Cell. Mol. Life Sci. 63 (2006) 1945–1961
1420-682X/06/171945-17
DOI 10.1007/s00018-006-6094-7

Cellular and Molecular Life Sciences

Review

Myelin basic protein: a multifunctional protein

J. M. Boggs

Department of Structural Biology and Biochemistry, Research Institute, Hospital for Sick Children, 555 University Ave., Toronto, Ontario, M5G 1X8 (Canada), Fax: +1 416 813 5022; e-mail: jmboggs@sickkids.ca, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, M5G 1L5 (Canada)

Received 2 March 2006; received after revision 12 April 2006; accepted 16 May 2006
Online First 23 June 2006

Abstract. Myelin basic protein (MBP), the second most abundant protein in central nervous system myelin, is responsible for adhesion of the cytosolic surfaces of multilayered compact myelin. A member of the 'intrinsically disordered' or conformationally adaptable protein family, it also appears to have several other functions. It can interact with a number of polyanionic proteins including actin, tubulin, Ca^{2+}-calmodulin, and clathrin, and negatively charged lipids, and acquires structure on binding to them. It may act as a membrane actin-binding protein, which might allow it to participate in transmission of extracellular signals to the cytoskeleton in oligodendrocytes and tight junctions in myelin. Some size isoforms of MBP are transported into the nucleus and thus they may also bind polynucleotides. Extracellular signals received by myelin or cultured oligodendrocytes cause changes in phosphorylation of MBP, suggesting that MBP is also involved in signaling. Further study of this very abundant protein will reveal how it is utilized by the oligodendrocyte and myelin for different purposes.

Keywords. Intrinsically unstructured, cytoskeleton, calmodulin, lipid vesicle, deimination, phosphorylation, oligodendrocyte, multiple sclerosis.

Introduction

The myelin sheath surrounding nerve axons has a unique multilamellar structure. In compact internodal myelin, the cytosolic surfaces of the plasma membrane adhere to each other, forming the major dense line, and the extracellular surfaces adhere to each other, forming the intraperiod line (Fig. 1) [see refs. 1, 2 for reviews]. The integrity of this compacted multilamellar structure determines the speed of transmission of action potentials along the axon. Some noncompacted regions containing cytosol are also present in the myelin sheath, such as the inner loop surrounding the axonal internodes, the outer loop, and the paranodal loops of myelin, and in peripheral nervous system (PNS) myelin, the Schmidt-Lanterman incisures which run through internodal myelin [1]. These regions of cytosol plus the presence of various enzymes, and cytoskeletal and signal transduction proteins in both compact myelin and noncompacted regions may allow myelin to perform dynamic functions and communicate with the axon [2, 4].

Myelin basic protein (MBP) is the second most abundant protein, after the proteolipid protein (PLP), in central nervous system (CNS) myelin: it comprises 30% of the total protein and about 10% of the dry weight of myelin. It is the only structural protein found so far to be essential for formation of CNS myelin, and has been called the 'executive molecule of myelin' [5]. The shiverer mutant mouse, a naturally occurring mutant in which a large portion of the MBP gene is deleted, lacks most compact myelin in the CNS [6]. The Long Evans shaker rat, with a mutation in the MBP gene causing aberrant transcription, also lacks compact CNS myelin [7]. Although MBP is also present in PNS myelin, it is not required for its formation due to the presence of other PNS myelin proteins such as PMP22, P0, and P2 [8], which may compensate for the lack of MBP. However, the shiverer PNS has morphological abnormali-

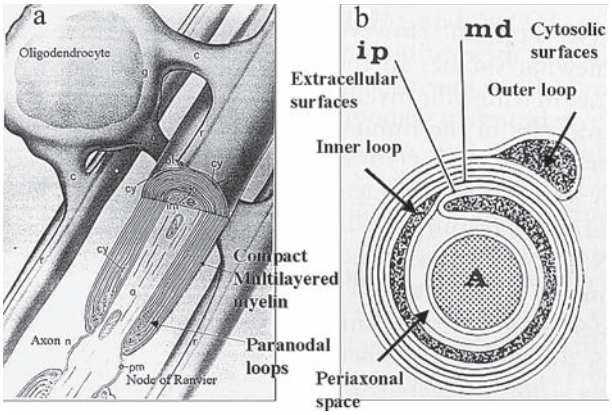

Figure 1. (*a*) One oligodendrocyte myelinates many axons. Multilayered compact myelin (arrow) containing some areas of cytosol (cy), with paranodal loops surrounding node (n) of Ranvier. Cytosol is retained in the paranodal loops, in the inner loop (inner mesaxon = im), and in the outer loop (ol). Reproduced from *The Journal of Cell Biology*, 1961, vol. 10, pp 67–94 [3] by copyright permission of The Rockefeller University Press. (*b*) Cross-section of myelin surrounding axon, A. Cytosolic surfaces of the oligodendrocyte plasma membrane come together to form the major dense line (md) seen by electron microscopy, and extracellular surfaces come together to form the intraperiod line (ip). Most of the cytosol and extracellular fluid are eliminated to form compact multilayered myelin. Cytosol is shown in the inner loop and outer loop. Extracellular fluid is present in the periaxonal space.

ties, such as a reduction in axon caliber and myelin sheath thickness, aberrant Schwann cell-axon contacts, and a twofold increase in the number of cytosol-containing Schmidt-Lanterman incisures throughout compact PNS myelin [9], indicating that MBP performs an important function in the PNS also. In addition to its importance in myelin formation, MBP is also of interest because immunization with it induces experimental allergic encephalomyelitis in animals, and immune response to it occurs in the demyelinating disease multiple sclerosis (MS).

MBP (pI ~ 10) is bound to the cytosolic surface of the oligodendrocyte (OL) membrane, primarily through electrostatic interactions with negatively charged lipids [reviewed in ref. 10]. It is present throughout compact internodal myelin and is acknowledged to be involved in adhesion of the cytosolic surfaces of the multilayered myelin sheath, where it has been localized by immunoelectron microscopy and X-ray diffraction [2, 6, 11]. However, it may have other functions as well. Roles in signaling [5, 12, 13], in the nucleus [14], in interactions with the cytoskeleton [15–18], and a regulatory function on the expression of other myelin proteins [7, 19] have been suggested.

Multifunctionality is being found for an increasing number of proteins [20, 21]. Hill et al. [22] [see Harauz et al. [23] for a recent review] have shown that MBP is a member of the family of intrinsically unstructured proteins [25, 26]. It has similarities to microtubule-associated proteins (MAP) such as tau and MAP2, to α-synuclein [27], and to proteins with disordered basic effector domains such as MARKS (myristoylated alanine-rich C kinase substrate) [28]. Their high net charge and low hydrophobicity maximize intramolecular electrostatic repulsion leading to an extended structure. Such proteins have sufficient flexibility to bind to charged surfaces and ligands and to acquire whatever local conformation is necessary to optimize binding to several different targets. They are often multifunctional regulatory proteins associated with signal transduction, adhesion, cell cycle regulation, gene expression, alternative splicing of pre-mRNAs, or chaperone action [20, 21, 27, 29, 30]. They are found primarily in eukaryotes, where there is an increased need for multifunctional proteins and complex protein-protein interactions, with only a small proportion in prokaryotes [31]. A greater increase in unfoldedness occurs with evolution in nonhousekeeping proteins such as MBP than in more ubiquitous housekeeping proteins, and disordered regions evolve at a faster rate than ordered regions [29, 32]. By increasing the native disorder of a protein, higher organisms may be able to accommodate a variety of new interactions, which may in turn aid in evolving novel functions. Disordered proteins with high surface charge which are involved in multiple interactions may integrate hubs of various biological activities [33, 34]. Thus, their deletion is more likely to lead to serious dysfunction or lethality [33].

A myelin sheath containing MBP is present in all vertebrates except the evolutionarily oldest, (hagfish and lampreys, members of *Agnatha cyclostomata*). The sequence of MBP is highly conserved from mammals (ten species sequenced) to chicken to clawed frog [23]. MBP is also present in fish, but shark MBP has only 44% homology with mammalian MBP [35, 36]. No homologues have been found in fruit fly, nematode, and Ascidian (*Ciona intestinalis*) genomes [37]. Since the latter is an invertebrate chordate derived from the common ancestor of extant vertebrates, this suggests that MBP evolved relatively late, along with myelin.

Size isoforms of MBP

The MBP found in myelin (classic MBP) is a product of a larger gene complex called Golli (Genes of OLigodendrocyte Lineage), which has 11 exons in mice and 10 in humans, including the 7 exons giving rise to classic MBP [38]. Various Golli proteins are produced in developing myelin and also immune and hemopoietic cells [38, 39]. They may be involved in signaling by regulation of intracellular Ca^{2+} levels [40]. Golli proteins contain only fragments of classic MBP. This review will focus on classic MBP which has been reported to occur only in myelin. Several size isoforms of classic MBP are formed by differential splicing of a single mRNA transcript. These are

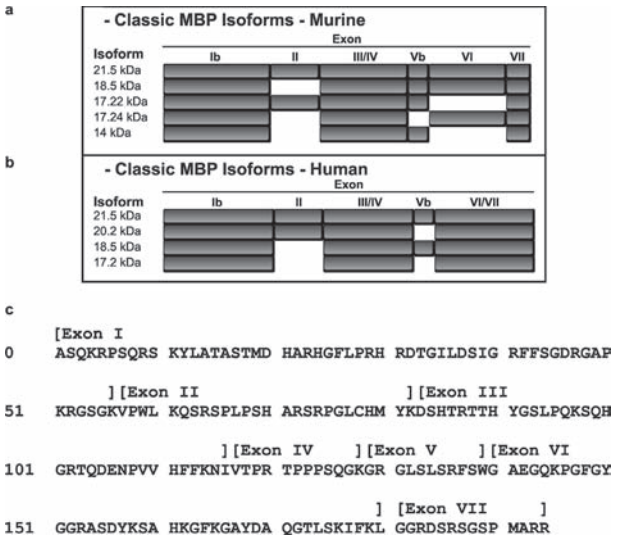

Figure 2. (*a*, *b*) Organization of the murine MBP gene (*a*) and the human MBP gene (*b*), showing the alternate splice variants of classic MBP. Adapted from Micron, v. 35, G. Harauz et al. [23]: Myelin basic protein – diverse conformational states of an intrinsically unstructured protein and its roles in myelin assembly and multiple sclerosis, pp. 503–542, Copyright (2004) with permission from Elsevier. (*c*) Sequence of murine 21.5-kDa MBP [24], showing regions encoded by different exons.

21.5, 20.2, 18.5, 17.24, 17.22, and 14 kDa in the mouse and 21.5, 20.2, 18.5, and 17.2 kDa in humans (Fig. 2) [23, 24, 41, 42]. The major MBP isoform in the adult human and bovine CNS is 18.5 kDa whereas that in adult mice and rats is 14 kDa. All isoforms contain protein domains encoded by exons I, III, IV, and VII, while only the 21.5-, 20.5-, and 17.22-kDa forms contain the domain encoded by exon II. The 21.5-, 20.2-, 18.5-, 17.24-, and 17.2-kDa forms also contain exon VI, whereas the 17.22- and 14-kDa forms do not. The greatest similarity between fish and rodent MBP is in exons I, III, and part of IV, which may be a functional core of MBP [2, 43]. Fish express two isoforms of MBP, one lacking exon II and the other lacking both exon II and exon Vb [35, 44].

Expression of these isoforms is developmentally regulated and they have different locations in cells and myelin, suggesting that they may have different functions. The isoforms containing the exon II-encoded domain are expressed at high levels early in myelination and in immature OLs in culture [42, 45, 46], while the forms lacking this domain are the primary forms expressed later in myelination, in mature OLs, and in adult myelin. Although the 18.5-kDa form is the primary form in adult human myelin, exon II-containing forms are expressed during fetal development and during remyelination [41]. In the aged rat, where many paranodal loops were retracted, MBP expression was decreased relative to that of other myelin proteins examined and the exon II-containing 21.5-kDa form was almost completely absent [47].

Interestingly, these different isoforms localize to different regions of the cell when expressed individually in transfected cells. Both in shiverer mouse OLs lacking endogenous MBP [48] or in HeLa cells [49], the exon II-containing forms were found distributed in the cytosol and in the nucleus but not at the plasma membrane, while the exon II-negative forms were confined to the plasma membrane. The exon II-containing forms were transported into the nucleus by active transport [45, 50]. However, in wild-type mouse OLs containing endogenous MBP, a transfected tagged exon II-negative 14-kDa form was found in the cytosol and nucleus in addition to being bound to the plasma membrane, leading to the suggestion that the presence of exon II-containing forms might help transport exon II-negative forms into the nucleus [19].

The domain encoded by exon II has a somewhat higher percentage of basic residues than other domains, and the exon II-positive 21.5-kDa isoform interacts with negatively charged lipid vesicles as efficiently as the exon II-negative 18.5-kDa form [51]. Indeed, all isoforms are found in compact myelin. However, the exon II-negative forms are excluded more from the radial component (a tight junctional specialization within internodal myelin) whereas exon II-positive forms are found equally in compact myelin and in the radial component [52]. Possible causes of localization of the exon II-positive isoforms in the nucleus of OLs may be the presence of an unrecognized nuclear targeting sequence in the protein [50] or in the mRNA. The mRNAs for exon II-negative forms of MBP are transported far into OL processes, whereas the mRNAs for exon II-positive forms have been detected only in the cell body [46].

