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The Classic Basic Protein of Myelin – Conserved Structural Motifs and the Dynamic Molecular Barcode Involved in Membrane Adhesion and Protein-Protein Interactions

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Abstract: The myelin basic protein (MBP) family comprises a variety of developmentally-regulated members arising from different transcription start sites, differential splicing, and post-translational modifications. The "classic" isoforms of MBP include the 18.5 kDa form, which predominates in adult human myelin and facilitates compaction of the mature myelin sheath in the central nervous system, thereby maintaining its structural integrity. In addition to membrane-association, the 18.5 kDa and all other classic isoforms are able to interact with a multitude of proteins, including Ca^{2+}-calmodulin, actin, tubulin, and SH3-domain containing proteins, and thus may be signalling linkers during myelin development and remodelling. All proteins in this family are intrinsically disordered, creating a large effective surface to facilitate multiple protein associations, and are post-translationally modified to various degrees by methylation, phosphorylation, and deimination. We have used spectroscopic (fluorescence, CD, EPR, and NMR) approaches to study MBP's conformational adaptability. A highly-conserved central domain presents an amphipathic α-helix in association with a phospholipid membrane, and contains a threonyl residue that is phosphorylated by MAP-kinases. In multiple sclerosis, this segment represents a primary immunodominant epitope. This helical structure is adjacent to a proline-rich region that presents a classic SH3-ligand, comprises a second MAP-kinase phosphorylation site, and forms a polyproline type II helix. This domain of the protein is thus essential to proper positioning of a protein-interaction motif, with the local conformation and accessibility being modulated by MAP-kinases. In addition, the C-terminus of 18.5 kDa MBP has been identified by NMR spectroscopy as a Ca^{2+}-calmodulin-binding site, and is of note for having a high density of post-translational modifications (protein kinase C phosphorylation, and deimination). For the most part, any classic protein isoform functions as an entropic spring that interacts in its entirety with membranes and cytoskeletal proteins, but the central and C-terminal motifs may represent molecular switches.

Keywords: Myelin basic protein, multiple sclerosis, intrinsically disordered protein, Golli (genes of oligodendrocyte lineage), membrane adhesion, lipid rafts, amphipathic α-helix, polyproline type II helix.

THE MYELIN BASIC PROTEIN FAMILY – DIVERSITY IN THE CENTRAL NERVOUS SYSTEM

Myelin is the lipid-rich structure wrapped in a tight spiral around nerve axons, enabling them to transmit nerve impulses efficiently [1]. In the central nervous system (brain and spinal cord), the multilamellar arrangement of the myelin sheath is maintained by myelin basic protein (MBP), one of the two predominant proteins, the other being proteolipid protein. The myelin basic protein family is a product of differential splicing of a single mRNA transcript arising from one of three transcription start sites of the gene complex called Golli (Genes of OLigodendrocyte LIneage) [2]. The structure of the murine Golli gene is depicted schematically in Fig. (**1**) (adapted from [3, 4]).

Various Golli proteins arising from transcription start site 1 are produced in developing myelin, and are called BG21, J37, and TP8 in the mouse [2]. The Golli-MBPs translocate between the nucleus and cellular processes, suggesting multiple roles in modulating the activity of signal transduction pathways in remyelination, T-cell activation, modulation of Ca^{2+} influx, or in gene regulation [5-10]. The classic (canonical) MBP isoforms arise primarily from transcription start site 3 (Fig. **1**). The major adult classic MBP isoform is 18.5 kDa, which is peripherally membrane-associated and found in cell processes and compact myelin [11, 12]. The full-length classic 21.5 kDa isoform has an additional 26 amino acids generated by exon-6/II, and is translocated to the nucleus [13]. Our focus in this review shall be on the 18.5 kDa classic MBP isoform, which has been the most studied. The degree of sequence conservation of this protein is extremely high, especially amongst mammals, as depicted by an aligned set of amino acid sequences (Fig. **2**) [14-16], and a phylogenetic tree constructed using these sequences (Fig. **3**) [17, 18].

The classic 18.5 kDa MBP isoform is extremely positively-charged, and exists as a number of charge components (sometimes called charge isomers) due to myriad post-translational modifications, representing considerable further diversification (Fig. **4**) [11, 19]. Each post-translational

