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Review

Myelin sheaths: glycoproteins involved in their formation, maintenance and degeneration

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Abstract. Myelin sheaths are formed around axons by extending, biochemically modifying and spiraling plasma membranes of Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS). Because glycoproteins are prominent components of plasma membranes, it is not surprising that they have important roles in the formation, maintenance and degeneration of myelin sheaths. The emphasis in this review is on four integral membrane glycoproteins. Two of them, protein zero (P0) and peripheral myelin protein-22 (PMP-22), are components of compact PNS myelin. The other two are preferentially localized in membranes of sheaths that are distinct from compact myelin. One is the myelin-associated glycoprotein, which is localized at the inside of sheaths where it functions in glia-axon interactions in both the PNS and CNS. The other is the myelin-oligodendrocyte glycoprotein, which is preferentially localized on the outside of CNS myelin sheaths and appears to be an important target antigen in autoimmune demyelinating diseases such as multiple sclerosis.

Key words. Glycoprotein; myelin; myelin-associated glycoprotein; myelin-oligodendrocyte glycoprotein; oligodendrocyte; peripheral myelin protein-22; protein zero; Schwann cell.

Introduction

Myelin sheaths surround large axons in the peripheral and central nervous systems (PNS and CNS, respectively) of vertebrates and facilitate rapid conduction of action potentials while conserving energy required for maintaining ion gradients [1]. In the PNS, the myelin sheath is formed as a spiraled extension of the Schwann cell plasma membrane around the axon, which is compacted to form the typical layered structure of mature myelin (figs 1 A, 2 A). Each Schwann cell forms a single segment of myelin around an axon. These segments of compact myelin are called internodes because they are separated by specialized structures called nodes where ion fluxes across the axonal membrane generate action potentials (fig. 1 B). In the CNS, the myelin-forming cell is the oligodendrocyte, and the myelin sheath is formed similarly as a spiraled extension of its plasma membrane (fig. 1 C). However, the relationship of oligodendrocytes to mature myelin sheaths is more complex because each oligodendrocyte can send out more than one process, each one of which forms a separate internode of myelin around different axons. The ultrastructure of myelin at the electron microscope level is characterized by alternating major dense and intraperiod lines that form when the spiraled cell surface membranes compact (figs 1, 2). Furthermore, although there are continuous membranes from the perikarya of myelin-forming cells to compact myelin, there are many specializations within the membranes which exhibit variability in terms of structure, function and biochemical composition. In addition to the compact myelin itself, the specialized domains include the outer (abaxonal) and inner (periaxonal or adaxonal) membranes of the sheath, lateral loops in the paranodal regions and incisures (fig. 1). When consider-

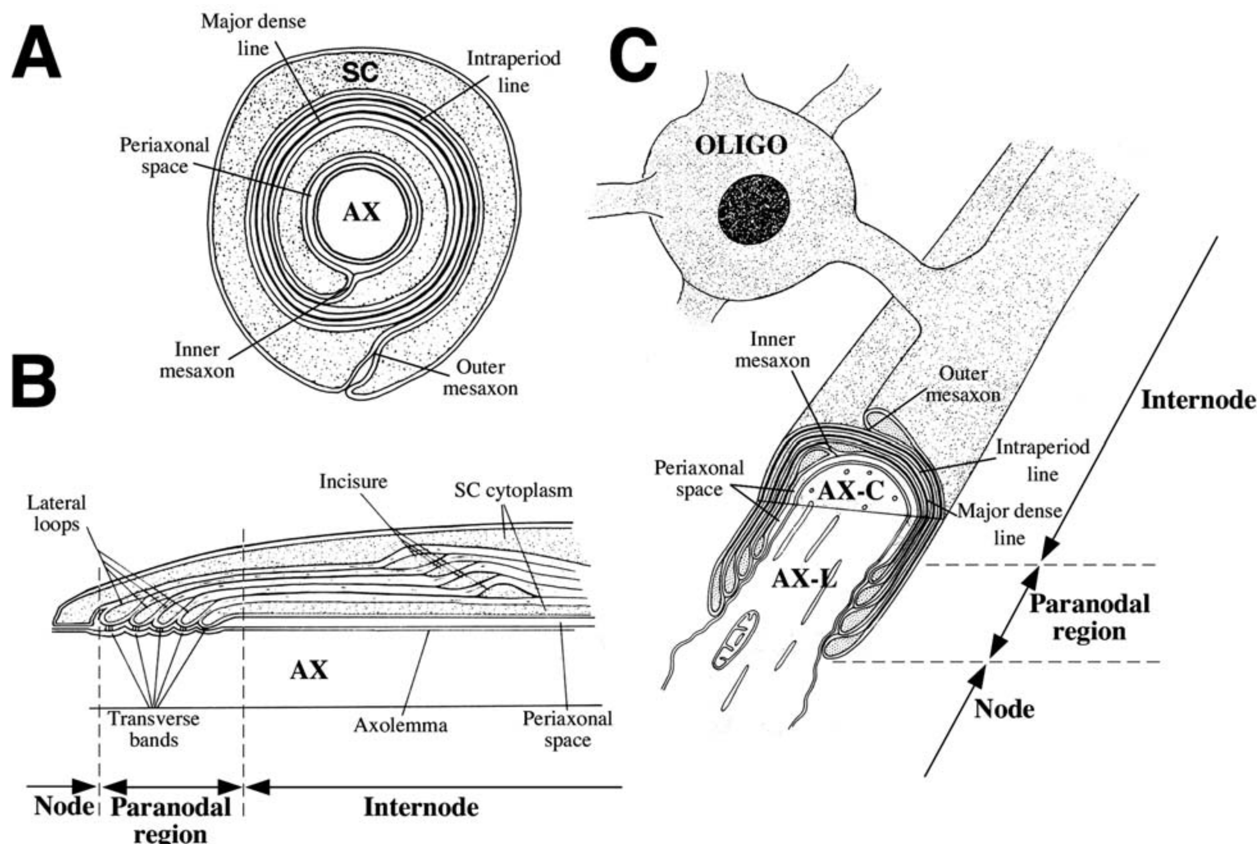

Figure 1. Schematic diagrams of the structures of myelin sheaths. These diagrams show only a few layers of compact myelin for simplification, but mature sheaths have many layers as shown in the micrographs in figure 2. (*A*) In the PNS, the Schwann cell (SC) surrounds the axon (AX), and the mesaxon where the SC plasma membrane comes in contact with itself is spiraled around the axon and tightly layered to form the compact myelin. The major dense lines represent the apposition of the cytoplasmic surfaces of the glial plasma membranes, whereas the intraperiod lines represent the apposition of the extracellular surfaces. Rings of SC cytoplasm (speckled areas) are retained inside and outside the compact myelin even in mature sheaths. The periaxonal space is the extracellular gap between the inner periaxonal SC membrane and the axonal surface membrane. (*B*) Longitudinal perspective of a myelinated axon in the PNS. At the end of each segment of compact myelin (internode) is a node of Ranvier, which is not covered by myelin. Between the node and the internode is the paranodal region with highly specialized structures, where the lateral loops of the Schwann cell form tight junctions (transverse bands) with the axon. The lateral loops and Schmidt-Lantermann incisures are regions containing SC cytoplasm where the membranes are not compacted. (*C*) In the CNS, myelin is formed in a similar way by spiraling of the plasma membrane of the oligodendrocyte (OLIGO), and in this diagram the myelinated axon is shown partly in cross section (AX-C) and partly longitudinal (AX-L). Whereas each SC forms only one internode of myelin, each oligodendrocyte sends out multiple processes each one of which forms an internode of myelin. This composite was adapted from reference [1] with permission of the American Society for Neurochemistry (ASN)/Lippincott Williams and Wilkins.

ing function or pathogenic mechanisms involving myelin sheaths, it is important to distinguish the constituents of compact myelin from those of these other myelin-related membranes in the sheaths. A detailed description of the structure, function and biochemistry of myelin sheaths and their relationship to myelin-forming glia is available elsewhere [1]. The main theme of this review is that because glycoproteins are prominent components of cell surface membranes, they have important roles in the formation, maintenance and degeneration of myelin sheaths.