Transfection of either the exon II-negative 14-kDa isoform or the exon II-positive 17.24-kDa isoform into the shiverer mouse restored myelin to a similar extent [53], indicating that the domain encoded by exon II is neither essential nor deleterious for myelination. However, in both cases myelin formation was less than that in the shiverer/wild-type heterozygote with similar MBP mRNA expression as the transfected mice, indicating that the other isoforms contribute in unique or synergistic ways to myelin formation. Shiverer OL precursor cells were not induced to differentiate in culture by growth factors, in contrast to those from normal mice [54]. Although they did respond to dibutyryl-cAMP, they produced abnormally high levels of myelin-associated glycoprotein (MAG). This suggests that MBP may be involved in regulation of the OL differentiation pathway.

Post-translational modifications of MBP

18.5-kDa MBP also occurs as a series of charge isomers, termed C1, C2, C3, C4, C5, C6, and C8 due to a va-

riety of post-translational modifications, which reduce their net positive charge, including deamidation, Ser/Thr phosphorylation [55, 56], and deimination of arginine (Arg) to citrulline (Cit), [57] also termed citrullination. Spiny dogfish MBP is also modified by phosphorylation and deamidation [58]. The N-terminal Ala is modified by acylation with chains of two to ten carbons in length, with four to six carbon chains being the dominant species [59]. Arg107 (human sequence) occurs as the methylated form, either mono-methyl Arg or symmetric dimethyl Arg [60–62]. Human MBP binds azido-GTP at Gln3 [63] and is ADP-ribosylated at Arg9 and Arg54 [64]. This high degree of modification [see ref. 23 for a summary] is consistent with its lack of structure, since regions of intrinsic disorder are usually preferred as sites of post-translational modification and protease digestion [65, 66].

Of the charge isomers of MBP, C1 is the least modified, most highly positively charged form. C2 is deamidated, while C3–C6 may be modified by combinations of phosphorylation, deamidation, and deimination [55, 56, 67]. C8 differs from C1 by deimination of 6–11 Arg to Cit [57, 67], decreasing its net positive charge from +19 at pH 7.4 by one for every deiminated Arg, and altering its hydrogen-bonding properties [68]. All charge isomers were ADP ribosylated at Arg9 and Arg54 except C8 [64]. These post-translational modifications occur both physiologically and pathologically. The phosphate groups of MBP turn over rapidly, and the highest turnover was in the most mature myelin fractions [69], suggesting phosphorylation of MBP plays a dynamic role in myelin structure and function [70]. Phosphorylation of MBP in the myelin sheath is altered in response to the nerve action potential [71–73] and in OLs in culture in response to extracellular ligands or to depolarization [74–77]. All size isoforms are phosphorylated with a similar turnover rate [69]. Possibly some post-translational modifications of the exon II-positive forms cause their targeting to the nucleus, although phorbol ester, which activates protein kinase C, inhibited nuclear targeting of MBP, suggesting that phosphorylation of MBP may inhibit nuclear targeting [50]. Effects of MBP phosphorylation on its functions will be addressed below.

MBP can be phosphorylated *in vitro* by protein kinase C, protein kinase A, glycogen synthase kinase (GSK), calmodulin-dependent kinase, and mitogen-activated protein kinase (MAPK), enzymes all present in myelin [4, 78–82]. The *in vitro* sites of phosphorylation differ in some cases from those found to be phosphorylated in myelin MBP *in vivo* [56, 67], but one of the *in vivo* sites, Thr97 (bovine sequence), is phosphorylated by MAPK and GSK *in vitro* [78, 79]. However, the sites of phosphorylation of MBP in OLs, as opposed to mature myelin, are not known and may include some of the *in vitro* phosphorylation sites. MBP from MS brain was less phosphorylated and less deamidated than normal MBP but contained more methylated Arg [67]. A peptidylarginine deiminase has been isolated from mammalian brain [83] and shown to deiminate purified MBP [84]. The amount of deiminated MBP, C8, in myelin is highest in children and decreases during development, suggesting that it may play a role in myelin formation rather than myelin function. However, C8 occurs in greater amounts in MS patients [57, 67, 85] and has been suggested to be involved in the pathogenesis of MS [86]. Furthermore, a greater number of Arg may be deiminated in MBP in MS patients than in normal individuals [67, 56]. In a patient with a rare case of acute MS known as Marburg's syndrome [87], 18 of the 19 arginines were deiminated [88].

Polyanionic ligand binding of MBP

Other polycationic proteins can interact with a number of polyanionic ligands, proteins and surfaces in functional ways [89]. Some proteins, such as MARCKS, gravin, K-Ras4B, and Src have an unstructured basic cluster which binds polyanionic ligands [90, 91], but other acidic domains, in contrast to MBP, whose basic and acidic residues are distributed throughout its sequence. Since it has more basic than acidic residues, it has a net positive charge. MBP has long been known to bind to negatively charged lipid surfaces. It can bind to two lipid surfaces at once, possibly by dimerizing, and cause the adhesion and aggregation of lipid vesicles [92–98] and multibilayer formation [99–103]. Its net positive charge distributed throughout the sequence may be better suited for binding two lipid surfaces together than would be a cluster of basic residues in one domain of the protein. MBP can also cause hemifusion of the contacting monolayers of apposed vesicles [104]. When added to lipid vesicles containing a mixture of a negatively charged and a neutral lipid, it binds the negatively charged lipid preferentially [105, 106]. Indeed, it binds to negatively charged lipids so tightly that it must be extracted with acid in order to delipidate it [107] and it can be isolated in a lipid-bound form by extraction with 3-[(3-cholamidopropyl)dimethyl ammonio]-1-propanesulfonate (CHAPS) [100, 108].

In addition to negatively charged lipids, MBP interacts with polyanions such as actin filaments [15, 17, 18, 109–112] and microtubules *in vitro* [113, 114] and binds to other proteins such as Ca^{2+}-calmodulin (CaM) [15, 16, 115–118], tropomyosin [119], and clathrin [120]. The fact that size isoforms of MBP containing the segment encoded by exon II have been found to also localize in the nucleus, suggests that they may bind to polynucleotides [45]. Such a variety of interactions may play a physiological role, especially for a protein as abundant in OLs and myelin as MBP.

Conformational adaptability of MBP and effect of post-translational modifications

All size isoforms and charge isomers of MBP have been predicted to be intrinsically unstructured [22, 23, 121] and circular dichroism (CD) spectroscopy has shown that MBP has little or no ordered structure in solution [reviewed in refs. 10, 23, 94]. However, it has been predicted to have a β sheet structure [122] and certain regions have been predicted to form amphipathic α helices [122–125]. Such structural elements may be important for recognition and stabilization of their structure by various ligands [27]. Phosphorylation increases the β sheet structure of MBP in solution, but deamidation had little effect on the secondary structure [126, 127]. C8 in solution is more susceptible to digestion by cathepsin D than is C1, suggesting that its conformation is even more disordered than that of C1 [128, 129].

Using the natural mixture of charge isomers, MBP was shown using CD spectroscopy to acquire α-helical and β sheet secondary structure on interacting with sodium dodecylsulfate (SDS) micelles [130] and lipids [108, 131]. The recognition of lipid-bound MBP by anti-MBP antibodies varied with lipid composition, also suggesting an altered conformation [132]. Electron microscopy studies of MBP bound to a lipid surface allowed construction of a three-dimensional (3D) model for MBP based on a single particle reconstruction; the structure had similarities but was not identical to the predicted β sheet structure referred to above [133, 134]. More recent studies using cysteine (Cys) substitution for Cys-specific spin labeling (Fig. 3) and electron paramagnetic resonance (EPR) spectroscopy have shown that the region 83–92 of recombinant murine 18.5-kDa C1 (rmC1) (corresponding to residues 86–95 of human MBP), formed an amphipathic α helix on binding to lipid vesicles (Fig. 4b) [135]. The helix was embedded into the lipid bilayer and tilted at an angle of 9° with respect to the membrane surface. Since other tilted amphipathic peptides have fusogenic properties [136], this may explain why MBP is able to cause hemifusion of lipid bilayers [104]. MBP contains two sets of Phe-Phe pairs at positions 42–43 and 86–87 (murine 18.5-kDa sequence numbering, Fig. 3) and both regions, including Phe87Cys, were embedded in the bilayer (Fig. 4). Substitution of Ala-Ala for Phe86–Phe87 caused this region to be less embedded in the bilayer (Fig. 4a) as found for the MARCKS basic peptide [137]. These two Phe-Phe pairs and the region 83–89 are conserved in fish MBP.

This region of MBP is of interest because it is an immunodominant epitope for both B and T cells [138, 139]. The linear polypeptide D82-ENPVVHFFKNIVTPR-T98 (human numbering), containing this segment has been used to induce immunologic tolerance in patients with

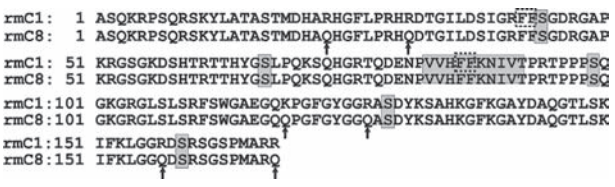

Figure 3. Sequence of 18.5-kDa rmC1 and rmC8 showing residues which were changed to Cys for spin labeling in gray boxes. The Phe-Phe pairs are shown in dashed boxes. The Arg/Lys changed to Gln to mimic citrullination in rmC8 are indicated by arrows. Adapted from Bates et al. [144].

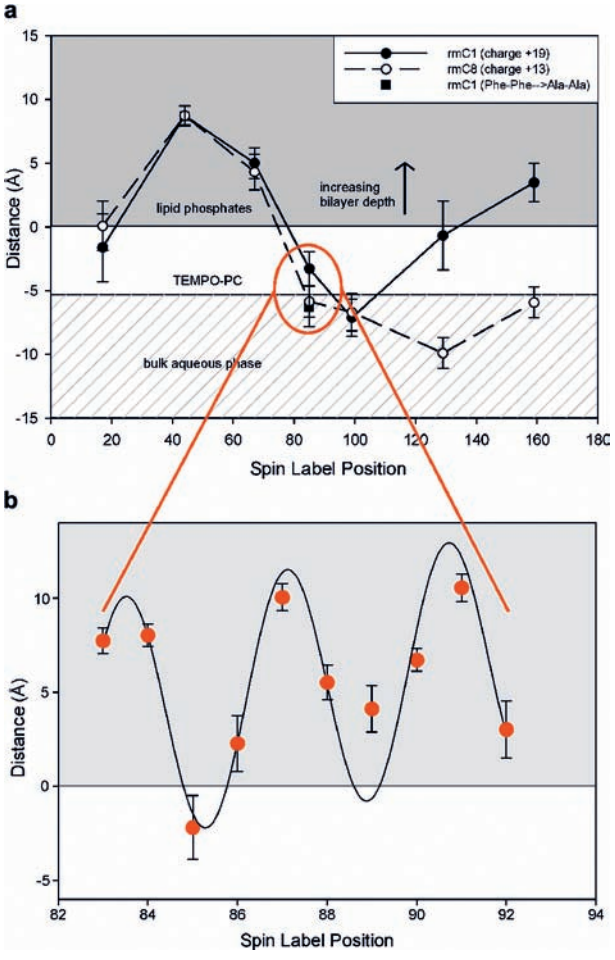

Figure 4. (*a*) Depth measurement of spin labels into Cyt-LUVs via power saturation EPR spectroscopy [144]. Depths of penetration of spin labels at indicated sites in rmC1 and rmC8 into the lipid bilayer of the Cyt-LUVs. The gray shading indicates values below the lipid phosphates (in the bilayer), whereas the cross-hatching specifies the region farther than 5 Å into the aqueous phase where the distance cannot be determined accurately. The horizontal line at –5 Å indicates the location of the nitrogen atom of the lipid headgroup spin label. (*b*) The region from Val83 to Thr92 was subsequently studied by Cys substitution of each residue, and site-directed spin labeling (SDSL)/EPR of each protein species in turn [135]. The periodicity of the penetration depth was fit to a sine function. The resulting fit revealed a periodicity of 3.6 residues, and amplitude of 10 Å, indicative of an amphipathic α helix. Moreover, the helix was tilted by 9º with respect to the plane of the bilayer. Adapted from Micron, v. 35, G. Harauz et al. [23]: Myelin basic protein – diverse conformational states of an intrinsically unstructured protein and its roles in myelin assembly and multiple sclerosis, pp. 503–542, Copyright (2004) with permission from Elsevier.

secondary progressive MS [140]. Molecular dynamics simulations showed this segment had a propensity to form an α helix in aqueous solution but this structure was transient in the absence of stabilizing factors [125, 135]. MBP acquired stable α-helical structure, including the region V83–T92, in 30% TFE, conditions believed to have similar structural effects on proteins as a membrane environment [135, 141]. Structure determination by solution nuclear magnetic resonance (NMR) of an 18-meric peptide containing this segment, Q78–T95 (murine numbering), confirmed it to be α-helical in 30% TFE-d_2 and dodecylphosphocholine micelles [142]. Accessibility of different residues to the micelle interior and aqueous phase was similar to results from EPR spectroscopy of spin-labeled rmC1 using lipid bilayers. The structure of several antigenic epitopes from MBP in aqueous solution, and when bound to class II major histocompatibility complex (MHC) proteins, has been reviewed recently [143].