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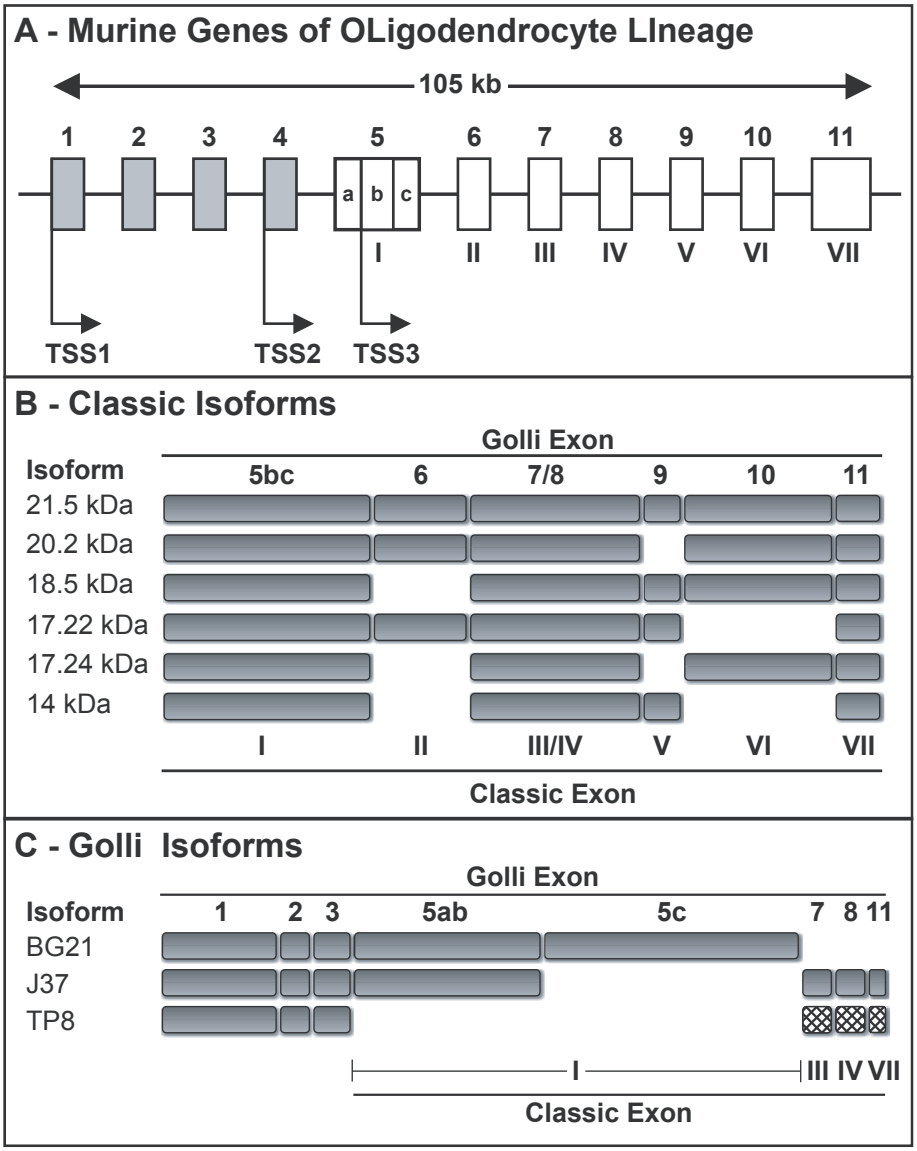

Fig. (1). (A) The exon distribution of the murine Golli-MBP gene. There are 11 exons spanning 105 kb. **(B)** The "classic" MBP isoforms arise primarily from transcription start site 3 and encode splice isoforms ranging in (nominal) molecular mass from 14 to 21.5 kDa. In human adult and bovine myelin, the 18.5 kDa isoform is predominant. The exons of the classic MBP isoforms have traditionally been denoted by Roman numerals; the Golli exon numbering with Sanskrit/Arabic numerals is now the preferable convention. The exon-6/II containing isoforms are translocated between the nucleus and the cytoplasm, whereas those lacking this exon are primarily found associated with distal membrane processes. **(C)** The Golli isoforms (murine BG21, J37, and TP8) arise from transcription start site 1 and encompass 133 amino acids arising from exons 1-3. Both BG21 and J37 also encompass some classic MBP sequences, but not TP8 due to a frameshift. Figure modified from references [3, 4].

modification reduces the net positive charge by 1 or 2. The charge components are named C1 to C8 according to their elution pattern from a cation exchange column. Component C1 is the most cationic (+19 at neutral pH) and the least modified; C8 is the flow-through fraction including the least-charged and most modified variants. Whereas genetic modifications in other myelin proteins are associated with a variety of dysmyelinating diseases [7], there is a varied pattern of post-translational modifications of MBP in early myelin development, and in the human demyelinating disease multiple sclerosis [19-22]. In other words, the classic protein isoforms are further diversified by a "dynamic molecular barcode" (an expression due to Yang [23]). It is inconceivable that these modifications are random or spurious, since they appear to be site-directed and require a considerable expenditure of cellular energy. Elucidating the purpose of this variety of post-translational modifications of MBP will help in understanding the mechanisms of myelin formation and homeostasis, and the pathogenesis of multiple sclerosis [24]. Other protein families such as histones [25] and tubulin [26] have their own "codes" as well.

The predominant modifications of the classic 18.5 kDa isoform are deimination, methylation, and phosphorylation (Fig. **4**) (summarised in [11]). Deimination is the conversion