Overview of glycoproteins in myelin and myelin-related membranes

This review will focus on specific glycoproteins that are important for the developmental process of myelination and for demyelination in neurological disorders. The first part of the review will consider myelin formed by Schwann cells in the PNS, whereas the latter part will consider the more complex situation by which oligodendrocytes form and maintain numerous internodes of myelin in the CNS. The emphasis will be on four integral membrane glycoproteins expressed in myelin internodes, which are either specific for myelin and myelin-forming cells or have been studied primarily in the context of myelination. Two of these, protein zero (P0) and peripheral myelin pro-

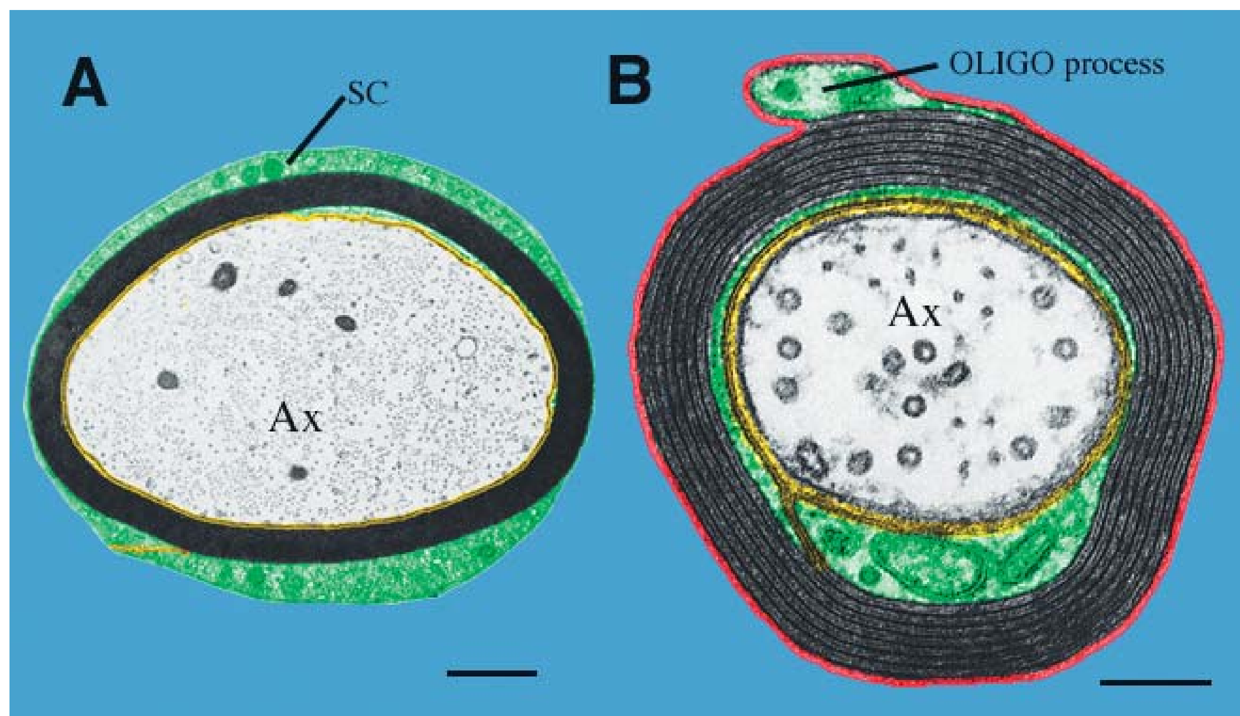

Figure 2. Electron micrographs of PNS (*A*) and CNS (*B*) myelin sheaths color coded to show the locations of glycoproteins. The PNS sheath is much larger than the CNS sheath, so the magnification in (*B*) is over 10-fold greater than in (*A*) (scale bars: *A*, 1.0 μm; *B*, 0.1 μm). The cytoplasm of the Schwann cell (SC) and oligodendrocyte (OLIGO) is highlighted in green, and the end of the OLIGO process leading to the myelin sheath is labeled. P0 and PMP-22 are components of the layered compact myelin (gray) in the PNS (*A*). Neither of these glycoproteins is in compact CNS myelin, in which the major integral membrane protein is non-glycosylated proteolipid protein (PLP). MAG (yellow) is localized in periaxonal Schwann cell and oligodendroglial membranes of both PNS and CNS myelin sheaths, where it projects into the periaxonal space and participates in glia-axon interactions. MOG (red) is specific to the CNS and is localized on the outside surfaces of myelin sheaths and oligodendrocytes, where it is accessible to interact with components of the extracellular environment. These micrographs were reproduced from reference [1] with permission of the ASN/Lippincott Williams and Wilkins, and color coded for the purpose of this review.

tein-22 (PMP-22) are components of compact peripheral nerve myelin. Other prominent proteins of compact myelin are not glycosylated and are reviewed elsewhere [1]. They include myelin basic protein (MBP) in both PNS and CNS myelin, P2 protein in the PNS and proteolipid protein (PLP), which is the major protein of compact CNS myelin. The other two glycoproteins reviewed here are not in compact myelin, but are preferentially localized in internodal membranes of myelin sheaths that are distinct from the compact myelin. The myelin-associated glycoprotein (MAG) is localized in periaxonal Schwann cell and oligodendroglial membrane, where it functions in glia-axon interactions in both the PNS and CNS. The fourth is the myelin-oligodendrocyte glycoprotein (MOG), which is specific to the CNS and appears to be important in the pathogenesis of autoimmune demyelinating diseases such as multiple sclerosis. Another glycoprotein with a similar name to MOG is the oligodendrocyte-myelin glycoprotein (OMgp). It was first characterized as a phosphatidyl inositol-linked glycoprotein in human white matter [2] and subsequently cloned [3, 4]. However, a more recent reference suggests that it is expressed primarily by neurons in the brain [5]. Its function with regard to myelination is unclear at this time, and it will not be considered further here. Comparisons of the locations and structures of the four glycoproteins to be emphasized in this article are summarized in figures 2 and 3, respectively. This review will concentrate on recent developments with regard to these glycoproteins, and more detailed descriptions of earlier research plus original references are available in previous reviews [6–8].

It is also important to note that numerous other glycoproteins, which are not considered in detail here, undoubtedly have important functions related to myelin formation and maintenance. For example, a very active current area of research concerns complex specialized axo-glial junctions in the paranodal regions of myelin sheaths (see figs 1B, C). Several components of these junctions are glycoproteins, including caspr/paranodin, F3/contactin and neurofascin 155. However, these glycoproteins will not be described in more detail here, because this area has been the subject of several excellent recent reviews [9–12]. Numerous well-known, glycosylated adhesion molecules, which mediate cell-cell or cell-substrate inter-

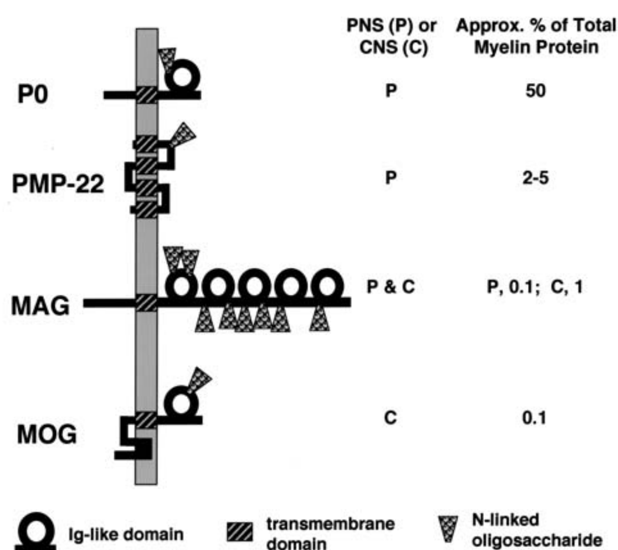

Figure 3. Comparisons of overall structures and expression of glycoproteins. P0, MAG and MOG are all members of the Ig superfamily with one or more Ig-like domains, whereas PMP-22 is in a different family of tetraspan proteins (see text). The extracellular part of MAG is much larger than the other proteins, containing five Ig-like domains and numerous sites for N-linked glycosylation, whereas P0, PMP-22 and MOG have smaller extracellular domains with only one site each for N-linked glycosylation. The proteins of compact PNS myelin (P0 and PMP-22) are present in larger amounts in isolated myelin than MAG and MOG, which are quantitatively minor proteins. However, it should be noted that both are present at substantially higher concentrations in the specialized myelin-related membranes in which they are localized. Also, there is ~10 fold more MAG in CNS sheaths than PNS sheaths, despite the fact that MAG is in incisures and lateral loops in addition to its periaxonal localization in the PNS.

actions in many different cells, are also expressed by myelin-forming glia and appear to have functions related to myelination. These include members of the immunoglobulin (Ig) superfamily such as neural cell adhesion molecule (N-CAM) and L1 [13], cadherins [9, 14] integrins [9, 15, 16] and dystrophin-dystroglycan complexes [9, 17]. The myelin-related functions of these proteins will not be described here, and the reader is referred to the above reviews or key references for more information.

Before describing the individual glycoproteins involved in myelination in more detail, it is necessary to comment on a sulfated carbohydrate structure found in many neural glycoconjugates that reacts with the HNK-1 antibody. This HNK-1 reactive structure has attracted considerable interest both with regard to cell-cell interactions and demyelinating diseases (reviewed in [6, 7]). It is expressed on glycoproteins, glycolipids and glycosaminoglycans, and the epitope involves sulfated glucuronic acid in those glycoconjugates in which its structure has been determined. The HNK-1 antibody was originally shown to react with natural killer cells and identifies an antigen shared by the immune system and the nervous system. In the nervous system, the HNK-1 epitope is expressed primarily on glycoproteins that have been implicated in adhesion, such as N-CAM, L1 and MAG, suggesting that it could have a functional role in cell-cell interactions. Furthermore, HNK-1 and the related L2 antibodies have been shown to block some interactions between neural cells. A functional role for the carbohydrate structure reacting with HNK-1 in peripheral nerve myelination is supported by the identification of a calcium-dependent binding site for this determinant in PNS membranes [18]. The HNK-1 antigen appears to be more important for some nerves than others because it is expressed by Schwann cells of motor nerves in mice, but not sensory nerves [19, 20]. Also, there is substantial variation between species with regard to the amount of the HNK-1 epitope on glycoproteins of nerve, with larger mammals such as humans, other primates, cows and cats generally expressing substantially more than rodents [21]. This species variation could be interpreted to indicate that the HNK-1 structure is not important for function. However, myelin glycoproteins of rodents with low or no HNK-1 reactivity do contain sulfated oligosaccharides [22, 23] that could perform the same function; i.e. the extent of structural similarity needed for conserving HNK-1 immunological reactivity may be more than that needed for retaining adhesive function. Each of the myelin-related glycoproteins reviewed in this article expresses this adhesion-related HNK-1 carbohydrate epitope in some species.