Conversion of six Arg/Lys (R25, R33, K119, R127, R157, R168) to Gln in rmC1, to mimic citrullination, yielding recombinant murine C8 (rmC8), resulted in dissociation of the C-terminal half of the intact spin-labeled protein from the lipid bilayer, whereas the N-terminal half remained bound and embedded in the bilayer (Fig. 4a) [144]. Four of the six Arg to Gln conversions are in the C-terminal half. The region V83–T92 in rmC8 formed a more highly surface exposed and shorter amphipathic helix consisting of H85–T92 than in rmC1 (Fig. 5) [145]. This was despite the fact that none of the Arg to Gln conversions are near this segment. These results suggested that C8 might be more accessible to enzymes and ligands when bound to membranes or myelin than C1. Indeed, cathepsin D digested lipid-bound rmC8 three times faster than rmC1; cleavage at both F42–F43 and F86-F87 occurred more readily in rmC8 than rmC1.

The segment consisting of residues 91–107 (murine sequence) contains TPRTPPP (residues 92–98) and may form a poly-proline helix. PRTP has been suggested to be a PXXP SH3-target consensus sequence [5] and both Thr are MAPK phosphorylation sites *in vitro* [78, 82]. Interestingly, this region is not conserved in fish MBP [44] suggesting that it might be involved in a more recently evolved function of MBP. Spin-labeled residue 99, just past this region, in membrane-bound rmC1 and rmC8 was exposed to the aqueous phase [144] suggesting that the Pro-rich region C-terminal to the amphipathic helix of both isomers should be accessible to enzymes and other proteins including those with SH3 domains.

The conformation of MBP when bound to CaM has also been investigated. 18.5-kDa MBP binds to CaM in a specific, Ca^{2+}-dependent manner, in a 1 : 1 stoichiometry, with sub-micromolar affinities. MBP had a significantly different interaction when deiminated, possibly with two binding sites, which was supported by further studies using N- and C-terminal deletion mutants of MBP [16, 118]. The CaM-binding tunnel has negatively charged extremities [146] and two Met-rich hydrophobic pockets [147]. The symmetry of this binding site often permits a reverse binding mode for a peptide, with roughly the same energy as the normal orientation. The latter is defined as having the C-terminal end buried in the tunnel. Regions of MBP with hydrophobic residues in the center and basic residues at one or both ends and thought to be capable of forming α helices on interaction with CaM are located at the C terminus (S149–R160) (murine sequence), in the central portion of the protein, P82–R94, (the region studied by spin labeling above), and close to the N terminus, R41–R52. These domains were modeled as α helices of 12–14 residues in length, and docking simulations were performed to investigate their interactions with the CaM peptide-binding tunnel [117]. Almost equally favourable CaM-binding modes were found for each of them, indicating that there are several plausible CaM-binding sites in MBP. These predictions were supported by EPR results for all three segments. These results indicated that all three domains of rmC1 inserted into the CaM-binding tunnel in the normal direction. However, in rmC8, the segment S149–R160 (with R157Q) inserted in the reverse direction. The results also indicated that the segment P83–R92 inserted as an α helix rather than the extended structure formed in the crystal structure of the complex of the peptide with the class II MHC protein.

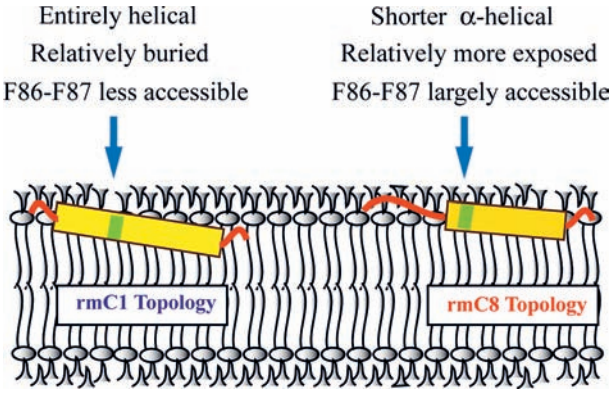

Figure 5. The region V83–T92 was found to form a more surface exposed and shorter helix in the deiminated form of MBP, rmC8, than in rmC1 [145]. The yellow rectangle represents this helical region embedded in the membrane bilayer with the N-terminal end to the left. The Phe86–Phe87 (indicated by the green band) are at or just below the level of the polar head groups in rmC1, but are somewhat above them in rmC8. The helix is tilted in rmC1 with the C-terminal end significantly more deeply embedded in the bilayer than for rmC8. The greater exposure caused membrane-bound rmC8 to be digested faster at Phe86–Phe87 by cathepsin D than rmC1. This could result in greater release of this immunodominant epitope. These results show that reduction in the net positive charge of MBP, which occurs more in MS patients, results in not only decreased adhesion of bilayers, which may destabilize myelin, but also could lead to release of antigenic peptides to initiate or sustain an immune response. The N terminus is at the left, C terminus at the right in both cases. Adapted from Musse et al. [145].

Interactions of MBP with lipids and effect of post-translational modifications

MBP probably causes adhesion of the cytosolic surfaces of myelin by binding to negatively charged lipids [6, 10, 11]. The only other membrane protein present in sufficient quantity to bind a significant amount of MBP is the transmembrane PLP. PLP is also positively charged at the cytosolic surface [148] and can be cross-linked to MBP in myelin [149]. Subsequent studies showed that it binds only to the least positively charged isomer of MBP, C8 [58]. Multilayer formation or aggregation of lipid vesicles containing negatively charged lipids by MBP is a useful model system to study factors which might affect its ability to cause adhesion of the cytosolic surfaces of myelin. This approach has been used to study the effect of post-translational charge modifications to the 18.5-kDa form on its ability to cause adhesion of apposed lipid surfaces [58, 95, 150]. At MBP/lipid ratios lower than those found in compact myelin, where there is an excess of lipid negative charge not neutralized by the MBP positive charge, there was a progressive decrease in the ability of charge isomers to adhere lipid bilayers with a decrease in the positive charge of MBP [58, 102, 150]. Phosphorylation of both C1 and C2 with rabbit muscle kinase decreased their ability to aggregate lipid vesicles [95] and also decreased their permeabilizing effect on vesicles, indicating decreased hydrophobic interactions with the bilayer [151]. Differential association of the charge isomers, which depended on protein concentration, with planar membranes has also been observed [152].

However, at higher MBP/lipid ratios similar to those found in compact myelin, all charge isomers except C8 aggregated large unilamellar vesicles (LUVs) containing phosphatidylcholine (PC) and 10–20% of a single negatively charged lipid such as phosphatidylserine (PS), to a similar degree [150]. There was even less variation in the ability of the charge isomers, including C8, to aggregate LUVs with a lipid composition resembling that of the cytosolic leaflet of myelin (Cyt-LUVs) than for PC/PS LUVs. These results indicate that charge modifications of MBP would probably not affect adhesion of the cytosolic surfaces of compact myelin, where the MBP to lipid ratio is high. However, charge modifications might affect adhesion in cytosol-containing regions of myelin such as the paranodal loops, where MBP concentration is low [11, 153].

Noncompacted regions of myelin contain K^+ channels [154–156] and may take up potassium released into the extracellular fluid and periaxonal space after the axonal action potential [157, 158]. At lower MBP to lipid ratios, where there was an excess of negatively charged lipid, physiological increases in K^+ (or Na^+) concentration up to about 100 mM greatly increased MBP-mediated aggregation of the LUVs by shielding the negative charge on the vesicle surface [98]. Salt increased the aggregation by all charge isomers to the same degree for all [150]. Thus, changes in K^+ concentration in the cytosolic regions of myelin, resulting from the axonal action potential, might be able to regulate MBP-mediated adhesion of the cytosolic myelin surfaces of noncompacted regions of myelin such as the paranodal loops. K^+ concentrations could thus regulate the volume of these cytosolic regions and is a mechanism by which MBP could have a dynamic function in myelin. An increase in the concentration of the acidic lipid PS in myelin from MS patients has been detected in normal-appearing white matter [159]. This could also reduce the ability of MBP to mediate adhesion of the cytosolic regions by increasing the net surface charge of the cytosolic membrane surface.

Neuronal activity also results in release of glutamate and ATP and causes shifts in extracellular pH [160, 161]. Myelin contains NMDA receptors and has been shown to take up Ca^{2+} [162]. Ca^{2+} uptake by OLs myelinating optic nerves also occurs following release of glutamate during axonal conduction due to activation of ionotropic glutamate receptors in OLs [161]. Release of ATP by axonal electrical activity activates P2Y purinoreceptors in OLs causing an increase in intracellular Ca^{2+}. pH fluctuations in the brain extracellular space are accompanied by rapid intracellular pH fluctuations in cells. An Na^+/H^+ exchanger, Na^+/HCO_3^- cotransporter and carbonic anhydrase II are present in OLs and can respond to shifts in pH of the extracellular space during neuronal activity [160]. pH microdomains in OLs occur which differ in pH by over 0.1 pH units. Similar pH fluctuations may occur in the cytosolic spaces of myelin. Changes in Ca^{2+} and/or proton concentrations could regulate MBP-mediated adhesion in these spaces by altering the surface charge of the lipid bilayer and the ionization state of MBP.

Vesicles with the cytosolic lipid composition interacted uniquely with MBP, resulting in greater aggregation, greater sensitivity to K^+ concentration, and resistance to dissociation of all charge isomers at high K^+ (400 mM) concentrations than PC vesicles containing a single negatively charged lipid [98]. The resistance to salt dissociation suggested that electrostatic interactions were not the only force involved in binding of MBP to the Cyt-LUVs. Hydrogen bonding of the protein to the lipid head groups [103] and hydrophobic interactions due to penetration of hydrophobic amino acid side chains into the bilayer, as indicated by the EPR and NMR studies described above [125, 142, 144], and suggested also by effects of MBP on lipid acyl chain order [reviewed in ref. 10], could also contribute to the binding affinity. The greater involvement of hydrophobic interactions of MBP with Cyt-LUVs compared with PC/PS LUVs was confirmed from greater covalent labeling of MBP bound to Cyt-LUVs by the hydrophobic photolabel 3-(trifluoromethyl)-3-(m-[^{125}I]iodophenyl)diazirine (TID) [150]. Cholesterol and

phosphatidylethanolamine together were found to be responsible for the greater MBP-mediated aggregation of Cyt-LUVs and the greater TID labeling of MBP bound to Cyt-LUVs compared with PC/acidic lipid LUVs. Thus, the lipid composition of the cytosolic surface of myelin is well suited to allow MBP to mediate adhesion of apposed cytosolic membranes and to respond in a dynamic way in some regions of myelin to changes in cation and proton concentration resulting from nerve conduction.

In contrast to the reduced aggregation of vesicles caused by C8 containing six citrullines, size exclusion chromatography and negative staining electron microscopy showed that the more highly deiminated isomer isolated from the brain of a patient with Marburg's MS caused fragmentation of large multilayered vesicles into much smaller vesicles [163]. Cryoelectron microscopy of these isoforms bound to a lipid monolayer revealed a larger and more open structure of the protein-lipid complex containing the more hydrophobic Marburg form of MBP compared with C1 and C8 [164]. The membrane fragmentation caused by this more highly citrullinated isomer is dramatically different from the effects of other forms of MBP from normal brain and may indicate a pathogenic effect of this charge isomer which contributed to the severity of Marburg's MS. Similar fragmentation of large vesicles into small ones or discs has been found for other proteins such as apolipoprotein A-1 which have an amphipathic structure [165]. Thus when most of the arginines of MBP are modified to an uncharged amino acid, the protein acquires properties similar to an apolipoprotein suggesting that it may take up an amphipathic structure when bound to lipid. The less deiminated form C8 may also have this apolipoprotein character to a lesser degree, which may be related to its role in normal immature myelin, where it is the predominant charge isomer. Such a lipoprotein-like lipid binding property could allow C8 to be involved in trafficking of lipid to the plasma membrane for synthesis of myelin.