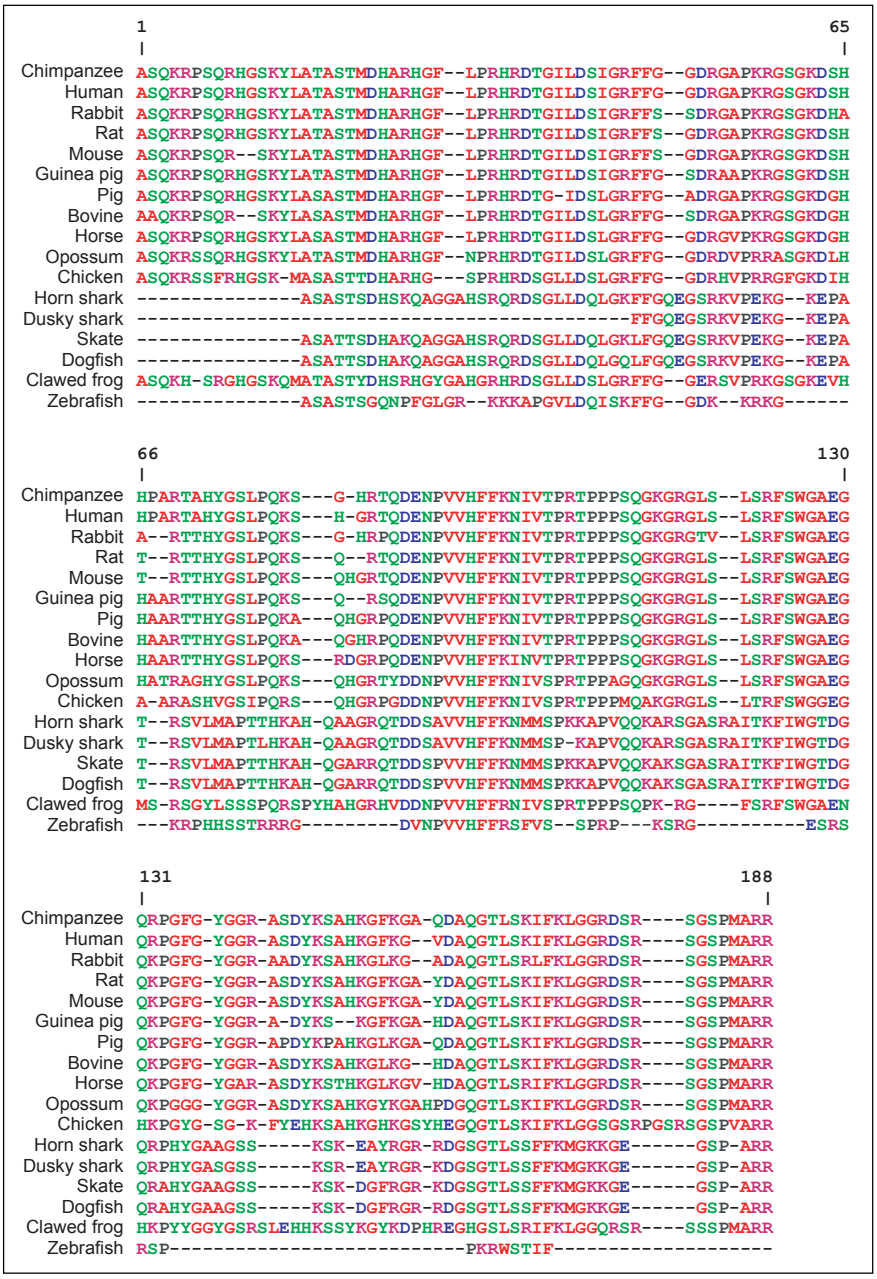

Fig. (2). A BLASTP/CLUSTALW [15, 16] alignment of sequences of 18.5 kDa MBP from various species: chimpanzee (*Pan troglodytes*), human (*Homo sapiens*), rabbit (*Oryctolagus cuniculus*), rat (*Rattus norvegicus*), mouse (*Mus musculus*), guinea pig (*Cavia porcellus*), pig (*Sus scrofa*), bovine (*Bos taurus*), horse (*Equus caballus*), opossum (*Monodelphis domestica*), chicken (*Gallus gallus*), horn shark (*Heterodontus francisci*), dusky shark (*Carcharhinus obscurus*), little skate (*Leucoraja erinacea*), spiny dogfish (*Squalus acanthias*), African clawed frog (*Xenopus laevis*), and zebrafish (*Danio rero*). The amino acids are coloured as follows: red (small and/or hydrophobic, *i.e.*, A, V, F, P, M, I, L, W, and G), blue (acidic, *i.e.*, D and E), magenta (basic, *i.e.*, R and K, and including H), green (polar, *i.e.*, S, T, N, Q, and C), and gray (imino, *i.e.*, P). The high degree of sequence conservation of the 18.5 kDa splice isoform had already been noted over a decade ago with the sequences then available [14]. The recombinant murine protein used in our studies (rmMBP) has an LEH_6 tag. Referring to numbering of the murine sequence, several segments are of notable interest: MBP(P82-P93) represents a surface-seeking, amphipathic α–helix [88-90]; MBP(T92-K102) represents a potential ligand of an SH3 (Src homology 3) domain [34]; both Thr92 and Thr95 are MAP-kinase sites [11, 19, 119, 120] and this domain of the protein may represent a molecular switch as discussed in the text; MBP(K119-G123) is similar in sequence to the tubulin-binding region of tau [191]; MBP(Y131-R167) represents the putative Ca^{2+}-CaM-binding domain [37, 170-173].

of arginine in MBP to citrulline by the enzyme peptidylarginine deiminase (PAD, EC 3.5.3.15, primarily the cytoplasmic isoform 2) [27]. As reviewed recently and extensively elsewhere, the degree of deimination correlates with the severity of multiple sclerosis and limits MBP's ability to maintain compact myelin, because its interactions with lipids and other proteins are altered significantly [28, 29]. Methylation (single or symmetric double) of MBP at a single arginyl residue (murine Arg104, human Arg107, bovine Arg106) is associated with healthy myelin [19, 30], but its purpose has