P0 glycoprotein, the major protein of PNS myelin

P0 is a 30-kDa glycoprotein that accounts for over half the total protein in compact PNS myelin. It was first identified as the major protein stained band on SDS gels of purified PNS myelin [24], and subsequently shown to be glycosylated by incorporation of radioactive sugars and periodic acid-Schiff staining [25, 26]. It is a type I transmembrane glycoprotein with a single extracellular Ig-like domain and one site for N-linked glycosylation (fig. 3). Recent detailed characterization of the oligosaccharide moieties of bovine P0 by mass spectrometry and nuclear magnetic resonance spectroscopy [27] extend and confirm earlier studies (reviewed in [8]and [28]) indicating that the glycans at the single glycosylation site are very heterogeneous. Many are sulfated and include oligosaccharides terminating in sialic acid or sulfated glucuronic acid. The sulfated glucuronic acid on P0 is a key part of the HNK-1 carbohydrate epitope that has been implicated in cell-cell adhesion [29]. In addition, there are developmental changes in the composition of the oligosaccharides on P0 [30] that could be important for its function in myelination.

P0 is believed to stabilize the intraperiod line of compact PNS myelin by homophilic interactions (fig. 4) [1]. The capacity of P0 to interact with itself was first demon-

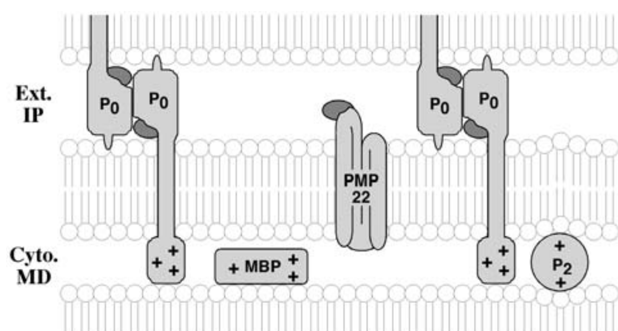

Figure 4. Schematic representation of current concepts about the molecular organization of compact PNS myelin. The apposition of the extracellular (Ext.) surfaces of the Schwann cell membranes to form the intraperiod line (IP) is shown in the upper half of the figure. The apposition of the cytoplasmic (Cyto.) surfaces of the membranes to form the major dense line (MD) is shown in the lower part of the figure. See text for summary of experimental evidence indicating that P0 stabilizes the intraperiod line by homophilic interactions. The blip at the apex of P0 represents a tryptophan residue, which X-ray analysis suggests might interact with the apposing bilayer, although the expected cis tetramerization of P0 is not shown for diagramatic simplification. The dark ovals on P0 and PMP-22 represent the oligosaccharide moieties. In view of the much lower amount and smaller extracellular domains of PMP-22 in comparison to P0, it may have a more dynamic function in the compact myelin rather than being an important structural component. The positively charged cytoplasmic domain of P0 also appears to play an important role in stabilizing the major dense line together with the positively charged extrinsic proteins, MBP and P2 protein. This figure was adapted from reference [1] with permission of the ASN/Lippincott Williams and Wilkins.

strated by the aggregation and homotypic interactions of nonneural cells transfected with P0 [31–33]. The homophilic interactions of P0 require glycosylation of the protein [34, 35]. Since the oligosaccharide moiety of P0 appears to be localized at the base of the extracellular domain near the lipid bilayer of the membrane (fig. 4), one function may be to stabilize the orientation of the protein in such a way as to promote its homophilic interactions [36]. However, other evidence suggests that part of the homophilic binding is due to protein-carbohydrate interactions between adjacent P0 molecules, some of which involve the adhesion related HNK-1 epitope [37, 38]. Overall, the evidence suggests that both protein-protein and protein-carbohydrate interactions are involved in the homophilic binding of P0, and the binding may occur both in a trans and cis manner with regard to the P0-containing membranes. Furthermore, the cytoplasmic domain of P0 is also important for the extracellular domain to exert its adhesive properties in cell-cell interactions, probably by interacting with the cytoskeleton [39, 40]. The crystal structure of the extracellular domain of P0 indicates that it probably exists on the membrane surface as a tetramer that can link to other tetramers from the apposing membrane to form an adhesive lattice [41].

Although much of the research on P0 has focused on the role of its extracellular domain in the formation and maintenance of the intraperiod line as discussed above, it is generally thought that its positively charged cytoplasmic domain also plays an important role in stabilizing the major dense line of compact PNS myelin (fig. 4) [1]. This is in contrast to the CNS, where it is well established that MBP is the most important component for stabilizing the major dense line. Part of the evidence for this difference is that although the major dense line does not compact normally in CNS myelin of mutant Shiverer mice that lack MBP, the structure of PNS myelin is relatively normal in these mutants. Also, there is substantially less MBP in PNS myelin than CNS myelin. Furthermore experimental data indicate that the cytoplasmic domain of P0 can induce membrane-membrane interactions by binding to acidic phospholipids such as phosphatidyl serine [42]. This interaction with phospholipids may be modulated by phosphorylation of the cytoplasmic domain [43]. On the other hand, a comparison of mice singly or doubly deficient for P0 and/or MBP indicates that both proteins contribute to the structure of the major dense line in PNS myelin [44]. It is likely that both the positively charged cytoplasmic tail of P0 and MBP can interact with acidic lipids to contribute to the stability.

Expression of antisense P0 messenger RNA (mRNA) in cultured Schwann cells impairs their capacity to myelinate dorsal root ganglion neurons in vitro [45]. The importance of P0 for stabilizing compact myelin in vivo is illustrated most clearly by the severe phenotype of P0-negative mice generated by homologous recombination [46]. These mice exhibit abnormal motor coordination, tremors, occasional convulsions and a severe hypomyelination. Furthermore, expression of the correct amount of P0 is also essential for normal myelin formation and maintenance. Young mice heterozygous for the P0-null mutation appear normal, but develop progressive demyelination with age which resembles chronic inflammatory demyelinating neuropathy and may involve autoimmune mechanisms [47, 48]. Furthermore, transgenic mice overexpressing P0 exhibit a dose-dependent dysmyelinating neuropathy ranging from a transient hypomyelination to a severe arrest of myelination and impaired sorting of axons [49]. The critical dosage of P0 required for normal myelin formation is similar to observations with other myelin proteins and may reflect the necessity for appropriate amounts of myelin proteins to form stoichiometric complexes in compact myelin. However, in the case of P0, the pathology that occurs with overexpression may also reflect a mistargeting of the protein and an interesting misuse of its obligate homophilic adhesive properties. Some of the extra P0 is inappropriately located in normally dynamic mesaxonal membranes, causing them to adhere like compact myelin and halting myelination [50].

It is well established that expression of most of the P0 in peripheral nerve during development parallels the accumulation of myelin, which is consistent with its important structural role in compact myelin. However, it should be noted that low basal levels of P0 are expressed in Schwann cells and neural crest cells early in embryonic development well before myelination, suggesting that P0 could be an early marker of the glial lineage and perform other functions that are unrelated to myelination. Because this review focuses on myelin, this early expression and other potential functions for P0 will not be covered comprehensively here, but are reviewed and discussed in more detail elsewhere [51, 52]. The other potential functions include Schwann cell-axon interactions and signal transduction. Indeed, P0 has been shown to promote neurite outgrowth [33, 53], and there is axonal degeneration in addition to dysmyelination in P0-null mice [46, 54]. The cytoplasmic domain of P0 is phosphorylated on serine and tyrosine residues [55, 56], and this might be indicative of signaling mechanisms within Schwann cells during early development as well as later during myelination.

Much research has been done to elucidate factors controlling expression of P0, both the low basal levels and the substantial upregulation associated with active myelination or remyelination. This regulation, which is reviewed elsewhere [51, 52, 57], is complex, involving interactions with the axon and basal lamina, rate of cell division, inhibitory and stimulatory growth factors, cyclic AMP (cAMP) levels and transcription factors. The massive upregulation of P0 by Schwann cells during active myelination appears to involve largely unknown signals from the axon, interaction with the basal lamina and elevated intracellular cAMP.

As the major protein of PNS myelin, P0 is generally reduced in a wide variety of neurological disorders with decreased myelin in peripheral nerve regardless of the cause. However, P0 has also been implicated directly in the pathogenesis of some inherited and acquired human neurological diseases. There are a growing number of reports of inherited human neuropathies affecting myelin in which the P0 gene is mutated [48, 58, 59]. These include severely affected Dejerine-Sottas patients and the less severely affected Charcot-Marie-Tooth (CMT) type 1 B patients. Research on animal models in which the expression of P0 has been genetically modified provide important clues about the pathogenesis of these human disorders [59, 60].

P0 may also be involved in immune-mediated neuropathies, because the protein or peptides derived from it can cause experimental allergic neuritis (EAN) [61–63]. Furthermore, antibodies or T cells sensitized to P0 have been detected in patients with inflammatory neuropathy [64, 65]. P0 has also been implicated as one of the target antigens in a form of autoimmune neuropathy associated with IgM gammopathy, in which the antibodies bind to the carbohydrate HNK-1 epitope shared by MAG and other neural glycoconjugates [66] (see later section on MAG). However, not all of the anti-MAG IgM antibodies in these patients cross react strongly with P0 [67], and it is not clear how important the reactivity with P0 is for the pathogenesis of this disorder.