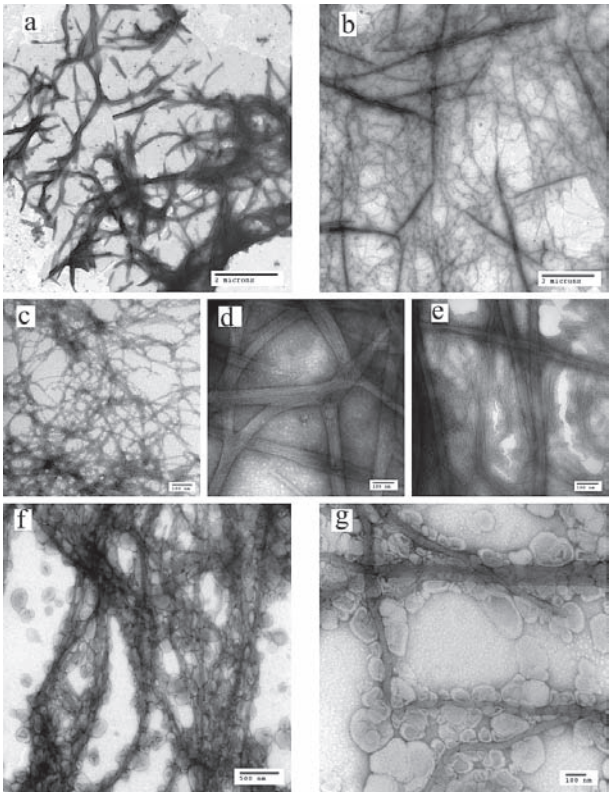

Figure 6. Phosphorylated C1 does not bundle actin filaments as tightly as unmodified C1. C1 can bind actin filaments to lipid vesicles. Electron micrographs of F-actin in the presence of equimolar C1 in F-buffer, bar, 2 μm (*a*); F-actin in the presence of equimolar phosphorylated-C1 in F-buffer, bar, 2 μm (*b*); F-actin in F-buffer, bar = 100 nm (*c*); as in (*a*) but higher magnification, bar, 100 nm (*d*); as in (*b*) but higher magnification, bar = 100 nm (*e*); F-actin in the presence of equimolar C1 and lipid vesicles, bar, 500 nm (*f*); as in (*f*) but higher magnification, bar = 100 nm (*g*). Reproduced from Biochemistry, 2006, vol. 45, pp. 391–401, with copyright permission from the American Chemical Society [18].

MBP-mediated interactions with actin and tubulin and effect of post-translational modifications

Another function of MBP, in addition to that of mediating adhesion of the cytosolic surfaces of myelin, may be to interact with the cytoskeleton in OLs, in cytosolic inclusions in myelin, and even in compact myelin. Actin and tubulin are present in compact myelin where they may be associated with MBP and the radial component, a series of tight junctions, containing claudin-11 [166], that pass through many layers of myelin [167]. MBP in solution causes polymerization of G-actin into filaments and bundling of the actin filaments [15, 17, 18, 109–112] (Fig. 6a, d). Ca^{2+}-CaM inhibits this activity, as it does for the basic domain of the actin-binding protein MARCKS [91]. Co-localization studies by immunohistochemistry in immature cultured OLs indicated that MBP is closely associated with microtubules and actin microfilaments [168–170]. MBP has been co-immunoprecipitated with tubulin from brain tissue [171].

MBP interacts with G-actin and F-actin through electrostatic interactions and the interaction is inhibited by an increase in salt concentration [17, 18, 112]. Thus, the interaction with actin might be expected to be affected by charge modification of MBP. The dissociation constant of the less charged isoform rmC8 for actin was a little greater than that of rmC1, and rmC8 had somewhat reduced ability to polymerize actin and bundle F-actin filaments than rmC1 [17]. Moreover, rmC8 was more readily dissociated from actin by Ca^{2+}-calmodulin than rmC1. In contrast to the effect of deimination, phosphorylation of C1 *in vitro* at Thr94 and Thr97 using MAPK [78, 82] significantly decreased the ability of MBP to polymerize actin and to bundle actin filaments (Fig. 6b, e) despite

having no effect on binding to F-actin or on the ability of Ca^{2+}-CaM to dissociate the complex [18]. The rate and extent of actin polymerization induced by naturally occurring MBP charge isomers C1–C6 decreased with reduction in positive charge [112].

The greater effect of deimination of MBP on its binding to actin compared with that of phosphorylation may be due to the fact that the deiminated Arg were distributed throughout the sequence while phosphorylated residues were in a localized region. Earlier studies indicated that MBP has at least two actin-binding sites [111]. Phosphorylation may interfere with only one of these, while deimination, which is spread over the entire sequence, may interfere with both but in a less severe way. The comparable rates of polymerization induced by rmC8 and rmC1 despite the reduced electrostatic interactions of rmC8 suggests that hydrophobic and/or hydrogen-bonding interactions may also contribute to actin binding by MBP. Indeed, the polymerizing activities of the different size 14 to 21.5-kDa recombinant isoforms also did not depend on their net charges [112]. Phenylalanyl residues in the MARCKS effector domain were found to contribute to its actin-binding affinity and rate of F-actin nucleation [172]. A common actin-binding motif is an amphipathic helix with hydrophobic side chains on one side which binds to a hydrophobic pocket in actin between actin subdomains 1 and 3 [173]. The two sets of Phe-Phe pairs at positions 42–43 and 86–87 in MBP may also contribute to its actin binding.

MBP also binds to tubulin and causes its polymerization and bundling of microtubules [113, 114]. It stabilized microtubules from depolymerizing in the cold and was one of two proteins found in rat brain with this STOP (Stable Tubule Only Polypeptide) activity [174]. Their STOP activity was inhibited by Ca^{2+}-CaM. MBP might also bind actin to microtubules. In shiverer mouse OLs which lack MBP, actin microfilaments were not colocalized with microtubular structures as they were in the wild-type OLs and did not form bundles [12]. The microtubular structures were also abnormal in size, and cell processes were usually smaller than normal with a larger cell body. In shiverer OLs transfected with the MBP gene, in which MBP expression was less than normal, a normal microtubule appearance and actin bundles were seen only in membrane sheets where MBP distribution appeared normal. They were not seen in membrane sheets where MBP distribution was abnormally low [175].

A reduction in positive charge due to deamidation in C2 decreased the ability of MBP to assemble tubulin, but further reductions in positive charge in naturally occurring charge isomers C2-C6, enhanced the ability of MBP to assemble tubulin, in contrast to their effects on actin polymerization [114]. rmC8 had a similar ability to assemble tubulin as rmC1, also in contrast to its decreased interaction with actin. Thus the interaction between MBP and tubulin is not solely dependent on electrostatics. Site-specific phosphorylation of MBP may be the modification in these charge isomers which enhances the interaction with tubulin, as is the case with tau phosphorylated at certain sites [176]. The rm21.5-, rm17.22- and rm18.5-kDa C1 had similar microtubule bundling and F-actin bundling activity, but that of rm14-kDa C1 was less [112, 114].

MBP-mediated binding of actin to membranes and effect of post-translational modifications.

MBP can bind actin filaments to the surface of negatively charged lipid vesicles, suggesting that it may be able to act as a membrane actin-binding protein [15] (Fig. 6f,g). In the case of the MARCKS effector domain, theoretical calculations showed that the electrostatic potential was quite positive above the peptide, even when bound to acidic lipids [177]. A similar effect for MBP would allow it to bind actin while simultaneously binding to the lipid negative surface charge. MBP was observed to be partially co-localized with actin at the edge of membrane sheets of OLs where it might bind actin to the membrane [18].

Many actin-binding proteins insert into the lipid bilayer allowing them to anchor actin filaments to the membrane and possibly to also sense signals transmitted through the membrane. This insertion into the lipid bilayer has been detected by labeling with hydrophobic photolabels such as TID [178] which react only with groups in the acyl chain region of the bilayer. The spin label studies described above indicate that hydrophobic side chains of MBP insert into the lipid bilayer and MBP can also be labeled by TID when bound to lipid [98, 179]. Like many actin-binding proteins which are myristoylated at the N terminus [180, 181], MBP is also acylated at the N terminus, although in MBP the chain length is heterogeneous and no longer than 10 carbons [59]. Other similarities, including analogous structural motifs, between MBP and the actin-binding MARCKS protein have been pointed out by Harauz et al. [28].

The ability of MBP to bind actin to the myelin or oligodendrocyte membrane may allow it to participate in signaling [74]. Insertion of hydrophobic side chains or its N-acyl group into the bilayer would allow it to sense mechanical signals transmitted through the membrane, e.g. disordering effects on the lipid bilayer or bilayer curvature strain, and respond through a structural change, which could then impact on the cytoskeleton. Actin binding to MBP decreased the labeling of MBP by TID, indicating that it decreased the hydrophobic interactions of MBP with the bilayer [15]. This may be due to transfer of hydrophobic residues of MBP, such as Phe, from the lipid bilayer to the hydrophobic pocket in actin. This change in interaction of MBP with the bilayer could then create a

cytosol-to-membrane signal caused by changes in interaction of the cytoskeleton with the membrane.

The amount of actin bound to the MBP-lipid vesicles decreased with increasing net negative surface charge of the lipid vesicles [15]. Ca^{2+}-CaM binding also caused dissociation of MBP and the MBP-actin complex from lipid vesicles [15, 17, 18] as it does for the basic domains of other proteins such as MARCKS [91]. Although basic domains of MBP are distributed throughout its sequence, in contrast to MARCKS, it also has several potential CaM-binding sites [117], which may allow different regions of MBP to be dissociated from the bilayer by CaM. The deiminated isomer, C8, and phosphorylated C1 (on Thr94 and Thr97 using MAPK) had significantly less ability to bind actin to lipid bilayers [17, 18]. The effect of phosphorylation was much greater than that of citrullination at six sites. Although average electrostatic forces are the primary determinant of the interaction of MBP with actin, phosphorylation may have an additional effect due to a site-specific electrostatic effect or to a conformational change. The 21.5-kDa isoform containing the exon II-encoded sequence bound actin to the lipid bilayer less well than the 18.5-kDa form lacking this sequence [17]. The ability of other size isoforms to bind actin to the lipid bilayer has not been investigated.

Thus, deimination, a post-translational modification of MBP, which occurs early in life and is increased in MS, and phosphorylation of MBP, which occurs in response to various extracellular signals in both myelin and OLs, attenuate the ability of MBP to polymerize and bundle actin, and to bind actin to a membrane surface. Phosphorylation of a number of membrane actin-binding proteins such as MARCKS, regulates their interaction with the cytoskeleton and the membrane [182].

MBP-cytoskeleton interactions *in vivo*

During the period of rapid myelination, in the first year of life in humans, the OL must produce prodigious amounts of membrane. Many events involved in myelination such as OL precursor migration in the CNS, differentiation, cell process extension, and membrane production may depend in part on the OL cytoskeleton. Its interactions with the cytosolic side of the plasma membrane would allow it to participate in transmission of signals between the extracellular environment and the cytosol [183]. Such signals are received from extracellular matrix, growth hormones, and the axon. Continued interaction and communication between the axon and the mature compact myelin sheath occurs throughout life [184, 185]. Interactions between the adhered membranes of myelin in the multilayered myelin sheath may also provide signals which are transmitted across the membrane and across many layers of myelin.

Interactions of MBP with the cytoskeleton may allow it to play a role in signaling in OLs. Anti-galactosylceramide (GalC) antibody added to OLs mediates signals which cause Ca^{2+} entry, lipid and protein redistribution in the membrane, phosphorylation changes of MBP, depolymerization of microtubules and other signal transduction events in cultured OLs [186, 187]. This may be a signal for compaction of the MBP-containing domains [13]. MBP is required for these effects, since they do not occur in shiverer OLs, suggesting that MBP is involved in transduction of the signal [74].

GalC and sulfatide may be ligands for each other which cause similar signaling effects [188] as found for GSLs in other cells [189]. Ca^{2+} can bridge sugars by binding sugar oxygens to its coordination sphere [190], and it causes adhesion of liposomes containing GalC to liposomes containing sulfatide [191]. Addition of GalC/sulfatide-containing liposomes to cultured OLs to interact with GalC and sulfatide in the OL membrane by these Ca^{2+}-mediated trans carbohydrate-carbohydrate interactions across apposed membranes had similar effects on lipid and protein distribution and depolymerization of the cytoskeleton as anti-GalC antibody [192]. Stabilization of the actin cytoskeleton with jasplakinolide prevented all effects of the liposomes including GalC and MBP redistribution and microtubule depolymerization, suggesting that the stability of microtubules in OLs depends on the integrity of the actin cytoskeleton [193]. The MBP redistribution caused by depolymerization of the actin cytoskeleton suggests that actin is linked directly or indirectly to MBP in OLs. In support of this suggestion, MBP is present in a Triton X-100-insoluble fraction from OLs, which resembled intact cytoskeleton and contained actin and tubulin [168].

MBP may also interact with actin and tubulin in myelin. Detergent extraction of myelin indicates that MBP is located in several different membrane domains or associated with different proteins. Some MBP distributes to two low-density glycosphingolipid/cholesterol-enriched Triton X-100-insoluble fractions, one of which is associated with actin, tubulin, caveolin, and kinases [167, 194–196]. Some MBP is also found in the Triton X-100 supernatant associated with MAG, PLP, MAPK, and some phospholipid [197]. Although these isolated fractions do not necessarily come from different membrane domains in myelin in situ [198], the presence of MBP in several different fractions suggests that it can interact with different contituents or complexes in myelin, some of which interact with kinases. This may allow MBP to participate in signaling in myelin also. The p42/p44 MAPK in myelin is active and phosphorylates exogenous MBP [194] and endogenous MBP, CNP, and tubulin [Y. Gong, W. Min and J. M. Boggs, unpublished data]. Different charge isomers and size isoforms of bovine MBP also are differentially associated with several detergent-insoluble fractions of

myelin. A CHAPS-resistant low-density fraction contained most of the phospho-Thr97-MBP whereas most of the citrullinated and methylated MBP were in a heavier CHAPS-insoluble fraction [199]. The 18.5-kDa MBP was in the low-density fraction and 21.5-kDa MBP was in the heavier CHAPS-insoluble fraction. This suggests that these different species of MBP associate preferentially with different myelin constituents and may perform different functions in these complexes.