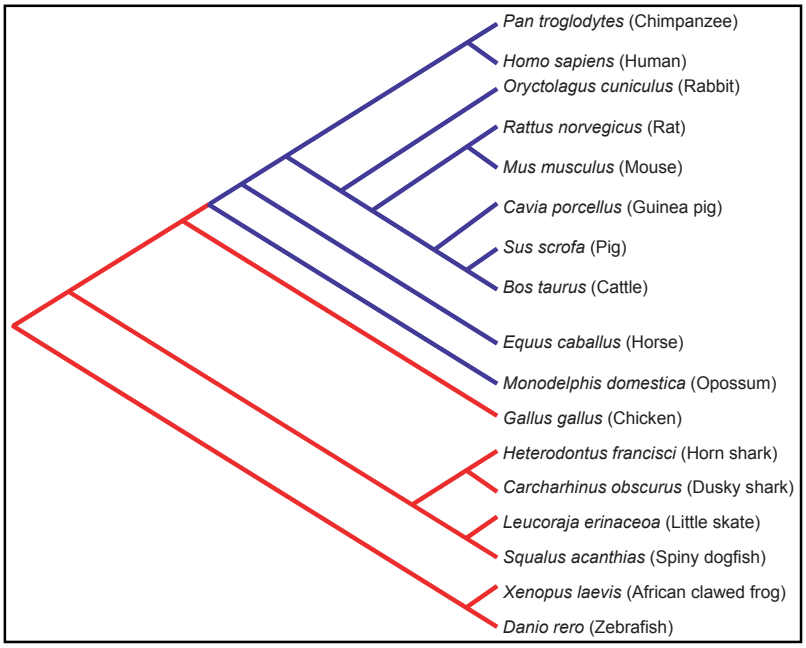

Fig. (3). Phylogenetic tree derived from known classic 18.5 kDa MBP sequences (Fig. **2**) using the program CLC Combined Workbench, v. 3.5.1 (CLC Bio, Aarhus, Denmark). The program used an *Unweighted Pair Group Method using Arithmetic averages* (UPGMA) algorithm to cluster the pairwise distance data [17, 18].

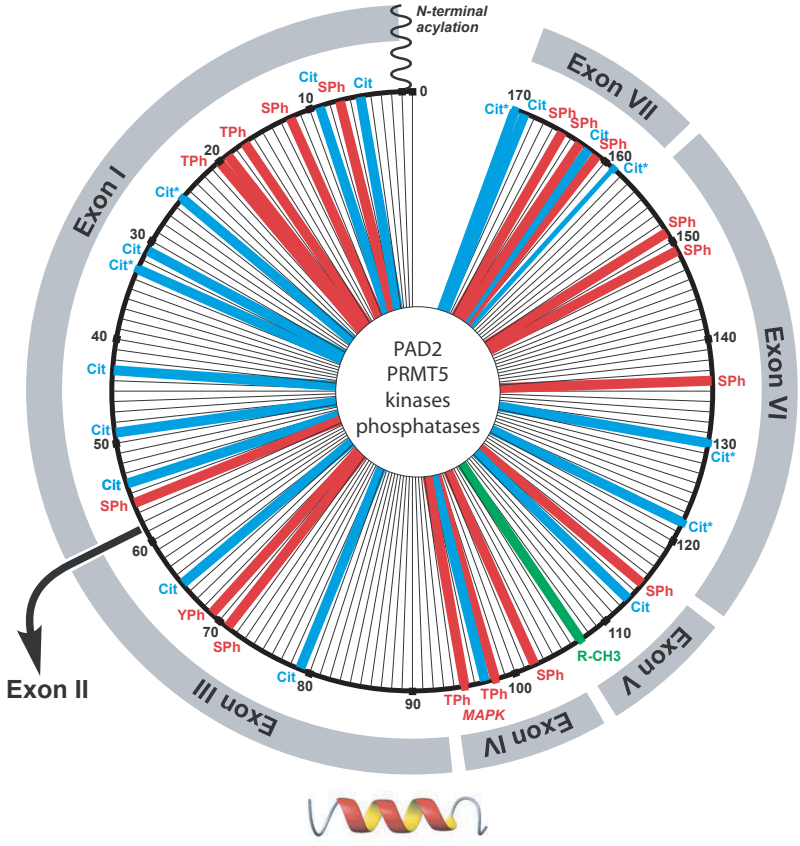

Fig. (4). Summary of significant sites of post-translational modification of the 18.5 kDa isoform of myelin basic protein, with reference to the murine 18.5 kDa sequence. The major modifications are the irreversible conversion of arginyl to citrullinyl residues by peptidylarginyl deiminase isoform 2 (PAD2), the irreversible mono- or di-methylation or Arg104 (murine 18.5 kDa sequence numbering) by protein-Arg-*N*-methyltransferase (probably PRMT5), the phosphorylation of Thr92 and Thr95 by MAP-kinases, and the phosphorylation of seryl and tyrosinyl residues by diverse other kinases, all of which can be reversed by phosphatases. The global pattern of modifications changes during development, with age, and with disease, and represents a "dynamic molecular barcode".

not yet been ascertained other than potentially modulating MBP-protein interactions (*e.g.*, as a deiminase antagonist [31]). Another major post-translational modification of MBP is phosphorylation by various kinases [11, 32], the overall level of which is concomitantly *de*creased in multiple sclerosis myelin [19]. The relative density of modifications is greatest at the N- and C-termini of the protein, *i.e.*, exon-5b/I and exon-11/VII, respectively, but also in exon-9/V and exon-10/VI (Fig. **4**).

