM20 (PLP gene) (610) has been localized to the ventral ventricular zone of the developing mammalian spinal cord. A group of cells in the ventricular and mantle zone of the ventral diencephalon of the E13 rat express mRNA for the PDGFR- $\alpha$  (478). A similar distribution of cells expressing DM-20 mRNA has also been described in the mouse (610). Nevertheless, this issue still remains unclear as both markers are not expressed by the same population of oligodendrocyte precursors, indicating a diversity in this population already at this stage. DM-20 protein is also expressed on embryonic cells that may become oligodendrocyte progenitors as shown by Dickinson et al. (144) in the spinal cord.

The initial restricted localization of oligodendrocyte precursors in the ventral plate of the neural tube appears not to be limited to the spinal cord (690) but is also observed in mid- and forebrain (576, 610). In addition, a similar restricted localization of the sites of oligodendroglialogenesis has been described in other vertebrate species, such as human (244), chick (440), and *Xenopus* (688). The population of oligodendrocytes derived from the restricted loci of cells appears to be regionally distributed in the brain. For instance, in the chick embryo, some of the oligodendrocytes of the optic nerve generate suprachiasmatic foci of precursors located in the medio-ventral epithelium of the third ventricle (440). The developing cerebral cortex of murine embryos could be populated by oligodendrocytes originating from precursor cells located in the laterobasal plate of the diencephalon and probably also in the telencephalon (475).

For comprehensive reviews of the different hypotheses concerning origin and development of cells of the oligodendrocyte lineage, see References 245, 510, 577.

## 3. Oligodendrocyte progenitors in the SVZ

The SVZ is a germinal matrix of the forebrain that first appears during the later third of murine embryonic development (149). It enlarges during the peak of gliogenesis, between P5 and P20, and then shrinks but persists into adulthood. Lineage tracing studies of perinatal SVZ cells using stereotactically injected retrovirus support the view that the majority of progenitors within this germinal matrix are glial precursors that generate astrocytes on the one hand and oligodendrocytes on the other hand (352, 476). Although the majority of cells give rise to homogeneous progeny, some SVZ cells give rise to both oligodendrocytes and astrocytes, and a rare cell will develop into both neurons and glia (329). The assumption that each cluster is a clone, i.e., the complete progeny of a single precursor, was based on the thought that the dispersion of cells in structures like embryonic cortex was purely



radial. However, other retroviral studies, together with other techniques, have shown that cells disperse considerably and also tangentially during postnatal corticogenesis and embryonic development (475). Thus two clones could occupy an overlapping space. Therefore, this is not yet a completely settled issue. Nevertheless, it does seem from tritiated thymidine, immunocytochemistry, and retroviral studies that the majority and possibly all oligodendrocytes originate from different cells in the SVZ than those that give rise to astrocytes. In the neonatal rat cerebrum, oligodendrocytes arise postnatally from the SVZ of the lateral ventricles (329, 695). Similarly, immunocytochemical studies have indicated that oligodendrocytes of the cerebellum arise postnatally from the SVZ of the fourth ventricle (505). Oligodendrocyte progenitors then migrate long distances away from these zones and populate the developing brain to form white matter throughout the brain, as shown by developmental (200, 329) and transplantation studies (177, 321). Because mature oligodendrocytes cannot migrate, preventing premature differentiation of progenitors is crucial for ensuring that they successfully make it to their final destination. Premature oligodendrocyte differentiation is effectively prevented by an inhibition mechanism recently shown to occur in gliogenesis, the Notch pathway (651) (see sect. III D2).

Oligodendrocytes, first found in the optic nerve around birth, continue to increase in number for six postnatal weeks in rodents (31, 567). Indirect evidence suggests that optic nerve oligodendrocytes are derived from precursor cells that migrate into the nerve from the brain rather than from neuroepithelial cells of the original optic stalk. For example, during late embryonic development in the rat (at about E15), cultures from the chiasma but not from the retinal end of the nerve contain significant numbers of oligodendrocyte progenitor cells (568).

#### 4. Oligodendrocyte migration: mechanisms and molecules involved

Oligodendrocyte progenitors migrate extensively throughout the CNS before their final differentiation into myelin-forming oligodendrocytes (568). Moreover, as in other developmental systems, oligodendrocytes have to extend processes in a similar fashion to the extension of neurites from neuronal cell bodies. As for neurons, a number of extracellular matrix (ECM) molecules play an instructive role in the control of migrating oligodendrocytes. For example, tenascin-C is expressed at high levels at the rat retina-optic nerve junction, an area through which oligodendrocytes do not migrate, so preventing access of myelin-forming cells to the retina (187). Therefore, it has been proposed that tenascin-C might provide a barrier to oligodendrocyte migration at the retinal end of the optic nerve (37). The inhibitory effect of tenascin-C is

dependent on the substrate: tenascin-C inhibits migration in response to fibronectin but not in response to merosin (200). Moreover, to find their way and to regulate the extension of their processes along the astrocyte-produced ECM, oligodendrocytes use metalloproteinases (MMP). In vivo, elevation of MMP protein expression in tissues rich in oligodendrocytes and myelin coincides with the temporal increase in myelination that occurs postnatally. Process formation is retarded significantly in oligodendrocytes cultured from MMP-9 null mice, as compared with controls, providing genetic evidence that MMP-9 is necessary for process outgrowth (434). On the contrary, MMP inhibitors decrease process extension of oligodendrocytes, supporting the key role of MMPs (623).

With the use of explant cultures of newborn rat neurohypophysis, it was shown that migrating oligodendrocyte progenitors express a polysialylated (PSA) form of NCAM (649). Removal of PSA-NCAM from the surface of progenitors by an endoneuraminidase treatment results in a complete blockade of the dispersion of the oligodendrocyte progenitor population from the explant. As shown for neurons and astrocytes, the expression of PSA-NCAM does not itself provide a specific signal for migration, but its expression appears to open a permissive gate (528) that allows cells to respond to external cues at the appropriate time and space. In contrast, in the chick, the migration of oligodendrocyte precursors along the optic nerve axons appears unaffected by removal of PSA-NCAM (440). Thus migrating oligodendrocyte progenitors may use distinct and discrete mechanisms to navigate specific migrational pathways.

Oligodendrocyte progenitors express a distinctive array of integrin receptors (394) that may mediate specific interactions with ECM components, such as thrombospondin-1, which could also be involved in the regulation of migration (553).

#### 5. Stages of maturation of oligodendrocytes

As described in section II, A and B, oligodendrocyte progenitors have been characterized in rodent species by their bipolar morphology and by the presence of specific markers: 1) glycolipids:GD3 (248) recognized by the monoclonal antibodies (MAbs) R24 and LB1; other glycolipids, not as yet fully characterized (198) recognized by the MAb A2B5; and 2) a chondroitin sulfate proteoglycan called NG2 (427). In vitro studies (127, 455) as well as in vivo experiments through transplantation techniques (26, 177, 226, 321) show that these cells are actively proliferating and possess migratory properties. They proliferate in vitro, in response to growth factors such as fibroblast growth factor (FGF) and PDGF (211, 249, 393, 489, 509) (see sect. II C8).

After their migration in the mammalian CNS, progenitors settle along fiber tracts of the future white matter



and then transform into preoligodendrocytes (Fig. 4), multiprocessed cells which keep the property of cell division and acquire the marker O4 (571). At this stage, they are less motile (442), or even postmigratory (455), and lose their mitogenic response to PDGF (208, 250, 478).

The preoligodendrocyte becomes an immature oligodendrocyte, characterized in the rat by the appearance of the marker GalC, and the loss of expression of GD3 and

A2B5 antigens on the cell surface. CNP is the earliest known myelin-specific protein to be synthesized by developing oligodendrocytes (505, 506, 579, 642). Other markers appear at this stage, such as RIP (199), CAII, and MBP (89) (see sect. II B). In vivo there seems to be a GD3/GalC intermediate stage (127). In rat cerebellum, CNP is expressed at the same time as GalC (505) and MBP is expressed 2–3 days later along with PLP, immediately

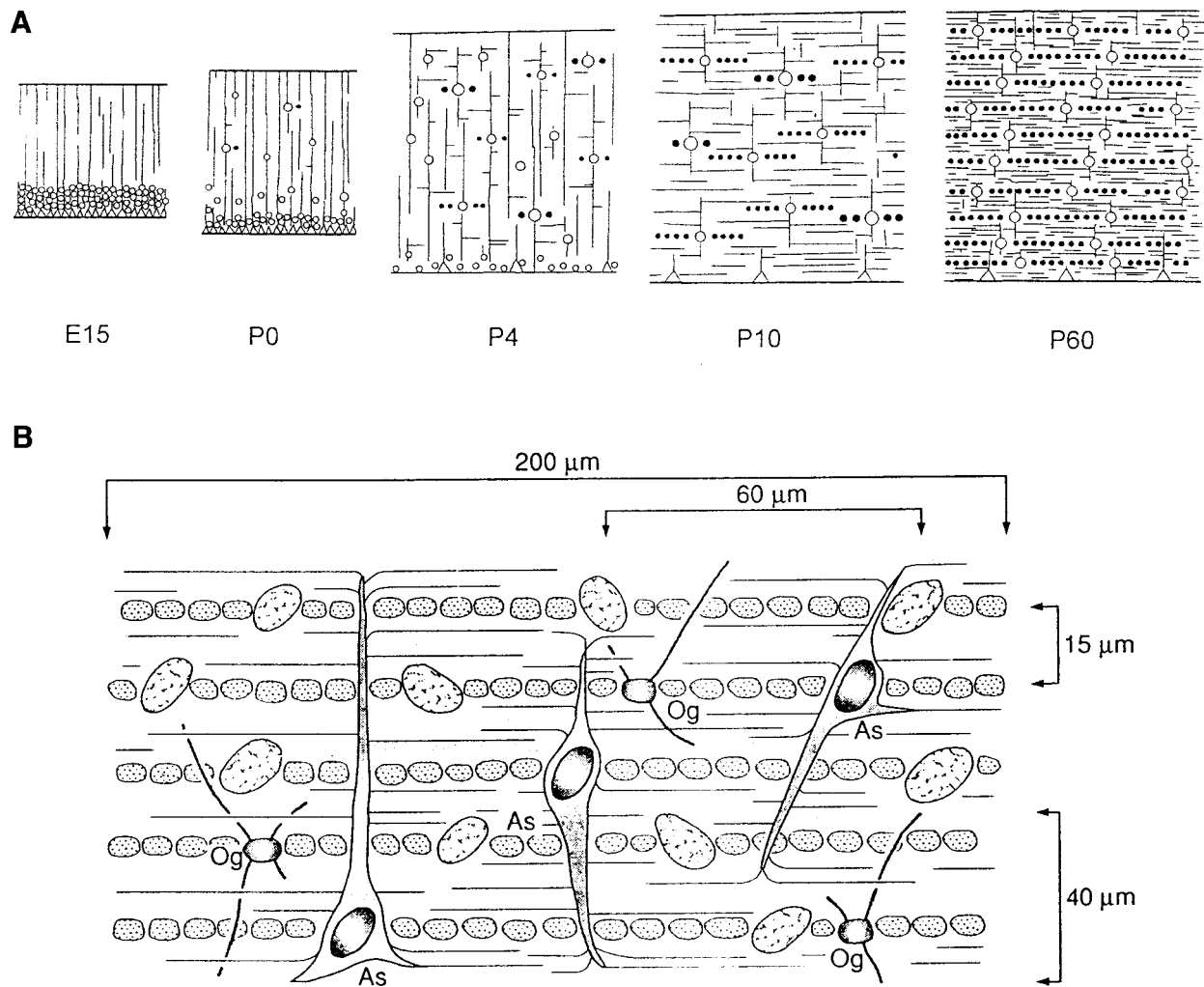


FIG. 4. A: migration and differentiation of cells of astrocyte and oligodendrocyte lineages, and multifocal pattern of development of glial rows in the fimbria between embryonic day 15 (E15) and postnatal day 60 (P60). (Lower surface, ependyma; upper surface, pia.) There is a relatively small increase in the thickness of the fimbria. The multicellular ventricular layer (small open circles at lower surface at E15 and P0) becomes reduced to a unicellular adult ependyma (triangles on ependymal surface). Cells migrating into the body of the fimbria, and supplemented by division of precursors there, become arranged in progressively longer interfascicular glial rows. The astrocytes (large, pale circles) change from predominantly radial processes to predominantly longitudinal. Oligodendrocytes are represented by small, black, filled contiguous circles. Myelination (not represented) largely occurs between P10 and P60. Axons (not represented) are present from the earliest developmental stage. [From Suzuki and Raisman (596). Copyright 1992, reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.] B: organization of the glial framework of central white matter tracts. Arrangement of radial and longitudinal processes of oligodendrocytes (Og) and astrocytes (As) forming a continuous meshwork of processes intermingled within the axonal tracts in the fimbria. Astrocytes are linked with each other by gap junctions, and also form gap junctions with oligodendrocytes, thus providing an indication for a functional as well as an anatomical functional syncytium. Myelination occurs on an asynchronous mode, with individual oligodendrocytes maturing independently within a cluster of adjacent oligodendrocytes, suggesting an interaction between axon maturation and oligodendrocyte differentiation. [From Raisman (492).]

before myelin formation. The same sequence occurs also *in vitro*; CNP is expressed at the same time as GalC in cultured oligodendrocytes (455). MBP, MAG, and PLP appear sequentially both *in vivo* and *in vitro* (154, 249, 399, 455) and signify a mature oligodendrocyte.

*In vitro* analyses suggest that maturation of oligodendrocytes from the precursor stage to the mature cell is identical in culture, even without neurons, as in intact tissue. Thus the capacity of oligodendrocyte progenitors to differentiate into oligodendrocytes is intrinsic to the lineage (605). In the absence of neurons, oligodendrocytes can clearly make a myelin-like membrane (533); nevertheless, coculture with neurons increases myelin gene expression, such as PLP, MBP, and MAG (358, 366). The presence of MOG correlates with late stages of maturation of the oligodendrocyte (570) (see sect. III C2).

A similar myelin-gene induction by neuronal contact with oligodendrocytes is also observed *in vivo* (295); the switch to PLP isoform expression in DM20-expressing premyelinating oligodendrocytes indicates the beginning of the process of myelination in individual myelinating processes (620). This myelin-gene induction parallels morphological modifications, i.e., *in vivo*, when axonal contact occurs, there is a dramatic modification of oligodendrocyte morphology with loss of oligodendrocyte processes that have not contacted axons (246).

#### 6. Oligodendrocyte-type 2 astrocyte (O-2A phenotype)

From the early 1980s glial research was centered around an oligodendrocyte-type 2 astrocyte progenitor (O-2A) first discovered in culture of the optic nerve (reviewed in Ref. 475). O-2A progenitors in culture generate oligodendrocytes constitutively but can give rise to process-bearing astrocytes (so-called type 2 astrocytes) when treated with 10% fetal calf serum. Attempts to find cells *in vivo* with the type 2 astrocyte phenotype during normal development have failed (248, 505), even after grafting O-2A cells into brain during development (177). Combination of tritiated thymidine and specific cell type immunocytochemistry indicate that most astrocytes are generated prenatally and during the first postnatal week, before oligodendrocyte formation (565), suggesting that there is not a second wave of type 2 astrocytes *in vivo* as suggested by the previous *in vitro* studies. "The apparent discrepancies between the data obtained *in vitro* and *in vivo* highlights an important aspect of the approach *in vitro*; when a progenitor cell is grown in tissue culture, the environments to which it can be exposed are restricted only by the imagination of the experimenter. By exposing the cell to a variety of signals, the full differentiation potentiality of the cell can be explored. In contrast, during development, a progenitor cell is exposed to a restricted sequence of signals that are spatially and temporally programmed" (196).

#### 7. The number of mature oligodendrocytes is regulated by apoptosis

The final number of mature myelinating oligodendrocytes is determined by the proliferative rate of their progenitors and by the process of programmed cell death that occurs during development (reviewed in Ref. 29). As oligodendrocytes differentiate late in the CNS, their development may be influenced by signals derived from other neural cell types, such as astrocytes and neurons (249, 510). Axon-to-oligodendrocyte signaling results in the generation of the precise numbers necessary to myelinate entirely a given population of axons (reviewed in Ref. 32).

Confocal microscopy analysis of rat brain tissue has clearly demonstrated that premyelinating oligodendrocytes that express DM-20 have two fates, programmed cell death (PCD) or myelination. PCD occurs during a process of axon matching to eliminate surplus cells. The cells that make contact with axons survive and begin myelination (620), as hypothesized earlier (28). The appearance of MOG (see sect. III C2E) in oligodendrocytes (119, 368, 455, 552), as in the CG4 oligodendrocyte cell line (570), is a reliable marker for fully differentiated myelin-forming oligodendrocytes.

#### 8. Factors necessary for oligodendrocyte maturation and survival

A) GROWTH FACTORS. Many growth factors have been found to be involved in the proliferation, differentiation, and maturation of the oligodendrocyte lineage (29, 99, 100, 248, 249, 377, 378, 455, 489, 509, 684). Most of these studies have been performed *in vitro*. It is extremely difficult to extrapolate to *in vivo* conditions, as multiple factors may act in concert to achieve the exquisitely fine regulation of the complex process of oligodendrocyte development and myelination. Combinations of factors often produce effects that are significantly different from those seen with any one factor alone (379). Furthermore, these factors have multiple effects during development. In contrast to experiments on rodent cells, growth factors that act on human cells have not as yet been fully determined.

**PDGF.** PDGF is synthesized during development by both astrocytes and neurons (412, 685). *In vitro*, PDGF, a survival factor for oligodendrocyte precursors (239), is a potent mitogen for oligodendrocyte progenitor cells, although it triggers only a limited number of cell divisions (208, 489). This developmental clock (605) is not related to the loss of PDGFR- $\alpha$  from the surface of oligodendrocyte progenitor cells but is due to the blockade of the intracellular signaling pathways from the PDGF receptor to the nucleus (250). The PDGF- $\alpha$  receptors disappear at the O4+ stage of oligodendrocyte maturation (173, 427). PDGF is also a survival factor for oligodendrocyte progenitors (28, 489), as recently demonstrated by the im-

paired oligodendrocyte development in the PDGF-A deficient mice (201). In these mice, there are profound reductions in the numbers of PDGFR- $\alpha$  progenitors and oligodendrocytes in the spinal cord and cerebellum, but less severe reductions of both cell types in the medulla. Infusion of PDGF into the developing optic nerve in vivo greatly reduces apoptotic cell death (33). PDGF also stimulates motility of oligodendrocyte progenitors in vitro and is chemoattractive.

**Basic FGF.** Basic FGF (bFGF) (also called FGF 2) is also a mitogen for neonatal oligodendrocyte progenitors (168). It upregulates the expression of PDGFR- $\alpha$  and therefore increases the developmental period during which oligodendrocyte progenitors or preoligodendrocytes are able to respond to PDGF (377). Preoligodendrocytes can even revert to the oligodendrocyte progenitor stage when cultured with both PDGF and bFGF (65). This inhibition of oligodendrocyte differentiation can be overridden by the presence of astrocytes (65, 371). bFGF is present in the developing nervous system in vivo (175). The levels of expression of mRNA for the high-affinity bFGF receptors-1, -2, and -3 are differentially regulated during lineage progression (22); this pattern of expression could provide a molecular basis for the varying response of cells to a common ligand that is seen during development.

**Insulin-like growth factor I.** Insulin-like growth factor I (IGF-I) stimulates proliferation of both oligodendrocyte progenitors and preoligodendrocyte O4+ positive cells, and IGF receptors have been shown to be present on cells of the oligodendrocyte lineage (378). IGF-I can be detected in the SVZ at a time when these cells are generated (34). Many commonly used defined culture media contain insulin at concentrations sufficient to activate IGF-I receptors and therefore should be considered as IGF-I supplemented (379). IGF-I is also a potent survival factor for both oligodendrocyte progenitors and oligodendrocytes in vitro (28). The morphology of myelinated axons and the expression of myelin specific protein genes have been examined in transgenic mice that overexpress IGF-I and in those that ectopically express IGF binding protein-1 (IGFBP-1), a protein that inhibits IGF-I action when present in excess (42, 107). The percentage of myelinated axons and the thickness of the myelin sheaths are significantly increased in IGF-I transgenics. An alteration in the number of oligodendrocytes is seen but cannot completely account for the changes in the increase in myelin gene expression. IGFBP-1 transgenic mice have a decreased number of myelinated axons and thickness of the myelin sheaths. IGF-I could be involved in both the increase in oligodendrocyte number and in the amount of myelin produced by each oligodendrocyte (107). However, it has recently been shown, in the IGF-I null mouse, that hypomyelination and depletion of oligodendrocytes

is directly proportionate to the primary decreased number of certain categories of neurons (115).