PMP-22

Similarly to P0, PMP-22 is a glycoprotein of compact PNS myelin with a single site for N-linked glycosylation. However, it has a very different overall structure from P0 and accounts for less than 5% of total PNS myelin protein. This 22-kDa glycoprotein has four hydrophobic, potential transmembrane domains and appears to be a tetraspan protein (fig. 3) like the major PLP of CNS myelin, but there is no sequence homology to PLP. It was originally identified biochemically as a glycoprotein in PNS myelin called PAS-II [68], but has gained much prominence since its cloning under the name PMP-22 (see [69] and [70] for detailed reviews]). Also, unlike P0, which is nerve specific, PMP-22 is expressed in other tissues including lung, gut and heart. PMP-22 was cloned as a gene whose expression is downregulated, similar to other myelin proteins, in association with Schwann cell proliferation following nerve transection. PMP-22 mRNA codes for the same protein as a growth arrest-specific mRNA (gas-3) that had been cloned previously from fibroblasts. It is in a highly homologous family of small hydrophobic tetraspan proteins that also include epithelial membrane proteins (EMP-1, -2 and -3) [71]. Actually, there are a growing number of tetraspan proteins in addition to PMP-22 and PLP in myelin and myelin-forming cells (reviewed in [72]). The oligosaccharide moieties on PMP-22 are of the complex type [73] and in some species include the adhesion-related HNK-1 carbohydrate epitope [74, 75]. The structure of a major HNK-1 reactive oligosaccharide on bovine PMP-22, containing a bisecting *N*-acetylglucosamine (GlcNAc) and sulfated glucuronic acid, is the same as a major oligosaccharide of P0 [73]. The stability of oligomers formed by a mutant form of PMP-22 lacking the carbohydrate moiety is less than those formed by the wild type [76]. Although the implications of this difference related to glycosylation are not known for certain, it might affect cellular transport or function of the protein.

PMP-22 has received much attention since its cloning because abnormalities of its gene cause the dysmyelinating phenotypes in trembler mice and several neuropathies in humans (see below). Therefore, it is clear that this protein must play a crucial role in the formation or maintenance of myelin. The trembler and less severe trembler-J murine phenotypes are caused by two different dominant point mutations in PMP-22 and are characterized by hy-

pomyelination, continued Schwann cell proliferation and partial paralysis. However, because PMP-22 is quantitatively a relatively minor component of compact myelin, it seems unlikely that it plays a major structural role like P0 glycoprotein, and it may have a more dynamic function. On the other hand, its primary localization within compact myelin suggests that an important aspect of its function occurs at this location. Furthermore, PMP-22 has been shown to form complexes with P0 in myelin membranes, and this interaction with P0 may be relevant to its function [70, 77]. The association of PMP-22 with growth arrest in Schwann cells and other cell types suggests that it may have an unknown role in regulation of growth or differentiation of Schwann cells.

The human neuropathies caused by abnormalities of the PMP-22 gene include CMT disease type 1A, which is usually caused by duplication of the gene and sometimes by point mutations. CMT1A usually has an onset in the 2nd or 3rd decade of life and is characterized by segmental demyelination and remyelination. A milder hereditary neuropathy with liability to pressure palsies is brought on by pressure or trauma to an affected nerve, and is caused by heterozygous deletion of the PMP-22 gene. These human neuropathies caused by over- and underexpression of PMP-22 [69, 70, 78], respectively, point to the fact that correct dosage of this protein is necessary for normal formation and maintenance of myelin. The importance of dosage is also clearly shown by a variety of genetically engineered mice expressing different amounts of PMP-22 and serving as useful models for the human neuropathies (see [69] for review). Studies on murine mutants and the human neuropathies suggest that an important aspect of the pathology may involve abnormal accumulation of PMP-22 in the Schwann cell endoplasmic reticulum rather than its being transported to the myelin membrane (reviewed in [69] and [70]).

PMP-22 may also be important as an antigen in immunologically mediated neuropathies. Immunization of Lewis rats with PMP-22 causes EAN [79]. Furthermore, antibodies to PMP-22 have been detected in a high proportion of patients with acute and chronic inflammatory neuropathies [80, 81]. The antibodies were also detected in patients with both CMT1 and CMT2 suggesting that secondary immune responses could contribute to inherited neuropathies. Also, because PMP-22 contains the HNK-1 epitope, it could be a target in the human autoimmune demyelinating disorder associated with anti-MAG antibodies [67, 81]. It is possible that immunological perturbation of the function of PMP-22 could have consequences similar to the genetic defects. However, one report indicates that PMP-22 in its natural endogenous site in the membranes is not accessible to antibodies, so the role of humoral immunity to PMP-22 in pathological changes remains to be established [81].

MAG

Another glycoprotein expressed by Schwann cells and located in myelin sheaths is MAG, but it is not in compact myelin as are P0 and PMP-22. Rather, it is concentrated in periaxonal Schwann cell membranes (fig. 2A) and other specialized structures in PNS myelin sheaths (see below). Also unlike P0 and PMP-22, it is expressed by oligodendrocytes in the CNS where it is similarly located in periaxonal glial membranes (fig. 2B) and absent from compact myelin. More detailed reviews of the earlier MAG literature are available [7, 82]. The term 'myelin-associated' was included in its name to reflect the fact that it is localized in glial membranes that are distinct from the compact myelin itself. It is a quantitatively minor component of isolated myelin accounting for less than 1% of the total protein and was originally detected by sensitive metabolic labeling experiments with radioactive fucose [83]. However, MAG is present at a substantially higher concentration in the periaxonal and other specialized glial membranes in which it is localized. This has been demonstrated biochemically by the use of centrifugation conditions and gradients designed to isolate myelin-related glial membranes enriched in MAG [84–86], which are normally discarded in standard methods for isolating large fragments of lipid-rich, compact myelin [87].

MAG is a 100-kDa glycoprotein with five extracellular Ig-like domains, a single transmembrane domain and a cytoplasmic domain that occurs in two developmentally regulated forms due to alternative mRNA splicing (figs 3, 5). The extracellular domain of MAG has eight sites for N-linked glycosylation and contains about 30% by weight carbohydrate. The oligosaccharides are very heterogeneous. Most are of the complex type and negatively charged due to sialic acid and/or sulfate, and many are bisected by GlcNAc. Like P0 and PMP-22, MAG in many species contains the adhesion-related, HNK-1 carbohydrate epitope.

The selective localization of MAG in the periaxonal membranes of myelin-forming Schwann cells and oligodendrocytes (fig. 2), as well as its belonging to the Ig superfamily, strongly suggests that it functions in glia-axon interactions. Early evidence supporting a role for MAG in glia-axon interactions included inhibition of neuron-oligodendrocyte adhesion by an anti-MAG antibody and the specific binding of MAG-containing liposomes to neurons. As with other members of the Ig superfamily involved in cell-cell interactions, MAG is thought to be involved in transmitting signals between cells in addition to having a simple adhesive role. The evidence supporting such functional roles for MAG has increased substantially in recent years as summarized below and represented schematically in figure 5. Actually, MAG may participate in signaling in both directions between axons and glia. It has long been thought that MAG was likely to be involved