In addition to kinases and phosphatases which can mediate rapid turnover of phosphate groups [reviewed in ref. 4], myelin also contains mechanisms which control kinase activity, e.g. phospholipase C to generate DAG, adenyl cyclase to generate cAMP, and MEK to activate p42/p44 MAPK [4, 197]. Myelin also contains relatively high amounts of the signal transduction lipids phosphatidylinositol (PI), phosphatidic acid, phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP_2) [200]. The lightest myelin fraction, which comes from compact myelin, was even more enriched in these signal transduction lipids. PIP_2 is 1.5% of the total phospholipid in myelin and 1.7% in the lightest fraction, compared with 1% reported for the plasma membrane of other cells [137]. MBP stimulates a PI-specific phospholipase C purified from human myelin, but deimination prevented this [201]. Thus the more highly charged isomers of MBP may be directly involved in a signaling system involving phosphoinositides in myelin. There is considerable turnover of phosphoinositides in myelin *in vivo* [202], which may be regulated by communication with the axon [185]. In addition to being a source of second messengers, PIP_2 is a potent regulator of many actin-binding proteins [203]. Thus mechanisms are present in both OLs and myelin to regulate the interaction of MBP with the membrane and with the cytoskeleton by phosphorylation of MBP and by changes in lipid composition.

Trans interactions between GSLs in membrane signaling domains in apposed cells trigger signaling and have been proposed to result in formation of a glycosynapse between the cells [189]. Glycosynapses could also form between the apposed extracellular surfaces in compact myelin through contact between GalC and sulfatide in signaling domains [188]. Ions and water released into the periaxonal space following the axonal action potential may be able to traverse throughout the extracellular space in compact myelin by passing through the tight junction pores of the radial component [13] and could regulate this interaction. The signals may in turn be transmitted across the membrane to MBP and the cytoskeletal elements in myelin, as found in cultured OLs for anti-GalC antibody and GalC/sulfatide-containing liposomes, possibly by causing Ca^{2+} entry into the cytosolic domains. Ca^{2+} accumulation into the cytosolic domains of compact myelin, mediated by NMDA receptors, has recently been detected within compact myelin [162]. Subsequent effects on the cytoskeleton may regulate opening and closing of the tight junction pores between the adjacent myelin layers [13] as occurs for intestinal epithelial cells [204]. This process may allow transmission of signals from the axon throughout compact myelin.

Conclusion

MBP, an intrinsically disordered basic protein which can bind to and adapt its structure to different polyanionic surfaces, such as the lipid bilayer, actin, tubulin, and clathrin, and possibly polynucleotides, may have several functions in OLs and myelin. In addition to causing adhesion of the cytosolic surfaces of compact myelin, it may interact with the cytoskeleton and bind it to the membrane surface and it may participate in transmission of extracellular signals to the cytoskeleton and/or to tight junctions. It has several different size isoforms and charge isomers, and a number of post-translational modifications, which could augment and regulate its multifunctionality. Binding of actin filaments to the membrane mediated by MBP could be regulated by changes in the ratio of negatively charged to neutral lipid which affect the membrane surface charge, in phosphorylation of MBP, and in intracellular Ca^{2+} levels to regulate calmodulin, all changes which occur during signaling. Deimination and size isoform expression, changes which occur during development and differentiation, could also regulate its interaction with the cytoskeleton.

Post-translational modifications also affect the interaction of MBP with the membrane and its conformation. The reduction in cationicity of charge isomers of MBP impedes the membrane adhesion and assembly activity of this protein. This may play a physiological role in membrane formation by the OL before axonal wrapping and myelin compaction is initiated, and in regulation of the volume of the cytosol-containing regions of the myelin sheath. The ability of MBP to cause adhesion of the cytosolic surfaces is also very sensitive to the membrane surface charge and the salt concentration, which may allow this function to be regulated by possible uptake of K^+ or Ca^{2+}, or pH fluctuations in the cytosolic regions during the neuronal activity. Contact between GSL-enriched membrane domains in apposed extracellular surfaces in compact myelin (glycosynapses) may trigger signals which are transmitted to MBP and the cytoskeleton. This may regulate the opening and closing of tight junctional pores and the ion and water content of the extracellular spaces of compact myelin.

Post-translational modifications may also have a pathologic role in destabilizing myelin. Deimination disorders the protein further, even when bound to lipid, and causes the C-terminal half to be more accessible to enzymes and proteases. One of the regions exposed to proteases is an

immunodominant epitope. Greater exposure on the cytosolic membrane surface may cause this highly encephalitogenic epitope to be cleaved and released to initiate or sustain immune response to MBP in MS.

Acknowledgement. The work from our laboratory discussed in this paper was supported by grants from the Canadian Institutes of Health Research and the Multiple Sclerosis Society of Canada. Gratitude is expressed to Drs G. Harauz (University of Guelph, Guelph, Ontario), R. Gould (University of Illinois at Chicago), and C. Husted (University of California at Santa Barbara) for reading the manuscript and providing helpful comments, and to G. Harauz and A. Musse for providing versions of Figures 2a, b, 4, and 5.

1 Raine, C. S. (1977) Morphological aspects of myelin and myelination. In: Myelin (Morell, P., Ed.), pp. 1–49, Plenum, New York.
2 Kirschner, D. A. and Blaurock, A. E. (1992) Organization, phylogenetic variations, and dynamic transitions of myelin. In: Myelin: Biology and Chemistry (Martenson, R. E., Ed.), pp. 1–78, CRC, Boca Raton.
3 Bunge, M. B., Bunge, R. P. and Ris, H. (1961) Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. J. Biophys. Biochem. Cytol. 10, 67–94.
4 Ledeen, R. W. (1992) Enzymes and receptors of myelin. In: Myelin: Biology and Chemistry (Martenson, R. E., Ed.), pp. 531–570, CRC, Boca Raton.
5 Moscarello, M. A. (1997) Myelin basic protein, the 'executive' molecule of the myelin membrane. In: Cell Biology and Pathology of Myelin: Evolving Biological Concepts and Therapeutic Approaches (Juurlink, B. H. J., Devon, R. M., Doucette, J. R., Nazarali, A. J., Schreyer, D. J. and Verge, V. M. K. Eds.), pp. 13–25, Plenum, New York.
6 Readhead, C., Takasashi, N., Shine, H. D., Saavedra, R., Sidman, R. and Hood, L. (1990) Role of myelin basic protein in the formation of central nervous system myelin. Ann. NY Acad. Sci. 605, 280–285.
7 Carre, J.-L., Goetz, B. D., O'Connor, L. T., Bremer, Q. and Duncan, I. D. (2002) Mutations in the rat myelin basic protein gene are associated with specific alterations in other myelin gene expression. Neurosci. Lett. 330, 17–20.
8 Martini, R. and Schachner, M. (1997) Molecular bases of myelin formation as revealed by investigations on mice deficient in glial cell surface molecules. Glia 19, 298–310.
9 Gould, R. M., Byrd, A. L. and Barbarese, E. (1995) The number of Schmidt-Lanterman incisures is more than doubled in shiverer PNS myelin sheaths. J. Neurocytol. 24, 85–98.
10 Boggs, J. M., Moscarello, M. A. and Papahadjopoulos, D. (1982) Structural organization of myelin: role of lipid-protein interactions determined in model systems. In: Lipid-Protein Interactions, vol. 2 (Jost, P. and Griffith, O. H. Eds.), pp. 1–51, Wiley, New York.
11 Omlin, F. X., Webster, H. D., Palkovits, C. G. and Cohen, S. R. (1982) Immunocytochemical localization of basic protein in major dense line regions of central and peripheral myelin. J. Cell Biol. 95, 242–248.
12 Dyer, C. A., Philibotte, T. M., Billings-Gagliardi, S. and Wolf, M. K. (1995) Cytoskeleton in myelin-basic-protein-deficient shiverer oligodendrocytes. Dev. Neurosci. 17, 53–62.
13 Dyer, C. A. (2002) The structure and function of myelin: from inert membrane to perfusion pump. Neurochem. Res. 27, 1279–1292.
14 Staugaitis, S. M., Colman, D. R. and Pedraza, L. (1996) Membrane adhesion and other functions for the myelin basic proteins. BioEssays 18, 13–18.
15 Boggs, J. M. and Rangaraj, G. (2000) Interaction of lipid-bound myelin basic protein with actin filaments and calmodulin. Biochemistry 39, 7799–7806.
16 Libich, D. S., Hill, C. M. D., Bates, I. R., Hallett, F. R., Armstrong, S., Siemiarczuk, A. and Harauz, G. (2003) Interaction of the 18.5 kDa isoform of myelin basic protein with Ca^{2+}-calmodulin: effects of deimination assessed by intrinsic Trp fluorescence spectroscopy, dynamic light scattering, and circular dichroism. Protein Sci. 12, 1507–1521.
17 Boggs, J. M., Rangaraj, G., Hill, C. M. D., Bates, I. R., Heng Y.-M. and Harauz, G. (2005) Effect of arginine loss in myelin basic protein, as occurs in its deiminated charge isoform, on mediation of actin polymerization and actin binding to a lipid membrane *in vitro*. Biochemistry 44, 3524–3534.
18 Boggs, J. M., Rangaraj, G., Gao, W. and Heng Y.-M. (2006) Effect of phosphorylation of myelin basic protein by MAPK on its interactions with actin and actin binding to a lipid membrane *in vitro*. Biochemistry 45, 391–401.
19 Hardy, R. J., Lazzarini, R. A., Colman, D. R. and Friedrich V. L. Jr. (1996) Cytoplasmic and nuclear localization of myelin basic proteins reveals heterogeneity among oligodendrocytes. J. Neurosci. Res. 46, 246–257.
20 Benmerah, A., Scott, M., Poupon, V. and Marullo, S. (2003) Nuclear functions for plasma membrane-associated proteins? Traffic 4, 503–511.
21 Tompa, P., Szasz C and Buday, L. (2005) Structural disorder throws new light on moonlighting. Trends Biochem. Sci. 30, 484–489.
22 Hill, C. M., Bates, I. R., White, G. F., Hallett, F. R. and Harauz, G. (2002) Effects of the osmolyte trimethylamine-N-oxide on conformation, self-association, and two-dimensional crystallization of myelin basic protein. J. Struct. Biol. 139, 13–26.
23 Harauz, G., Ishiyama, N., Hill, C. M., Bates, I. R., Libich, D. S. and Farès, C. (2004) Myelin basic protein – diverse conformational states of an intrinsically unstructured protein and its roles in myelin assembly and multiple sclerosis. Micron 35, 503–542.
24 de Ferra, F., Engh, H., Hudson, L., Kamholz, J., Puckett, C., Molineaux, S. and Lazzarini, R. A. (1985) Alternative splicing accounts for the four forms of myelin basic protein. Cell 43, 721–727.
25 Uversky, V. N. (2002) Natively unfolded proteins: a point where biology waits for physics. Protein Sci. 11, 739–756.
26 Dyson, H. J. and Wright, P. E. (2002) Coupling of folding and binding for unstructured proteins. Curr. Opin. Struct. Biol. 12, 54–60.
27 Tompa, P. (2005) The interplay between structure and function in intrinsically unstructured proteins. FEBS Lett. 579, 3346–3354.
28 Harauz, G., Ishiyama, N. and Bates, I. R. (2000) Analogous structural motifs in myelin basic protein and in MARCKS. Mol. Cell. Biochem. 209, 155–163.
29 Pandey, N., Ganapath, M., Kumar, K., Dasgupta, D., Das Sutar, S. K. and Dash, D. (2004) Comparative analysis of protein unfoldedness in human housekeeping and non-housekeeping proteins. Bioinformatics 20, 2904–2910.
30 Haynes, C. and Iakoucheva, L. M. (2006) Serine/arginine-rich splicing factors belong to a class of intrinsically disordered proteins. Nucleic Acids Res. 34, 305–312.
31 Ward, J. J., Sodhi, J. S., McGuffin, L. J., Buxton, B. F. and Jones, D. T. (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J. Mol. Biol. 337, 635–645.
32 Brown, C. J., Takayama, S., Campen, A. M., Vise, P., Marshall, T. W., Oldfield, C. J., Williams, C. J. and Dunker, A. K. (2002) Evolutionary rate heterogeneity in proteins with long disordered regions. J. Mol. Evol. 55, 104–110.
33 Uversky, V. N., Oldfield, C. J. and Dunker, A. K. (2005) Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. J. Mol. Recog. 18, 343–384.
34 Patil, A. and Nakamura, H. (2006) Disordered domains and high surface charge confer hubs with the ability to interact