**Neurotrophin-3.** Neurotrophin-3 (NT-3) is a mitogen for optic nerve oligodendroglial precursors only when added with high levels of insulin, with PDGF, or with their combination (25, 31). Astrocytes express NT-3 in optic nerve. NT-3 promotes also oligodendrocyte survival in vitro (25, 28). The TrkC receptor for NT-3 is expressed in oligodendrocytes (317). Mice lacking NT-3 or its receptor TrkC exhibit profound deficiencies in CNS glial cells, particularly in oligodendrocyte progenitors; there is an important reduction in the spinal cord diameter, thereby suggesting that cell populations other than neurons are affected (289).

It was recently shown that NT-3 in combination with brain-derived neurotrophic factor (BDNF) is able to induce proliferation of endogenous oligodendrocyte progenitors and the subsequent myelination of regenerating axons in a model of contused adult rat spinal cord (380). These findings may have significant implications for chronic demyelinating diseases or CNS injuries. A reduction of the axonal caliber has been observed in BDNF-deficient knock-out mice as an indirect effect of the reduced size of retinal ganglion cell axons and hypomyelination (108).

**Glial growth factor.** The glial growth factor (GGF), a member of the neuregulin family of growth factors generated by alternative splicing, including Neu, heregulin, and the acetylcholine receptor-inducing activity (ARIA), is a neuronal factor, mitogenic on oligodendrocyte precursors (100, 393); it is also a survival factor for these cells. It delays differentiation into mature oligodendrocytes (99). In mice lacking the family of ligands termed neuregulins, oligodendrocytes in spinal cord failed to develop (632). This failure can be rescued in vitro by the addition of recombinant neuregulin to explants of spinal cord. In the embryonic mouse spinal cord, neuregulin expression by motoneurons and the ventral ventricular zone is likely to exert an influence on early oligodendrocyte precursor cells. Neuregulin is a strong candidate for an axon-derived promoter of myelinating cell development (32).

**Ciliary neurotrophic factor.** The ciliary neurotrophic factor (CNTF) can also act as comitogen with PDGF. Animals deficient in CNTF have a reduced number of mitotic glial progenitors. CNTF also promotes oligodendrocyte survival in vivo (33).

**Interleukin-6.** Interleukin (IL)-6 may also act on oligodendrocyte survival (33) as well as leukemia inhibitory factor (LIF) and a related molecule (210).

**Transforming growth factor.** In vitro, transforming growth factor (TGF)- $\beta$  inhibits PDGF-driven proliferation and promotes differentiation of oligodendrocyte progenitors (379).



*IL-2*. IL-2 directly affects the function of both neurons and glia in the nervous system, inducing proliferation and differentiation or inducing oligodendrocyte cell death (444).

Hormones act also on myelinating cells and are described in section III F.

#### B) NEUROTRANSMITTERS AND OLIGODENDROCYTE DEVELOPMENT.

Oligodendrocyte progenitors are equipped with a variety of ligand- and voltage-gated ionic channels that are expressed *in vitro* as *in vivo* (572, 586). They are not detailed here.

Recent data suggest that glutamate, the most abundant excitatory neurotransmitter in the mammalian brain, acting on its ionotropic receptors, is involved in the shaping of the oligodendrocyte population. The main ionotropic glutamate receptors expressed by oligodendrocytes belong to the dl- $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate classes (369). It has been shown that non-NMDA glutamate receptor agonists are able to inhibit oligodendrocytes progenitor proliferation in cell cultures (207). In cerebellar slice cultures, glutamate is an antimitotic signal at all proliferative stages of the oligodendrocyte lineage, i.e., for both oligodendrocyte progenitors and precursors. Interestingly, the glutamate effects are receptor and cell specific, because they are selectively mediated through AMPA receptors, whereas in the same context, astrocyte proliferation and number are not affected (691).

The dopamine D3 receptor (D3R) has been found to be expressed by precursors and immature oligodendrocytes, but absent from mature oligodendrocytes (68). By confocal microscopic analysis, it was shown that D3R was associated with cell bodies and cell membranes, but not with the processes emanating from cell soma. Immunohistochemistry revealed the presence of D3R in some oligodendrocytes located mainly within parts of the corpus callosum during myelinogenesis and was shown to modulate the timing of oligodendrocytes maturation and elaboration of myelin sheath. Treatment of glial cultures with a dopamine agonist altered the normal pattern of oligodendrocyte differentiation. Another dopamine receptor, D2R, is also present in a subset of mature interfascicular oligodendrocytes in the rat corpus callosum (262).

GABA<sub>A</sub> receptors have also been reported in oligodendrocytes (45).

Oligodendrocytes express opioid receptors,  $\mu$ -receptors are apparent at the earliest stages of oligodendrocyte development, while  $\kappa$ -receptors are detected later at the time that MBP is expressed (306). There is a proliferative response to  $\mu$ -receptor stimulation.

These data indicate that these neurotransmitter receptors are involved in processes other than nerve conduction and may have additional, perhaps trophic functions in glial and neuronal differentiation.

## D. The Glial Network

In a review of cell junctions among the supporting cells of the CNS, E. Mugnaini wrote in 1986 (413): "Cell junctions require careful analysis because they reflect not only the biology of individual cells, but also their sociology; that is, the cooperativity with other cells, and the relation to the environment." *In situ*, morphological studies have shown that astrocyte gap junctions are localized between cell bodies, between processes and cell bodies, and between astrocytic end-feet that surround brain blood vessels (679). *In vitro*, junctional coupling between astrocytes has also been observed (189, 635). Although less frequently observed than junctions between astrocytes, gap junctions also occur between oligodendrocytes, as observed *in situ* (90, 519, 574) and *in vitro* (634). Moreover, astrocyte-to-oligodendrocyte gap junctions have been identified between cell bodies, cell bodies and processes, and between astrocyte processes and the outer myelin sheath (654; reviewed in Ref. 218). Thus the astrocytic syncytium extends to oligodendrocytes, allowing glial cells to form a generalized glial syncytium (413), also called "panglial syncytium," a large glial network that extends radially from the spinal canal and brain ventricles, across gray and white matter regions, to the glia limitans and to the capillary epithelium (498) (Fig. 1).

Gap junctions are channels that link the cytoplasm of adjacent cells and permit the intercellular exchange of small molecules with a molecular mass <1–1.4 kDa, including ions, metabolites, and second messengers (reviewed in Refs. 81, 218). In addition to homologous coupling between cells of the same general class, heterologous coupling has been observed not only between astrocytes and oligodendrocytes, but also between ependymal cells and retinal Muller glia. Homologous coupling could serve to synchronize the activities of neighboring cells that serve the same functions. Such coupling would extend the size of a functional compartment from a single cell to a multicellular syncytium, acting as a functional network (reviewed in Ref. 218).

Gap junctions are now recognized as a diverse group of channels that vary in their permeability, voltage sensitivities, and potential for modulation by intracellular factors; thus heterotypic coupling may also serve to coordinate the activities of the coupled cells by providing a pathway for the selective exchange of molecules below a certain size. In addition, some gap junctions are chemically rectifying, favoring the transfer of certain molecules in one direction versus the opposite direction. The main gap junction protein of astrocytes is connexin (Cx) 43 (review in Ref. 218), whereas Cx32 is expressed in oligodendrocytes in the CNS (539) as well as another type of connexin, Cx45 (140, 141, 318, 332, 419). Heterologous astro-oligodendrocyte gap junctions may be composed of Cx43/Cx32, if these connexins form functional junctions.



Heterocoupling between astrocytes and oligodendrocytes has been proposed to serve  $K^+$  buffering around myelinated axons (332; reviewed in Ref. 692).

### E. Functions of Oligodendrocytes

The main and evident function of oligodendrocytes is the formation of a myelin sheath around most of axons in the CNS. The function of myelin is studied in section III, including roles newly ascribed to myelin itself, such as clustering of sodium channels at the node of Ranvier during axogenesis, participation to development and regulation of axonal caliber (sect. III G2), and maintenance of axons, but also inhibition of axonal growth and regeneration (sect. III G3). Myelin morphology, composition, and specific roles are treated in specific sections (sect. III, B, C, and G), as well as experimental and human diseases involving oligodendrocyte/myelin (sect. IV).

## III. MYELIN

The myelin sheath around most axons constitutes the most abundant membrane structure in the vertebrate nervous system. Its unique composition (richness in lipids and low water content allowing the electrical insulation of axons) and its unique segmental structure responsible for the saltatory conduction of nerve impulses allow the myelin sheath to support the fast nerve conduction in the thin fibers in the vertebrate system. High-speed conduction, fidelity of transfer signaling on long distances, and space economy are the three major advantages conferred to the vertebrate nervous system by the myelin sheath, in contrast to the invertebrate nervous system where rapid conduction is accompanied by increased axonal calibers.

The importance of myelin in human development is highlighted by its involvement in an array of different neurological diseases such as leukodystrophies and multiple sclerosis (MS) in the CNS and peripheral neuropathies in the PNS.

### A. Phylogeny

In terms of evolution of the nervous system, the myelin sheath is the most recent of nature's structural inventions. Myelin is found in all vertebrate classes except the evolutionarily oldest agnathan cyclostomata, i.e., the jawless fish (hagfish and lampreys) in which axons, however, are surrounded by glial cells. All other vertebrates, including cartilaginous fish, bony fish, and tetrapods as well as lungfish and coelacanth manifest extensive myelination in both the CNS and PNS. So, the first myelin-like ensheathed axons may have appeared about 400 million years ago. Although compact myelin is a

uniquely vertebrate feature, a form of glial ensheathment of axons exists in invertebrates (such as annelids, crustaceans, mollusks, and arthropods), forming a superficially resembling vertebrate myelin sheath (reviewed in Refs. 122, 645). Ontogenetically, PNS myelin appears before CNS myelin; however, it has not been demonstrated to occur phylogenetically.

### B. Morphological Structure of the Myelin Sheath and of the Node of Ranvier

#### 1. Myelin

Myelin, named so by Virchow (640), is a spiral structure constituted of extensions of the plasma membrane of the myelinating glial cells, the oligodendrocytes in the CNS (82, 452). These cells send out sail-like extensions of their cytoplasmic membrane (Figs. 2 and 3), each of which forms a segment of sheathing around an axon, the myelin sheath (reviewed in Refs. 41, 404, 453, 457). Several structural features characterize myelin. Its periodic structure, with alternating concentric electron-dense and light layers, was already shown in 1949, in the optic nerve myelin of guinea pig (563). The major dense line (dark layer) forms as the cytoplasmic surfaces of the expanding myelinating processes of the oligodendrocyte are brought into close apposition. The fused two outer leaflets (extracellular apposition) form the intraperiodic lines (or minor dense lines) (Figs. 2 and 5). The periodicity of the lamellae is 12 nm. Each myelin sheath segment or internode appears to be 150–200  $\mu\text{m}$  in length (90); internodes are separated by spaces where myelin is lacking, the nodes of Ranvier (84). It does seem that oligodendrocytes form thicker myelin around larger axons (657).

A) THE SCHMIDT-LANTERMAN CLEFTS. The Schmidt-Lanterman clefts (also called Schmidt-Lanterman incisures) correspond to cytoplasmic faces of the myelin sheath that have not compacted to form the major dense line; thus they constitute pockets of uncompacted glial cell cytoplasm within the compact internode myelin. They are oblique, funnel-like clefts that extend across the entire thickness of the sheath, providing a pathway through which cytoplasm on the outside of the sheath is confluent with that of the inside part. Schmidt-Lanterman clefts are common in the PNS, but rare in the CNS (Fig. 5). The myelin sheath is separated from the axonal membrane by a narrow extracellular cleft, the periaxonal space.

B) THE PARANODAL LOOPS. Myelin is less compacted at the inner and outer end of the spiral, forming inner and outer loops that retain small amounts of oligodendrocyte cytoplasm. The myelin lamellae end near the node of Ranvier in little expanded loops containing cytoplasm. The loops are arranged on a roughly regular and symmetric pattern on each side of the node. This sequence of loops is named the paranodal region or paranode. Each paranodal loop

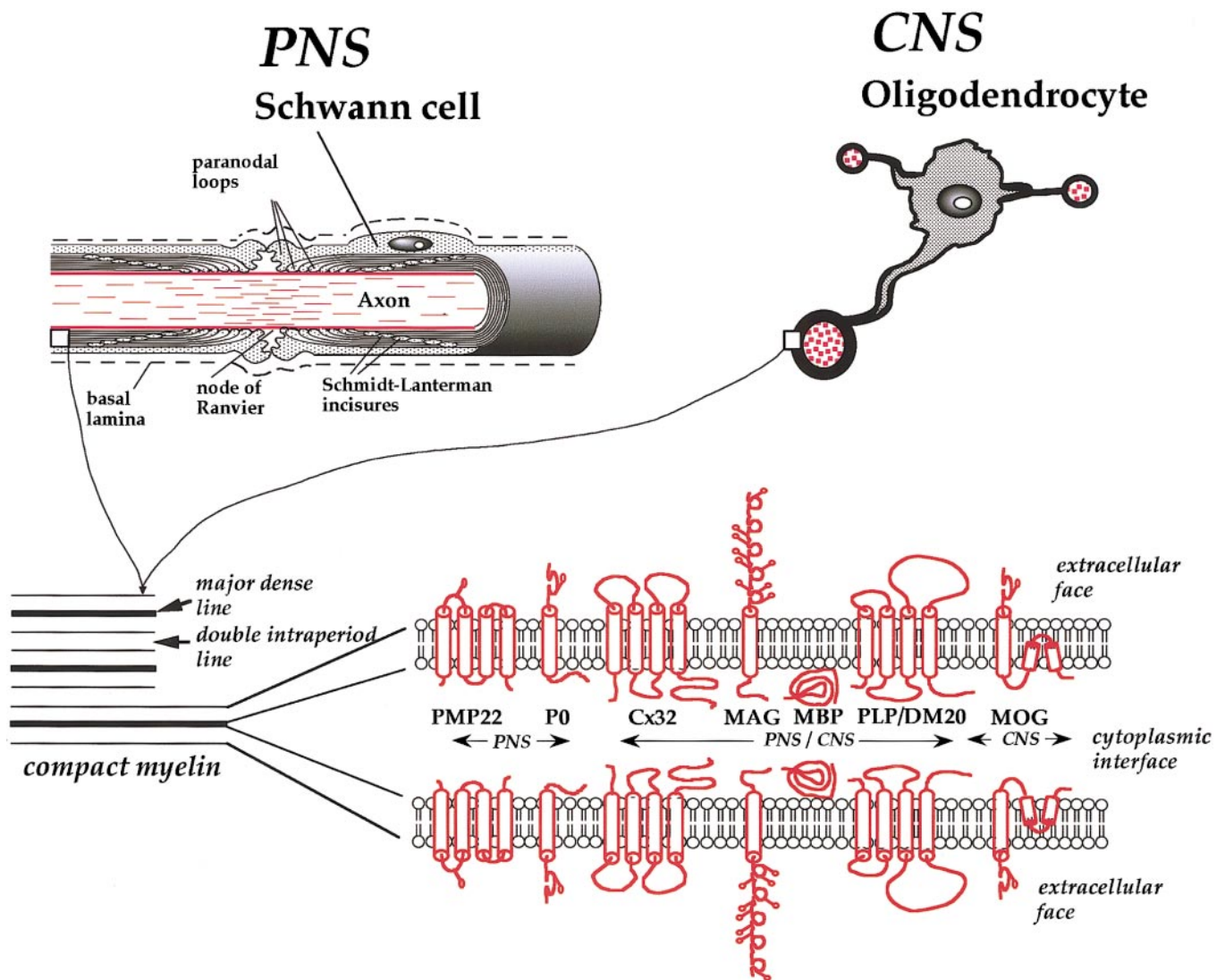


FIG. 5. Myelinating glial cells, myelin structure, and composition in the peripheral nervous system (PNS) and in the CNS. In the PNS, the myelinating Schwann cell myelinates only one segment of axon (top left corner), whereas in the CNS (top right corner), the oligodendrocyte is able to myelinate several axons. The compact myelin is formed by the apposition of the external faces of the membrane of the myelinating cell, forming the “double intraperiodic line”; the apposition of the internal faces followed by the extrusion of the cytoplasm, form the “major dense line.” The myelin proteins are schematically described; they differ between PNS and CNS. [Adapted from Pham-Dinh (457).]

makes contact with the axon. Freeze-fracture studies have shown distinct membrane morphologies in the axolemma (reviewed in Ref. 655), corresponding to the different domains of the myelinated fibers: the internode, the paranodal region, and the node of Ranvier. These anatomically different regions of the axolemma are formed by specific interactions between the axon and the myelinating glial cell. The interface between the lateral loops and the axon membrane exhibits a row of 15 nm regularly spaced densities, the transverse band (256). The transverse bands, which comprise rows of regularly spaced particles in glial and axonal membranes, are associated with cytoskeletal filaments and appear to tighten the ax-

onal-paranodal apposition (270). Molecular structure and organization of the axolemma at the paranode and node of Ranvier have recently come to light (135, 171, 293, 382; reviewed in Refs. 530, 653) (see below and sect. III E).

## 2. The node of Ranvier

Along myelinated fibers, adjacent internodes are separated by the nodes of Ranvier where the axolemma is exposed to the extracellular milieu (84) (Fig. 2). The nodes of Ranvier play a major role in nerve impulse conduction. They allow the fast saltatory conduction, the impulse jumping from node to node, rather than

progressing slowly along the axon as in unmyelinated or demyelinated fibers. Thin axons appear to possess tiny node gaps, and thick axons exhibit spacious node gaps, as observed at nodes of Ranvier in rat white matter (56). Astrocytic processes may be seen in close proximity to the axon membrane at the node of Ranvier (58) (see sect. III E for the molecular organization of the node). Another cell type characterized by the NG2 antigen has recently been shown to contact the node of Ranvier in adult CNS white matter (88), and this cell is thought to be a glial precursor.

### C. Myelin Isolation and General Composition

Myelin is the essential constituent of white matter in the CNS which contains ~40–50% myelin on a dry weight basis. The composition of myelin was widely studied more than 20 years ago, when methods of myelin isolation became available (431, 432); its low density allows its easy preparation as a fraction after sucrose gradient centrifugation. Although developed for fresh rat brain, it has been widely used for human specimens and gives a well-defined fraction, even from frozen myelin (39). Contaminants may increase when myelin is in low quantities, such as during development, and in pathological conditions such as hypomyelination and demyelination (689). Most biochemical analyses refer to the work of the group of Norton (reviewed in Ref. 404). Myelin is a poorly hydrated structure containing 40% water in contrast to gray matter (80%). Myelin dry weight consists of 70% lipids and 30% proteins. This lipid-to-protein ratio is very peculiar to the myelin membrane. It is generally the reverse in other cellular membranes. The insulating properties of the myelin sheath, which favor rapid nerve conduction velocity, are largely due to its structure, its thickness, its low water content, and its richness in lipids.

The specific constituents of myelin, glycolipids, and proteins are formed in the oligodendrocyte.

#### 1. Myelin lipids

Lipids found in oligodendrocytes and myelin are also present in other cellular membranes, but in a different proportion. In every mammalian species, myelin contains cholesterol, phospholipids, and glycolipids in molar ratios ranging from 4:3:2 to 4:4:2 (reviewed in Ref. 404). Therefore, the molar ratio of cholesterol is greater than that of any other lipid. Cholesterol esters are not present in normal myelin. There are no major peculiarities in myelin phospholipids that represent 40% of total lipids, except for the high proportion of ethanolamine phosphoglycerides in the plasmalogen form which account for about one-third of the phospholipids. Diphosphoinositides and triphosphoinositides are nonnegligible components, respectively, 1–1.5 and 4–6% of myelin phosphorus.

One of the major characteristics of oligodendrocyte and myelin lipids is their richness in glycosphingolipids, in particular galactocerebrosides, i.e., galactosylceramides (GalC) and their sulfated derivatives, sulfatides, i.e., sulfogalactosylceramides. These lipids have been immunolocalized in oligodendrocytes and tissue sections of myelin (490, 693). Even though there are no “myelin lipids,” GalC are the most typical lipids of myelin in which they are specifically enriched (reviewed in Ref. 404) and represent 20% lipid dry weight in mature myelin. These glycosphingolipids represent a family of components, as there is a great variability in the ceramide moiety, both in its sphingosine content (18–20 carbon atoms) and in its long-chain fatty acids content. The ceramide core of these glycosphingolipids is particularly rich in very-long-chain fatty acids, 22–26 carbon atoms, saturated, mono-unsaturated, and  $\alpha$ -hydroxylated. During development, the concentration of galactocerebrosides in brain is proportional to the amount of myelin present (432). This is also the case for the very-long-chain fatty acids (276). The biosynthesis for myelin very-long-chain fatty acids occurs in the microsomal compartment and not in mitochondria (72). Abnormalities of lysosomal enzymes related to degradation of sulfatides or galactocerebrosides, or proteins involved in the transport of these fatty acids to the peroxisomes, give rise to leukodystrophies (see sect. IV A2).