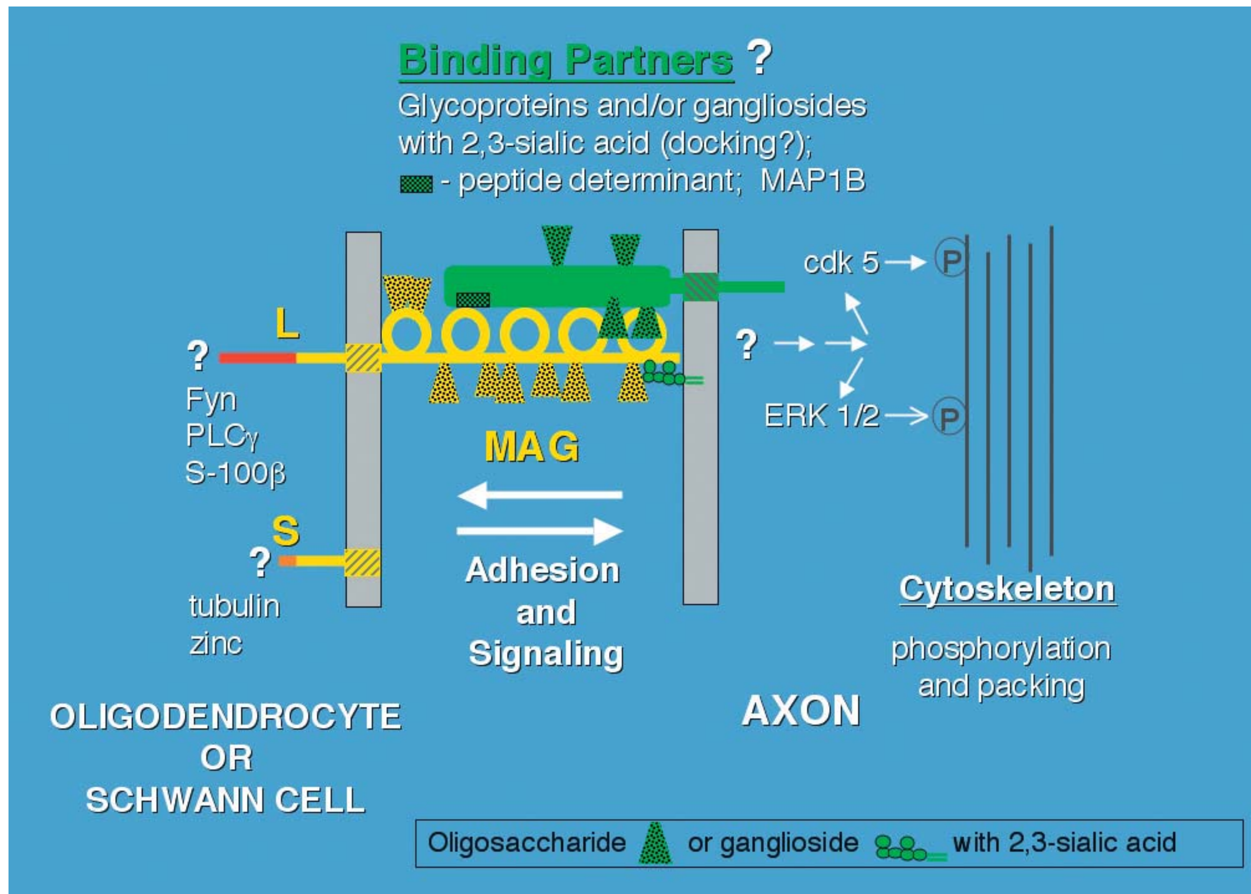

Figure 5. Schematic summary of biochemical mechanisms by which MAG may mediate interactions and signaling between myelin-forming cells and axons. The question marks in the figure indicate that little is known for certain about the other molecules that are involved in the function of MAG (yellow), although some experimental evidence for involvement of the components shown has been reported (see text). The extracellular domain of MAG mediates axon-glia interactions by binding to an unknown component(s) (green) on the axonal surface membrane. Because MAG has lectin properties and binds to oligosaccharides with 2,3-SA, its axonal binding partner is likely to be glycoprotein or ganglioside. However, some evidence suggests that the carbohydrate binding reflects a docking mechanism and that interaction with a specific peptide determinant (checkered rectangle) is needed for signal transduction. A novel form of MAP1B on the axonal surface is one specific protein that has been shown to interact with MAG. In the PNS, the primary role of MAG in the Schwann cell periaxonal membrane appears to be as a ligand that binds to an axonal receptor, which activates a signal transduction cascade that is necessary for the normal maintenance of myelinated axons. The signaling causes increased phosphorylation of neurofilaments by cdk5 and ERK 1/2, leading to greater axonal caliber. In the CNS, the most important signaling appears to be in the reverse direction with MAG acting as a receptor to enhance the vitality of oligodendrocytes and their capacity to form and maintain myelin. The different C-termini of the L and S isoforms of MAG are shown in red and orange, respectively, and may interact selectively with the various glial components shown as part of MAG's function.

in glial recognition of axons ready to be myelinated and serve as a receptor for an axonal signal that promotes myelination. Although some findings do support such a role, especially in the CNS, more recent research has indicated that a very important function of MAG is to act as ligand for a receptor on the axonal surface of PNS axons that affects axonal properties. In PNS myelin sheaths, but not CNS sheaths, MAG is also present in Schmidt-Lanterman incisures, lateral loops, and the inner and outer mesaxons. This suggests that MAG may have a function in addition to axon-glia interactions which is related to its location in these membranes associated with pockets of Schwann cell cytoplasm.

Research on two lines of MAG knockout mice have substantially influenced and altered concepts of MAG function [88, 89]. Another relatively recent review on MAG [90], which emphasizes these MAG-null mice, includes representative electron micrographs that illustrate some of the key points described here. These mice exhibit subtle structural abnormalities in the periaxonal region of myelin sheaths, supporting a role for MAG at the glia-axon junction. However, they myelinate relatively normally, showing that MAG is not essential for myelination. Behavioral studies on MAG-null mice have produced variable results [88, 89, 91], but as a whole they suggest some functional cognitive and/or locomotor deficits. Al-

though the formation of compact myelin in the knockouts is largely unaffected, there are some other abnormalities in addition to the changes in periaxonal structure. In the CNS, these include aberrant or redundant myelin loops, supernumerary myelin sheaths and a significant delay of myelination [88, 89, 92–94]. Thus in the absence of MAG, some oligodendrocytes do not seem to be efficient at determining when, where and how much myelin to form. Furthermore, there is degeneration of periaxonal oligodendroglial processes in 8-month-old MAG-null mice, suggesting the occurrence of a dying-back oligodendrogliopathy [90, 95]. Biochemical studies on the brains of 14-month-old MAG-null mice [96] revealed significant reductions in several oligodendroglial proteins consistent with an oligodendrogliopathy, although the proteins of compact myelin were not significantly decreased, indicating relatively normal levels of compact myelin. Furthermore, significant reductions of the oligodendroglial proteins were present as early as 2 months, indicating that the oligodendroglial abnormalities begin at a relatively early age. Overall, the CNS findings suggest that the absence of MAG causes oligodendrocytes to form myelin less efficiently during development and become dystrophic with aging. It may be that MAG-mediated signaling from axons to oligodendrocytes provides a trophic signal needed for efficient myelination and maintenance of healthy mature oligodendrocytes.

Myelin formation in the PNS during development seems to be more normal in the absence of MAG. However, as the mice age they exhibit a neuropathy characterized by degeneration of myelinated axons [97–99]. The amount of compact myelin in the nerves appears normal by morphological and biochemical criteria. Rather, the pathology is associated with decreased axonal caliber, increased neurofilament density, reduced expression and phosphorylation of neurofilaments, and eventually axonal degeneration [98, 99]. Because neurofilament phosphorylation is thought to be important for determining neurofilament spacing and axonal caliber [100], these findings suggested an essential role for MAG in signaling from Schwann cells to axons that is necessary for the maintenance of normal myelinated axons of the PNS. Electrophysiological evaluation of 1-year-old MAG-null mice revealed a mild, but statistically significant, reduction of conduction velocity and a nonsignificant mild decrease in compound muscle action potential amplitudes [99]. This constellation of findings is consistent with an axonopathy rather than a demyelinating neuropathy. In contrast to the PNS, axons appear to be normal in the CNS of aging MAG-null mice [91, 95] [M. D. Weiss and R. H. Quarles, unpublished observations]. Overall, the studies on the knockout mice have indicated that the most important functions of MAG are different in the CNS and PNS. In the CNS its primary role appears to be in signaling from axons to oligodendrocytes to promote myelin formation and oligodendroglial health, whereas in the PNS it is essential for signaling from Schwann cells to axons that is needed for the normal maintenance of myelinated axons.

Although the neurological deficit in MAG-null mice is mild, double knockouts in which the absence of MAG is combined with the genetic ablation of other proteins results in more severe phenotypes than either knockout alone and have provided further insights into MAG function. One example is mice in which both MAG and Fyn tyrosine kinase are absent [101]. It is well established that Fyn has an important role in the formation of myelin in the CNS, and the CNS of Fyn-null mice is hypomyelinated. However, in the absence of both Fyn and MAG, there is a much more severe hypomyelination of the CNS. This may relate to the report of a direct Fyn-MAG interaction in signal transduction [102] (see later), but could also be explained by independent complementary roles for these two proteins in myelination. Another example, in which combining a MAG deficiency with the absence of another oligodendroglial component exacerbates CNS pathology, is the MAG/UDP-galactose:ceramide galactosyl transferase (CGT) double knockout [103]. CGT-null mice are unable to synthesize galactocerebroside (GalC) or sulfatide, which are characteristic lipids of oligodendrocytes and myelin. Similarly to the MAG-null mice, myelination is not dramatically impaired in these mutants, but they exhibit more severe pathology in the CNS with aging and die at about 90 days. Particularly noteworthy are defects in axo-glial interactions such as splitting of the periaxonal space along the internode and also a severe disorganization and breakdown of the tight glia-axon junctions in the paranodal regions (see fig. 1). The normal developmental increase in the amount of the small MAG isoform relative to the large isoform in the CNS (see later) is impaired in the CGT knockouts [104]. In MAG/CGT double knockouts, the defects of axon-glia interactions are much more severe, and the degeneration in the paranodal region progresses much more rapidly leading to death by about 3 weeks of age. Interestingly, careful examination of the paranodal region in the CNS of single MAG knockouts revealed mild degeneration of these structures, but they did not progress to the extent that occurs in the CGT single knockout or the double knockout. It is of interest that the paranodal junctions in the PNS of CGT and MAG/CGT double knockouts are only mildly affected. Thus there appears to be some overlap of function between MAG and galactolipids in glia-axon interactions of the CNS, despite the very different structure and properties of these molecules. Furthermore, because MAG is localized in the internodal periaxonal membrane, it is easy to understand how its absence contributes to splitting at this location. However, it is not in the paranodal region of the CNS where the degeneration of the loops and tight junctions occur, so it is harder to understand its contribution to the pathology at this location. It is feasible that

weakened paranodal interactions in the absence of the glycolipids alone deteriorate earlier because of elevated physical stress caused by reduced adhesion along the internode in the absence of MAG in the double knockout. Alternatively, if MAG-mediated signaling provides trophic support that increases the vigor of oligodendrocytes in general, as suggested above, its absence may exacerbate the structural or functional defects caused by a variety of other deficiencies such as Fyn or galactolipids. Interestingly, culturing oligodendroblasts on a substratum containing N-CAM or MAG itself increased both cell survival and the generation of myelin-like membranes [105]. The effect of MAG suggests the possibility of autotypic MAG-mediated signaling between adjacent, loosely spiraled, oligodendroglial membranes that enhances the myelination process. Similarly, there is evidence to support the possibility of autotypic contact-mediated promotion of PNS myelinogenesis by adjacent loosely spiraled Schwann cell membranes [106, 107].