CYTOSKELETAL ASSEMBLY BY CLASSIC MBP ISOFORMS

The classic 18.5 kDa MBP isoform has been shown to interact *in vitro* (and also in some *in vivo* studies) with a wide variety of proteins, primarily cytoskeletal ones, as recently reviewed [11, 12]. New MBP-protein interaction studies and examples appear frequently [33-37].

With respect to the cytoskeleton *per se*, oligodendrocytes in the central nervous system undergo dramatic morphological changes during myelination [38-41]. By remodelling their internal microfilament and microtubule networks, these cells extend membranous processes which contact and spiral around nearby neuronal axons to produce the multilamellar myelin sheath [42-44]. Several lines of evidence implicate the MBPs as additional regulators of the cytoskeleton in addition to their main function of maintaining the spacing of myelin lamellae, as recently reviewed [12]. First of all, MBP polymerises and bundles actin, both associations being modulated by post-translational modifications and binding to calmodulin [45-48]; it also links actin to the membrane [12, 45, 49]. Secondly, MBP polymerises and bundles tubulin in similar ways [4, 49]. It is possible that MBP may cross-link actin and tubulin to each other [12], and further studies of cytoskeletal associations within different regions of myelin are required [50].

Thus, given the large number of developmentally-regulated splice isoforms of the protein, some targeted to the plasma membrane and others to the nucleus, and also given its myriad protein-protein interactions and extensive post-translational modifications, it is probable that MBP must play further roles in myelin development and maintenance beyond mere membrane adhesion. This possibility was recognised already in 1980 [51]. Moreover, the exon-6/II-containing isoforms of the classic protein are karyophilic, and have been suggested to have further roles in gene regulation, although these also remain to be determined more fully [13, 52]. What are the structural properties of MBP isoforms that allow them to do so many things, at least *in vitro*?

STRUCTURE-FUNCTION RELATIONSHIPS OF INTRINSICALLY-DISORDERED PROTEINS – CONFORMATIONAL FLEXIBILITY AND MULTIFUNCTIONALITY OF THE MBP FAMILY

A fundamental tenet of structural molecular biology has been that a protein's three-dimensional structure determines its function, a concept derived from the successes of X-ray crystallography since the 1950s, and the correlation between the activity and (relatively) rigid architecture of enzymes, with their lock-and-key mechanisms of substrate recognition and modification. Despite many attempts, it has not been possible to crystallise MBP with a high degree of order [11, 53, 54]. The reason is that the cellular proteome comprises many intrinsically disordered proteins (IDPs), with properties similar to MBP, whose lack of globular (folded) structure may be directly part of their inherent function (*e.g.*, springs or linkers – entropic chains), or is required for recognition and binding [55-59]. They are designed to be pliable (*i.e.*, natively unfolded or intrinsically disordered) to present a large effective surface area in order to associate rapidly (but still specifically) with one or multiple binding partners [60-62]. These intermolecular associations may involve pre-formed structural elements such as α-helices, and/or an induced fit, *i.e.*, coupled folding and binding [*ibid*.]. Dunker and colleagues have encapsulated this idea in their MoRF (Molecular Recognition Fragments, specifically α-MoRF) hypothesis [63, 64]. One intriguing example has been published where two intrinsically disordered protein partners (cellular co-activators) fold together synergistically [65]. It appears that IDPs often function as hubs or linkers in protein interaction networks [66, 67], or as molecular switches in transcriptional and translational control [68-71]; many IDPs are important in signal transduction, where their binding affinities can be regulated by post-translational modifications such as phosphorylation [62, 70, 72-74].

All known Golli and classic members of the MBP family are IDPs [11, 54, 75, 76]. The classic 18.5 kDa isoform, in particular, has long been known to be flexible [77] and is an extended, extreme polycation whose net charge is reduced by combinatorial post-translational modifications representing a molecular barcode, an idea introduced above. We may envisage MBP's various functions to be twofold in nature. First, MBP can be considered to be in the entropic chain category (as an adhesive of the cytoplasmic leaflets of the oligodendrocyte membranes, potentially defining various domains of distinct lipid composition, and linked also to proteolipid and other proteins in compact myelin, and the underlying cytoskeleton in the paranodal regions of myelin). Studies with various classic splice isoforms and deletion mutants of MBP have failed to reveal distinct motifs that interact with the cytoskeletal proteins [4, 46, 47] – the entire polypeptide chain was involved in the association, as appeared also to be the case with MBP with calmodulin [37], although exon-6/II and/or exon-10/VI seemed to be required for effective microfilament bundling. These observations were consistent with the paradigm of intrinsically disordered proteins that function as springs or as linkers (*i.e.*, entropic chains).

Second, MBP may participate in molecular recognition and networking in signalling pathways (potentially with SH3-domain containing proteins; SH3 – Src homology 3). In general, it has been suggested that some forms of MBP may be components of signalling pathways, perhaps in regulation of gene expression and/or cytoskeletal assembly [11-13, 40, 78-81]. We have shown that MBP sequesters $PI(4,5)P_2$ (phosphatidylinositol-4,5-bis-phosphate) in oligodendrocytes and membrane vesicles [82], with which it interacts strongly [83, 84], providing further evidence that it may be involved directly in signalling functions. These sorts of associations may be representative of the MoRF paradigm, where we need to consider specific target motifs on MBP.