There are also several minor galactolipids (3% of total galactose) in myelin such as fatty esters of cerebroside, i.e., acylgalactosylceramides (608) and galactosyldiglycerides (463). Monogalactosyl diglyceride appears also to be a marker for myelination (541). This may be also the case for acylgalactosylceramides (608).

A sialylated derivative of galactocerebroside (sialosylgalactosylceramide), the ganglioside GM4, is present mainly in murine and primate myelin, including human, but not, for instance in bovine brain (117). Ganglioside GM1, although a minor component of myelin, is greatly enriched in myelin, compared with polysialogangliosides. It has been localized in myelin and some glial cells using specific monoclonal antibodies (313).

Furthermore, a number of major CNS myelin proteins, such as PLP, MAG, CNP (see below) are also covalently acylated (3, 448) (see sect. III C2), which gives them hydrophobic properties.

#### 2. Myelin proteins

Myelin proteins, which comprise 30% dry weight of myelin, are for most of the known ones, specific components of myelin and oligodendrocytes (97). The major CNS myelin proteins MBP and PLP (and isoform DM-20) are low-molecular-weight proteins and constitute 80% of the total proteins. Another group of myelin proteins, insoluble after solubilization of purified myelin in chloroform-methanol 2:1, have been designated as the Wolfgram



proteins, since their existence was suspected already in 1966 by Wolfgarm (670). These proteins comprise the CNP and other proteins.

Several glycoproteins are present in myelin (reviewed in Ref. 486), among which are MAG and MOG. Other proteins have also been identified, some of which have enzyme activities.

A) MBP. MBP (296) is one of the major proteins of CNS myelin and constitutes as much as 30% of protein. In fact, it is a family of proteins, as there are many isoforms of different molecular masses. The amino acid composition of the major MBPs was determined by Eylar et al. (178) from bovine brain, and by Carnegie (104) in humans. The major protein isoforms can be separated by SDS-PAGE. The molecular masses of the major forms are 21.5, 18.5, 17, and 14 kDa in the mouse and 21.5, 20.2, 18.5, and 17.2 kDa in humans (for review, see Ref. 97). In the adult, the major isoforms are 18.5- and 17.2-kDa isoforms in humans and 18.5 and 14 kDa in the mouse, constituting ~95% of the MBPs (582). The MBP isoforms are coded by alternative transcripts from the MBP gene which consists of seven exons (516). The MBP gene is distributed over a 32-kb stretch in the mouse on chromosome 18. In the human, the MBP gene has been assigned to 18q22-qter (535, 575) and is distributed over a length of 45 kb (291). In the mouse, at least seven transcripts are expressed from this gene through alternative splicing of exons 2, 5A, 5B, and 6 (15, 137, 424, 600). A number of other mRNA splicing variants of the mouse MBP gene predominantly expressed in embryonic stage have also been characterized (365, 421). One of these variants is expressed at the protein level in embryonic nervous system at a time when other MBP isoforms are not detected. By *in situ* hybridization, the expression of the MBP gene has been observed as early as E14.5 in the spinal cord in the mouse (454).

The MBP gene is contained within a larger transcription unit (98, 238, 474) that contains three unique exons spanning a region 73 kb upstream of the classic MBP transcription start site in the mouse (98). This transcription unit, called the Golli-MBP gene, is 195 kb in mice and 179 kb in humans (98, 474) and produces a number of alternative transcripts. The Golli-MBP gene consists of 10 exons, 7 of which constitute the MBP gene. The acronym Golli has been chosen for "gene expressed in the oligodendrocyte lineage." In fact, transcripts are also found in cells of the immune system (237, 473, 474, 694) and in some neurons (472). Corresponding Golli-MBP proteins have been found in the same tissues (290). Both Golli and MBP families of transcripts are under independent developmental regulations. Regulatory elements responsible for the specific expression of the MBP gene in the CNS (versus the PNS) have been identified using transgenic mice (227). A MBP promoter region of 256 bp that has been shown to contain regulatory elements for efficient

transcription in glial cells is sufficient to direct oligodendrocyte-specific expression (224).

It is of note that the 17- and 21.5-kDa isoforms, which contain exon 2, appear earlier during development in the mouse. In the human, the exon 2-containing isoforms of 20.2 and 21.5 kDa are also mainly expressed during myelination. They are reexpressed in chronic lesions of MS, and their reexpression correlates with remyelination (101) (see sect. IIIH). The active transport of exon 2-containing MBPs from the cytoplasm to the nucleus suggests a regulatory role in myelination for these karyophilic MBPs (447) (details on immunolocalization of MBP are reported further, see sect. IIII).

Posttranslational modifications including NH<sub>2</sub>-terminal acetylation, phosphorylation, and methylation can occur on the MBPs; indirect evidence suggests that methylation of MBP may be important for compaction of the membrane during myelin maturation (97). Citrullination can also occur (673).

Direct evidence that MBPs play a major role in myelin compaction in the CNS was provided from studies of the shiverer mutant mouse (see sect. IVA), which presents a large deletion of the MBP gene (517) and in which the major dense line is absent from myelin (480).

B) PROTEOLIPID PROTEINS (PLP AND DM-20). In 1951, Folch and Lees (190) discovered that a substantial amount of protein from brain white matter could be extracted by organic solvent mixtures. Because these proteins were apparently lipid-protein complexes, they were given the generic name of proteolipids to distinguish them from water-soluble lipoproteins. They are mainly myelin constituents. Although other myelin proteins can be solubilized this way, the name has been given to a major component of myelin, named PLP. This protein corresponds to 50% of myelin proteins. The predominant isoform commonly named PLP has an apparent molecular mass on SDS-PAGE of 25 kDa; a second isoform DM-20 (10–20% PLP in the adult) migrates as a 20-kDa band on electrophoresis. The authentic molecular masses appear to be slightly higher as PLP and DM-20 are both acylated by covalent linkage of mainly palmitic, oleic, and stearic acids (3, 606) on numerous cysteine residues localized on the cytoplasmic side of the myelin membrane (659). There is also a nonenzymatic glycosylation of extracytoplasmic domains (660). The complete amino acid sequence of bovine PLP has been elucidated by Stoffel et al. (592) and Lees et al. (326).

The gene coding for PLP is located on the X chromosome, in humans (667) at position Xq22 (367) and in mice in the H2C area (131). Its size is 15 kb, and it is organized in seven exons. PLP and DM-20 are coded by the same gene (405), and the two isoforms are formed by alternative splicing (423). The mRNA coding for PLP comprises all 7 exons, while a sequence corresponding to the 5'-part of exon 3 (exon 3B) is spliced out of the mRNA coding for



DM-20, leading to a 35-amino acid deletion in the protein sequence. There is a 100% sequence identity between murine and human PLP proteins (146, 356). PLP comprises four hydrophobic  $\alpha$ -helices spanning the whole thickness of the lipid bilayer, two extracytoplasmic and three cytoplasmic domains (including the NH<sub>2</sub> and COOH termini) localized, respectively, at the intraperiodic and major dense lines of myelin. The theoretical tridimensional topographical model developed by Popot et al. (470) has been confirmed experimentally by Weimbs and Stoffel (659). The respective distribution of PLP and DM-20 isoforms during development has been mentioned in section *II B 1*.

Spontaneous mutations involving the PLP gene occur, among which are the jimpy (*jp*) mouse (see sect. *IV A 1*) and a number of other animal models, as well as human dysmyelinating diseases (sect. *IV A 2*). PLP null alleles have been recently engineered in mice by an antisense transgenic approach (67), or by a knock-out strategy (305). Without PLP/DM-20 expression, oligodendrocytes are still competent to myelinate axons and to assemble compacted myelin sheaths. However, the ultrastructure of myelin showed condensation of the intraperiodic lines (as also observed in natural PLP mutants), correlating with a reduced physical stability; these observations suggest that PLP forms a stabilizing membrane junction after myelin compaction (66), similar to a "zipper" (305). On the other hand, the unexpected consequence of the absence of PLP/DM-20 is an early occurrence of widespread focal axonal swellings, followed later by axonal degeneration associated with impairment of motor performance in 16-mo-old mice (235) (see sect. *IV A 1 A*).

Two mammalian proteins of unknown functions (M6a and M6b) show 56 and 46% sequence identity with DM-20 protein (680), suggesting the existence of a family of DM-20 genes. M6a is a neuronal-specific protein, and M6b is a neuronal and myelin-associated protein (681). Several other members of this gene family have been characterized in sharks and rays. The "DM" proteins not only are highly homologous to each other, but also contain regions bearing similarities with segments of channel-forming regions of the nicotinic acetylcholine receptor and the glutamate receptor (122, 303).

**C) CNP.** CNP represents 4% of total myelin proteins. This protein hydrolyzes artificial substrates, 2',3'-cyclic nucleotides into their 2'-derivatives. However, the biological role of this enzyme activity is obscure since 2',3'-nucleotides have not been detected in the brain (642). The association of CNP with one of the major Wolfram proteins has been known for a long time (580). CNP appears on SDS-PAGE as a doublet of two peptides of molecular masses varying from 48 to 55 kDa, according to species. In general, there are two closely spaced protein bands at 48

and 46 kDa referred to CNP2 and CNP1, respectively (579).

The structure for the mouse and human CNP genes have been determined. The gene is located on chromosome 11 in the mouse (49) and on chromosome 17q21 in the human (152). The gene consists of four exons spanning 7 kb. The two CNP isoforms are produced by alternative splicing from two transcription start sites; the 5'-exon (exon 0) contains an upstream initiation codon; a splice site is present on exon 1 (319). Interestingly, two translational start sites can be used on the mRNA encoding the CNP 2 isoform, giving rise to CNP1 and CNP2 protein isoforms (439).

CNP is at high concentration not only in myelin, but also in photoreceptor cells in the retina (642). CNP mRNAs are detected in mouse spinal cord during embryonic stages (454, 690).

CNP, although isolated with the myelin fraction, is not found immunocytochemically localized in compact myelin; it is present in the cytoplasm of noncompacted oligodendroglial ensheathment of axons and in the paranodal loops (618). The protein is posttranslationally modified, acylated, and phosphorylated, especially the larger isoform (642). CNP is associated with the cytoplasmic plasma membrane of the oligodendrocyte by isoprenylation (74), mainly CNP1. CNP possesses two of the three binding domains for GTP (75). In addition, a COOH-terminal domain is analogous to the GTP-binding proteins of the Ras family. These properties are not related to the catalytic site of the enzyme. Thus CNP may function in a way unrelated to this enzyme activity for which no physiological substrate has been found.

Overexpression of CNP in transgenic mice perturbs myelin formation and creates aberrant oligodendrocyte membrane expansion (233).

**D) MAG.** MAG (487) is quantitatively a minor constituent, representing 1% of the total protein found in myelin isolated from the CNS and 0.1% in the PNS. The MAG gene spans 16 kb in length and includes 13 exons; it is located on chromosome 7 in mouse and 19 in human (35, 142). MAG has an apparent molecular mass of 100 kDa on SDS-PAGE, of which 30% is carbohydrate. Two MAG proteins have been identified (194, 531), large MAG (L-MAG) and small MAG (S-MAG). These proteins correspond to polypeptides of 72 and 67 kDa in the absence of glycosylation. MAG proteins have both a membrane-spanning domain and an extracellular region that contains five segments of internal homology that resemble immunoglobulin domains (531) and are strikingly homologous to similar domains of the NCAM and other members of the immunoglobulin gene superfamily. Both share identical extracellular and transmembrane domains but differ in their cytoplasmic domains, corresponding to the alternative splicing of exon 12 from a single RNA transcript. The deduced amino acid sequence of the human S-MAG

COOH terminus shows conservative substitutions of 4 of the 10 residues compared with the rodent peptide (387).

MAG is also glycosylated and bears the L2/HNK1 epitope (374, 466). The 72-kDa L-MAG can be phosphorylated and acylated (169). It was shown in the mouse that L-MAG is involved in the activation of Fyn, a protein tyrosine kinase of the src family, which suggested that the tyrosine phosphorylation cascade might be implicated in signal transduction for myelinogenesis, as evidenced by the impaired myelination observed in fyn-deficient mice (625). Moreover, it has recently been demonstrated that morphological differentiation of oligodendrocytes is dependent on fyn tyrosine kinase activation (443).

In the adult rat CNS, MAG is confined to the periaxonal collar of the myelin sheath (617), contrasting with a larger distribution across the different regions of PNS myelin (see sect. III C3). A differential intracellular sorting of MAG isoforms has been characterized, suggesting that L-MAG contains a basolateral sorting signal, whereas the sorting of S-MAG is dependent on extrinsic factors, such as coexpression by adjacent cells; altogether, these features indicate that the isoforms perform different functions (395).

MAG function has been studied by generating MAG gene knock-out mice (331, 400, 686). Surprisingly, CNS myelin forms almost normally in MAG-deficient mice; however, a prominent defect of the mutant myelin sheaths is an abnormal formation of the periaxonal cytoplasmic collar that is lacking in most of the internodes; myelin sheaths contain cytoplasmic organelles between lamellae, indicating a delay or block of myelin compaction; ~10% of axons, versus 3% in wild-type mice, received two to four sheaths around a single axon, suggesting that MAG may have a role in helping oligodendrocyte processes to distinguish between myelinated and unmyelinated axons in the CNS (331, 400). Moreover, a "dying-back oligodendrogliopathy" was further observed (323), which seems to be a toxic death of oligodendrocytes induced by an abnormality at the inner mesaxon, i.e., far from the cellular body of the cell. The differential role of S-MAG and L-MAG was further studied by generating mutant mice that express a truncated form of the L-MAG isoform, eliminating the unique portion of its cytoplasmic domain, but that continue to express S-MAG (203). CNS myelin of the L-MAG mutant mice displays most of the pathological abnormalities reported for the total MAG null allele mice, whereas PNS axons and myelin of older L-MAG mutant animals do not degenerate, in contrast to the peripheral neuropathy observed in full null MAG mutants (103, 202). These data indicate that S-MAG is sufficient to maintain PNS integrity, whereas L-MAG is the critical MAG isoform in the CNS (203). It is of note that during CNS myelination in rodent, L-MAG is expressed earlier than S-MAG, which is the predominant form in adult CNS myelin, whereas S-MAG is always prominent in

PNS myelin (194, 531). These differential developmental patterns of expression may explain the different phenotypes presented by the full- and the L-MAG-mutant deficient mice, demonstrating that the cytoplasmic domain of the large MAG isoform is needed for proper CNS but not PNS myelination (203). In contrast to rodents, the L-MAG predominates in adult human brain while, like in rodents, S-MAG is most abundant in peripheral nerves (387). Moreover, in the MAG-deficient mice, axonal caliber is significantly decreased in the PNS (686).

For a long time MAG has been considered as a potential receptor for an axonal ligand important for the initiation and progression of myelination (reviewed in Refs. 188, 384). Indeed, MAG is expressed exclusively by myelinating cells where it is enriched in the periaxonal membranes of the myelin internodes, thus in direct contact with the axon (622). Moreover, in vitro studies of transfected Schwann cells correlated MAG overexpression with accelerated myelination, whereas MAG underexpression was associated with hypomyelination (see reviews in Refs. 188, 384). The fact that MAG belongs to a family of glycoproteins sharing a common L2/HNK1 carbohydrate epitope led to the suggestion that MAG could be involved in cell surface recognition (466). Indeed, in vitro experiments with anti-MAG antibodies showed that MAG is involved in neuron-oligodendrocyte interactions (466). Recently, the discovery that MAG belongs to the family of sialic acid binding lectins, the sialoadhesins, as shown by the binding of MAG to neurons in a sialic acid-dependent manner, has led to the study of gangliosides, prominent neural cell surface sialoglycolipids, as MAG ligands (683). The sialic acid-dependent binding of MAG to neurons is trypsin sensitive, indicating that MAG binds to a neuronal sialo-glycoprotein rather than to a sialo-glycolipid and that the sialic acid binding site of MAG with the axon has been shown to be located at Arg-118 in the MAG amino acid sequence (601).

Another proposed function of MAG is to inhibit neurite outgrowth, i.e., axon regeneration in the CNS after lesion (reviewed in Refs. 188, 485) (see sect. III G3D). On the other hand, it has recently been shown that, in the CNS, MAG can be proteolyzed near its transmembrane domain by a calcium-activated protease and that the soluble proteolytic product can be found in the cerebrospinal fluid (583). The conversion of 100-kDa MAG to its soluble derivative dMAG of 90 kDa occurs more rapidly in myelin from human and other primates than in myelin from rat brain. Soluble dMAG may be involved in the molecular mechanism of demyelination, as the rate of formation of dMAG is increased even more in myelin from white matter of patients with MS (398).

E) MOG. MOG was first identified by a polyclonal antibody directed against an antigen called M2 that induces autoimmune encephalomyelitis in the guinea pig. MOG was first identified as the antigen responsible for the

demyelination observed in animals injected with whole CNS homogenate; it was later identified as a minor glycoprotein specific for CNS myelin (324, 325). MOG was further characterized by immunological methods, immunohistochemistry, and Western blot, using a mouse monoclonal antibody against glycoproteins of rat cerebellum (337). Both M2 and MOG migrate at the 52- to 54-kDa and 26- to 28-kDa levels on SDS gels. The purified protein is a minor protein of myelin of 25 kDa with some glycosylation resulting in molecular mass 26–28 kDa that can form dimers of 52–54 kDa (9, 54). MOG is only present in mammalian species (54) and is highly conserved between species. In humans, MOG expresses the L2/HNK1 epitope (86). The NH<sub>2</sub>-terminal, extracellular domain of MOG has characteristics of an immunoglobulin variable domain and is 46% identical to the NH<sub>2</sub> terminus of bovine butyrophilin (BT) expressed in the mammary gland, and chick histocompatibility BG antigens (212, 461). These proteins form a subset of the immunoglobulin superfamily. They colocalize to the human major histocompatibility complex (MHC) locus, on chromosome 6 (6p21.3-p22) in the human, and on chromosome 17 in the mouse (461). In both species, the MOG gene is localized at the distal end of the MHC class Ib region (460). The mouse and human MOG genes have been cloned and sequenced (130, 255, 459, 525). The mouse and the human MOG gene are 12.5 and 19 kb long, respectively. There are eight exons and at least six transcripts generated by alternative splicing in the human (459). MOG protein is mostly located on the outermost lamellae of compact myelin sheaths in the CNS (79). The topology of MOG indicates that there may be only one transmembrane domain (138, 316) as other members of the immunoglobulin superfamily. MOG is located on the plasma membrane of the oligodendrocyte, especially on oligodendrocyte processes, and on the outermost lamellae of myelin sheaths (79). It is a surface marker of oligodendrocyte maturation (54, 119, 461, 552). Its presence correlates with late stages of maturation, possibly restricted to the myelinating oligodendrocytes, as shown using the CG4 cell line (570). At present, MOG is the only CNS protein that can induce both a T cell-mediated inflammatory immune reaction and a demyelinating antibody-mediated response in an animal model of demyelinating diseases, the experimental autoimmune encephalomyelitis (EAE) (see sect. IVB2).

F) OTHER PROTEINS OF MYELIN. I) *Small basic proteins. Myelin-associated oligodendrocyte basic protein.* Myelin-associated/oligodendrocyte basic protein (MOBP) is a small highly basic protein that has been recently isolated (678). Three isoforms are generated by alternative splicing. The three corresponding polypeptides are of 8.2, 9.7, and 11.7 kDa. MOBPs are located in the major dense line of myelin (259, 678) where they could play a similar role to MBP in myelin compaction. MOBP has also been described in the mouse, where the abundance of MOBP transcripts is less

than PLP but greater than that of the CNP gene (401). The MOBP mRNA is located initially in the cell bodies of the oligodendrocytes and moves distally into the processes when myelination proceeds, as does the MBP mRNA (401). The gene has been mapped to chromosome 9 in the mouse (372), at a region syntenic with the human chromosome 3 (3p22) (258).

P2. P2, a basic protein of 13.5 kDa, has also been found in the CNS (619), although it is mainly a PNS myelin protein. In the CNS, it is present essentially in the spinal cord of the rabbit and the human, but not in the rat and the mouse. The reason for this regional and species variation has not been determined.

II) *Members of the tetraspan-protein family.* Besides PLP in the CNS, PMP22 and Cx32 in the PNS, an increasing number of four- $\alpha$ -helix transmembrane proteins have been found to be associated with myelin.

*Rat myelin and lymphocyte protein.* rMAL (r for rat, MAL for myelin and lymphocyte protein), an homolog of human MAL, a protein expressed in various T cell lines in human, was identified in myelin by Schaeren-Wiemers et al. (536); it was further characterized in the mouse (362). On the other hand, the biochemical properties of a purified protein-lipid complex were used by Pfeiffer and co-workers (298) to identify MVP17 ("myelin vesicular protein" of 17 kDa), homologous to rMAL. Plasmalogen, also a myelin-oligodendrocyte proteolipid protein, was cloned by Gillen and coll. (220).