with multiple proteins in interactions networks. FEBS Lett. 580, 2041–2045.
35 Saavedra, R. A., Fors, L., Aebersold, R. H., Arden, B., Horvath, S., Sanders, J. and Hood, L. (1989) The myelin proteins of the shark brain are similar to the myelin proteins of the mammalian peripheral nervous system. J. Mol. Evol. 29, 149–156.
36 Milne, T. J., Atkins, A. R., Warren, J. A., Auton, W. P. and Smith, R. (1990) Shark myelin basic protein: amino acid sequence, secondary structure, and self-association. J. Neurochem. 55, 950–955.
37 Gould, R. M., Morrison, H. G., Gilland, E. and Campbell, R. K. (2005) Myelin tetraspan family proteins but no non-tetraspan family proteins are present in the Ascidian (*Ciona intestinalis*) genome. Biol. Bull. 209, 49–66.
38 Givogri, M. I., Bongarzone, E. R., Schonmann, V. and Campagnoni, A. T. (2001) Expression and regulation of golli products of myelin basic protein gene during *in vitro* development of oligodendrocytes. J. Neurosci. Res. 66, 679–690.
39 Marty, M. C., Alliot, F., Rutin, J., Fritz, R., Trisler, D. and Pessac, B. (2002) The myelin basic protein gene is expressed in differentiated blood cell lineages and in hemopoietic progenitors. Proc. Natl Acad. Sci. USA 99, 8856–8861.
40 Jacobs, E. C., Pribyl, T. M., Kampf, K., Campagnoni, C., Colwell, C. S., Reyes, S. D., Martin, M., Handley, V., Hiltner, T. D., Readhead, C., Jacobs, R. E., Messing, A., Fisher, R. S. and Campagnoni, A. T. (2005) Region-specific myelin pathology in mice lacking the Golli products of the myelin basic protein gene. J. Neurosci. 25, 7004–7013.
41 Kamholz, J., Toffenetti, J. and Lazzarini, R. (1988) Organization and expression of the human myelin basic protein gene. J. Neurosci. Res. 21, 62–70.
42 Barbarese, E., Carson, J. H. and Braun, P. E. (1978) Accumulation of the four myelin basic proteins in mouse brain during development. J. Neurochem. 31, 779–782.
43 Brosamle, C. and Halpern, M. E. (2002) Characterization of myelination in the developing zerbrafish. Glia 39, 47–57.
44 Martenson, R. E. (1992) Myelin basic protein isoforms: structural and evolutionary implications of alternative exon splicing. In: Myelin: Biology and Chemistry (Martenson, R. E., Ed.), pp. 387–412, CRC, Boca Raton.
45 Pedraza, L. (1997) Nuclear transport of myelin basic protein. J. Neurosci. Res. 50, 258–264.
46 de Vries, H., de Jonge, J. C., Schrage, C., van der Haar, M. E. and Hoekstra, D. (1997) Differential and cell development-dependent localization of myelin mRNAs in oligodendrocytes. J. Neurosci. Res. 47, 479–488.
47 Sugiyama, I., Tanaka, K., Akita, M., Yoshida, K., Kawase, T. and Asou, H. (2002) Ultrastructural analysis of the paranodal junction of myelinated fibers in 31-month-old rats. J. Neurosci. Res. 70, 309–317.
48 Allinquant, B., Staugaitis, S. M., D'Urso, D. and Colman, D. R. (1991) The ectopic expression of myelin basic protein isoforms in Shiverer oligodendrocytes: implications for myelinogenesis. J. Cell Biol. 113, 393–403.
49 Staugaitis, S. M., Smith, P. R. and Colman, D. R. (1990) Expression of myelin basic protein isoforms in nonglial cells. J. Cell Biol. 110, 1719–1727.
50 Pedraza, L., Fidler, L., Staugaitis, S. M. and Colman, D. R. (1997) The active transport of myelin basic protein into the nucleus suggests a regulatory role in myelination. Neuron 18, 579–589.
51 Boggs, J. M., Rangaraj, G., Koshy, K. M. and Mueller, J. P. (2000) Exon-2-containing 21.5 kD isoform of myelin basic protein causes adhesion of acidic lipid vesicles. Biochim. Biophys. Acta 1463, 81–87.
52 Karthigasan, J., Garvey, J. S., Ramamurthy, C. V. and Kirschner, D. A. (1996) Immunolocalization of 17 and 21.5 kDa MBP isoforms in compact myelin and radial component. J. Neurocytol. 25, 1–7.
53 Kimura, M., Sato, M., Akatsuka, A., Saito, S., Ando, K., Yokoyama, M. and Katsuki, M. (1998) Overexpression of a minor component of myelin basic protein isoform (17.2 kDa) can restore myelinogenesis in transgenic shiverer mice. Brain Res. 785, 245–252.
54 Seiwa, C., Kojima-Aikawa, K., Matsumoto, I. and Asou, H. (2002) CNS myelinogenesis *in vitro*: myelin basic protein deficient shiverer oligodendrocytes. J. Neurosci. Res. 69, 305–317.
55 Chou, F. C.-H., Chou, C.-H. J., Shapira, R. and Kibler, R. F. (1976) Basis of microheterogeneity of myelin basic protein. J. Biol. Chem. 251, 2671–2679.
56 Zand, R., Li, M. X., Jin, X. and Lubman, D. (1998) Determination of the sites of posttranslational modifications in the charge isomers of bovine myelin basic protein by capillary electrophoresis-mass spectroscopy. Biochemistry 37, 2441–2449.
57 Wood, D. D. and Moscarello, M. A. (1989) The isolation, characterization, and lipid-aggregating properties of a citrulline containing myelin basic protein. J. Biol. Chem. 264, 5121–5127.
58 Zand, R., Jin, X., Kim, J., Wall, D. B., Gould, R. and Lubman, D. M. (2001) Studies of posttranslational modifications in spiny dogfish myelin basic protein. Neurochem. Res. 26, 539–547.
59 Moscarello, M. A., Pang, H., Pace-Asciak, C. R. and Wood, D. D. (1992) The N terminus of human myelin basic protein consists of C2, C4, C6, and C8 alkyl carboxylic acids. J. Biol. Chem. 267, 9779–9782.
60 Deibler, G. E. and Martenson, R. E. (1973) Chromatographic fractionation of myelin basic protein: partial characterization and methylarginine contents of the multiple forms. J. Biol. Chem. 248, 2392–2396.
61 Brostoff, S. and Eylar, E. H. (1971) Localization of methylated arginine in the A1 protein from myelin. Proc. Natl. Acad. Sci. USA 68, 765–769.
62 Baldwin, G. S. and Carnegie, P. R. (1971) Isolation and partial characterization of methylated arginines from the encephalitogenic basic protein of myelin. Biochem. J. 123, 69–74.
63 Chan, C. K., Ramwani, J. and Moscarello, M. A. (1988) Myelin basic protein binds GTP at a single site in the N terminus. Biochem. Biophys. Res. Commun. 152, 1468–1473.
64 Boulias, C. and Moscarello, M. A. (1994) ADP-ribosylation of human myelin basic protein. J. Neurochem. 63, 351–359.
65 Iakoucheva, L. M., Radivojac, P., Brown, C. J., O'Connor, T. R., Sikes, J. G., Obradovic, Z. and Dunker, K. A. (2004) The importance of intrinsic disorder for protein phosphorylation. Nucleic Acids Res. 32, 1037–1049 .
66 Tarcsa, E., Marekov, L. N., Mei, G., Melino, G., Lee, S. C. and Steinert, P. M. (1996) Protein unfolding by peptidylarginine deiminase: substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. J. Biol. Chem. 271, 30709–30716.
67 Kim, J. K., Mastronardi, F. G., Wood, D. D., Lubman, D. M., Zand, R. and Moscarello, M. A. (2003) Multiple sclerosis: an important role for post-translational modifications of myelin basic protein in pathogenesis. Mol. Cell. Proteom. 2, 453–462.
68 Jantz, D. and Berg, J. M. (2003) Expanding the DNA-recognition repertoire for zinc finger proteins beyond 20 amino acids. J. Am. Chem. Soc. 125, 4960–4961.
69 Ulmer, J. B. and Braun, P. E. (1986) *In vivo* phosphorylation of myelin basic proteins: single and double isotope incorporation in developmentally related myelin fractions. Dev. Biol. 117, 502–510.
70 DesJardins, K. C. and Morell, P. (1983) Phosphate groups modifying myelin basic proteins are metabolically labile; methyl groups are stable. J. Cell Biol. 97, 438–446.

71 Murray, N. and Steck, A. J. (1984) Impulse conduction regulates myelin basic protein phosphorylation in rat optic nerve. J. Neurochem. 43, 243–248.
72 Atkins, C. M., Chen S-J., Klann, E. and Sweatt, J. D. (1997) Increased phosphorylation of myelin basic protein during hippocampal long-term potentiation. J. Neurochem. 68, 1960–1967.
73 Atkins, C. M., Yon, M., Groome, N. P. and Sweatt, J. D. (1999) Regulation of myelin basic protein phosphorylation by mitogen-activated protein kinase during increased action potential firing in the hippocampus. J. Neurochem. 73, 1090–1097.
74 Dyer, C. A., Philibotte, T. M., Wolf, M. K. and Billings-Gagliardi, S. (1994) Myelin basic protein mediates extracellular signals that regulate microtubule stability in oligodendrocyte membrane sheets. J. Neurosci. Res. 39, 97–107.
75 Vartanian, T., Szuchet, S., Dawson, G. and Campagnoni, A. T. (1986) Oligodendrocyte adhesion activates protein kinase C-mediated phosphorylation of myelin basic protein. Science 234, 1395–1398.
76 Vartanian, T., Szuchet, S. and Dawson, G. (1992) Oligodendrocyte-substratum adhesion activates the synthesis of specific lipid species involved in cell signaling. J. Neurosci. Res. 32, 69–78.
77 Soliven, B., Takeda, M. and Szuchet, S. (1994) Depolarizing agents and tumor necrosis factor-α modulate protein phosphorylation in oligodendrocytes. J. Neurosci. Res. 38, 91–100.
78 Erickson, A. K., Payne, D. M., Martino, P. A., Rossomando, A. J., Shabanowitz, J., Weber, M. J., Hunt, D. F. and Sturgill, T. W. (1990) Identification by mass spectrometry of threonine 97 in bovine myelin basic protein as a specific phosphorylation site for mitogen-activated protein kinase. J. Biol. Chem. 265, 19728–19735.
79 Yu, J. S. and Yang, S. D. (1994) Protein kinase FA/glycogen synthase kinase-3 predominantly phosphorylates the *in vivo* site Thr97-Pro in brain myelin basic protein: evidence for Thr-Pro and Ser-Arg-X-X-Ser as consensus sequence motifs. J. Neurochem. 62, 1596–1603.
80 Kishimoto, A., Nishiyama, K., Nakanishi, H., Uratsuji, Y., Nomura, H., Takeyama, Y. and Nishizuka, Y. (1985) Studies on the phosphorylation of myelin basic protein by protein kinase C and adenosine 3′:5′-monophosphate-dependent protein kinase. J. Biol. Chem. 260, 12492–12499.
81 Martenson, R. E., Law, M. J. and Deibler, G. E. (1983) Identification of multiple *in vivo* phosphorylation sites in rabbit myelin basic protein. J. Biol. Chem. 258, 930–937.
82 Hirschberg, D., Radmark, O., Jornvall, H. and Bergman, T. (2003) Thr94 in bovine myelin basic protein is a second phosphorylation site for 42-kDa mitogen-activated protein kinase (ERK2). J. Protein Chem. 22, 177–181.
83 Kubilus, J. and Baden, H. P. (1983) Purification and properties of a brain enzyme which deiminates proteins. Biochim. Biophys. Acta 745, 285–291.
84 Lamensa, J. W. E. and Moscarello, M. A. (1993) Deimination of human myelin basic protein by a peptidylarginine deiminase from bovine brain. J. Neurochem. 61, 987–996.
85 Moscarello, M. A., Wood, D. D., Ackerley, C. and Boulias, C. (1994) Myelin in multiple sclerosis is developmentally immature. J. Clin. Invest. 94, 146–154.
86 Mastronardi, F. G. and Moscarello, M. A. (2005) Molecules affecting myelin stability: a novel hypothesis regarding the pathogenesis of multiple sclerosis. J. Neurosci. Res. 80, 301–308.
87 Mendez, M. F. and Pogacar, S. (1988) Malignant monophasic multiple sclerosis or 'Marburg's disease'. Neurology 38, 1153–1155.
88 Wood, D. D., Bilbao, J. M., O'Connors, P. and Moscarello, M. A. (1996) Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. Ann. Neurol. 40, 18–24.
89 Jones, L. S., Yazzie, B. and Middaugh, C. R. (2004) Polyanions and the proteome. Mol. Cell. Proteomics 3, 746–769.
90 Murray, D., Ben-Tal, N., Honig, B. and McLaughlin, S. (1997) Electrostatic interaction of myristoylated proteins with membranes: simple physics, complicated biology. Structure 5, 985–989.
91 McLaughlin, S., Hangyas-Mihalyne, G., Zaitseva, I. and Golebiewska, U. (2005) Reversible – through calmodulin – electrostatic interactions between basic residues on proteins and acidic lipids in the plasma membrane. Biochem. Soc. Symp. 72, 189–198.
92 Smith, R. (1977) Non-covalent cross-linking of lipid bilayers by myelin basic protein: a possible role in myelin formation. Biochim. Biophys. Acta 470, 170–184.
93 Lampe, P. D., Wei, G. J. and Nelsestuen, G. L. (1983) Stopped-flow studies of myelin basic protein association with phospholipid vesicles and subsequent vesicle aggregation. Biochemistry 22, 1594–1599.
94 Smith, R. (1992) The basic protein of CNS myelin: its structure and ligand binding. J. Neurochem. 59, 1589–160.
95 Cheifetz, S. and Moscarello, M. A. (1985) Effect of bovine basic protein charge microheterogeneity on protein-induced aggregation of unilamellar vesicles containing a mixture of acidic and neutral phospholipids. Biochemistry 24, 1909–1914.
96 Surewicz, W. K., Epand, R. M., Epand, R. F., Hallett, F. H. and Moscarello, M. A. (1986) Modulation of myelin basic protein-induced aggregation and fusion of liposomes by cholesterol, aliphatic aldehydes and alkanes. Biochim. Biophys. Acta 863, 45–52.
97 ter Beest, M. B. A. and Hoekstra, D. (1993) Interaction of myelin basic protein with artificial membranes: parameters governing binding, aggregation and dissociation. Eur. J. Biochem. 211, 689–696.
98 Jo, E. and Boggs, J. M. (1995) Aggregation of acidic lipid vesicles by myelin basic protein: dependence on potassium concentration. Biochemistry 34, 13705–13716.
99 Hu, Y., Doudevski, I., Wood, D., Moscarello, M., Husted, C., Genain, C., Zasadzinski, J. A. and Israelachvili, J. (2004) Synergistic interactions of lipids and myelin basic protein. Proc. Natl. Acad. Sci. USA 101, 13466–13471.
100 Riccio, P., Bobba, A., Romito, E., Minetola, M. and Quagliariello, E. (2000) A new detergent to purify CNS myelin basic protein isoforms in lipid-bound form. Neuroreport 5, 689–692.
101 Mateu, L., Luzzati, V., London, Y., Gould, R. M. and Vossenberg, F. G. (1973) X-ray diffraction and electron microscope study of the interactions of myelin components: the structure of a lamellar phase with a 150 to 180 Å repeat distance containing basic proteins and acidic lipids. J. Mol. Biol. 75, 697–709.
102 Brady, G. W., Murthy, N. S., Fein, D. B., Wood, D. D. and Moscarello, M. A. (1981) The effect of basic myelin protein on multilayer membrane formation. Biophys. J. 34, 345–350.
103 Sedzik, J., Blaurock, A. E. and Hochli, M. (1984) Lipid/myelin basic protein multilayers: a model for the cytoplasmic space in central nervous system myelin. J. Mol. Biol. 174, 385–409.
104 Cajal, Y., Boggs, J. M. and Jain, M. K. (1997) Salt-triggered intermembrane exchange of phospholipids and hemifusion by myelin basic protein. Biochemistry 36, 2566–2576.
105 Boggs, J.M., Moscarello, M.A. and Papahadjopoulos, D. (1977) Phase separation of acidic and neutral phospholipids induced by human myelin basic protein. Biochemistry 16, 5420–5426.
106 Sankaram, M. B., Brophy, P. J. and Marsh, D. (1989) Selectivity of interaction of phospholipids with bovine spinal cord myelin basic protein studied by spin-label electron spin resonance. Biochemistry 28, 9699–707.
107 Lowden, J. A., Moscarello, M. A. and Morecki, R. (1966) The isolation and characterization of an acid-soluble protein from myelin. Can. J. Biochem. 44, 567–577.