Thus, we must consider the idea of "structure" in the MBP family of proteins in a variety of environments, and/or when they are interacting with other biological molecules or cofactors. In this review, we focus primarily on the topology of 18.5 kDa MBP in association with membranes and with calmodulin, one of its major protein partners. We explore particularly some of the structural motifs of the classic 18.5 kDa MBP isoform that may be involved in its multifunctionality, and how post-translational modifications (*i.e.*, the molecular barcode) affect the conformations and interactions of these regions of the protein.

MEMBRANE-ASSOCIATED CONFORMATION OF 18.5 KDA MBP – DEPTHS OF PENETRATION AND IDENTIFICATION OF ORDERED/DISORDERED SEGMENTS

In electron micrographs of sectioned myelin, MBP is localised in the major dense lines of the myelin sheath [1]. These electron dense lamellae are formed by the tight apposition of the cytoplasmic leaflets of the oligodendrocyte membrane, wherein MBP serves as an adhesive molecule. The *in vitro* reconstitution of MBP with unilamellar vesicles containing anionic lipids results in the formation of multilamellar structures similar to those seen in myelin sheaths [85, 86], and these assemblies can then be probed by a variety of biochemical and biophysical techniques [11, 87].

One approach that has been very fruitful to determine the topology and conformation of 18.5 kDa MBP on the bilayer surface is site-directed spin-labelling (SDSL) in conjunction with electron paramagnetic resonance (EPR) spectroscopy [88-90]. The SDSL procedure involves first introducing cysteinyl residues at specific positions in the protein, marking the site with a sulphydryl-specific spin label such as methanethiosulphonate [91, 92], and reconstituting the spin-labelled protein with cytoplasmic large unilamellar vesicles (of composition mimicking the cytoplasmic leaflet of the myelin sheath: 44 mol% cholesterol, the rest being zwitterionic or negatively-charged phospholipids). Then EPR spectroscopy can be used to measure spin label mobility and solvent accessibility, particularly the depth of penetration of each site into the membrane bilayer [93].

In Fig. **(5A**), the Ångstrom level depths of spin label penetration are depicted for the 18.5 kDa isoform of MBP [88]. The N-terminal third of the protein, particularly around the region of the first Phe-Phe pair (Fig. **2**), is embedded in the hydrophobic portion of the bilayer. In the central region, the protein appears to be associated with the phospholipid headgroups, and then penetrates the hydrophobic core of the membrane again at its C-terminus. These data are shown for comparison with a prediction of the disordered segments of the protein (Fig. **5B**) [94, 95], and a depiction of the protein sequence using hydrophobic cluster analysis (Fig. **5C**) [96]. Although post-translational modifications of the 18.5 kDa MBP isoform are distributed throughout the sequence (Fig. **4**), their relative density appears greater in regions of higher predicted disorder (Fig. **5B**), *e.g.*, exon-VII/11. This observation is consistent with the paradigm that intrinsically disordered regions of proteins are "hot spots" for phosphorylation [72, 97].

MEMBRANE-ASSOCIATED CONFORMATION OF 18.5 KDA MBP – STRUCTURE AND TOPOLOGY OF AN IMMUNODOMINANT EPITOPE *IN SITU*

The segment Pro85-Pro96 of human MBP, corresponding directly to the murine residues Pro82-Pro93 (Fig. **2**), is the minimal B cell epitope of MBP, and is also an important T cell recognition site [98]. The amino acid sequence of this region of the protein is highly conserved amongst mammals [11, 99], and is post-translationally unmodified except for the threonyl 92 residue, which is phosphorylated by mitogen-activated protein (MAP) kinase (as discussed below, see Fig. **4**). It is also curious that all three valines and both asparagines in the protein are clustered in this region. Experimental treatments for multiple sclerosis based on polypeptide mimetics of MBP have focussed on this and neighbouring regions of the protein [100-102]. Several linear and cyclic analogues of hMBP(V87-P99) have been designed, analysed structurally by NMR and molecular modelling, and evaluated for their ability to induce and/or inhibit experimental autoimmune encephalomyelitis in rats [103-108]. The cyclic analogues, in particular, showed promise as potential antagonist mimetics for treating multiple sclerosis as artificial regulators of the immune response. The linear polypeptide D82-ENPVVHFFKNIVTPR-T98 (human numbering) has been used to induce immunologic tolerance in patients with progressive multiple sclerosis [102], and clinical efficacy is under evaluation in a phase II/III clinical trial that is currently enrolling patients (www.biomsmedical.com) [109, 110]. Thus, it is worthwhile to compare and understand the tertiary structures of this epitope under various conditions.

We have probed the structure of this region by SDSL-scanning through the segment Val83-Thr92, and examining the depth of each residue in the lipid bilayer [89]. Here, secondary structure was revealed by the periodicity of the depth of penetration of the spin label. The results demonstrated conclusively that this portion of the protein formed an amphipathic α-helix *in situ*, since it had a depth profile that could be modelled by a sinusoidal function, with a periodicity of approximately 3.6 residues (Fig. **6A**). The α-helix was embedded into the lipid bilayer, and tilted at an angle of 9° with respect to the membrane surface. This tilt was a result of the larger hydrophobic potential profile around the C-terminal end of the helix.