*Oligodendrocyte-specific protein.* Oligodendrocyte-specific protein (OSP) is a single 22-kDa protein present in CNS myelin and oligodendrocytes; it is structurally related to other tetraspan proteins and particularly to PMP-22 found in PNS myelin, with which it presents 48% amino acid similarity and 21% identity. The gene for OSP has been localized in the mouse and in humans on the 3q26.2–26.3 region of chromosome 3. It has recently been shown that OSP is the third most abundant protein in CNS myelin, after PLP/DM20 and MBP, accounting for ~7% of the protein content (76). The analysis of the OSP-deficient mice indicated that OSP is a tight junction protein, claudin-11 (408), which is the mediator of parallel-array tight junction strands in CNS myelin as in testis (231).

*Cx32.* Cx32 has recently been found on some areas of CNS myelin and corresponding myelinating oligodendrocytes (140, 141, 318, 332, 539). These data suggest a specialized role of gap junctions composed of Cx32 along myelinated fibers belonging to subpopulations of neurons.

*Tetraspan-2.* Tetraspan-2, a novel rat tetraspan protein in cells of the oligodendrocyte lineage has recently been identified (55).

III) *Other minor proteins. Oligodendrocyte-myelin glycoprotein.* Oligodendrocyte-myelin glycoprotein (OMgp) is a glycosylated protein first isolated from human myelin. It is bound to myelin through a glycosyl phosphatidylinositol. Its apparent molecular mass is 120 kDa. Its oligo-



saccharidic moiety bears also the HNK-1 epitope (388). The gene contains only two exons and is located in the human on chromosome 17 q11–12 band. Interestingly, the OMgp gene is located in the intronic site of the gene of type 1 neurofibromatosis (641). The gene has also been identified in the mouse and is highly conserved. OMgp location in myelin is limited to the paranodal areas. Recently, it has been shown in the mouse CNS that OMgp is not confined to oligodendrocytes and myelin but is also expressed on the plasma membrane of the neuronal perikarya and processes (243).

*Myelin/oligodendrocyte specific protein.* Myelin/oligodendrocyte specific protein (MOSP) is a 48-kDa protein identified with a monoclonal antibody (165). It is located on the extracellular surface of oligodendrocytes and myelin.

*RIP antigen.* RIP antigen (see sect. II B) labels both oligodendrocyte processes and myelin sheath (44, 199). Two bands of 23 and 160 kDa have been found by Western blot, the nature of which have not been elucidated.

*NI-35/250 proteins.* NI-35/250 proteins are membrane-bound proteins highly enriched in mammalian CNS myelin and oligodendrocytes, but minor in PNS myelin (105) (see sect. II B2). Their role in axonal growth inhibition conditions has been well documented (see sect. III G3c). The cDNA encoding the bovine NI-220 inhibitor has been recently characterized (578); this will allow an accurate analysis of the specific functions of this myelin inhibitory molecule during neurogenesis as well as in pathophysiological conditions.

Nevertheless, as pointed out by Colman et al. (123), there are still today a great number of unidentified myelin proteins.

G) OLIGODENDROCYTE/MYELIN ENZYMES. UDP-galactose:ceramide galactosyltransferase (CGT), which catalyzes the transfer of galactose to ceramide to form galactosylceramide, has been found in the myelin fraction. The human gene encoding CGT has five exons distributed over >40 kb; it has been cloned and assigned to human chromosome 4, band q26 (69), and is highly conserved during evolution (70). Expression of CGT transcripts in brain is time regulated and parallels that of MBP (544). In situ hybridization has shown that in cerebrum and cerebellum, CGT is restricted to the oligodendrocyte-containing cell layers that also express MBP (544). However, CGT is also weakly expressed in some subtypes of neurons in the adult CNS (537).

Surprisingly, in CGT-knock-out (KO) mice (71, 118), ultrastructure of myelin is apparently normal, except for slightly thinner sheaths in some regions of the CNS. Glucocerebroside, previously not identified in myelin, is present in these galactocerebroside- and sulfogalactosylceramide-deficient mice. However, the apparent normal structure is associated with impaired insulator function, as shown by a block of saltatory conduction, especially in

the CNS; severe generalized tremor and ataxia develop with age with extensive vacuolization of the ventral region of the spinal cord, indicating that galactosylceramide and sulfatides play an indispensable role in the insulator function of myelin and its stability. The CGT-KO mice generally die at the end of the myelination period. Recently, using electron microscopic techniques, specific ultrastructural myelin abnormalities in the CNS that are consistent with the electrophysiological deficits were demonstrated (163). These abnormalities include altered nodal lengths, an abundance of heminodes, an absence of transverse bands, and the presence of reversed lateral loops. In contrast to the CNS, no ultrastructural abnormalities and only modest electrophysiological deficits were observed in the PNS. Taken together, these data indicate that GalC and sulfatide are essential in proper CNS node and paranode formation and that these lipids are important in ensuring proper axon-oligodendrocyte interactions (163; reviewed in Ref. 468).

Many enzyme activities have been found when myelin is isolated by ultracentrifugation (reviewed in Ref. 425): neuraminidase, cholesterol ester hydrolase, phospholipid synthesizing enzymes and catabolizing enzymes, phosphoinositide metabolizing enzymes, proteases, protein kinases, and phosphatases. Recently, neutral sphingomyelinase activity has also been found in myelin, which is stimulated by tumor necrosis factor- $\alpha$  (110).

### 3. Differences in lipid and protein composition between CNS and PNS

Except for ganglioside GM4, the specific galactolipids of CNS myelin are present in peripheral nerve. Thus leukodystrophies involving the degradation of these sphingolipids such as Krabbe disease, adrenoleukodystrophy, and metachromatic leukodystrophy affect also the peripheral nerve. On the other hand, some glycolipids such as sulfated glucuronyl paragloboside and its derivatives are specific of peripheral nerve myelin (116).

Several major CNS myelin proteins are also present in the peripheral nerve (Fig. 5), generally in smaller quantities, although their function may not be identical.

MBP is present in peripheral nerve myelin, but probably does not play a major role in myelin compaction, as the major dense line of PNS myelin is not affected in the shiverer mutant mouse which is devoid of MBP (302). Moreover, P0 and MBP can play interchangeable roles during the process of myelin formation as shown from the work on P0 knock-out and P0/MBP double knock-outs (363).

The PLP mRNA and protein have been identified in Schwann cells (482). In the sciatic nerve, DM-20 (messengers as well as protein) is present at a much higher level than PLP (458), and both are present in the myelin membrane (2). In the same context, it has recently been



shown in humans that PLP/DM-20 protein is necessary in peripheral as well as central myelin, since patients with Pelizaeus-Merzbacher disease caused by absence of PLP gene expression (due to a premature stop codon) also present an associated peripheral neuropathy (209). In contrast, PLP null mice do not present any peripheral defect (235).

MAG is a very minor component of PNS myelin, in which it represents 0.1% total proteins. In rodents, L-MAG is the major isoform during development and declines in the adult CNS myelin, whereas S-MAG is the predominant isoform during development as in the adult PNS myelin (448). In contrast to rodents, the L-MAG splice variant predominates in adult human brain while, like in rodents, S-MAG transcripts are most abundant in peripheral nerve. In contrast to the CNS where MAG is confined to the periaxonal region of the myelin sheath (622), in the adult rat PNS, MAG present a large distribution in myelin internodes and is enriched in paranodal loops, Schmidt-Lanterman incisures, and inner as outer mesaxon membranes (622; see for review Ref. 384).

PMP 22, a peripheral nerve myelin protein, plays an important role in Schwann cell metabolism and PNS myelin compaction (reviewed in Ref. 418); its mRNA are also found in some categories of motoneurons in the CNS (446), even at embryonic stages (445).

Cx32 is not a typical PNS myelin component since it is widely expressed in the organism. Nevertheless, it seems to have a special role in relation to myelin formation and/or maintenance in PNS, as recently shown by the deleterious effect of mutations of Cx32 leading to Charcot-Marie-Tooth neuropathies (46). Recently, Cx32 has also been found in oligodendrocytes of rat brain together with Cx45 (318). Expression in oligodendrocytes of another type of connexin may prevent dysmyelinating effects of Cx32 mutations in the CNS of Charcot-Marie-Tooth X type patients.

P2 protein is a myelin-specific protein of peripheral nerve, although it is found in some areas of the CNS (see sect. *III C2*). It has a molecular mass of 13.5 kDa and contains a high percentage of basic amino acids. P2 is a true fatty acid-binding protein isoform (543).

#### *4. Relations between oligodendrocyte/myelin proteins and the immune system*

Recent works have shown that several oligodendrocyte/myelin protein genes are expressed in the immune system.

In humans, Northern blot analysis reveal that Golli-MBP transcripts are also expressed in fetal thymus, spleen, and human B-cell and macrophage cell lines, clearly linking the expressions of genes coding for an encephalitogenic protein to cells and tissues of the immune system (98, 237, 694). Some Golli-MBP proteins

have been found expressed in the embryo (365, 421) and have also been found expressed in the thymus (473), in the thymic macrophages. Recently, Kalwy et al. (290) have found that a 25-kDa band, composed at least of four proteins, is present in brain, thymus, and spleen and not in the tissues that do not express the novel MBP transcripts.

PLP transcripts are also expressed in peripheral blood lymphocytes (102), probably by a process of illegitimate transcription, very useful for genetic diagnostic purposes. On the other hand, PLP/DM-20 proteins have also been found in macrophages of the human thymus (473). Thus PLP/DM-20 proteins are expressed in antigen-presenting cells in the human immune system and are not shielded from the immune system behind the blood-brain barrier. These observations have to be interpreted in the context of acquisition of tolerance against encephalitogenic antigens such as PLP and MBP, which may not simply be related to their sequestration from a "naive" immune system (473).

In the rat, an mRNA for CNP has been demonstrated in thymus and in lymphocytes (48). CNP has recently been implicated in the humoral response in MS; indeed, anti-CNP antibodies have been found in large amount in serum and CSF from MS patients, the antibody response being exclusively restricted to CNP1. Both CNP1 and CNP2 isoforms bind the C3 complement fraction, providing a plausible mechanism for opsonization of myelin membrane in the MS brain (647).

There is an amino acid sequence homology between MAG and immunoglobulin-like molecules, especially NCAM (531). The immunoglobulin gene family is a family of proteins that shares a common extracellular subunit termed the immunoglobulin homology unit. Among the members of this superfamily expressed in the nervous system are L1, NCAM, P0, MOG, and CD22. MAG most closely resembles the leukocyte adhesion molecule CD22, which is a sialic acid binding protein (557).

The antibody HNK1 has been used to identify a subpopulation of human lymphocytes with natural killer function. Thirty percent of all MAG molecules isolated from mouse brain carry the L2/HNK1 epitope, which is also expressed in a group of NCAM and L1 molecules (466), on glycolipids (paraglobosides) with sulfated glucuronic acid (116), and on other myelin proteins from CNS, such as MOG, OMgp (see sect. *III C2*), or PNS, such as P0 (643) and PMP-22 (569). Thus several myelin proteins share an epitope with cells of the immune system.

The recently shown association of myelin proteins, MOG and CNP, with different elements of the complement cascade (282, 647), may be relevant to connections between the immune system and the nervous system and may have some physiopathological implications in demyelinating disease (587). Also the MOG gene is located in the MHC locus (460, 461) (see sect. *III C2E*).

## D. Myelination

Myelination consists of the formation of a membrane with a fixed composition and specific lipid-protein interactions allowing membrane compaction and the formation of the dense and intraperiodic lines of myelin. Therefore, myelination also needs activation of numerous enzymes of lipid metabolism necessary for the synthesis of myelin lipids, of synthesis and transport of specific protein components of myelin or their mRNAs to the oligodendrocyte processes.

### 1. Steps and timing of myelination

Little is known about the mechanism of myelination or the signals that regulate this complex process. There are sequential steps involving the following: 1) the migration of oligodendrocytes to axons that are to be myelinated, and the fact that axons and not dendrites are recognized; 2) the adhesion of the oligodendrocyte process to the axon; and 3) the spiraling of the process around the axon, with a predetermined number of myelin sheaths and the recognition of the space not to be myelinated, i.e., the nodes of Ranvier.

In the first step, the preoligodendroglial multiprocessed cells settle along the fiber tracts of the future white matter, maintaining the ability to divide. Indeed, mitoses are present in the interfascicular longitudinal glial rows (507). Second, these preoligodendrocytes become immature oligodendrocytes, characterized by the acquisition of specific markers (see sect. III C) and ready for myelination.

Myelination occurs caudorostrally in the brain and rostrocaudally in the spinal cord. The sequence of myelination is a strictly reproducible process for a given species. In the mouse, it starts at birth in the spinal cord. In brain, myelination is achieved in almost all regions around 45–60 days postnatally. In humans, the peak of myelin formation occurs during the first year postnatally, although it starts during the second half of fetal life in the spinal cord. It can continue until 20 years of age in some cortical fibers, especially associative areas (677) (Fig. 6). More precise data on the topographical sequence of myelination in the human have been recently obtained using magnetic resonance imaging (MRI) (630).

Myelination requires an extraordinary capacity for oligodendrocytes to synthesize membranes at a given time, specific for a species and a region of the CNS, and concerns only certain nerve fibers and tracts (547). The timing of CNS myelination pathways is so precise and characteristic that the age of human fetuses can be determined accurately simply by assessing which pathways have myelinated. These data suggest that a highly localized signaling mechanism regulates the timing of oligodendrocyte differentiation and myelination.

### 2. Oligodendrocyte and axon interactions related to myelin formation

In vivo, Barres and Raff (30) have shown that proliferation of oligodendrocyte precursor cells depends on electrical activity in ganglion retinal cells. Oligodendrocyte number is also dependent on the number of axons, as has been shown in transgenic mice expressing Bcl2 under the control of a neuron-specific enolase promoter (87). In these mutant mice, the oligodendrocyte cell population is increased due both to the decrease of oligodendrocyte apoptosis and to the increase in the number of axons. Interestingly, it was shown in an early work that sectioning the optic nerve postnatally, a few days after the appearance of mature oligodendrocytes, induces apoptosis in these cells, although astrocytes remain intact (205).

It is well known in vivo that oligodendrocytes myelinate solely axons, although oligodendrocytes are able to form uncompacted myelin or myelin-like figures in the absence of neurons (533, 674). Lubetzki et al. (343) have established long-term dissociated cultures of neurons and oligodendrocytes from mouse embryos that myelinate in culture solely axons after 13–14 days, as shown by the use of specific monoclonal antibodies recognizing differently the somatodendritic domain and axonal neurofilament epitopes.

The signal indicating which axon should be myelinated remains unclear. Studies in the CNS support the idea of a critical diameter for myelination, but individual axons are not myelinated along their entire length simultaneously, indicating that other factors are involved (597) (Fig. 4B). On the other hand, using intracellular dye injections, Butt and Ransom (90) showed that, during the markedly asynchronous development of the whole population, individual oligodendrocytes matured all their processes simultaneously to an equal length. Using MBP immunolabeling, Suzuki and Raisman (596, 597) studied the rat fimbria and found, first, patches of cells of the oligodendrocyte lineage along the axonal tract that finally form unicellular rows. Differentiation of immature oligodendrocytes into myelinating cells occurs in a multifocal mode. Within each cell cluster, a single oligodendrocyte gives rise simultaneously to a complement of varicose myelinating processes. This quantal mode of differentiation of the oligodendrocyte population suggests that over adjacent areas of the fimbria, all the clusters are responding simultaneously to the same overall stimulus but that there exists an internal regulation within each cluster so that only one cell is triggered. Such a highly localized “mosaic-like” pattern of differentiation, and the subsequent asynchronous myelination, could be generated by an interaction between axon maturation and oligodendrocyte differentiation (596, 597) (Fig. 4B). Recently, indirect evidence suggests that, in the rat optic nerve, the timing of oligodendrocytes differentiation and myelination is con-

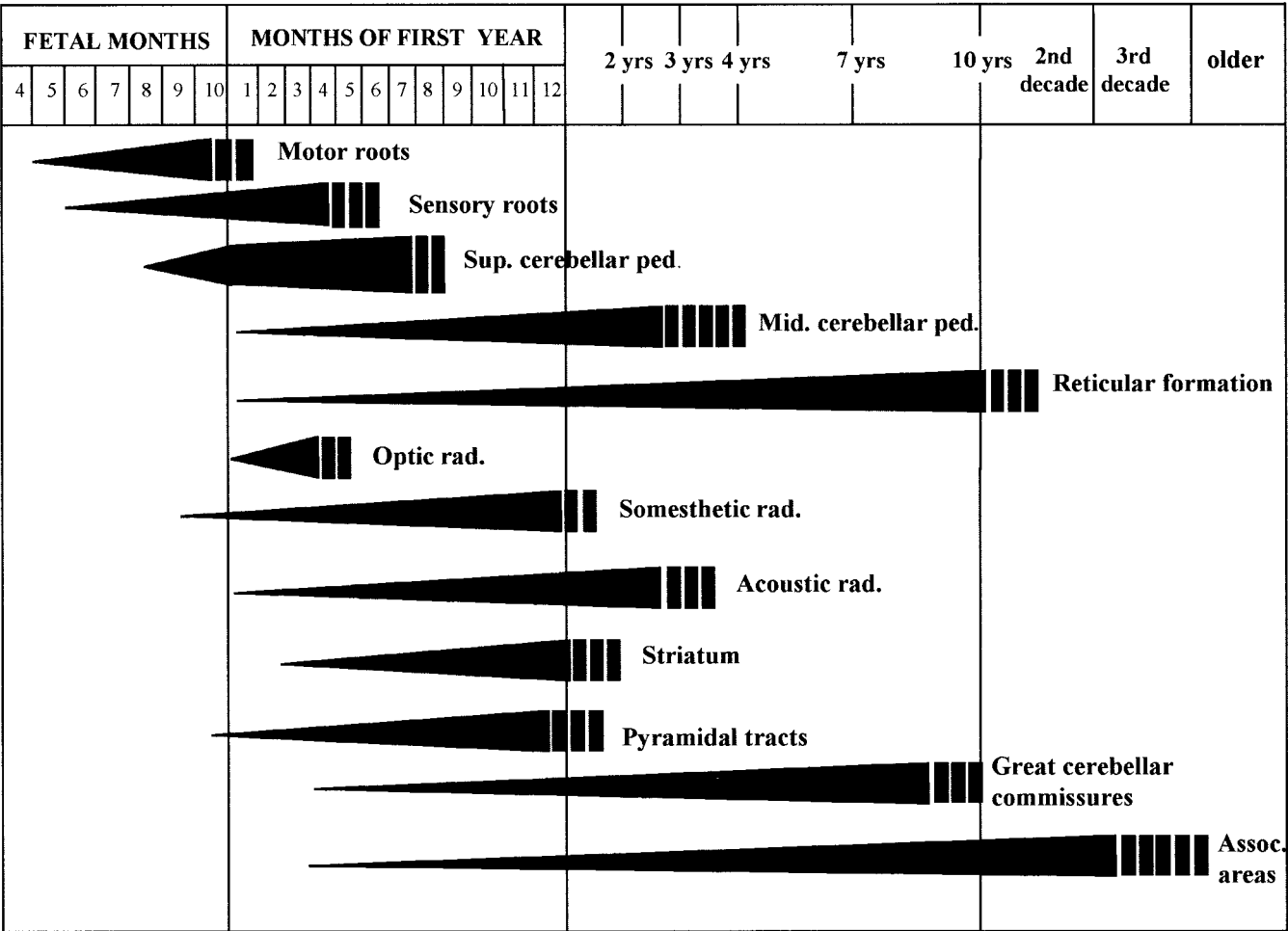


FIG. 6. Cycles of myelination in the CNS during development. The width and the length of graphs indicate progression in the intensity of staining corresponding to the density of myelinated fibers; the vertical stripes at the end of the graphs indicate approximate age range of termination of myelination estimated from comparison of the fetal and postnatal tissue with tissue from adults in the third and later decades of life. [Adapted from Yakovlev and Lecours (677).]

trolled by the Notch pathway. Whereas the Notch pathway was thought to specifically regulate neuronal development (14), it was also shown that Notch receptors are present on oligodendrocytes and their precursors. Retinal ganglion cells express Jagged 1, a ligand of Notch1 receptor, along their axons (651). Jagged1 is developmentally downregulated in axons of retinal ganglion cells with a time course that parallels myelination, suggesting that Jagged1 signals to Notch1 on surfaces of oligodendrocyte precursors to inhibit their differentiation into oligodendrocytes. The downregulation of Jagged1 expression by retinal ganglion cells correlates well with the onset of myelination in the optic nerve, as well as with the maturation of immature astrocytes. Myelination of axons in a given pathway generally occurs a few days after they reach their target (547), raising the question of whether downregulation of Jagged1 in axons occurs in response to target innervation, subsequently triggering the signal to begin myelination.