108 Polverini, E., Fasano, A., Zito, F., Riccio, P. and Cavatorta, P. (1999) Conformation of bovine myelin basic protein purified with bound lipids. Eur. Biophys. J. 28, 351–355.

109 Barylko, B. and Dobrowolski, Z. (1984) Ca^{2+}-calmodulin-dependent regulation of F-actin-myelin basic protein interaction. Eur. J. Cell Biol. 35, 327–335.

110 Dobrowolski, Z., Osinska, H., Mossakowska, M. and Barylko, B. (1986) Ca^{2+}-calmodulin-dependent polymerization of actin by myelin basic protein. Eur. J. Cell Biol. 42, 17–26.

111 Roth, G. A., Gonzalez, M. D., Monferran, C. G., De Santis, M. L. and Cumar, F. A. (1993) Myelin basic protein domains involved in the interaction with actin. Neurochem. Int. 23, 459–465.

112 Hill, C. M. D. and Harauz, G. (2005) Charge effects modulate actin assembly by classic myelin basic protein isoforms. Biochem. Biophys. Res. Commun. 329, 362–369.

113 Modesti, N. M. and Barra, H. S. (1986) The interaction of myelin basic protein with tubulin and the inhibition of tubulin carboxypeptidase activity. Biochem. Biophys. Res. Commun. 136, 482–489.

114 Hill, C. M., Libich, D. S. and Harauz, G. (2005) Assembly of tubulin by classic myelin basic protein isoforms and regulation by post-translational modification. Biochemistry 44, 16672–16683.

115 Grand, R. J. A. and Perry, S. V. (1980) The binding of calmodulin to myelin basic protein and histone H2B. Biochem. J. 189, 227–240.

116 Chan, K.-F. J., Robb, N. D. and Chen, W. H. (1990) Myelin basic protein: interaction with calmodulin and gangliosides. J. Neurosci. Res. 25, 535–544.

117 Polverini, E., Boggs, J. M., Bates, I. R., Harauz, G. and Cavatorta, P. (2004) Electron paramagnetic resonance spectroscopy and molecular modelling of the interaction of myelin basic protein (MBP) with calmodulin (CaM) – diversity and conformational adaptability of MBP CaM-targets. J. Struct. Biol. 148, 353–369.

118 Libich, D. S., Hill, C. M., Haines, J. D. and Harauz, G. (2003) Myelin basic protein has multiple calmodulin-binding sites. Biochem. Biophys. Res. Commun. 308, 313–319.

119 Dobrowolski, Z., Barylko, B. and Drabikowski, W. (1986) Interaction of tropomyosin with myelin basic protein and its effect on the ATPase activity of actomyosin. Eur. J. Cell Biol. 41, 65–71.

120 Prasad, K., Barouch, W., Martin, B. M., Greene, L. E. and Eisenberg, E. (1995) Purification of a new clathrin assembly protein from bovine brain coated vesicles and its identification as myelin basic protein. J. Biol. Chem. 270, 30551–30556.

121 Hill, C. M. D., Haines, J. D., Antler, C. E., Bates, I. R., Libich, D. S. and Harauz, G. (2003) Terminal deletion mutants of myelin basic protein: new insights into self-association and phospholipid interactions. Micron 34, 25–37.

122 Stoner, G. L. (1984) Predicted folding of beta-structure in myelin basic protein. J. Neurochem. 43, 433–447.

123 Mendz, G. L., Brown, L. R. and Martenson, R. E. (1990) Interactions of myelin basic protein with mixed dodecylphosphocholine/palmitoyllysophosphatidic acid micelles. Biochemistry 29, 2304–2311.

124 Randall, C. S. and Zand, R. (1985) Spectroscopic assessment of secondary and tertiary structure in myelin basic protein. Biochemistry 24, 1998–2004.

125 Bates, I. R. and Harauz, G. (2003) Molecular dynamics exposes α helices in myelin basic protein. J. Mol. Model. 9, 290–297.

126 Ramwani, J. J., Epand, R. M. and Moscarello, M. A. (1989) Secondary structure of charge isomers of myelin basic protein before and after phosphorylation. Biochemistry 28, 6538–6543.

127 Deibler, G. E., Stone, A. L. and Kies, M. W. (1990) Role of phosphorylation in conformational adaptability of bovine myelin basic protein. Proteins 7, 32–40.

128 Pritzker, L. B., Joshi, S., Gowan, J. J., Harauz, G. and Moscarello, M. A. (2000) Deimination of myelin basic protein. 1. Effect of deimination of arginyl residues of myelin basic protein on its structure and susceptibility to digestion by cathepsin, D. Biochemistry 39, 5374–5381.

129 Bates, I. R., Libich, D. S., Wood, D. D., Moscarello, M. A. and Harauz, G. (2002) An Arg/Lys → Gln mutant of recombinant murine myelin basic protein as a mimic of the deiminated form implicated in multiple sclerosis. Protein Expr. Purif. 25, 330–341.

130 Anthony, J. S. and Moscarello, M. A. (1971) A conformation change induced in the basic encephalitogen by lipids. Biochim. Biophys. Acta 243, 429–433.

131 Keniry, M. A. and Smith, R. (1979) Circular dichroic analysis of the secondary structure of myelin basic protein and derived peptides bound to detergents and to lipid vesicles. Biochim. Biophys. Acta 578, 381–391.

132 Boggs, J. M., Clement, I. R., Moscarello, M. A., Eylar, E.H. and Hashim, G. (1981) Antibody precipitation of lipid vesicles containing myelin proteins – dependence on lipid composition. J. Immunol. 126, 1207–1211.

133 Beniac, D. R., Luckevich, M. D., Czarnota, G. J., Tompkins, T. A., Ridsdale, R. A., Ottensmeyer, F. P., Moscarello, M. A. and Harauz, G. (1997) Three dimensional structure of myelin basic protein. I. Reconstruction via angular reconstitution of randomly oriented single particles. J. Biol. Chem. 272, 4261–4268.

134 Ridsdale, R. A., Beniac, D. R., Tompkins, T. A., Moscarello, M.A. and Harauz, G. (1997) Three-dimensional structure of myelin basic protein. II. Molecular modeling and considerations of predicted structures in multiple sclerosis. J. Biol. Chem. 272, 4269–4275.

135 Bates, I. R., Feix, J. B., Boggs, J.M. and Harauz, G. (2004) An immunodominant epitope of myelin basic protein is an amphipathic α helix. J. Biol. Chem. 279, 5757–5764.

136 Brasseur, R. (1991) Differentiation of lipid-associating helices by use of three-dimensional molecular hydrophobicity potential calculations. J. Biol. Chem. 266, 16120–16127.

137 McLaughlin, S. and Murray, D. (2005) Plasma membrane phosphoinositide organization by protein electrostatics. Nature 438, 605–611.

138 Warren, K.G., Catz, I. and Steinman, L. (1995) Fine specificity of the antibody response to myelin basic protein in the central nervous system in multiple sclerosis: the minimal B-cell epitope and a model of its features. Proc. Natl. Acad. Sci. USA 92, 11061–11065.

139 Wucherpfennig, K.W., Catz, I., Hausmann, S., Strominger, J. L., Steinman, L. and Warren, K.G. (1997) Recognition of the immunodominant myelin basic protein peptide by autoantibodies and HLA-DR2-restricted T cell clones from multiple sclerosis patients: identity of key contact residues in the B-cell and T-cell epitopes. J. Clin. Invest. 100, 1114–1122.

140 Warren, K. G. and Catz, I. (2000) Kinetic profiles of cerebrospinal fluid anti-MBP in response to intravenous MBP synthetic peptide DENP(85)VVHFFKNIVTP(96)RT in multiple sclerosis patients. Mult. Scler. 6, 300–11 .

141 Libich, D. S., Robertson, V. J., Monette, M. M. and Harauz, G. (2004) Backbone resonance assignments of the 18.5 kDa isoform of murine myelin basic protein (MBP). J. Biomol. nmR 29, 545–546.

142 Farès, C., Libich, D. S. and Harauz, G. (2006) Solution nmR structure of an immunodominant epitope of myelin basic protein (MBP) – conformational dependence on environment of an intrinsically unstructured protein. FEBS J. 273, 601–614.

143 Tzakos, A. G., Troganis, A., Theodorou, V., Tselios, T., Svarnas, C., Matsoukas, J., Apostolopoulos, V. and Gerothanassis, I. P. (2005) Structure and function of the myelin proteins: current status and perspectives in relation to multiple sclerosis. Curr. Med. Chem. 12, 1569–1587.