Double knockouts have also provided insights about MAG's function in the PNS. For example, careful comparison of the spiraled, uncompacted Schwann cell membranes in the P0 knockout and the P0/MAG double knockout suggests that MAG has a functional role in the spiraling process that cannot be duplicated fully by other molecules [108]. Also, axonal degeneration in peripheral nerves of MAG/N-CAM double knockouts begins about 4 weeks earlier than in MAG single knockouts [109], indicating that N-CAM can partially substitute for MAG in maintaining the integrity of myelinated axons of the PNS. As mentioned above, similar axonal degeneration has not been observed in the CNS of MAG-null mice. Moreover, it appears that PLP is particularly important for CNS axon maintenance because there is axonal degeneration with aging in PLP-null mice. However, it is of interest that degeneration of CNS axons begins earlier in the PLP/MAG double knockout than in the PLP single knockout, suggesting that MAG does have some role in promoting axonal stability in the CNS [91].

The abnormalities of myelinated axons of the PNS that occur in vivo in the absence of MAG strongly suggest that MAG is part of signal transduction systems that mediate axon-glia interactions. However, the structural disruption of the periaxonal region in myelinated axons of MAG-null mice raises the possibility that a general loosening of the Schwann cell-axon junction in vivo, caused by the absence of MAG, could interfere with other signaling systems in which MAG does not participate directly. In order to circumvent that disadvantage of in vivo studies, in vitro models were used in which dorsal root ganglion neurons or PC12 cells were cocultured with MAG-transfected COS cells or treated with a soluble MAG-Fc chimera [110]. The presence of MAG resulted in increased expression and phosphorylation of neurofilament subunits and microtubule-associated proteins by the neurons. Furthermore, the decreased phosphorylation of neurofilaments in vivo in the absence of MAG is associated with decreased activities of two proline directed kinases, cyclin dependent kinase-5 (cdk5) and extracellular signal regulated kinases 1 and 2 (ERK 1/2), and both of these kinases were upregulated in vitro in the presence of MAG. These in vitro results substantially reinforce the hypothesis that MAG itself is a component of a signaling system that affects the axonal cytoskeleton (see fig. 5).

Another active area of recent research demonstrating the capacity of MAG to affect neuronal properties concerns its identification as one of the principal components of CNS white matter that may inhibit neuronal regeneration following neural injury. This area has been reviewed elsewhere [111, 112] and will not be considered in detail here. Suffice it to say that MAG can promote or inhibit the outgrowth of neurites in vitro depending on the intrinsic state of the neuron, including factors such as cyclic nucleotide levels. The findings are consistent with a MAG-mediated signaling mechanism that could be important for the normal maintenance of myelinated axons. A physiologically important signal promoting the stability of mature myelinated axons could be interpreted inappropriately by a plastic developing neurite in vitro or a regenerating neurite in vivo, thereby inhibiting its growth. Thus, information obtained about the molecular mechanisms involved in MAG's effects on neurite outgrowth may be relevant to its physiological role in the maintenance of myelinated axons. Whether or not the inhibition of neurite outgrowth by MAG is a significant factor in regeneration in vivo following neural injury has been controversial, as reviewed in [112]. In normal CNS tissue, MAG is sequestered in the periaxonal space and would not be expected to affect neurite outgrowth, but it may be released during degeneration following tissue injury so it could be accessible to regenerating neurons. Indeed, a proteolytic derivative of MAG (dMAG) [113], consisting of its soluble extracellular domain [114, 115], is released in some neurological disorders [116] and has been shown to inhibit neurite outgrowth in vitro [117].

The capacity of myelination to increase the caliber of axons in internodes underlying compact myelin has been known for many years [100]. Evidence for an effect of myelination on axonal caliber includes comparisons of myelinated and unmyelinated regions of the same axon [118] and studies on dysmyelinating mutants. It was first shown by grafting segments of hypomyelinated trembler sciatic nerve into normal host sciatic nerve and showing that reduced axonal caliber was restricted to the local hypomyelinated trembler environment [119]. Later studies [120, 121] revealed that the reduced axonal caliber in the absence of compact myelin was associated with an increased packing density of neurofilaments and decreased neurofilament phosphorylation. At that time, it was proposed that this effect could be mediated by the binding of

a Schwann cell ligand at the inside of myelin sheaths to an axonal receptor, and it was even suggested that MAG was a candidate ligand because of its periaxonal localization [121]. Clearly, the more recent findings on MAG-null mice and the effects of MAG on neurons in vitro support this suggestion.

A very important unanswered question about MAG is the identity of the putative axonal binding-partner(s) with which it interacts (fig. 5). Schwann cells appear to upregulate the MAG-binding partner on sensory neurons in culture by production of nerve growth factor [122]. An important clue about its identity emerged from the determination that MAG is in a subgroup of the Ig superfamily, whose members exhibit high homology in the first two amino-terminal V and C2 type Ig-like domains and bind to sialic acid-containing oligosaccharides [123]. This is the 'siglec' family for 'sialic acid-binding immunoglobulin-like lectins' [124], and members differ in their affinity for sialyloligosaccharides depending on the carbohydrate configurations. There are now at least 10 members of this family, including sialoadhesin, CD22 and CD33, most of which are expressed primarily on cells of the immune system [125]. MAG (Siglec-4 a) was reported to bind best to oligosaccharides with α2,3-linked sialic acid (2,3-SA) on a core structure of Galβ1-3GalNAc [123]. As such, MAG showed a preference for binding to O-linked oligosaccharides on glycoproteins and to some gangliosides, such as the major GD1 a and GT1 b brain gangliosides [123, 126] as well as some minor ones [127, 128]. However, an investigation of glycoproteins in a neuroblastoma cell line that bound to MAG demonstrated that most binding was to N-linked oligosaccharides with terminal 2,3-SA, so the reported specificity for glycoproteins with O-linked oligosaccharides is not absolute [129]. Other detailed studies of the specificity of MAG binding have been reported [130–132]. In general, however, the fact that MAG binds to oligosaccharides on both glycoproteins and gangliosides indicates that there are likely to be many binding partners on the axolemma. Furthermore, the physiologically functional glycoconjugate binding partner(s) for MAG has not yet been identified with certainty. A body of evidence suggesting that gangliosides are the functional binding partners was summarized recently [133]. A particularly interesting aspect of this argument is that mice engineered to lack complex gangliosides exhibit some of the same abnormalities of myelinated axons as MAG-null mice [134]. Some evidence also suggests that MAG binding to gangliosides is involved in inhibition of neurite outgrowth [133]. For example, antibody cross-linking of GT1 b ganglioside on the surface of neurites inhibits outgrowth similarly to MAG via activation of Rho kinase in the neurons [135]. However, other results suggest that the receptor mediating this effect is a sialoglycoprotein [136]. Furthermore, there are indications that sialic acid-binding by MAG may reflect only a docking mechanism and that a separate peptide binding site on MAG is crucial for the signaling that inhibits outgrowth [137]. A substantial number of neuronal membrane glycoproteins have been shown to interact with MAG [129, 136, 138], but these studies do not show which are physiologically important. Recently, a novel glycosylated form of microtubule-associated protein 1 B (MAP1 B) expressed at the surface of axonal membranes [139] was identified as a binding partner for MAG [140], which could be relevant to the effects of MAG on the microtubular cytoskeleton and stability of myelinated axons.

The binding partners discussed above are those on neuronal membranes that could interact with MAG in the periaxonal glial membranes, but MAG localized in mesaxons, lateral loops and incisures of Schwann cells may interact with the same or different molecules on adjacent Schwann cell membranes. These structures are very dynamic during the process of active myelination, and there is evidence from dysmyelinating mutants that MAG in these locations may modulate the plasticity of these membranes (see later). Also, MAG has been shown to bind to extracellular matrix components such as heparin [141], collagen [142, 143], tenascin-R [144] and fibronectin [145]. However, the physiological relevance of its binding to components of the extracellular matrix is not clear, because most MAG is localized in sequestered periaxonal and other spiraled glial membranes. On the other hand, a small amount of MAG has been detected in the extracellular matrix of sciatic nerve [146], and this may represent the soluble extracellular domain of MAG formed by proteolysis (see above). Therefore binding to these molecules could be relevant to MAG's effect on neurite outgrowth, and tenascin-R was shown to neutralize the inhibition of neurite outgrowth by MAG [144].