The proliferation of oligodendrocyte precursor cells depends in vivo on electrical activity, as shown in ganglion retinal cells by Barres and Raff (30). The role of electrical activity in the myelination process has been suggested by the fact that animals maintained without light since birth have a retardation in myelination (242); conversely, premature opening of the eyelids accelerates myelination in optic nerve (603). Demerens et al. (139) have shown in an in vitro myelination system using dissociated cultures from embryonic brain, that tetrodotoxin (TTX) blocks the initiation of myelination.  $\alpha$ -Scorpion toxin, which induces repetitive electrical activity by slowing  $Na^+$  channel inactivation, has an opposite effect. This group has shown also in vivo in the developing optic nerve of mice that the intraocular injection of TTX inhibits myelination. Different results, possibly under slightly different experimental conditions, have been observed by Colello et al. (120) and by Shrager and Novakovic (561). One of the important steps for triggering myelination

is the oligodendrocyte process extension that favors axonal contacts. Recently, it has been shown that astrocytes induce oligodendrocyte process to align with and adhere to axons, providing a novel role for astrocytes in controlling the onset of myelination (385).

Interestingly, cells of the oligodendrocyte lineage exclusively express a novel form of microtubule-associated proteins (MAP) called MAP 2c localized in the cell body of preoligodendrocyte that may assist in the initiation of process extension (644). There is a developmental pattern of several other MAPs suggesting that during myelination, there is a reorganization of the cytoskeleton of the oligodendrocyte that may interact with the growing myelin membrane. An isoform of neurofascin, comprising the third fibronectin type 3 (FNIII) repeat but lacking the mucinlike domain, is specifically expressed by oligodendrocytes at the onset of myelinogenesis and decreases rapidly once oligodendrocytes have engaged their targeted axons (121). Given its unique transient pattern of expression in oligodendrocytes at the early stages of myelination, this cell adhesion molecule protein may have a role in mediating axon recognition but also signals axonal contact through its links with the actin cytoskeleton. It is of note that later on a different neurofascin isoform (lacking FNIII but comprising the mucinlike domain) is specifically expressed in the axolemma at the node of Ranvier (135). On the other hand, in MAG-null mutant transgenic mice (400), axons may be myelinated several times, indicating that MAG may be involved in the recognition of myelinated axons from the nonmyelinated ones by the oligodendrocyte processes (330).

### 3. Axon regulation of myelin thickness

The axon probably participates in the regulation of myelin thickness as single oligodendrocytes can myelinate several axons with different diameters; they form thicker myelin along the larger axons (657). This suggests that the axon specifies, in a localized way, the number of myelin lamellae formed by a single oligodendrocyte process. Studies of the female carrier of the jimpy mutation (see sect. IV A1A) exemplify well the plasticity of neuroglial cells and the mechanisms of quantitative production of oligodendrocytes and myelin. Oligodendrocytes have considerable flexibility in the amount of myelin they can make, and a reduction in the number of oligodendrocytes does not necessarily involve a reduction in the amount of myelin (566).

### 4. Astrocytes and myelination

Outside of providing trophic factors to oligodendrocytes, astrocytes may be important in relation to myelination. Suzuki and Raisman (596, 597) have studied the rat fimbria and found, first, patches of cells of the oligodendrocyte lineage along the axonal tract which finally

form unicellular rows in which astrocytes are interspersed singly between stretches of 5–10 continuous oligodendrocytes. Mitosis is present in the interfascicular longitudinal glial rows (507). Radial and longitudinal processes of the oligodendrocytes and the astrocytes are interwoven to form a rectilinear meshwork along which the tract axons run (Fig. 4B). The presence of junctional coupling (see sect. II D) between the glial cells suggest that this glial array constitutes a functional as well as an anatomical unit (597).

An increase in glial fibrillary acidic protein (GFAP) level has been found in the mouse CNS during the early stages of myelinogenesis (277); whether it is functionally related to myelinogenesis could not be determined. Recently, mice carrying a null mutation for GFAP were generated (334). These mice display abnormal myelination; nonmyelinated axons are numerous in the optic nerve and spinal cord anterior column; the altered white matter vascularization, possibly linked to abnormal astrocytic processes, may be related to the dysmyelination observed. It is of note that the vimentin-GFAP transition in the rat neuroglia cytoskeleton occurs at the time of myelination (128). Moreover, transgenic mice carrying several copies of the human GFAP gene die by the second postnatal day by a fatal encephalopathy with astrocyte inclusion bodies similar to the Rosenthal fibers found in Alexander's disease (75a). Astrocytes in these mice are hypertrophic and upregulate small heat shock proteins (383). These studies suggest major links between astrocyte function, myelination, and its maintenance. Interestingly, these hypotheses are supported by data obtained in several human demyelinating conditions; e.g., in Alexander's leukodystrophy, the primary target cell is the astrocyte whose dysfunctions are characterized by high levels of expression of the heat shock protein,  $\alpha$ -B-crystallin (251, 275). In adrenoleukodystrophy (ALD), the mutated protein, adrenoleukodystrophy protein (ALDP), is expressed in white matter oligodendrocytes, but also in astrocytes and microglial cells everywhere in the brain, suggesting that dysfunction of these cells may indirectly affect oligodendrocytes early in the disease (16).

### 5. Difference between PNS and CNS myelinating glial cells and process of myelination

Oligodendrocytes embrace an array of axons while Schwann cells myelinate single axons. In contrast to the CNS node, where there is contact of the axon with the extracellular space (84), the myelinating Schwann cell processes interdigitate over the node. Moreover, the Schwann cell soma is circumferentially covered by a basement membrane. This basal lamina is continuous with that of the adjacent internode; thus the Schwann cell processes and basal lamina cover the PNS node. In fact, whether the Schwann cells myelinate or not, they share a



common basal lamina that forms a glial channel along the nerve fiber. PNS myelinated fibers are separated from each other by an extracellular compartment, the endoneurium, whereas the CNS myelinated fibers are in close contact. Interestingly, electron microscopy and immunohistochemistry have shown that rat CNS fibers do not exhibit a strict relation between nodal complexity and fiber size, as in the PNS (56). Furthermore, some of the components of PNS myelin, lipids, and proteins are different (see sect. III C3) (Fig. 5).

#### 6. Formation of the node of Ranvier

The internodes are separated along myelinated axons by intervals where the axolemmal membrane is exposed, the node of Ranvier (Figs. 2, 3, 5, and 8). Nodes of Ranvier comprise a membrane specialization (see sect. III E) of the axon (Fig. 6) (reviewed in Refs. 253, 655). Premyelinated axons exhibit a low density of sodium channels that appear to be distributed uniformly along the length of the fiber, whereas during maturation of myelinated tracts, sodium channels become segregated into regularly spaced gaps in myelin, i.e., at the nodes of Ranvier, thereby allowing saltatory conduction in myelinated fibers. Moreover, potassium channels are enriched in the paranodal regions where they may contribute to the repolarization and ionic homeostasis. This molecular specialization enables the regeneration of the action potential at the node of Ranvier and is responsible for its unique function. The clustering of sodium channels was first ascribed to the presence of astrocytic processes at the node of Ranvier, since previous studies have demonstrated a close spatial and temporal correlation between astrocyte-axon contact and the appearance of a high density of intramembrane particles (reviewed in Ref. 655). However, it was recently demonstrated, first in the PNS, that the contact with Schwann cells induces clustering of sodium channels along axons of peripheral nerves, *in vivo* as *in vitro* (155). In a same way, the remarkable enrichment, i.e., clustering, of voltage-gated sodium channels in the nodal axolemma of myelinated CNS tracts has been shown to be due to signals from the oligodendrocyte, as shown in the optic nerve (293). Ultrastructural studies indicated that the axon begins to differentiate and displays foci of membranes with nodal properties before the onset of myelin compaction (656). Indeed, Kaplan et al. (293) found that clustering of the sodium channel occurs concomitantly as oligodendrocytes wrap axons with insulating myelin sheaths. The induction but not the maintenance of sodium channel clustering along the axons was demonstrated to depend on a protein secreted by oligodendrocytes in coculture with rat retinal ganglion cells. Furthermore, *in vivo*, mutant md rats (see sect. IV A1A), deficient in oligodendrocytes but not in neurons or astrocytes, develop few axonal sodium channel clusters (293).

These data indicate that oligodendrocytes are necessary for clustering of sodium channels *in vivo* as *in vitro*. As clusters are regularly spaced even in the absence of direct axon-glial cell contact, with an average intercluster distance similar to that predicted for the Ranvier nodes *in vivo*, this suggests that there is an intrinsic mechanism, within the axon, that participates in the determination of spacing between nodes (reviewed in Refs. 530, 653). Glia induce ankyrin-G clustering in a pattern identical to that of sodium channel clustering; thus it is likely that cytoskeletal interactions play an important role in the induction and maintenance of sodium channel clusters (312). These data demonstrate that oligodendrocytes are specialized to induce saltatory conduction by inducing clustering of sodium channels and providing myelin insulation, and furthermore by regulation of axonal caliber and other properties (see sect. III G) (see discussion in Ref. 293).

### E. Biochemical Aspects of Myelin Assembly and of the Node of Ranvier

#### 1. Specific processing of myelin proteins during synthesis by oligodendrocytes

Preceding myelination, myelin specific genes are activated. Immature oligodendrocytes express GalC and CNP and are clearly arborized; later, mature nonmyelinating oligodendrocytes express MBP, PLP, and MAG (154, 399). Interestingly, in the postnatal period, the peak of mRNA is probe dependent and not region dependent for CNP, PLP, and MBP, suggesting a common signal occurring simultaneously in all areas, regardless of the caudo-rostral gradient of myelination in brain (292). Although these proteins appear in oligodendrocytes regardless of the presence of neurons, these data as well as others indicate that the presence of neurons clearly enhances the quantity of myelin specific proteins being synthesized (358). In oligodendrocytes in culture, there is a spatial organization of the protein synthetic machinery in granules that may provide a vehicle for transport and localization of specific mRNAs within the cell (4, 24).

A) MBP. The MBPs are a set of membrane proteins that function to adhere the cytoplasmic leaflet of the myelin bilayer. Because their biophysical properties may render MBP nonspecifically adhesive to any organelle membranes, oligodendrocytes have developed a mechanism for the transport of MBP mRNAs selectively to intracellular regions where MBP proteins will be necessary for myelin compaction to occur (123). Indeed, *in situ* hybridization has shown that MBP mRNAs are first localized in the cytoplasm of oligodendrocytes, even before myelination (589), and then dispersed in the oligodendrocyte processes at the beginning of myelination; the myelin sheaths stain for MBP mRNAs because the messages are

in part transported within the cytoplasmic channels that surround and infiltrate the sheaths. Spatial segregation of MBP messages begins to function only after oligodendrocytes have differentiated into fully myelinating cells, cellular extensions being in place first, before the MBP transport machinery is activated (636). Before that period, MBP is expressed throughout the cytoplasm and surprisingly in the nucleus. All the isoforms do not behave in the same way when individually expressed, as has been shown transfecting individual isoform cDNA in the shiverer oligodendrocyte devoid of functional MBP (6); the 14- and 18.5-kDa forms have a plasma membrane distribution in the oligodendrocyte, whereas the exon 2-containing 17- and 21.5-kDa MBPs (MBP exII) distribute diffusely through the cytoplasm and accumulate in the nuclei. The transport of MBP exII is an active process that may suggest that these karyophilic MBPs are involved in a regulatory function in implementing the myelination program (447). The 14-kDa isoform that lacks exon 2 is sufficient to create myelin compaction in the CNS, as transgenic mice expressing only this form of MBP in the shiverer mutant (see sect. IV A1A) have a compacted myelin (299). Once the cytoplasmic extensions form, the exon 2 lacking MBPs are upregulated, and the mRNA movement mechanism is activated. MBP is exceptionally present in the nuclei of apparently mature oligodendrocytes (247) that may be remodeling or remyelinating some of the myelin sheaths that they support (447). Cytoskeletal elements may be involved in dynamic process of MBP mRNA transport (4).

B) PLP. There may be a vesicular transport of myelin PLP and sulfogalactosyl ceramides (78). Studies of DM-20/PLP proteins show that specific sequences of PLP gene and protein are necessary for correct trafficking and that abnormal proteins are readily eliminated in the endoplasmic reticulum (230). Abnormal level of misfolded PLP proteins as seen in Pelizaeus-Merzbacher disease represent a tremendous burden to the oligodendrocyte, as PLP mRNA represents 10% total mRNA of the oligodendrocyte at the time of myelination (see sect. IV A2).

Proteins involved in vesicular trafficking such as GTP-binding proteins (5, 264) and rab3a (360) have been identified in oligodendrocytes. SNARE complex proteins (359) have also been identified in oligodendrocytes.

## 2. Lipid enzyme activation

The peak of myelination is extremely precise in rat and mouse, at 18 days postnatal. The enzyme activities, which peak during that particular period, appear to be related to the myelination process. This is the case for enzymes related to lipid metabolism. Mutations in the mouse affecting the myelination process show massive reductions in these enzyme activities, in relation to the degree of reduction in myelin. These aspects were de-

scribed in the 1980s (38). PAPS cerebroside-sulfotransferase, an enzyme required for the synthesis of sulfated galactosylceramide, is inducible in CNS in relation to myelination; its activity is constitutively stable in the kidney, even in dysmyelinated mutants such as jimpy (534). CGT activity also increases rapidly during myelination and decreases markedly when myelination ceases. Expression of CGT mRNA (544) correlates clearly with myelination and agrees with previously determined enzyme activities. Very-long-chain fatty acid (VLCFA) biosynthesis (C24) follows the same pattern in the endoplasmic reticulum; interestingly, although mitochondria synthesize also VLCFA, there does not seem to be a relation to myelination, in contrast to VLCFA synthesized in the microsomal fraction that comprises the endoplasmic reticulum (72). HMG-CoA reductase seems also to be activated in oligodendrocytes at the time of myelination, as has been shown using a transgenic mouse expressing the promoter of this enzyme colinked to the marker chloramphenicol acetyltransferase (320). Peroxisomal enzymes involved in plasmalogen biosynthesis are necessary for myelination; the key enzymes are acyl CoA: dihydroxyacetone phosphate acetyltransferase (DHAP-AT) and alkyl dihydroxyacetone phosphate (alkyl DHAP) synthase (545).

## 3. Myelin assembly by oligodendrocytes: formation of lipid and protein complexes

It has been proposed that proteins destined for the apical surface of polarized epithelial cells cocluster with glycolipid-rich microdomains during sorting and transport from the *trans*-Golgi network. Glycolipid-rich oligodendrocytes may adopt this mechanism for myelinogenesis. Protein-lipid complexes from oligodendrocytes and myelin were isolated utilizing detergent insolubility and two-dimensional gel electrophoresis, leading to the identification of a developmentally regulated protein, myelin vesicular protein of 17 kDa (MVP17), highly homologous to human T-cell MAL protein. (298). In relation to the processes of myelin deposition and assembly, it is interesting to note that the family of MAL-related proteins (see sect. III C2F), highly expressed in myelinating glial cells (362), present a lipidlike behavior and are components of detergent-insoluble myelin membranes (450). Furthermore, it was shown that rMAL, found to interact with glycosphingolipids, may lead to the formation and maintenance of stable protein-lipid microdomains in myelin and apical epithelial membranes, thus contributing to specific properties of these highly specialized plasma membranes (195). In the same context, it was discovered that, in contrast to oligodendrocyte progenitors, in maturing oligodendrocytes and myelin, GPI-anchored proteins form a complex with glycosphingolipids and cholesterol, suggesting sorting of this macromolecular complex to

myelin (314). The recently described presence of caveolae, which are membrane microdomains containing high levels of GPI-anchored proteins, in oligodendrocytes as in astrocytes (93) may be relevant to the mechanisms of myelin assembly.

#### 4. Molecular organization of the myelin membrane

Electron microscopy data visualize myelin as a series of alternating dark and less dark lines that should be protein layers, separated by unstained zones constituted by lipid hydrocarbon chains (404) (Fig. 5). In fact, the currently accepted view is that there are integral membrane proteins embedded in the lipid bilayer, although there are proteins attached to one surface or the other by weaker links. Individual components form macromolecular complexes with themselves and each other (538). Such an orderly structure could require a precise stoichiometric relationship of the individual components so that either increasing or decreasing the amount of one component could perturb the entire structure and therefore perturb myelination (see sect. IV).

The lipid compositions of the two halves of the lipid bilayer of biological membranes, including myelin, are strikingly different. The glycolipids, as in other membranes, are preferentially located at the extracellular surfaces, in the intraperiodic line. Almost all the lipid molecules that have choline in their head group (phosphatidylcholine and sphingomyelin) are in the outer half of the lipid bilayer, i.e., as the glycolipids. Almost all the phospholipid molecules that contain a terminal primary amino group (e.g., phosphatidylethanolamine, mainly plasmalogens, and phosphatidylserine) are in the inner half; they are negatively charged. This lipid asymmetry is functionally important in the compaction mechanisms of the mature myelin.

PLP and MBP are the most abundant proteins of myelin. Thus it is widely believed that the two proteins play complementary roles in myelin and are required for intra- and extracellular myelin compaction; this has been well demonstrated in the double mutant mice for PLP and MBP, viable and fertile (305, 591), which display an absence of fusion at the major dense line (typical of the homozygous shiverer mice) and a more compacted structure at the intraperiodic lines that appears as a single fused line (as in PLP mutants). PLP is an integral membrane protein with four transmembrane domains; in mutant mice that lack expression of the PLP gene, intraperiodic lines have reduced physical stability, indicating that PLP may form a stabilizing membrane junction similar to a zipper (305). It is widely accepted that the positively charged cytoplasmic proteins such as MBP in the CNS, or the cytoplasmic domain of P0 in the PNS, are responsible for compaction at the major dense line of myelin, and most likely as a result of an interaction with the acidic

lipids, such as phosphatidylserine, located in the opposing membrane (113, 147).

#### 5. Molecular organization of the node of Ranvier

Details of the molecular organization of the node of Ranvier have recently started to emerge (see Fig. 7). Spacious nodal gaps present in thick spinal axons are labeled by antibodies against HNK-1, chondroitin sulfate, and tenascin, but tiny node gaps along thin callosal axons are not labeled (56). The filamentous network subjacent to the nodal membrane contains the cytoskeletal proteins spectrin and isoforms of ankyrin (135, 322). Among the proteins binding to ankyrin at the node are the sodium channels, the  $\text{Na}^+\text{-K}^+\text{-ATPase}$ , specific forms of the NCAMs and the neurofascin isoform containing mucin and a third fibronectin III domain (135). Each node is flanked by paranodal regions where the axolemma is in close interaction with the membrane of the terminal loops of myelinating glial cells. Potassium-dependent channels are mainly localized in the axolemma at the level of these paranodal loops (57). Paranodin, a prominent 180-kDa transmembrane neuronal glycoprotein, was purified and cloned from adult rat brain and found in axonal membranes at their junction with myelinating glial cells, in paranodes of central and peripheral nerve fibers (382). Simultaneously, other authors isolated the same protein they called Caspr (171). Paranodin/Caspr may be a critical component of the macromolecular complex involved in the tight interactions between axons and the myelinating glial cells at the level of the paranodal region (163, 468). The glial ligand of Paranodin/Caspr has yet to be determined.

### F. Factors Influencing Glial Cell Maturation Leading to Myelin Formation

The influence of growth factors on oligodendrocytes at different stages of their differentiation has been reported previously (see sect. II C8).