Secondly, a helical wheel representation of this segment revealed it to be amphipathic (Fig. **6B**). Amphipathic peptides with fusogenic properties are also tilted with respect to the membrane plane, but at greater angles [111]. The smaller tilt angle for this MBP domain may explain why MBP is able to cause hemifusion of lipid bilayers without causing mixing of the vesicle compartments [112]. Thus, the degree of tilt for this segment of MBP is sufficient for deep penetration and firm membrane adhesion, but is not large enough to destabilise the bilayer. This study provided the first direct experimental evidence of specific, local secondary structure in MBP when bound to a lipid bilayer. In general, amphipathic α-helices are an essential component of almost all peripheral and many integral membrane-associated proteins, involved not only in membrane interactions, but also in surface remodelling [84, 113].

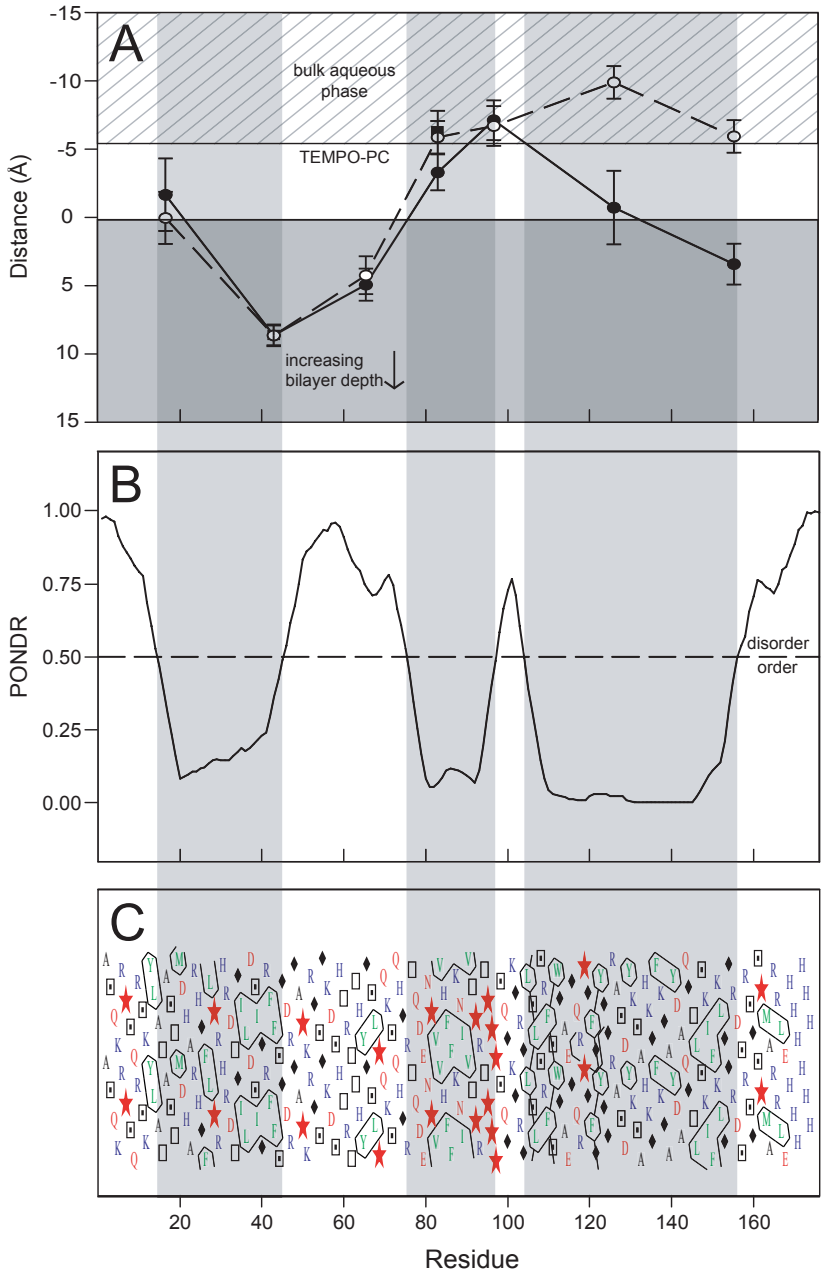

Fig. (5). (A) Depths of penetration of 18.5 kDa MBP (recombinant, based on the murine sequence) into the lipid bilayer of large unilamellar vesicles with lipid composition resembling that of the cytoplasmic face of oligodendrocytes. The gray shading indicates values below the lipid phosphates (*i.e.*, within the membrane), whereas the cross-hatching specifies the tentative region of distance determination. The horizontal line near 5 Å indicates the location of the nitrogen atom of the TEMPO-phosphatidylcholine nitroxide [88]. The filled circles represent data obtained with the unmodified, highly-charged rmC1 (+19 at neutral pH) form of the protein. The open circles represent data obtained with a quasi-deiminated, less-charged rmC8 (+13 at neutral pH) variant of the protein. **(B)** Depiction of the degree of intrinsic disorder of 18.5 kDa rmMBP using PONDR® (Predictor Of Natural Disordered Regions – VL-XT Predictor) [94, 95], which uses a neural network to predict regions of order/disorder in IDPs. The dashed line represents the order/disorder threshold; based on this prediction, MBP has three major regions of potential order, denoted by gray bars. **(C)** A visually-enhanced sequence representation using hydrophobic cluster analysis [96], which visually enhances primary sequence data using symbols (□, T; ⊡, S; ◆, G; ★, P), and colours (red – P, acidic residues D and E, and N, Q; blue – basic residues K and R, and H; green – hydrophobic residues V, L, I, F, W, M, Y; and black for all other residues – G, S, T, C, A). The hydrophobic clusters in MBP occur within the PONDR®-predicted ordered regions. Figures adapted and reproduced with permission from references [37, 88].