As described above, there is a substantial amount of evidence suggesting that MAG also is involved in signaling from axons to glia that influences their capacity to form and maintain myelin sheaths, especially in the case of oligodendrocytes. In this situation, signal transduction is likely to involve the cytoplasmic tail of MAG, which occurs in two developmentally regulated forms in rodents due to alternative splicing [7]. The mechanism of alternative splicing results in two isoforms that share a common sequence of 37 amino acids adjacent to the inner surface of the plasma membrane in their cytoplasmic domains, but differ at the C-terminal tails (fig. 5). The large isoform (L-MAG) has a unique sequence of 53 amino acids at the C-terminus, whereas the small isoform (S-MAG) has a different 9-amino acid sequence at the C-terminus. In the CNS, L-MAG is the predominant form early in myelinogenesis, whereas S-MAG increases with maturation, so the two isoforms are present in approximately equal amounts in adults. In the PNS, S-MAG is the predominant form at all ages, although small amounts of L-MAG are present early. Furthermore, the phenotype of mice engi-

neered to lack L-MAG, because of truncation of the cytoplasmic domain, are consistent with a primary importance of L-MAG for the CNS [147]. The CNS of these mice lacking L-MAG exhibits the same abnormalities as in total MAG knockouts, but they do not show the degeneration of myelinated axons that occurs with aging in the PNS of total knockouts. Also, the isoforms have been reported to differ with regard to the types of oligodendrocytes in which they are expressed in adult rat anterior medullary velum [148]. Type I and II oligodendrocytes, which myelinate numerous relatively small axons, express both isoforms. However, only S-MAG was detected in type III and IV oligodendrocytes, which are larger and have only a few processes that myelinate larger axons.

It is likely that the cytoplasmic domains of the two isoforms of MAG differ in their interactions with components in glial cytoplasm. Indeed, numerous examples of such cytoplasmic interactions have now been reported and include proteins that are involved in signal transduction and cytoskeletal structure (fig. 5). The cytoplasmic binding partners for L-MAG include Fyn tyrosine kinase [102, 149], phospholipase Cγ [149] and S-100β [150, 151], whereas S-MAG interacts with tubulin [152] and zinc [153]. The cytoplasmic domains of both S- and L-MAG are phosphorylated [reviewed in [8], and this may modulate the interactions of MAG with components of the glial cytoplasm. Protein kinase C appears to be especially important for the phosphorylation of MAG on serine and threonine moieties. Phosphorylation of a consensus sequence for protein kinase A in the cytoplasmic domain of L-MAG may be downregulated by its interaction with S-100β [151]. The interaction of MAG with an SH2 domain in phospholipase Cγ requires phosphorylation of tyrosine 620, which is specific for L- MAG [149]. The interaction with Fyn appears to be mediated by a different mechanism, but is also strongest for the L-MAG isoform [102, 149]. In view of MAG's selective localization in periaxonal and other noncompacted glial membranes, one might expect specific signals affecting its intracellular transport could reside in the cytoplasmic domains. However, a recent study with Madin-Darby canine kidney (MDCK) epithelial cells, which are widely used to investigate protein targeting to apical or basolateral membranes, revealed little evidence for selective targeting of either MAG isoform [154] and challenged an earlier report showing some evidence for basolateral sorting [155]. It may be that because of the complex structure of myelinating cells, there are specialized sorting signals for MAG and some other glial proteins that are not recognized in MDCK cells.

Unlike the situation with P0 and PMP-22, no naturally occurring murine mutants or inherited human neuropathies involving the MAG gene have been identified. The reason for this may be the relatively mild neurological and behavioral deficits observed in the absence of MAG. If such a disorder does exist, it is likely to present as an axonal, rather than a demyelinating, neuropathy in view of the electrophysiological studies on aging MAG-null mice, which resemble those in Charcot-Marie Tooth disease type II [99]. However, the physiological consequences of a mutation resulting in an incorrect dose or abnormal form of MAG could be quite different and more severe.

Studies on some dysmyelinating murine mutants suggest that MAG may play an important role in the pathology, even though the MAG gene is not affected directly. Generally, MAG is not reduced as much as the proteins of compact myelin in hypomyelinated mutants, because the myelin-related glial membranes containing MAG are preserved relative to compact myelin [156]. However, a clear exception to that generalization is the taiep rat, in which MAG is decreased much more than the other myelin proteins [157–160]. This is a mutant in which an early hypomyelination is followed by a progressive severe demyelination of the CNS, apparently caused by a microtubule defect which impedes the transport of myelin proteins and mRNAs along oligodendroglial processes. The much greater reduction of MAG in comparison with other myelin proteins is probably related to the fact that it has the greatest distance to be transported to the distal periaxonal membrane. Interestingly, the deficit of MAG results in structural pathology at the periaxonal region similar to that in MAG-null mice [159].

Another murine mutant in which abnormalities of MAG are likely to have an important role is the quaking mouse, which is inherited as an autosomal recessive characterized by a myelin deficiency in both the CNS and PNS. It is caused by mutation of a gene that produces a diverse set of proteins (QKIs) by alternative splicing and links signal transduction to RNA metabolism [161, 162], but the mechanisms that cause the dysmyelination are not clearly understood. With regard to MAG, it is of interest that quaking mutants express almost exclusively S-MAG and very little L-MAG in the CNS [163–166]. In fact, it was shown very recently that a nuclear localized QKI-5 isoform directly represses alternative splicing of MAG pre-mRNA that is necessary for the formation of L-MAG [167]. The scarcity of L-MAG in this mutant may be related both to reduced synthesis and to endocytic depletion of L-MAG from the periaxonal oligodendroglial membrane [168]. Severely reduced L-MAG is likely to cause some CNS pathology as indicated by studies on mice engineered to express truncated L-MAG without the novel C-terminus [147]. Furthermore, the MAG that is expressed in the CNS and PNS of quaking mutants contains an abnormally high content of 2,3-SA in comparison to control MAG [166]. Similarly, 2,3-SA is also elevated in MAG and other glycoproteins in the PNS of trembler mutants [169], which exhibit a dysmyelination restricted to the PNS caused by a mutation in PMP-22. Although the total amount of MAG is approximately normal in quaking and trembler sciatic nerves [170, 171], it is distributed ab-

normally [172–174]. MAG accumulates in loosely spiraled, incompletely compacted Schwann cell membranes, and is only weakly immunostained or undetectable in the periaxonal membranes of many fibers where communication with the axon would take place. Because MAG binds to glycoproteins containing 2,3-SA, the elevated content of 2,3-SA in the mutant nerves could contribute to its abnormal distribution. It was hypothesized that the failure of myelin compaction in these mutants is related to their inability to remove MAG from the plastic, loosely spiraled mesaxon membranes and replace it with P0 glycoprotein [173]. An explanation for this could be that binding of MAG to itself, caused by the abnormally high 2,3-SA, impairs its removal from the spiraled membranes. Furthermore, binding and signal transduction to adjacent cells by MAG and other members of the siglec family are known to be impeded by elevated cis sialic acid [175–178]. Therefore, even signaling to the axon by MAG appropriately located in the periaxonal Schwann cell membrane could be impaired by the increased 2,3-SA. Furthermore, the similar cytoskeletal abnormalities that occur in myelinated axons of trembler and MAG-null mice may be caused in the trembler both by the reduced content and less efficient signaling of hypersialylated MAG in the periaxonal Schwann cell membrane. The altered sialylation observed in these mutants may reflect a general response of Schwann cells to pathological circumstances that could occur in other neuropathies as well, and preliminary results in our laboratory suggest that this is the case.

Axonal abnormalities and/or loss in the PNS have been observed in mice deficient for other myelin or myelin-related proteins, including P0 [54], PMP-22 [179] and connexin-32 [180]. P0-deficient mice also exhibit a reduced content of periaxonal MAG and its accumulation in loosely spiralled Schwann cell membranes [109, 181] similarly to trembler and quaking mice, so dysfunction of MAG could contribute to the axonal pathology in this mutant as well [182]. Furthermore, as recently reviewed in detail [182], axonal abnormalities and degeneration also occur in the different forms of CMT disease in humans caused by mutations of the genes for some of the proteins mentioned above. This axonal pathology may be caused, in part, by the absence or disruption of Schwann cell-to-axon signaling. The extent to which dysfunction of MAG, the mutated proteins and/or other Schwann cell mediators responsible for glia-axon signaling contribute to axonal pathology in inherited neuropathies remains to be established.

MAG has been implicated more definitively in some acquired neurological disorders thought to be caused by autoimmune mechanisms. The best-documented example of this is patients with a demyelinating sensorimotor neuropathy occurring in association with IgM gammopathy. This disorder has been reviewed recently [66, 183, 184] and will be summarized only briefly here. The patients have high levels of a monoclonal IgM antibody in their serum that reacts with a carbohydrate epitope in MAG. The specificity of these human antibodies is similar to that of HNK-1, and they react with other glycoconjugates that share the carbohydrate epitope with MAG, including P0, PMP-22 and glycolipids such as sulfate-3-glucuronyl paragloboside. The capacity of these antibodies to cause demyelination is now well documented, but the importance of the different potential target antigens for the pathology is not established. However, some immunocytochemical and ultrastructural studies on sural nerve biopsies from these patients suggest that reactivity with MAG is an important aspect of the pathology [185–187]. Furthermore, the fine specificities of the monoclonal antibodies differ from patient to patient with regard to their strength of binding to MAG and the other potential antigens [67, 188–190], and this may correlate with different pathological mechanisms.