#### 1. Thyroid hormone

Hyperthyroidism accelerates the myelination process, and hypothyroidism decreases it (648). Nevertheless, at an advanced age, hyperthyroid animals have a myelin deficit. The differentiation of oligodendrocytes as well as the degree of myelin synthesis are increased by thyroid hormone (7). Hypothyroidism coordinately and transiently affects myelin protein gene expression in most brain regions during postnatal development (269). In culture, oligodendrocyte progenitors stop dividing and differentiate in the presence of 3,3',5-triiodothyronine ( $\text{T}_3$ ); although this process may occur even in the absence of this signal, thyroid hormone may be involved in regulating



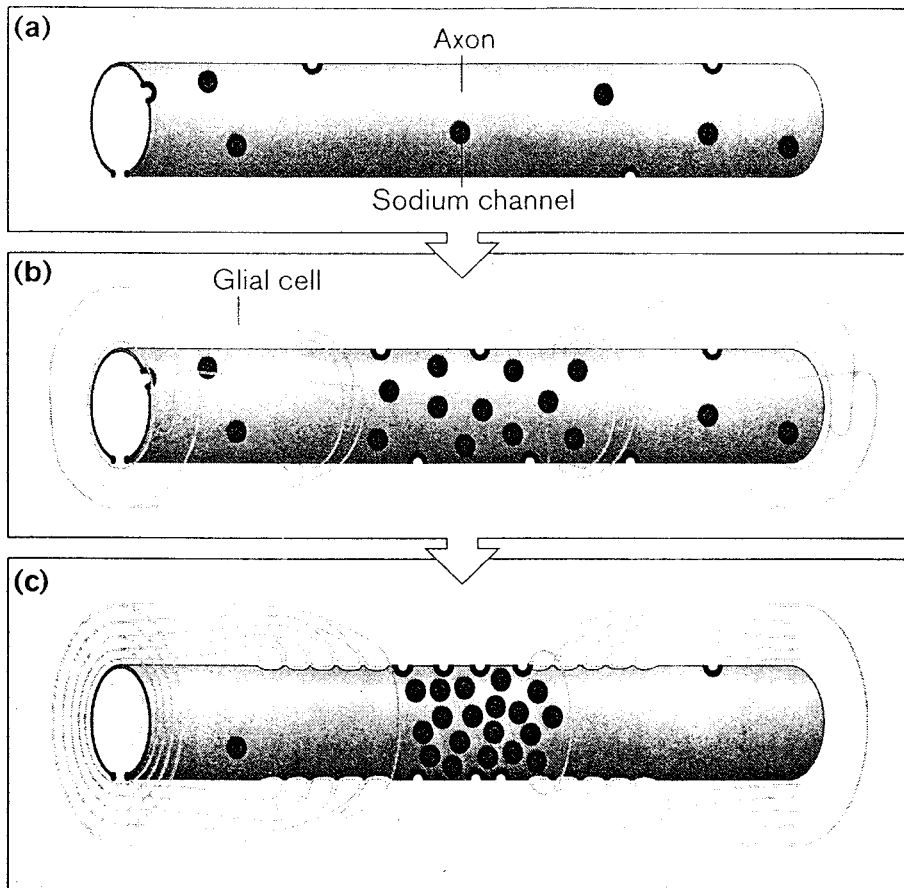


FIG. 7. Sequential steps in the formation of the node of Ranvier. Before glial ensheathment, sodium channels are distributed uniformly, and at low density. At the time of glial ensheathment but before the formation of compact myelin, loose clusters of sodium channels develop at sites that will become nodes. With formation of compact myelin and mature paranodal axon-glial cell junctions, well-defined nodal clusters of sodium channels are established, and sodium channels are eliminated from the axon membrane beneath the myelin sheath. [From Waxman (653). Copyright 1997, with permission from Elsevier Science. See also Kaplan et al. (293).]

the timing of differentiation of this cell population (29).  $T_3$  promotes also morphological and functional maturation of postmitotic oligodendrocytes, thus the number of mature oligodendrocytes (19, 269).

The effect of  $T_3$  is mediated by the interaction of this hormone with specific receptor isoforms (TR), three of which, only, are functional,  $\alpha_1$ ,  $\beta_1$ , and  $\beta_2$ . Progenitor cells express  $\alpha_1$ , whereas mature oligodendrocytes express  $\alpha_1$  and  $\beta_1$ , although  $\beta_2$  may also be expressed on progenitor cells (29). Myelin gene expression correlates with a striking increase in  $\beta_1$ -expression in the developing brain (19). The expression of different receptors in relation to the maturation state of the oligodendrocytes may mean that these effects are independently regulated by thyroid hormone.

TR are ligand-activated transcription factors that modulate the expression of certain target genes in a developmental and tissue-specific manner. These specificities are determined by the tissue distribution of the TR isoforms, the structure of the thyroid hormone responsive element (TRE) bound by the receptor and heterodimerization partners such as retinoic acid receptor (64). Addition of glucocorticoids, thyroid hormone, or retinoic acid all increase galactocerebroside synthesis in oligodendroglia (465) and sulfolipid biosynthesis (51). Among the

known target genes are MBP (182), PLP, MAG, and CNP (269).

Progress has been made in identifying some of the molecules involved in the transcriptional regulation of myelination (265). Huo et al. (267) have identified a nuclear protein that binds to TR isoform  $\beta_1$ , which forms a specific complex on the MBP-TRE. The expression of this brain nuclear factor is restricted to the brain perinatal period when myelination is sensitive to  $T_3$ .

## 2. Other hormones

A) GROWTH HORMONE. Growth hormone has an effect on proliferation and maturation of both glial and neuronal cells (430).

B) NEUROSTEROIDS. Baulieu and co-workers (287) have shown that steroids can be synthesized in the CNS and PNS. In the CNS, pregnenolone, progesterone, and their sulfated metabolites are synthesized by oligodendrocytes (287) and activate the synthesis of specific proteins.

## 3. Other factors

Factors that may act within the nucleus at early stages of the myelination program in the upregulation of transcription for myelinogenesis have been described.

The DNA binding protein MyT1 (297), which binds to the promoter of the PLP gene (265), may play a role in specification toward the glial lineage; as do SCIP (402), cerebellum-enriched nuclear factor 1 (CTF-NF) (274), and TR transcription factor (182). Both CTF-NF and TR activate the MBP promoter. The karyophilic MBPs, containing exon 2, once in the nucleus, may also act as regulatory factors in myelination (447).

## G. Roles of Myelin

### 1. Saltatory conduction of nerve impulses

As described in section III B, a myelinated fiber appears as myelinated membrane segments, termed internodes, separated by regions of unsheathed (naked) nerve membrane (Figs. 1, 2, 3, 5, and 8). It is this disposition by segments that induces the major characteristic of the so-called "saltatory conduction" of nerve impulses in myelinated tracts. As myelin is found almost exclusively in vertebrates, it can be considered as an essential element in the evolution of higher nervous functions (559).

The extremely dense clustering of sodium channels in the node of Ranvier accounts for the specialization of this region of the axolemma (see sect. III D6). In myelinated fibers, at the nodes of Ranvier, the sites where impulses are generated, the sodium channels are clustered at a density of some  $120,000/\mu\text{m}^2$ , the highest density in the nervous system. The myelin sheath has a high resistance and low capacitance that makes the current tend to flow down the fiber to the next node rather than leak back across the membrane. Therefore, the impulse may jump from node to node, and this form of propagation is therefore called saltatory conduction (Fig. 8) (559).

### 2. Role of myelin on axonal development and maintenance

A) DEVELOPMENT AND REGULATION OF AXONAL CALIBER. As early as 1951, Rushton (527) noted that the conduction velocity in a myelinated fiber is linearly correlated with diameter and proposed that, at a given diameter, there is a particular myelin thickness that maximizes conduction velocity. However, today it is still not yet fully understood how the mechanisms of morphogenesis of the myelinated nerve fibers are regulated. Although it was first proposed that myelination occurs only on fibers with a minimum diameter, it has been actually shown that signals from the myelinating glial cell, independent of myelin formation, are sufficient to induce full axon growth, primarily by triggering local accumulation and organization of the neurofilament network, as this can still occur in dysmyelinated mutant mice (532). Therefore, myelinating cells and myelin itself are important actors in the internal organization of the axonal structure and the subsequent

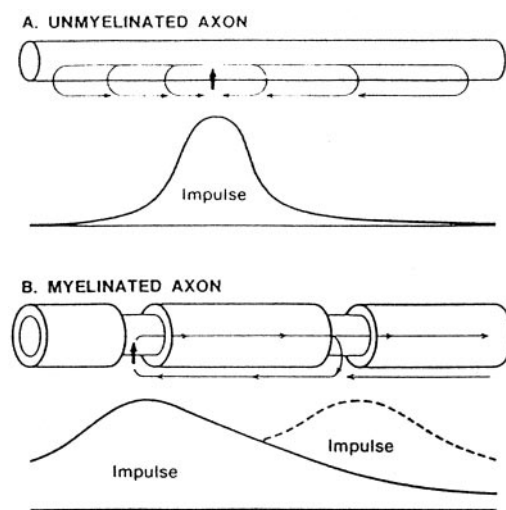


FIG. 8. Mechanisms for spread of the nerve impulse. A: continuous conduction in an unmyelinated axon. Amplitude scale is in millivolts. B: discontinuous (saltatory) conduction from node to node in a myelinated axon. In the diagrams, the impulses are shown in their spatial extent along the fiber at an instant of time. The extent of the current is governed by the cable properties of the fiber. [From Shepherd (559).]

growth of axon caliber (669; reviewed in Ref. 653). The final axonal caliber is influenced by other factors, such as the neurofilaments themselves.

Indeed, it has been demonstrated recently that oligodendrocytes are able to promote the radial growth of axons, by induction of accumulation of neurofilaments, independently of myelin formation (532, 581). The extrinsic effect of myelination on axonal caliber was demonstrated in the developing optic nerve (532) and by comparing axonal caliber in myelinated regions with the nonmyelinated initial segment of dorsal root ganglion axons (263). Reduced axon caliber in nonmyelinated regions correlated with a reduced neurofilament spacing and a decrease of neurofilament phosphorylation.

B) MAINTENANCE AND SURVIVAL OF AXONS. In absence of the PLP/DM-20 proteins, in the PLP-deficient mice which make a near-normal myelin sheath (305) (see sect. III G2B), morphological abnormalities are surprisingly observed in axons, i.e., axonal swellings and degeneration, and particularly by axonal "spheroids" (235). The axonal spheroids contain numerous membranous bodies and mitochondria and are often located at the distal paranode, suggesting an impairment of axonal transport; these spheroids were detected predominantly in regions of myelinated small-caliber nerve fibers and were never observed in unmyelinated fibers. To demonstrate that the absence of PLP and DM-20 was fully responsible of these axonal abnormalities, PLP null mice were interbred with transgenic mice expressing an autosomal PLP transgene (500): full complementation was observed in offspring that were totally rescued from the defect. To distinguish the ability of each isoform, PLP and DM-20, to rescue a normal

phenotype, PLP null mice were crossed with the PLP or DM-20 cDNA-based transgenes (416); the presence of one or the other of these isoforms allowed a near-complete restoration of a normal phenotype for the PLP, and at a lesser level for the DM-20 transgene, suggesting a possible similar role for the two isoforms in maintenance of axonal integrity. It is of note that PLP abnormal overexpression in transgenic lines (288, 500) causes significant axonal degeneration with predilection for specific tracts (13) and is toxic for myelinating oligodendrocytes and leads to different degrees of dysmyelination or demyelination. Altogether, these data demonstrate a pivotal role of glial-axonal communication for PLP in maintenance of axonal integrity and function, although the molecular mechanisms involved have yet to be determined.

### 3. Myelin and inhibition of axonal growth and regeneration

A major problem following mammalian CNS injury, and particularly spinal cord traumatic insult in humans, is that lesioned axons do not regenerate. It has been suggested that myelin and oligodendrocytes (reviewed in Refs. 20, 61, 188, 222, 485, 546), as well as reactive astrocytes and the molecules they secrete (reviewed in Ref. 512), participate in inhibition of regeneration within the adult mammalian CNS.

A) INHIBITORY PROPERTIES OF MYELIN FOR LATE-DEVELOPING AXONAL TRACTS. Oligodendrocytes and myelin may play an inhibitory role on neurite growth, e.g., for late-developing tracts and inhibition of plasticity in the adult brain. Because myelin formation starts at different times in different regions and tracts of the CNS, in a so-called "mosaic-like pattern," the inhibitory property of myelin could serve boundary and guidance functions for late-growing fiber tracts. Oligodendrocyte-associated neurite growth inhibitors could channel late-growing CNS tracts by forming boundaries and by exerting a "guard-rail" function (548). The different candidate proteins for a major role in inhibition of neurite growth are described below.

B) MYELIN INHIBITORY ROLES IN CNS REGENERATION. The limited potential for axonal regrowth and regeneration of nerve fibers in the adult CNS from higher vertebrates contrasts with recovery that happens in the PNS of these species. It was long recognized that the permissiveness of the environment was a determinant for CNS axonal repair (496, 604). Later, Aguayo and collaborators demonstrated that most CNS neurons are able to regenerate a lesioned axon in the environment of a peripheral nerve transplant (508), whereas peripheral nerves fail to regenerate in a CNS glia environment (132). Recently, the use of multiple grafts of peripheral nerve tissue, bridging the lesion site and the gray matter, was shown to permit extensive CNS axon growth and to restore partial function to a completely transected spinal cord (reviewed in Refs. 183, 438). Invest-

igations of the biological mechanisms that could be responsible for this difference in neuronal regeneration between CNS and PNS tissue led to a new concept, that of a neurite growth inhibitor (549). Central myelin and differentiated oligodendrocytes in the adult CNS were therefore recognized as responsible for inhibition of neurite outgrowth and shown to cause growth-cone collapse in culture (21).

C) MYELIN-ASSOCIATED INHIBITORY PROTEINS, NI-35 AND NI-250. Two related protein fractions of 35 and 250 kDa (NI-35 and NI-250) were found to contain the myelin-associated inhibitory activity. NI-35 and NI-250 are membrane-bound proteins highly enriched in mammalian CNS myelin and oligodendrocytes, but minor in PNS myelin (105). A monoclonal antibody (Mab IN-1) has been generated and shown in vitro to have neutralizing properties against the inhibitory activity of oligodendrocytes and myelin (106). In vivo, application of Mab IN-1, after the complete bilateral transection of the corticospinal tracts in spinal cord, allowed some regeneration on a short length of a small percentage of cortical tract axons (542). On the other hand, in a recent work (607), IN-1 treatment after unilateral transection of the corticospinal tract in the brain stem, above the pyramidal decussation, was shown to induce a significant collateral sprouting of both intact and lesioned corticospinal and corticobulbar nerve fibers, above and below the lesion site. This remarkable structural plasticity was associated with a functional recovery of forelimb function contralateral to the lesion (607). A similar form of recovery, through sprouting in gray matter rather than through severed axon regrowth in white matter, has been described in another lesion model (reviewed in Ref. 438).

D) MAG, ANOTHER INHIBITORY MYELIN PROTEIN. A well-characterized myelin protein produced by oligodendrocytes but also by Schwann cells (albeit at 10-fold less level), MAG (see sect. III C2), has been recently identified as a major CNS inhibitory protein (376, 414), for a number of different types of neurons. MAG is an inhibitory rather than a nonpermissive protein; MAG is able to cause growth cone collapse in vitro (333), and soluble extracellular domain of MAG (dMAG), released in abundance from myelin and found in vivo (398, 583), has been shown to inhibit axonal regeneration in vitro (602; reviewed in Ref. 485).

## H. Plasticity of the Oligodendrocyte Lineage: Demyelination and Remyelination

### 1. Fate of glial precursors and progenitors in the adult CNS

In the adult, the subventricular germinative zone persists around the brain lateral ventricles, along the striatum. These cells, although mostly quiescent, can be amplified in vitro in the presence of epidermal growth factor



(EGF) and give rise to neurons, and to astrocytes and oligodendrocytes, when grown in the presence of FGF2, LIF or BDNF (339, 503), or T<sub>3</sub> (281). The intrathecal infusion of EGF and FGF-2 activates the mitotic and migratory potential of these cells (126). CNS stem cells are also present in the adult spinal cord and ventricular neuroaxis (661). Recently, a major glial cell population, suggested to be adult oligodendrocyte progenitors, has been described in the CNS; these cells can be identified by the expression of two cell surface molecules, the NG2 proteoglycan and the PDGF- $\alpha$  receptor (reviewed in Ref. 426). They could represent a third type of glial cells in the CNS, with still unknown functions (reviewed in Ref. 339a).

Recently, mouse oligodendroglial cells derived from totipotent embryonic stem cells were transplanted in a myelin-deficient rat model; these oligodendrocytes were able to interact with host neurons and efficiently myelinate axons in brain and spinal cord of the mutant animal, suggesting that similar transplantations might help patients with Pelizaeus-Merzbacher disease or other demyelinating conditions (80).

## 2. Remyelination capacities of cells of the oligodendrocyte lineage

Plasticity of oligodendrocytes is particularly important for remyelination, mainly after lesions of demyelination. A number of genetic and/or inflammatory diseases may affect myelin formation (dysmyelinating diseases) or its maintenance (demyelinating diseases) (see sect. iv, A and B). The potential of glial cells to support spontaneous remyelination, as well as cellular or genetic therapeutic approaches aimed at myelin repair and improvement of conduction block in lesioned myelinated tracts, has focussed a number of studies.

Very little is known about spontaneous remyelination, as only a few early studies deal with the subject of myelin turnover. It appears that under normal conditions, the turnover of myelin is very slow and that each component has a different turnover rate, possibly different according to its location in myelin and in the myelinating cell (404).

Spontaneous remyelination was observed for the first time by Bunge et al. (83), after an experimental lesion in the cat spinal cord. It has also been reported in other experimental models (347, 349). The ways to stimulate the endogenous oligodendrocyte population to expand and regenerate myelin may be an important strategy. For the moment, the growth factor responses have not as yet been fully determined in humans, and no myelinating cell line has as yet been obtained analogous to the CG4 cell line isolated from rodents (340). So far, no studies have identified growth factor stimulation of division in the human oligodendrocyte lineage. Studies determining the

effect of growth factors on remyelinating capacities in vivo are few (379, 675). In immune-mediated demyelination, related to Theiler's virus infection, monoclonal antibodies of natural origin (266) may stimulate remyelination through a mechanism as yet unknown. Interestingly, inflammation seems to enhance migration of oligodendrocyte and thus remyelination in EAE (615).

Interestingly, in chronic MS (see sect. ivB1) lesions, oligodendrocyte precursor cells are present (671, 672); however, they appear to be quiescent, not expressing a nuclear proliferation antigen. In MS, remyelination also occurs, but it is incomplete and poorly sustained (477, 491). After a demyelinating lesion, remyelinated myelin never regains its normal thickness, and the normal linear relationship between axon and sheath thickness is also never regained (349). It is not clear whether the mechanism of remyelination is identical to myelination. At least in part, it could be similar to mechanisms at work in myelinogenesis; for example, it appears that MBP transcripts containing exon 2 are widely expressed in remyelinating oligodendrocytes in vivo and in vitro (101, 221). The molecular basis of the remyelination process has not been studied extensively.

The origin and identity of endogenous cells that affect remyelination is still an unsolved issue. There are immature cycling cells endogenous to white matter, which respond to experimental demyelination, by differentiating into myelinating oligodendrocytes (216). Proliferation of mature oligodendrocytes can also occur after a trauma of the nervous system (348) and in an experimental model of stab wound (8). However, no one has demonstrated that mature oligodendrocytes become involved in remyelination.

Glial transplantation techniques have proven to be a useful tool for the study of glial cell biology, as it is possible to label the transplanted cells and follow them throughout the whole myelination (321) and remyelination process. It is also possible to determine the amount of myelin being formed by transplanting myelin-producing cells into the CNS of myelin-deficient mutants. With the use of such techniques, it is also possible to determine the influence of the in vivo environment on these capacities. The experimental conditions, myelin-deficient models, markers for transplanted cells, and use of myelinating cell lines have been extensively reviewed recently (156). Even adult brain retains the potential to generate oligodendrocyte progenitors with extensive myelination capacity (696). The potentiality for remyelination can be exerted by many types of cells of the oligodendrocyte lineage. Cells present in the SVZ could be able to migrate toward a demyelinating lesion and to differentiate into myelinating oligodendrocytes (420). It appears that migration is reduced or very scarce under normal conditions (196, 197), except in genetic dysmyelinating mutants (321). There seem to be signals in a demyelinated lesion

that attract myelinating cells (638). PSA-NCAM, which is a migration-supporting signal, has been observed in areas of demyelination (420). Inflammation may favor migration (615). Analogous results have not been obtained by other groups (278) in the rat without X-irradiation (197). It is possible to enhance the proliferating capacities of progenitor cells to be transplanted by producing oligospheres (18, 415), or using the oligodendrocyte progenitor cell line, i.e., the CG4 cells (612). Nevertheless, up to now, no extensive remyelination has been obtained experimentally, although, in some cases, a functional electrophysiological recovery has been obtained (627).

Remyelination by transplantation of potentially myelinating cells may be a therapeutic approach. Although oligodendrocytes derived from fetal human white matter implanted into the dysmyelinating rodent CNS can synthesize myelin even after prolonged cryopreservation (554), harvesting aborted fetuses on a therapeutic scale is unlikely to be ethically or socially acceptable (550). Furthermore, there is a risk of immunological rejection. Thus it could be envisaged to use tissues from the patient itself, to obtain donor cells, such as Schwann cells (17, 260) or olfactory glia which both can be expanded (273, 494). The ability of a transplanted cell to migrate is obviously also of great importance in its remyelinating capacity after a lesion (196, 197).

### 3. Neuron-glia interactions during demyelination and remyelination

Recent experimental data show that axonal loss or perturbations may be more responsible than myelin deficiency for permanent disability (133). This has recently been observed in an experimental model: mice sensitive to Theiler's virus develop secondary demyelination. In a transgenic mouse deficient in MHC gene class I, Theiler's virus demyelination still occurs, but the animal does not present any functional deficit (515). This may be as a result of the persistence of sodium channels at the site of the Ranvier node, implicating possibly MHC class I molecules in the development of neurological deficits after demyelination.

Loss of myelin, even from a single internode, is sufficient to block conduction. If sufficient axons are affected in this way, severe symptoms can arise. The loss of insulation along the axon has as consequence that the action current is no longer focused at the very excitable nodal membrane. The axon may adapt to its demyelinated state over several days, by a redistribution of sodium channels. Possibly, cell contact involving astrocytes may be required to initiate channel rearrangement and permit restoration of nerve function (155), although, under these conditions, conduction is very slow and highly susceptible to external disturbances. Fast potassium channels are located in the internodal axon membrane; being un-

masked, they may also interfere with conduction in demyelinated axons (reviewed in Ref. 653).