144 Bates, I. R., Boggs, J. M., Feix, J. B. and Harauz, G. (2003) Membrane-anchoring and charge effects in the interaction of myelin basic protein with lipid bilayers studied by site-directed spin labeling. J. Biol. Chem. 278, 29041–29047.
145 Musse, A. A., Boggs, J. M. and Harauz, G. (2006) Deimination of membrane-bound myelin basic protein in multiple sclerosis exposes an immunodominant epitope. Proc. Natl. Acad. Sci. USA 103, 4422–4427.
146 Afshar, M., Caves, L. S., Guimard, L., Hubbard, R. E., Calas, B., Grassy, G. and Haiech, J. (1994) Investigating the high affinity and low sequence specificity of calmodulin binding to its targets. J. Mol. Biol. 244, 554–571.
147 Yang, C., Jas, G. S. and Kuczera, K. (2004) Structure, dynamics and interaction with kinase targets: computer simulations of calmodulin. Biochim. Biophys. Acta 1697, 289–300.
148 Weimbs, T. and Stoffel, W. (1992) Proteolipid protein (PLP) of CNS myelin: positions of free, disulfide-bonded, and fatty acid thioester-linked cysteine residues and implications for the membrane topology of PLP. Biochemistry 31, 12289–12296.
149 Golds, E. E. and Braun, P. E. (1978) Protein associations and basic protein conformation in the myelin membrane: the use of difluoronitrobenzene as a cross-linking reagent. J. Biol. Chem. 253, 8162–8170.
150 Boggs, J. M., Yip, P. M., Rangaraj, G. and Jo, E. (1997) Effect of posttranslational modifications to myelin basic protein on its ability to aggregate acidic lipid vesicles. Biochemistry 36, 5065–5071.
151 Cheifetz, S., Boggs, J. M. and Moscarello, M. A. (1985) Increase in vesicle permeability mediated by myelin basic protein: effect of phosphorylation of basic protein. Biochemistry 24, 5170–5175.
152 Shanshiashvili, L. V., Suknidze, N. C., Machaidze, G. G., Mikeladze, D. G. and Ramsden, J. J. (2003) Adhesion and clustering of charge isomers of myelin basic protein at model myelin membranes. Arch. Biochem. Biophys. 419, 170–177.
153 Brunner, C., Lassmann, H., Waehneldt, T. V., Matthieu, J.-M. and Linington, C. (1989) Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase in the CNS of adult rats. J. Neurochem. 52, 296–304.
154 Chiu, S. Y. (1991) Functions and distribution of voltage-gated sodium and potassium channels in mammalian Schwann cells. Glia 4, 541–558.
155 Cherksey, B., Durrie, R., Braun, P. E. and Sapirstein, V. S. (1994) *In vitro* analysis of ion channels in periaxolemmal-myelin and white matter clathrin coated vesicles: modulation by calcium and GTP gamma S. Neurochem. Res. 19, 1101–1106.
156 Butt, A. M. and Kalsi, A. (2006) Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir4.1 in glial functions. J. Cell. Mol. Med. 10, 33–44.
157 Kettenmann, H., Sonnhof, U. and Schachner, M. (1983) Exclusive potassium dependence of the membrane potential in cultured mouse oligodendrocytes. J. Neurosci. 3, 500–505.
158 Lev-Ram, V. and Grinvald, A. (1986) Ca^{2+}- and K^{+}-dependent communication between central nervous system myelinated axons and oligodendrocytes revealed by voltage-sensitive dyes. Proc. Natl. Acad. Sci. USA 83, 6651–6655.
159 Ohler, B., Revenko, I. and Husted, C. (2001) Atomic force microscopy of nonhydroxy galactocerebroside nanotubes and their self-assembly at the air-water interface, with applications to myelin. J. Struct. Biol. 133, 1–9.
160 Ro, H. and Carson, J. H. (2004) pH microdomains in oligodendrocytes. J. Biol. Chem. 279, 37115–37123.
161 Butt, A. M., Pugh, M., Hubbard, P. and James, G. (2004) Functions of optic nerve glia: axoglial signalling in physiology and pathology. Eye 18, 1110–1121.
162 Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., Yin, X., Trapp, B. D., McRory, J. E., Rehak, R., Zamponi, G. W., Wang, W. and Stys, P. K. (2005) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439, 988–992.
163 Boggs, J. M., Rangaraj, G., Koshy, K. M., Ackerley, C., Wood, D. D. and Moscarello, M. A. (1999) Highly deiminated isoform of myelin basic protein from multiple sclerosis brain causes fragmentation of acidic lipid vesicles. J. Neurosci. Res. 57, 529–535.
164 Beniac, D. R., Wood, D. D., Palaniyar, N., Ottensmeyer, F. P., Moscarello, M. A. and Harauz, G. (2000) Cryoelectron microscopy of protein-lipid complexes of human myelin basic protein charge isomers differing in degree of citrullination. J. Struct. Biol. 129, 80–95.
165 Brouillette, C. G., Jones, J. L., Ng, T. C., Kercret, H., Chung, B. H. and Segrest, J. P. (1984) Structural studies of apolipoprotein A-I/phosphatidylcholine recombinants by high-field proton nmR, nondenaturing gradient gel electrophoresis, and electron microscopy. Biochemistry 23, 359–367.
166 Gow, A., Southwood, C. M., Li, J. S., Pariali, M., Riordan, G. P., Brodie, S. E., Danias, J., Bronstein, J. M., Kachar, B. and Lazzarini, R. A. (1999) CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 99, 649–659 .
167 Karthigasan, J., Kosaras, B., Nguyen, J. and Kirschner, D. A. (1994) Protein and lipid composition of radial component-enriched CNS myelin. J. Neurochem. 62, 1203–1213 .
168 Wilson, R. and Brophy, P. J. (1989) Role for the oligodendrocyte cytoskeleton in myelination. J. Neurosci. Res. 22, 439–448.
169 LoPresti, P., Szuchet, S., Papasozomenos, S. C., Zinkowski, R. P. and Binder, L. I. (1995) Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. Proc. Natl. Acad. Sci. USA 92, 10369–10373.
170 Muller, R., Heinrich, M., Heck, S., Blohm, D. and Richter-Landsberg, C. (1997) Expression of microtubule-associated proteins MAP2 and tau in cultured rat brain oligodendrocytes. Cell Tissue Res. 288, 239–249.
171 Taketomi, M., Kinoshita, N., Kimura, K., Kitada, M., Noda, T., Asou, H., Nakamura, T. and Ide, C. (2002) Nogo-A expression in mature oligodendrocytes of rat spinal cord in association with specific molecules. Neurosci. Lett. 332, 37–40.
172 Wohnsland, F., Schmitz, A. A. P., Steinmetz, M. O., Aebi, U. and Vergères, G. (2000) Interaction between actin and the effector peptide of MARCKS-related protein: identification of functional amino acid segments. J. Biol. Chem. 275, 20873–20879.
173 Dominguez, R. (2004) Actin-binding proteins – a unifying hypothesis. Trends Biochem. Sci. 29, 572–5780.
174 Pirollet, F., Derancourt, J., Haiech, J., Job, D. and Margolis, R. L. (1992) Ca^{2+}-calmodulin regulated effectors of microtubule stability in bovine brain. Biochemistry 31, 8849–8855.
175 Dyer, C. A., Phillbotte, T., Wolf, M. K. and Billings-Gagliardi, S. (1997) Regulation of cytoskeleton by myelin components: studies on shiverer oligodendrocytes carrying an MBP transgene. Dev. Neurosci. 19, 395–409 .
176 Feijoo, C., Campbell, D. G., Jakes, R., Goedert, M. and Cuenda, A. (2005) Evidence that phosphorylation of the microtubule-associated protein Tau by SAPK4/p38delta at Thr50 promotes microtubule assembly. J. Cell Sci. 118, 397–408.
177 Murray, D., Arbuzova, A., Hangyas-Mihalyne, G., Gambhir, A., Ben-Tal, N., Honig, B. and McLaughlin, S. (1999) Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment. Biophys. J. 77, 3176–3188.
178 Isenberg, G. and Goldmann, W. H. (1995) Actin-binding proteins – lipid interactions. In: The Cytoskeleton, Structure and Assembly, vol. 1 (Hesketh, J. and Prynne, I., Eds.), JAI Press, Greenwich, Conn., pp. 169–204.
179 Boggs, J. M., Rangaraj, G. and Koshy, K. M. (1999) Analysis of the membrane-interacting domains of myelin basic protein

by hydrophobic photolabeling. Biochim. Biophys. Acta 1417, 254–266.
180 Behrisch, A., Dietrich, C., Noegel, A. A., Schleicher, M. and Sackmann, E. (1995) The actin-binding protein hisactophilin binds *in vitro* to partially charged membranes and mediates actin coupling to membranes. Biochemistry 34, 15182–15190.
181 Isenberg, G. and Goldmann, W. H. (1992) Actin-membrane coupling: a role for talin. J. Musc. Res. Cell Motil. 13, 587–589.
182 Bubb, M. R., Lenox, R. H. and Edison, A. S. (1999) Phosphorylation-dependent conformational changes induce a switch in the actin-binding function of MARCKS. J. Biol. Chem. 274, 36472–36478.
183 Lunn, K. F., Baas, P. W. and Duncan, I. D. (1997) Microtubule organization and stability in the oligodendrocyte. J. Neurosci. 17, 4921–4932.
184 Chakraborty, G., Drivas, A. and Ledeen, R. (1999) The phosphoinositide signaling cycle in myelin requires cooperative interaction with the axon. Neurochem. Res. 24, 249–254.
185 Witt, A. and Brady, S. T. (2000) Unwrapping new layers of complexity in axon/glial relationships. Glia 29, 112–117.
186 Dyer, C. A. (1993) Novel oligodendrocyte transmembrane signaling systems. Mol. Neurobiol. 7, 1–22.
187 Dyer, C. A. and Benjamins, J. A. (1989) Organization of oligodendroglial membrane sheets. II. Galactocerebroside: antibody interactions signal changes in cytoskeleton and myelin basic protein. J. Neurosci. Res. 24, 212–221.
188 Boggs, J. M., Wang, H., Gao, W., Arvanitis, D. N., Gong, Y. and Min, W. (2004) A glycosynapse in myelin? Glycoconj. J. 21, 97–110.
189 Hakomori, S. (2002) The glycosynapse. Proc. Natl. Acad. Sci. USA 99, 225–232.
190 Cook, W. J. and Bugg, C. E. (1975) Calcium-carbohydrate bridges composed of uncharged sugars: structure of a hydrated calcium bromide complex of α-fucose. Biochim. Biophys. Acta 389, 428–435.
191 Stewart, R. J. and Boggs, J. M. (1993) The carbohydrate-carbohydrate interaction between galactosylceramide-containing liposomes and cerebroside sulfate-containing liposomes: dependence on the glycolipid ceramide composition. Biochemistry 32, 10666–10674.
192 Boggs, J. M. and Wang, H. (2001) Effect of liposomes containing cerebroside and cerebroside sulfate on cytoskeleton of cultured oligodendrocytes. J. Neurosci. Res. 66, 242–253.
193 Boggs, J. M. and Wang, H. (2004) Co-clustering of galactosylceramide and membrane proteins in oligodendrocyte membranes on interaction with polyvalent carbohydrate and prevention by an intact cytoskeleton. J. Neurosci. Res. 76, 342–355.
194 Arvanitis, D. N., Min, W., Gong, Y., Meng Y.-M. and Boggs, J. M. (2005) Two types of detergent-insoluble, glycosphingolipid/cholesterol-rich membrane domains in isolated myelin. J. Neurochem. 94, 1696–1710.
195 Gillespie, C. S., Wilson, R., Davidson, A. and Brophy, P. J. (1989) Characterization of a cytoskeletal matrix associated with myelin from rat brain. Biochem. J. 260, 689–696.
196 Pereyra, P. M., Horvath, E. and Braun, P. E. (1988) Triton X-100 extractions of central nervous system myelin indicate a possible role for the minor myelin proteins in the stability in lamellae. Neurochem. Res. 13, 583–595.
197 Arvanitis, D. N., Yang, Y. and Boggs, J. M. (2002) Myelin proteolipid protein, basic protein, the small isoform of myelin-associated glycoprotein, and p42 MAPK are associated in the triton X-100 extract of central nervous system myelin. J. Neurosci. Res. 70, 8–23.
198 Lichtenberg, D., Goni, F. M. and Heerklotz, H. (2005) Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem. Sci. 30, 430–436.
199 DeBruin, L. S., Haines, J. D., Wellhauser, L. A., Radeva, G., Schonmann, V., Bienzle, D. and Harauz, G. (2005) Developmental partitioning of myelin basic protein into membrane microdomains. J. Neurosci. Res. 80, 211–225.
200 Deshmukh, D. S., Kuizon, S., Bear, W. D. and Brockerhoff, H. (1980) Distribution of phosphoinositides among subfractions of rat brain myelin. Lipids 15, 14–18.
201 Tompkins, T. A. and Moscarello, M. A. (1993) Stimulation of bovine brain phospholipase C activity by myelin basic protein requires arginyl residues in peptide linkage. Arch. Biochem. Biophys. 302, 476–483.
202 Kahn, D. W. and Morell, P. (1988) Phosphatidic acid and phosphoinositide turnover in myelin and its stimulation by acetylcholine. J. Neurochem. 50, 1542–1550.
203 Janmey, P. A., Xian, W. and Flanagan, L. A. (1999) Controlling cytoskeleton structure by phosphoinositide-protein interactions: phosphoinositide binding protein domains and effects of lipid packing. Chem. Phys. Lipids 101, 93–107.
204 van Itallie, C. M. and Anderson, J. M. (2004) The molecular physiology of tight junction pores. Physiology 19, 331–338.