MAG has also been suggested to have a key role in the molecular pathogenesis of multiple sclerosis (MS), based on immunocytochemical and biochemical demonstrations that it is reduced more than other myelin proteins at the edges of some developing plaques (reviewed in [7]). However, it seems unlikely that MAG is a primary target antigen in the putative immunological aspects of MS since it is sequestered in the periaxonal region of myelin sheaths (fig. 2). Only low levels of humoral or cellular immunity to MAG have been observed in MS patients (reviewed in [7]). A recent report showed that multiple MAG peptides are recognized by T and B lymphocytes in MS and polyneuropathy, but there was no disease-specific immunodominance for any of the peptides [191]. The authors suggested that this could represent a secondary response to myelin damage, which might contribute to disease progression or just indicate tolerization rather than autoaggressive immunity. Recent reports also demonstrate that there are peptide epitopes in MAG capable of sensitizing T cells and inducing experimental allergic encephalomyelitis (EAE) in rats [192] and mice [193]. It may be that an immune response to MAG contributes to secondary demyelination in MS after degeneration of myelin has already begun.

The high susceptibility of human MAG to a myelin-associated neutral protease that cleaves it to a soluble 90-kDa derivative (dMAG) may be involved in its selective loss from the edge of MS lesions. Earlier studies had suggested that the enzyme responsible for this proteolysis was m-calpain, but more recent studies have indicated that it is a cathepsin-L like protease [114, 115]. This enzyme cleaves MAG at a lys-ala peptide bond, which is situated extracellularly just four amino acid residues from the transmembrane domain. A possible instigating factor for the proteolysis and preferential loss could be that an early aspect of the pathogenesis involves a dying back oligoden-

drogliopathy in which the first pathology occurs in the most distal periaxonal oligodendroglial membranes for which MAG is a specific marker. Indeed, it has been reported that the first pathology observed in some early MS lesions is in the periaxonal regions of myelin sheaths [194]. The recent paper by Lucchinetti et al. [195] has reemphasized this concept of preferential MAG loss at the edge of some acute plaques and interpreted it in the context of heterogeneous patterns of pathology among different MS patients [196, 197]. Preferential MAG loss was not observed in patterns exhibiting similarities to T-cell-mediated or T cell plus antibody-mediated autoimmune encephalomyelitis. However, it was characteristic of a type of pathology also including oligodendrocyte apoptosis and reminiscent of viral- or toxin-induced demyelination. Although the importance of oligodendroglial apoptosis in MS lesions is controversial [198, 199], it is likely that oligodendrocyte death in MS should be envisioned as a continuum between apoptotic and necrotic death [200]. Whatever the primary cause of oligodendroglial injury in MS or other disorders, it is easy to understand how this could lead to an early failure to maintain the most distal periaxonal oligodendroglial processes. Because of MAG's specific periaxonal location, its early loss in MS could simply be a biochemical marker for the degeneration of periaxonal membranes in a dying back oligodendrogliopathy. On the other hand, the morphological [95] and biochemical [96] evidence for similar oligodendrocyte dystrophy in MAG-null mice suggests that the loss of MAG could contribute directly to oligodendroglial pathology in MS or other disorders. It may be that MAG-mediated signaling from myelinated axons to oligodendrocytes provides a needed trophic signal, without which oligodendroglial degeneration occurs.

MOG

MOG is a 26–28-kDa glycoprotein that was originally detected by a monoclonal antibody raised to rat cerebellar glycoproteins [201], and research designed to elucidate its structure and function was reviewed in detail [202]. Similarly to MAG, it is a member of the Ig superfamily that is a quantitatively minor component of myelin sheaths that is localized in membranes distinct from compact myelin. However, unlike MAG it is not expressed in the PNS, and its localization in the CNS is very different from periaxonal MAG. It is expressed preferentially on the outside surface of myelin sheaths and oligodendrocytes (fig. 2), where it is well situated to interact with components in the extracellular environment. It has a single Ig-like variable region domain, one site for N-linked glycosylation, and two hydrophobic, potential transmembrane domains (fig. 3). However, topographical studies have indicated that the second hydrophobic domain does not transverse the membrane completely, so the carboxy terminus of MOG is in the oligodendroglial cytoplasm. It is of interest that the cytoplasmic domain of MOG contains targeting signals that direct it to the basolateral domain of MDCK cells and presumably account for its selective localization on the surface of oligodendrocytes and myelin sheaths [154, 203]. Alternatively spliced forms of mRNA for MOG were not detected in rodents, but studies on humans have demonstrated several alternatively spliced mRNAs, some of which would encode isoforms of MOG truncated at various positions in the protein. However, none of these isoforms have been detected at the protein level, indicating either that they are not translated or translated at very low levels. The oligosaccharide moieties on MOG are primarily of the complex type, and a subset of the molecules contains the adhesion-related HNK-1/L2 epitope.

The function of MOG is not known, although its localization on the surface of oligodendrocytes and myelin strongly suggests that it could function in the transmission of signals from the extracellular environment to oligodendrocytes. The developmental pattern of MOG is delayed somewhat in comparison to other myelin proteins, indicating that its primary functional role may relate to mature oligodendrocytes. In their relatively recent review [202], Johns and Bernard suggest three possible functions. The first is that it could be an adhesion protein, perhaps mediating an interaction between the surfaces of neighboring mature myelin sheaths, which does not occur in the PNS. The second is a role in modulating the stability of the oligodendroglial microtubules. They summarize experimental results suggesting that the interaction of MOG with an unknown extracellular ligand could regulate microtubular stability with MBP acting as an intermediary molecule. The third suggested function was that MOG binding to the C1q component of complement is responsible for the capacity of CNS myelin to activate complement. Although the role of this phenomenon in normal physiology is not obvious, it could be related to inflammatory processes occurring in demyelinating diseases such as multiple sclerosis. So far no one has reported the effect of deleting the MOG gene, which may provide important clues about its normal function. In general, it seems fair to say that the function of MOG is very poorly understood at this time. On the other hand, much of the recent research on MOG has built a strong case that it may play a very key role in the autoimmune aspects of MS, as discussed below.

Certainly, the surface location of MOG suggests that it could be an important primary antigen in autoimmune demyelinating diseases of the CNS, and much recent research supports this hypothesis both in animal models and MS (reviewed in detail in several recent reviews [204–206]). MOG is highly immunogenic, and immunization with this protein alone can induce severe EAE in

rodents and primates. Both MOG-specific antibodies and T cells sensitized to MOG appear to be involved in the pathology. In the rat and marmoset models, the demyelination is antibody mediated and T cell dependent. Furthermore, the pathology closely resembles that seen in many cases of MS. Marmoset EAE in particular provides a good model for MS. It is characterized by relapsing-remitting or primary progressive clinical courses and is being used to evaluate potential therapies for MS. However, it should be noted that antibodies and T cells sensitized to MOG are only detected in a subset of MS patients. Therefore, although immunity to MOG has the capability of causing a demyelinating disorder resembling MS and may be important in many MS patients, it is likely that other antigens are also involved in this major human demyelinating disease.

Conclusions and perspectives

It is clear that expression of normal amounts of P0 and PMP-22 is essential for the formation and/or maintenance of myelin in the PNS. P0 obviously has a major structural role needed for the stability of compact myelin, but it is likely to have additional functional roles in myelination and other aspects of nerve development that remain to be elucidated. PMP-22 appears to have been recruited from another growth arrest-related function in a variety of cells to take on a very important role in myelination, but the exact nature of that role is not clear at this time. MAG is not essential for myelination, but it seems to function as a receptor that promotes efficient myelination by oligodendrocytes in the CNS and as a ligand that is crucial for the maintenance of myelinated axons in the PNS. Important areas for future research include identifying with certainty its physiological binding partners, which participate in these axon-glia interactions, and the signal transduction pathways that are activated by MAG in axons and glia. It is also important to determine factors controlling the relative expression and phosphorylation of the L- and S-MAG isoforms, how this affects MAG's interaction with cytoplasmic components in Schwann cells or oligodendrocytes, and what effects these interactions have on myelin formation or maintenance. The physiological function of MOG is really unknown at this time and should be a fruitful area for future research. Even after the individual roles of each of these four glycoproteins are determined with more certainty, the next important challenge will be to understand how they interact with each other and numerous other glial or axonal proteins to generate the complex structure of a myelinated axon.

All four glycoproteins reviewed here have been implicated in the pathogenesis of neurological diseases. Mutations affecting the genes for P0 and PMP-22 cause inherited peripheral neuropathies, whereas MAG and MOG have been implicated in acquired demyelinating diseases, including MS. However, much additional research is needed to understand precisely how these proteins are involved in the biochemical pathogenesis affecting myelinated axons. Hopefully, such research will lead to information useful for ameliorating or curing some of these debilitating neurological disorders.

Note added in proof. Since submitting this review, two important papers have appeared implicating GD1a and GT1b gangliosides and/or a GT1b/neurotrophin receptor p75 complex as functional MAG-binding partners involved in the inhibition of neurite outgrowth [Vyas A. A., Patel H. V., Fromholt S. E., Heffer-Lauc M., Vyas K. A., Dang J., Schachner M. and Schnaar R. L. (2002) Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc. Natl. Acad. Sci. USA **99:** 8412–8417; and Yamashita T., Higuchi H. and Tohyama M. (2002) The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho J. Cell Biol. **157:** 565–570]. However, another very recent paper reported that MAG inhibits neurite outgrowth by acting as an additional ligand for the glycosylphosphatidylinositol-anchored receptor for a different oligodendroglial inhibitor of neurite outgrowth called Nogo [Domeniconi M., Cao Z., Spencer T., Sivasankaran R., Wang K., Nikulina E., Kimura N., Cai H., Deng K., Gao Y., He Z. and Filbin M. (2002) Myelin-associated glycoprotein interacts with the nogo66 receptor to inhibit neurite outgrowth. Neuron. **35:** 283–291].

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