## IV. EXPERIMENTAL AND HUMAN DISEASES OF MYELIN

### A. Genetic Diseases of Myelin

#### 1. Myelin mutants

The recent characterization of a number of mutants and determination of physical maps for mammalian genomes have greatly improved our ability to characterize a number of animal models for human diseases. The term *myelin mutants* designates animal mutants in which myelin formation or maintenance is affected. Myelin mutants are often named according to their phenotypes, e.g., shiverer, rumpshaker, twitcher, etc. They allow not only the identification and cloning of genes possibly related to human diseases, but also analysis of myelin development and biology of myelin-forming cells. They are furthermore essential for the development of therapeutic strategies useful for human diseases.

A) MUTATIONS OF MYELIN STRUCTURAL PROTEINS. 1) *PLP*. Because they are recessive X-linked diseases, the PLP mutation disorders are expressed only in males that are hemizygous (they possess only one allele). Because they are "mosaic" at the cellular level as a result of the random inactivation of one of the two X-chromosomes in each cell (355), heterozygous females present generally with a wild-type phenotype. This group of mutants contains the higher number of alleles within and across species. The PLP mutations occur in different regions of the PLP protein, yet they produce qualitatively similar phenotypes.

The jimpy,  $jp^{msd}$ , and jimpy-4j mice, as the md rat, share similar qualitative and quantitative neurological abnormalities with each other. All develop tremor, tonic seizures, with death occurring in the fourth postnatal week, when myelination is at its maximum.

The jimpy mutation, the first discovered (462), has been the most extensively studied. In the CNS of jimpy affected males, the number of mature oligodendrocytes is reduced by ~50% (307), due to an extensive premature oligodendroglial cell death; thus only 1–3% of jimpy axons are surrounded by myelin sheaths compared with normal littermates (52, 157). Myelin sheaths produced by surviving oligodendrocytes are generally clustered in patches around vessels, are thinner than normal, and present a fusion of the extracellular leaflets composing the double intraperiodic line. This abnormal compaction is attributed to the total absence of PLP proteins in the mutants, leading to the lack of intraperiodic lines where PLP is located in wild-type animals (157). Although it was recognized that a developmental disturbance of the oligoden-

droglial lineage was the cause of the decreased number of mature oligodendrocytes and the nearly total lack of myelin (381, 518), it was also hypothesized that the primary defect could be due to other cellular abnormalities. Indeed, a reduction of axon caliber (518) and axonal swellings were observed in jimpy mice (524). There is also a prominent astrogliosis, especially in the spinal cord (180), with a considerable increase of GFAP protein content both in the detergent-extractable pool and its soluble pool (277). As a probable consequence of absence of a stable interaction with the axons, a protracted proliferation of oligodendroglial cells is also observed (481) that can be considered as an effort to compensate for a shortened life span of mature oligodendrocytes (564). Interestingly, although in jimpy the mutation affects only the PLP gene, there is a considerable decrease in microsomal VLCFA biosynthesis (72) and in sulfogalactosylceramide biosynthesis; the latter occurs only in brain and not in kidney, although sulfogalactosylceramides are important components of this tissue (534). There are also consequences on the synthesis of MBP isoforms, especially a drastic decrease of the 14-kDa isoform of MBP (96).

The "myelin synthesis deficiency" or jimpy<sup>msd</sup> mutant (170) closely resembles the jimpy (52). However, jimpy<sup>msd</sup> has twice as much myelin as jimpy. The recently described jimpy-4j mouse (53) has the sharpest reduction in CNS myelin, oligodendrocyte number, and the shortest life span, with death usually occurring before postnatal day 24.

The myelin deficient (md) rat presents a similar severe phenotype, and the myelin formed also generally lacks a normal intraperiodic line (158). Recently, a long-lived md rat has been described (161), which lives to 75–80 days of age. Both mutants have similar numbers of myelinated fibers and frequency of seizures. However, the total glial cell number was markedly reduced in long-lived md rats compared with younger md rats. It is of note that short- and long-lived md rats present the same PLP gene mutation (161), but they are not on an inbred strain, which may explain these differences.

The canine shaking pup, described by Griffiths et al. (234), is reduced in weight and size and shows gross generalized tremor, particularly during early stages of the disease, at ~10–12 days of age. However, after this early crisis, shaking pups overcome and are able to live for an extended period of time (up to 23 mo of age). Shaking pups present considerable more myelin than jimpy alleles, albeit many axons are either nonmyelinated or are surrounded by thin myelin sheaths. Shaking pup oligodendrocyte number is normal, but their differentiation is impaired, as shown by the overexpression of DM-20 (417). Interestingly, it was recently shown that Schwann cells invade the CNS of shaking pup and that P0-positive myelin is present in the spinal cord of this mutant (159).

The rumpshaker mouse (236) and the paralytic tremor (pt) rabbit also present CNS hypomyelination and astrogliosis; however, they are strikingly different from other X-linked myelin mutants in that they have a normal life span and breed normally. In the two mutants, the ratio of DM-20 to PLP is higher than in age-matched controls (236, 613), suggesting an impairment of differentiation of the oligodendroglial cell lineage. Moreover, rumpshaker oligodendrocyte number is slightly increased in several regions of the CNS (179).

The molecular basis of the X-linked dysmyelinating mutants has been characterized. A deletion in the myelin PLP and DM-20 transcripts was first characterized in jimpy brain (131), suggesting that they were encoded by the same gene (405). These preliminary results were confirmed by the detection of a 74-bp deletion in the PLP/DM-20 mRNAs, causing a frameshift in the open reading frame and resulting in an altered COOH terminus for jimpy PLP/DM-20 proteins (423). A single nucleotide mutation in the splice acceptor signal preceding exon 5 leads to the skipping of the fifth exon of the PLP gene in jimpy (357, 407, 423). Diverse point amino acid substitutions were found in other PLP mutants (reviewed in Ref. 422). The rumpshaker mutation, Thr36His, is located in the first extracellular loop of PLP/DM-20 molecules; the identical mutation has been found in one SPG-2 patient (see sect. IV A2).

The functional consequences of the PLP mutations have been analyzed. Previous results had shown that the jimpy mutation blocks the cellular trafficking of PLP in the endoplasmic reticulum in jimpy oligodendrocytes (526); therefore, the transport of mutated PLP/DM-20 molecules was studied by transient expression in eukaryotic cells. A common mechanism of misfolding of the mutated PLP/DM-20 (229, 286) and the defective transport of mutated molecules to the cell surface (228, 230, 614) may explain the spectrum of phenotypes associated with the different mutations. This is also the case for PMD and its variant forms. Thus a direct correlation can be made between the severity of the phenotype and the transport of the abnormal proteins to the cell surface. Interestingly, although DM-20 is expressed at early stages of embryogenesis (see sect. II B), cells of the oligodendrocyte lineage develop normally in the jimpy mouse; it is only at the time of myelin deposition that the oligodendrocytes die (454, 566, 668).

**II) MBP.** The shiverer mouse and its less affected allele shi<sup>ml</sup> (150, 151) are clinically characterized by unstable locomotion, intention tremor appearing from P10 to P12, followed later on by tonic seizures. Shiverer mice usually die 4 or 5 mo after birth. The shiverer mutant lacks MBP (162, 390, 516) in both the PNS and CNS. However, the resulting defects are confined essentially to the CNS where hypomyelination, reduced numbers of lamellae, myelin uncompaction, myelin breakdown, and



morphological anomalies of myelin, such as a characteristic absence of the major dense line, have been observed (480).

To determine the primary target of the shiverer mutation, chimeric mice (wild type/shiverer) were produced, in which the presence of CNS MBP-negative fibers (presenting an absence of the major dense line), and MBP-positive fibers indicated that the cause of the demyelination is primarily in the oligodendrocyte itself and is not due to neuronal, humoral (391), or astrocytic origin. When observed on the same genetic background, at P21, defects of *shi<sup>mld</sup>* myelination are comparable to those in shiverer with respect to number of myelinated axons, sheath thickness, and myelin abnormalities. However, the major dense line is present in a few *shi<sup>mld</sup>* myelin sheaths positive for MBP, whereas neither is seen in shiverer at this age. *Shi<sup>mld</sup>* mice survive their disease significantly better than shiverer (558).

In the PNS in shiverer mice, in which there are no phenotypic manifestations, the major peripheral myelin glycoprotein, P0, which is thought to form the stable link between apposed cytoplasmic surfaces in peripheral myelin, probably compensates for the absence of MBP. On the other hand, recent reexamination of shiverer PNS revealed a two- to threefold increased number of Schmidt-Lanterman incisures in sciatic nerves, suggesting a compensation for a defect in Schwann cell-axon communication (225).

The MBP gene was identified as the shiverer gene by Southern blot analysis revealing that the last five of the seven exons constituting the wild-type gene are deleted in shiverer (397, 517). As a consequence, MBP transcripts in shiverer brain are severely truncated, leading to a total lack of all MBP isoforms. Complementation of the pathological phenotype can be rescued by expressing the wild-type MBP gene in transgenic mice (499) leading to the expression of the major four MBP isoforms. It is of note that restoration of myelin formation can be obtained by a single type of MBP, the 14-kDa isoform, as well as the minor 17.2-kDa isoform (299, 300).

In contrast, in the allelic mutation *shi<sup>mld</sup>*, a reduced level of normal length MBP transcripts, to ~5% of the wild type, is present (436), indicating that the defect is not caused by the deletion of a large portion of the MBP gene. Southern blot analysis revealed that the 5'-portion of the MBP gene is duplicated in *shi<sup>mld</sup>* and that a large portion of the duplication is inverted upstream of the intact copy. As a consequence, in *mld*, the reduced expression of MBP transcripts could be due to formation of RNA duplex between antisense RNA transcribed from the inverted segment, and RNA transcribed from the normal gene located downstream (389). Interestingly, MBPs were expressed in a mosaic fashion in the CNS of *mld* mice, as three types of internodes could be observed: 1) a wild-type compact myelin comporting a major dense line, 2) a

shiverer-type myelin with no major dense line, and 3) a mixed-type myelin, in which, within a myelin lamella, the major dense line abruptly disappears. Such cell-to-cell variation may result in mosaic expression of sense and antisense MBP transcripts (390). As in the shiverer transgenic mice (499), expression of a wild-type MBP gene in transgenic *shi<sup>mld</sup>* mice greatly reduced the mutant phenotype (469).

Despite the important CNS myelin defects, shiverer mice survive to adulthood, suggesting that central nerve conduction is retained in some parts. In adult shiverer, CNS hypomyelinated fiber tracts, sodium (429) as well potassium (650) channel expression is upregulated in both axons and glia, possibly reflecting a compensatory reorganization of ionic currents, allowing impulse conduction to occur in these dysmyelinating mutants. Moreover, no axonal implications have been observed in these MBP mutations, in contrast to the jimpy alleles (428). These data may account for the relatively mild degree of neurological CNS impairment in the shiverer animals.

Transplantation techniques have shown that neural stem cells can repair dysmyelination in shiverer; it appears to be a shift in the fate of these multipotent cells toward an oligodendroglial fate (682).

Another mutation in the MBP gene has recently been identified in the "Long Evans shaking" (*les*) rat, characterized by the insertion of a retrotransposon in the intronic region which disrupts mRNA splicing and myelination (433).

**B) MUTATIONS IN A REGULATORY PROTEIN: THE QUAKING MOUSE.** The quaking mutation comprises two classes of recessive alleles, with strikingly different phenotypes. Indeed, quaking mutations present pleiotropic effects on myelination and embryogenesis. The original "quaking viable" mutation is a spontaneous, recessive mutation, presenting a hypomyelination of both CNS and PNS, associated with a large deletion of one megabase on chromosome 17 (167). Homozygous quaking viable mice survive to adulthood and exhibit a characteristic tremor or "quaking" of the hindlimbs (562). The identification of oligodendrocytes as the affected cells, the abnormal composition and compaction of myelin, with pockets of oligodendrocyte cytoplasm, suggested that an arrest of myelin assembly is causative of the quaking viable mutation. Interestingly, myelin is not reduced in all fascicles (43). Other developmental abnormalities have also been observed, such as an increased number of noradrenergic neurons in the locus ceruleus that is likely to be involved in the convulsions of this mutant mouse (370).

A second group of recessive quaking alleles, ethylnitrosourea induced, are embryonic lethal around 9–10 embryonic days of gestation, 10 days before myelination begins; because these mutants do not present the deletion found in quaking viable, it was supposed they are due to point mutations in the quaking gene.

The mouse quaking gene was identified using a positional cloning strategy by Artzt and co-workers (166). Two classes of transcripts generated by alternative splicing are expressed; class I (Qk1) is expressed in the earliest cells of the embryonic nervous system, and class II (Qk2) is expressed in the myelinated tracts of the neonatal brain. Qk1 transcripts implicated in embryonic developmental stages present point mutations in the ethylnitrosourea-induced qk mutants, whereas Qk2 transcripts, implicated in myelination, are truncated at their 5'-terminal end, which is coded by the part of the gene included in the deletion at the quaking locus.

The loss of fertility of quaking viable affected males could be due to the expression of the quaking gene during spermatogenesis as recently demonstrated for an ortholog of the mouse quaking gene in chick spermatogenesis (386).

The quaking gene products comprise a so-called K homology (KH) domain, a RNA-binding motif conserved throughout all taxonomic groups (reviewed in Ref. 697). Some of the quaking products could be located in the cytoplasm or in the nucleus of glial cells, suggesting that the quaking transcripts could combine features of RNA-binding and signal transduction proteins (166). The abnormal expression ratio and glycosylation of MAG isoforms in quaking mutants (36) could be explained by abnormal sorting, transport, or targeting of L-MAG or S-MAG (63), or alternatively, as a defect in the relative expression ratio of the two MAG isoforms, according to the putative function of the quaking proteins in regulation of alternative splicing (166). In this context, one indirect consequence of the quaking mutation might be the abnormally little L-MAG production, participating to the morphological alterations of CNS myelin in quaking mice, which could be compared with similar defects observed in the engineered L-MAG mutant mice (203) (see sect. III C2).

C) MUTATIONS INVOLVING ENZYMES OF LIPID METABOLISM. The twitcher mouse was early recognized as an authentic model of Krabbe's disease (599). The twitcher mouse exhibits a leukodystrophy characterized by ataxia ("twitching") and abnormal periodic acid-Schiff (PAS)-positive cells, often multinucleated ("globoid cells"), in both CNS and PNS. On C57BL/6j background, death occurs at 40–45 days. Loss of myelin and accumulation of globoid cells, often clustered around blood vessels, are prominent in the white matter spinal cord and brain stem of twitcher mice. However, compacted myelin appears structurally normal, with an orderly periodicity of the dense major line and intraperiodic line. Crystalloid or slender tubular inclusions (GLD inclusions) are present in macrophages, oligodendrocytes, and astrocytes. Extensive astrocytosis is detected both in white and gray matter. Axonal loss is not prominent. The PNS is also severely affected. Infiltration of macrophages, similar to those

seen in the CNS, is apparent between nerve fibers. Many nerves are demyelinated, and the presence of thinly myelinated fibers indicates remyelination. Schwann cells of myelinated fibers contain the characteristic GLD inclusions, whereas nonmyelinating Schwann cells present unusually elongated and branching processes. Both Schwann cell types possess numerous GFAP-immunoreactive processes.

Myelin and degenerating oligodendrocytes are removed and phagocytosed by globoid cells, indicating that they actively participate in the demyelinating process. As such, twitcher is a unique model of congenital demyelination.

The globoid cells invading the nervous system are exogenous macrophages of mesenchymal phagocytic origin, positive for vimentin and Mac-1 but negative for GFAP. It has long been known that GalC has a specific capacity to elicit infiltration of macrophages into the brain, as shown by the production of globoid-like cells obtained by injecting cerebroside directly into rat brain white matter. Once in the brain, macrophages are transformed to multinucleated globoid cells. The characteristic inclusions in the globoid cells appear to be GalC itself. Psychosine, the other substrate of the defective enzyme, is consistently and progressively increased in tissues of twitcher mice; the degree of psychosine accumulation correlates well with the severity of pathological lesions (see review in Ref. 595). Hematopoietic cell transplantation prolongs survival in the twitcher mouse and improves biochemical parameters, especially increasing galactocerebrosidase (GalCase) level (271).

The twitcher cDNA encoding the 668 amino acids GalCase revealed a nonsense mutation at codon 339 (Trp339stop) (529). The mutation is homozygous in the twitcher and heterozygous in the carrier. A number of naturally occurring other animal mutants associated with a deficiency of GalCase have been characterized.

D) MYELIN MUTANTS WITH UNKNOWN GENETIC DEFECTS. The taiep rat presents a developmental defect in CNS myelination, leading to a progressive neurological disorder (160). "Taiep" is an acronym for trembling (t), ataxia (a), immobility episodes (i), epilepsy (e), and paralysis (p). The myelin defect correlates with an abnormal and progressive accumulation of microtubules associated with endoplasmic reticulum membranes in taiep oligodendrocytes. These morphological abnormalities suggest a blockage of protein trafficking (125). Glial cell number is normal (351). Myelin yield decreases continuously from 2 wk until it reaches a stable level of ~10–15% in the affected brain and 20–25% in the spinal cord, compared with control. Thus the myelination defect in taiep has features of both hypomyelination and demyelination.

The zitter rat (501) presents two main neurological abnormalities: a spongiform encephalopathy in gray matter and a hypomyelination in the brain stem and cerebel-

lum. Zitter oligodendrocytes contain abnormal structures associated with nuclear membranes, resembling stacks of split myelin lamellae. These abnormalities are prominent at 3 wk of age, then decrease transiently and then again increase slightly with advancing age. The spongy degeneration observed in the gray matter gradually extends to the entire CNS; however, no inflammatory nor phagocytic cell infiltrations are detected. GFAP immunoreactivity increases transiently in the vacuolated areas from 2 to 15 wk of age, then decreases, suggesting an astrocytic hypofunction in response to tissue damage. A yet unknown (see NOTE ADDED IN PROOF) genetic abnormality, probably related to cell membrane biosynthesis, produces both hypomyelination and spongy degeneration in the zitter rat (311).

The hindshaker mouse shows tremors that decrease, then cease with age (301). Decreased numbers of oligodendrocytes and amount of myelin are observed in the spinal cord. Although most sheaths remain thin, there is a marked clinical improvement with time. Thus hypomyelination could result, at least in part, from a deficiency of mature oligodendrocytes able to elaborate myelin membranes or to a delayed differentiation or maturation of oligodendrocytes that are unable to synthesize sufficient myelin at the appropriate time.

Spontaneous myelin mutations are extremely good tools for the study of the myelination process and its consequences; furthermore, it is possible to study the animals before the onset of the clinical disease. They are physiological models of human diseases that may be of further help for therapeutic trials.

## 2. *Leukodystrophies in humans*

Inherited myelin diseases in humans, leukodystrophies, may be the result of dysmyelination, hypomyelination, or demyelination. Dysmyelination and hypomyelination are failure to myelinate occurring during fetal life or early infancy, as observed in different forms of Pelizaeus-Merzbacher disease. Demyelination, breakdown of myelin, is characteristic of metabolic leukodystrophies, such as Krabbe's disease, metachromatic leukodystrophy, ALD, Canavan disease, Alexander disease, orthochromatic leukodystrophy, or mitochondrial disorders (reviewed in Ref. 309). Dysmyelination and demyelination can be combined in some forms of leukodystrophies.

Myelin formation and maintenance require also a balance between synthesis and degradation of lipids. Although alterations of cerebroside, sulfatide, and long-chain fatty acid degradation possibly involve other cell types than oligodendrocytes, they give rise to leukodystrophies (reviewed in Ref. 309). These complex lipids and their VLCFAs are also present in the PNS. Therefore, symptoms associate both central demyelination visible by MRI and PNS demyelination with slowing of nerve con-

duction velocity. These leukodystrophies associate defective lysosomal enzymes or lipid transporters and accumulation of the undegraded lipid which is easily tested in fibroblast cell cultures.

In Krabbe's disease (1916), an autosomal recessive disorder, there is a defect in the lysosomal galactocerebrosidase (598), the enzyme which degrades GalC into ceramide and galactose. This disease occurs mainly during infancy, but some adult cases manifesting only as spastic paraplegia have been diagnosed (reviewed in Ref. 310). The degeneration of myelin and oligodendrocytes is due to the accumulation of psychosine, a toxic metabolite also normally degraded by this lysosomal enzyme (reviewed in Ref. 595). Exogenous macrophages, the globoid cells, invade the CNS and have given the name of globoid cell leukodystrophy to this disease. The gene is located on chromosome 14 and contains 17 exons (353, 354). There is a spontaneous mouse mutant, the twitcher mouse (see sect. IV A1), which constitutes an experimental model of Krabbe's disease (reviewed in Ref. 599).

Metachromatic leukodystrophy is also an autosomal recessive inherited lysosomal disorder. It is caused by a defect in the arylsulfatase A (ASA) gene (reviewed in Ref. 219). ASA, together with an activator (saposin B), degrades sulfogalactosylceramide into GalC and sulfate. In most of the cases, it is the deficiency of ASA that leads to the accumulation of sulfogalactosylceramide and provokes a lethal progressive demyelination. The ASA gene has been cloned; the coding sequence is divided into eight exons (315); the entire coding sequence is of 1.5 kb. The leukodystrophy generally develops during infancy, and there is a destruction of the newly formed myelin, but it can also occur later, even at adulthood (40). The phenotype of ASA-deficient mice shows morphological, biochemical, but not functional relationships to human metachromatic leukodystrophy (252), possibly in relation to other compensating metabolic pathways in the mouse.

Sphingolipid activator proteins (saposins) are necessary to the enzymatic hydrolysis of myelin galactolipids. They originate from a prosaposin gene. Targeted disruption of the mouse sphingolipid activator protein gene gives rise to a complex phenotype including a severe leukodystrophy (204).

In X-linked adrenoleukodystrophy (ALD), there is an impairment in VLCFA  $\beta$ -oxidation that is normally degraded in peroxisomes. There is an accumulation of VLCFA cholesterol esters and other lipids mainly in white matter of the brain and in the adrenal gland. There are two major forms (410): 1) cerebral childhood ALD (which is associated with inflammation in the brain) and multifocal demyelination and 2) adrenomyeloneuropathy which affects mainly the spinal cord and peripheral nerves. The ALD locus has been mapped to Xq28. The gene involved is called the ALD protein (ALDP) gene and consists of 21 kb and 10 exons. ALDP is a 75-kDa protein



(411) that belongs to the ATP-binding cassette (ABC) protein family. ALDP has been localized to the peroxisomal membrane (411) and might be involved in the import of cofactors necessary to VLCFA-CoA synthase activity or import of VLCFA-CoA into the peroxisomes. In the brain, ALDP is expressed in oligodendrocytes but also in astrocytes and microglia (16). Mice defective in the ALDP gene have been generated (192, 308, 342). Unexpectedly, there are no neurological symptoms even in aged mice, although there is impaired  $\beta$ -oxidation of VLCFA that accumulate in CNS white matter, adrenal cortex, and testis. Therefore, the accumulation of VLCFA alone is probably not sufficient to cause demyelination in the nervous system (reviewed in Refs. 16, 410).

Zellweger disease is a complex peroxisomal syndrome (reviewed in Ref. 409) that also affects white matter in brain and gives rise to an accumulation of VLCFA; there is also a defect in plasmalogen biosynthesis (545), compounds known to be enriched in myelin and other abnormalities of this cerebro-hepato-renal syndrome. It is a group of genetically heterogeneous disorders that affects peroxisome peroxidation.

Another enzyme, aspartoacylase, which degrades *N*-acetylaspatic acid, is possibly also a myelin enzyme (284). The enzyme deficiency is the cause of Canavan's disease, which is also characterized biochemically by *N*-acetylaspatic aciduria. Canavan's disease gives rise to dysmyelination and spongy degeneration of the brain (reviewed in Ref. 364). Significant levels of *N*-acetylaspatic acid (NAA) are detected in cultures of immature oligodendrocytes; these metabolic abnormalities in the development of oligodendroglia may be related to the physiopathology of this disease (626). In the adult, NAA is neuronal and a useful neuronal marker in NMR spectroscopy. Recently, it has been shown that mature oligodendrocytes can express NAA in vitro (50) and possibly in vivo contribute to the NAA signals observed in spectroscopy.

Mutations in the PLP gene cause a rare leukodystrophy, the Pelizaeus-Merzbacher disease (PMD). PMD presents in several forms: connatal, infantile, and more recently a different phenotype with spastic paraplegia type 2 (SPG-2), either isolated or associated to clinical features of the classical PMD (reviewed in Refs. 422, 555). More than 30 point mutations have been found in the PLP gene from PMD and SPG-2 patients.

Another rare leukodystrophy, the 18q-syndrome, presents a deletion which includes the MBP gene (213, 309, 520).

An astrocyte pathology may also give rise to leukodystrophies such as Alexander disease. Recently, sequence analysis of DNA samples from patients representing different Alexander disease phenotypes revealed that most cases are associated with nonconservative mutations in the coding region of GFAP. Alexander disease

represents the first example of a primary genetic disorder of astrocytes, one of the major cell types in the CNS (75a).

A number of leukodystrophies, most of which are genetically inherited, have also no known etiology. A new leukoencephalopathy termed "childhood ataxia with diffuse central nervous system hypomyelination" (CACH syndrome) or "leukoencephalopathy with vanishing white matter" was recently identified on clinical and neuroradiological grounds (540, 629). In addition to the cavitating white matter lesions found in this orthochromatic leukodystrophy, neuropathological data recently obtained showed that this new characterized entity is associated with an unusually increased oligodendrocyte density (521, 522).

### 3. Leukoencephalopathies

If the pathology of myelin or myelinating cells dominates in leukodystrophies, some genetic diseases may give rise to leukoencephalopathies in which demyelination is secondary to vascular, mitochondrial, or neuronal alterations or may be linked to a metabolic disease that may have ubiquitous signs.

White matter is vulnerable to ischemic damage (143, 186). White matter diseases can also be posttoxic, postinfectious, and postradiological.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant cerebral arteriopathy. MRI evidences multiple subcortical infarcts, with a demyelination of white matter that can be more or less extensive (616; reviewed in Ref. 136).

MELAS means mitochondrial myopathy, encephalopathy, lactic acidosis, strokelike episodes. Most of the patients present a lactic acidosis with an increase of the lactate-to-pyruvate ratio in serum and CSF. MRI shows white matter modifications are present together with cortical atrophy (148).

Refsum disease is characterized biochemically by an accumulation of phytanic acid in tissues. This acid is only present in food (green vegetables, and also grass-eating animals) and normally degraded by phytanate oxidase (585). MRI evidences demyelination in the brain stem and the cerebellum.

### 4. Other metabolic diseases

It is well known that phenylketonuria can be associated with demyelination. The diet may not always be sufficient to repair cerebral alterations, especially if it is not well observed or set up too late. Abnormalities of intermediary metabolism may also cause demyelination.

Some neuronal genetic diseases can affect myelin (GM2 gangliosidosis, Wilson's disease, and degenerative diseases of CNS). Oligodendrocyte express AMPA/kainate-type receptors, and they share with neurons a high

vulnerability to the AMPA/kainate receptor-mediated death, a mechanism that may contribute to white matter CNS disease (369, 373, 435).

## B. Inflammatory Demyelinating Diseases

Breakdown of the blood-brain barrier is a primary event in pathological manifestations of demyelinating disease of the CNS, such as MS, demyelinating forms of EAE (reviewed in Ref. 663), and virus-induced demyelination (reviewed by Ref. 184). Neural and even nonneural activated T cells play a pivotal role in this process (336, 665). Moreover, the opening of the blood-brain barrier allows the entry of circulating antibodies into the CNS, in particular demyelinating antibodies, such as anti-MOG antibodies (214, 215, 336; see also review in Ref. 77). Access of activated T cells to the CNS is responsible for release by inflammatory cells, macrophages, and microglia and of proinflammatory cytokines, such as TNF- $\alpha$  and interferon- $\gamma$  (reviewed in Ref. 77). However, the toxic action of TNF- $\alpha$  on oligodendrocytes (556) remains debatable. Activated macrophages engulf myelin debris or internalize myelin by Fc receptor and complement receptor-mediated phagocytosis after binding to myelin-specific antibodies (403, 628).

Other different mechanisms may be involved in the process of demyelination. Oligodendrocytes have a high iron content, making them especially prone to oxidative stress by reactive oxygen species (240). Neurotransmitters, especially glutamate, may also exert a direct deleterious effect on oligodendrocytes and lead to demyelination (369, 464; reviewed in Ref. 588). Heat shock proteins may also be involved (77), among which  $\alpha$ -B crystallin, which may be a candidate encephalitogenic antigen in demyelination (631). Matrix metalloproteinases (MMP) are a group of zinc-dependent enzymes that can degrade extracellular matrix components (reviewed in Ref. 687). Among other activities, they can degrade MBP (111). MMPs are increased in active and chronic MS (361), especially MMP-7 and MMP-9 (124). More and more, there is thought to be a viral triggering of autoimmune diseases (664). More than one mechanism may be present in the same disease or even in the same lesion (349).

There are many causes of demyelination in human diseases. The main causes of primary demyelination are genetic (such as in ALD, see sect. IV A2), immune mediated, viral such as HIV, and toxic (reviewed in Ref. 349); they may also be secondary to neuronal dysfunction. In some cases, white matter thinning is visualized on MRI; it is called leuko-araiosis, and it can be seen in normal, even young individuals, and more frequently in aging; its significance remains obscure. There may be a relationship between leuko-araiosis at MRI, myelin pallor, and white matter atrophy, which are as yet poorly understood (637).

Diffuse myelin pallor is seen in some dementia patients (471).

### 1. MS

MS is a chronic and inflammatory demyelinating disease of the CNS (112). Storch and Lassmann (593) have shown that there is a profound heterogeneity of pathology and immunopathogenesis of the lesions. There is a high interindividual but a low intraindividual variety of MS lesions. The spectrum of pathology appears to be the following (344, 345): 1) primary demyelination with little oligodendrocyte damage; 2) extensive oligodendrocyte loss in the course of demyelination; 3) primary oligodendrocyte damage with secondary demyelination; and 4) extreme macrophage activation together with rather non-selective tissue damage, involving not only myelin and oligodendrocytes, but also axons and astrocytes. Axonal loss is also a prominent feature of MS and may be the pathological correlate of the irreversible neurological impairment in this disease (185, 341, 375, 621). Current evidence shows that the etiology of MS and other demyelinating diseases may involve a combination of viral and autoimmune factors.

Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of MS but fail to repair demyelinated regions (551, 671, 672).

### 2. Experimental models of demyelinating diseases

There are several experimental models of demyelination: toxic, viral, and immune mediated. Among the toxic models, ethidium bromide (676) and cuprizone (59) alter mitochondrial DNA leading to abnormal respiration and necrosis (347); lysophosphatidylcholine (60) is also widely used.

A) VIRUS MODELS OF MS. The most useful virus models of MS are the experimental infections of rodents that give rise to an inflammatory demyelinating disease in the CNS. The most studied are Theiler's virus, mouse hepatitis virus, and Semliki Forest virus.

Theiler's murine encephalomyelitis virus (TMEV) is a natural pathogen of mice in which CNS infection is a rare event. To study the virus experimentally, the virus is injected into the CNS. The inflammatory response is recruited into the CNS and in some mouse strains eradicates the infection. In other mouse strains the virus persists within oligodendrocytes, albeit at low titer. Persistence gives rise to inflammation in the brain and cord and results in paralysis; susceptibility has been linked to a number of loci including MHC and TCR as well as MBP genes. In some mouse strains, infection gives rise to autoreactive T cells, demonstrating that viruses can give rise to a myelin-specific pathogenic response. Demyelination is observed with the Theiler's virus (523) in which a secondary immune-mediated disease gives rise to

oligodendrocyte destruction, following a primary infection in neurons. There are susceptibility genes and resistance genes to viral persistence and demyelination (85).

Like TMEV, the mouse hepatitis virus (MHV) strains JHM, A59, and MHV-4 are neurotropic and may induce CNS inflammation after either intracranial or intranasal infection. With the JHM strain virus, replication is confined to an intermediate stage between O2-A progenitors and fully differentiated oligodendrocytes, both of which are resistant to infection. Host genetics also play a role in susceptibility as seen in the resistant SJL mouse and susceptible BALB/c mice. Although in some strains virus can directly infect and destroy oligodendrocytes, this is again dependent on the host background. Infection with JHM may lead to the generation of autoreactive T cells to myelin proteins.

Another experimental virus infection is Semliki Forest virus of mice. The most commonly used strains include L10, M9, and the avirulent A7 (73), which are neuroinvasive as well as neurotropic, with the advantage being that the virus can be injected intraperitoneally and thus enable the study of the blood-brain barrier. Virus infection gives rise to large plaques of demyelination in the brain and cord. The absence of demyelination in immunocompromised athymic mice or in SCID mice in which virus persists in the brain demonstrates the role of the immune response in disease. In addition to the CNS lesions, SFV-infected animals display abnormalities in their visual evoked responses and have changes in axonal transport and optic nerve lesions. Although some studies demonstrate that SFV gives rise to autoreactive T cells (S. Amor, personal communication), others have demonstrated that similarities between the myelin protein MOG and SFV peptides are responsible for the demyelination observed in this model (396).

In contrast to the experimental viral models, Visna and canine distemper are naturally occurring inflammatory diseases of sheep and dogs, respectively, and both have been studied as experimental infections from the point of view of investigating mechanisms of viral damage to myelin important for the study of MS.

B) EAE. EAE, also called experimental allergic encephalomyelitis, is provoked by injection of CNS tissue, whole spinal cord, purified myelin, or myelin proteins or peptides in susceptible animals (reviewed in Ref. 73). The incubation period between sensitization and onset of the disease as well as the severity and course of the clinical disease are variable and depend on the genetic background of the animal (species and strain differences); on environmental factors, like the mode of sensitization; the dose and nature of the sensitizing antigen; or exogenous influences on immune functions, such as intercurrent infections or treatment with immunosuppressive or immunomodulatory drugs. In many cases, it is also possible to transfer EAE by sensitized T lymphocytes to a naive

recipient. For a long time, MBP has been considered as the main encephalitogenic antigen (296). Later, it was recognized that PLP may lead to a very similar disease (92). Immunodominant encephalitogenic epitopes of MBP and PLP have been characterized and found to be different in various animal species and strains, in relation to MHC phenotypes (12, 304, 646; review in Ref. 268).

Many other minor myelin and myelin-associated proteins, OSP, MAG and the heat shock protein  $\alpha$ -B-crystallin have been demonstrated to be encephalitogenic in mice (590, 609, 658). Actually, MOG is one of the most encephalitogenic proteins and is widely used to induce EAE. MOG by itself is able to induce both an encephalitogenic T-cell response and an autoantibody response in a large range of susceptible animals. MOG-induced EAE constitute a relevant model for MS, presenting different clinical phenotypes depending on MHC haplotype and other susceptibility genes, as environmental factors, with both inflammation and demyelination specifically in the CNS (1, 47, 283, 294, 335, 584, 594, 662). Interestingly, anti-MOG antibodies have been shown to be very important in the demyelination processes; in an experimental model in which MBP is the encephalitogenic antigen T cell mediated. In this model there is only inflammation, unless anti-MOG antibodies are also injected intravenously (336), which dramatically increase the degree of demyelination. The important role of anti-MOG antibodies has been also well demonstrated in a transgenic mice engineered to produce high titers of autoantibodies against MOG (338); in this transgenic mouse, the presence of numerous plasma cells secreting MOG-specific IgG antibodies accelerates and exacerbates EAE, independently of the identity of the initial autoimmune insult (PLP or MOG peptides). Circulating antibodies against MOG have also been shown to facilitate MS-like lesions in a nonhuman primate, the marmoset, and have been observed in MS (214, 215). Moreover, tolerization assays in the marmoset model induce a lethal encephalopathy after cessation of the treatment (214). These data suggest that such autoreactivity to MOG may be significant in the development of MS.

Amor et al. (10) were the first to describe T-cell encephalitogenic epitopes of MOG that induce both clinical and histologic signs of EAE in mice. Furthermore, chronic relapsing EAE with a delayed onset and an atypical clinical course can be induced in PL/J mice by specific MOG peptides (294). Interestingly, epitopes of MBP, PLP, and MOG for EAE induction in Biozzi ABH mice share an amino acid motif (11).

One of the mechanisms participating in autoimmunity could be molecular mimicry, due to recognition by the immune system of analogous sequences shared by viral or bacterial proteins and myelin proteins (PLP, MBP, or MOG) able to induce EAE (see review in Ref. 437).



### C. Tumors: Oligodendrogliomas

The most common CNS gliomas traditionally are thought to arise from mature astrocytes and oligodendrocytes. Oligodendrogliomas, which appear to arise from oligodendroglial cells, have been thought for long times to be uncommon gliomas (<10% of all intracranial tumors) (254). However, reappraisal of recent data indicates that they may be more common than generally thought (193).

Recently, the possibility that gliomas may arise from a population of glia that has properties of oligodendrocyte progenitors has been examined. These glial cells express the NG2 chondroitin sulfate proteoglycan and the  $\alpha$ -receptor of PDGFR- $\alpha$  *in vivo*. NG2 and the PDGFR- $\alpha$  high levels of expression have been identified in tissue from seven of seven oligodendrogliomas, three of three pilocytic astrocytomas, and one of five glioblastoma multiform, raising the possibility that the NG2+/PDGF  $\alpha$ R+ cells in the mature CNS contribute to glial neoplasm. These data provide evidence that glial tumors arise from glial progenitor cells. Molecules expressed by these progenitor cells should be considered as targets for novel therapeutics (426, 560).

### V. CONCLUSIONS

The importance of glia has become more and more clear with the development of techniques of molecular biology and cell cultures. Culturing neurons and glia separately or together has contributed to the understanding of the role of oligodendrocytes in the development of neuronal pathways as well as in synaptic functions. The study of spontaneous mutations, as well as transgenic mice with overexpression or no expression of specific markers, has shown the role of specific glial components in brain constitution and function. More and more, the molecules involved, in developmental processes and in the adult, are being identified. Molecular studies of developmental mutants and human pathology have led to the identification of the involvement of glia in numerous developmental diseases.

Axonal guidance seems in many cases to involve preformed glial pathways that may remain and create glial boundaries. Myelin plays an inhibitory role to growing axons and participates in the stability of the mature, intact nervous system, by preventing random regrowth of axon sprouts once pathways have been established and myelinated. Although it may present the unwanted side effect of impairing axonal reparation after CNS injury, it gives new areas of research for brain repair (134, 607). Without glia, and especially the oligodendrocyte, nerve conduction velocity would not develop normally because these glial cells are necessary for the clustering of sodium channels at the Ranvier node essential to fast nerve con-

duction velocity in myelinated fibers (293). The role of the oligodendrocyte and neuronal pairing is not only essential in relation to myelin formation but also in demyelinating diseases. If neurons are needed for myelination, impaired myelin function may induce profound cytoskeletal alterations leading to axonal degeneration, as observed in myelin protein-deficient animal models (235, 686). Altogether, these data enlighten the role of myelinating glial cells-axon interactions, the "functional axon-glial unit," in the pathophysiology of myelin-related disorders. Furthermore, these studies and other works related to remyelination show the unexpected intrinsic plasticity of glial cells in the CNS, commonly recognized in neonates, but surprisingly still present in adults (reviewed in Ref. 61). Furthermore, experimental models such as EAE and studies of cellular markers in human brain seem to indicate that there are different ways of inducing demyelination, thus possibly different types of MS.

More and more these neuroglial interactions are being identified in relation to neuronal functions. By their mobility and plasticity, glial cells appear to be intricately involved in the functions of the neuronal network. As mentioned by Ullian and Barres (624), "glia are not listening to synaptic transmission but participating in the conversation as well." Synapses throughout the brain are ensheathed by glial cells. Astrocytes help to maintain synaptic functions by buffering ion concentrations, clearing released neurotransmitters, and providing metabolic substrates to synapses. Recent findings suggest that oligodendrocytes may have such a role as well.

The dysfunction of glial cells is possibly at the origin of many of the degenerative diseases of the nervous system and of the major brain tumors (glioma). Nevertheless, there are still many mysteries that are not unraveled. Although growth factors and cytokines are secreted by astrocytes and oligodendrocytes *in vitro*, their relevance *in vivo* remains to be elucidated, as neurons are also able to synthesize them. Some cytokines may be useful or harmful. Receptors for these substances have not as yet been well identified *in vivo*, especially in pathological states.

Although myelin repair and synaptic remodeling and regeneration can occur, many enigmas are still to search for, especially in the human in which growth factors may be different from the rodent species. Thus studies in primates, and *in vivo* systems, cannot be omitted at this stage, in view of therapeutic implications.

No doubt we will understand more and more the language between glial cells and neurons in normal and pathological states. Already abnormal astrocytes and oligodendrocytes appear to be involved in cognitive functions as seen in the leukodystrophies.

As mentioned by Peschanski (451), time has come for "neurogliobiology" in which neurons and glia (including microglia) in the nervous system are inseparable partners.

## NOTE ADDED IN PROOF

Recently, the genetic defect involved in the Zitter rat mutation has been characterized as a small deletion near a splicing site in the "attractin" gene. A mutation in attractin is also responsible for the myelin defect in a previously described mutant, the Mahogany mouse. Therefore, attractin, which plays multiple roles in regulating different physiological processes, also has a critical role in normal myelination in the CNS (318a).

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