Another significant consequence of this work concerned the role of Lys88, which was the deepest penetrating residue on the polar face of the helix in this study (5.5 Å into the bilayer). Thus, this residue was in an ideal position for snorkelling, *i.e.*, positioning the positively-charged group of the amino acid in the polar region, whereas the aliphatic part was in the hydrophobic portion of the bilayer (Fig. **6C**). In peripheral membrane proteins, snorkelling is thought to allow

the long and bendable side chain of lysine to place the charged amino group in the more polar interface region, while keeping the hydrocarbon part of the side chain inside the hydrophobic part of the membrane, thus resulting in stronger binding [114].

STRUCTURE OF THE CENTRAL IMMUNODOMINANT EPITOPE BY SOLUTION NMR SPECTROSCOPY

Although EPR spectroscopy was useful for defining the topology of MBP and a specific segment of it in a membrane environment, it could not provide atomic level detail of the structure. Solution NMR spectroscopy enabled a detailed structural investigation of this highly conserved segment of MBP and confirmed its amphipathic α-helical character [99]. Standard ^{1}H homonuclear correlation experiments were employed to assign the resonances and provide the semi-quantitative distance restraints for the calculation of the structure of a polypeptide comprising the immunodominant epitope (Q78-T95, murine sequence, Figs. (**2** and **6D**)) in aqueous solution, in membrane-mimetic solvent (trifluoroethanol), and in dodecylphosphocholine (DPC) micelles [99].

The secondary fold of the peptide was assessed using the chemical shifts of the H^{α} and C^{α} atoms. A database of chemical shift indices has been compiled [115-117] for identification of residues involved in ordered secondary structures. Typically, α-helical structures are identified by an uninterrupted segment of four or more residues that have downfield C^{α} and upfield H^{α} chemical shift differences, relative to the random coil chemical shift values for the same residue [115, 116]. In our study [99], the chemical shift index analyses indicated an α-helical conformation of a 10-residue segment of the polypeptide (residues V83-T92) when

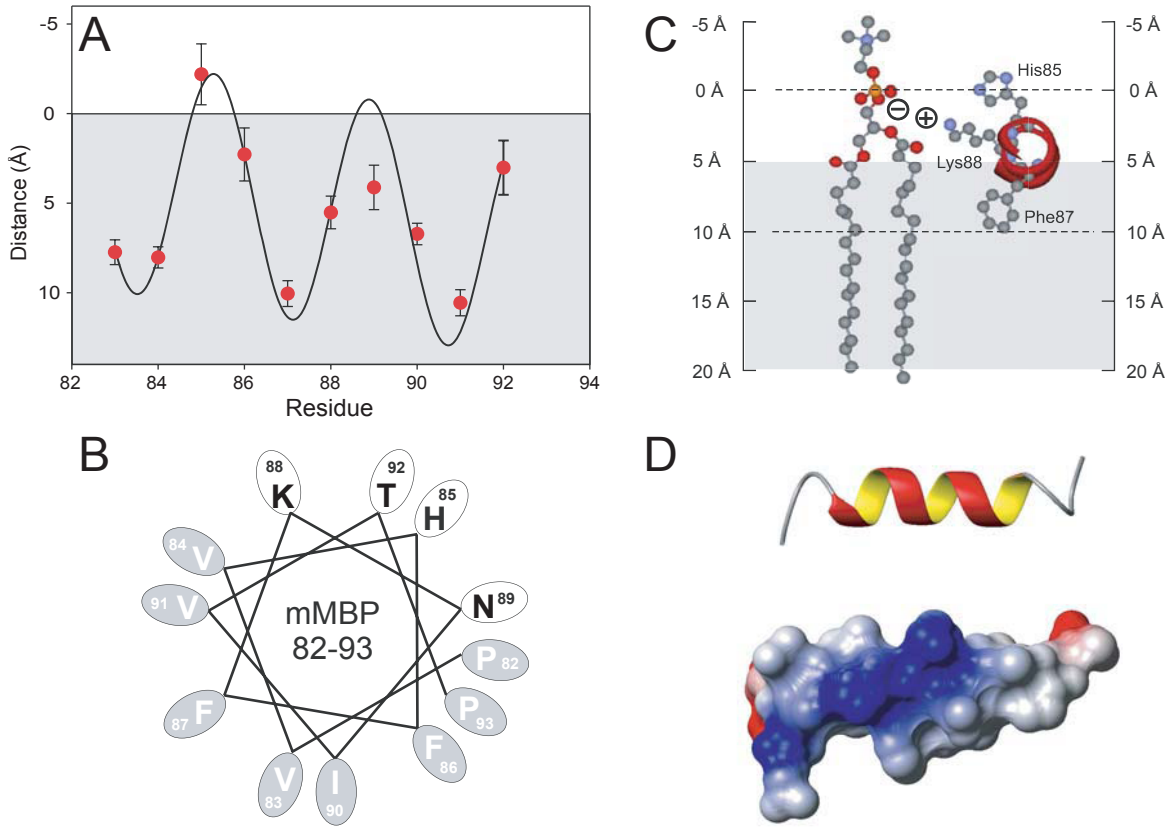

Fig. (6). (A) The region of recombinant murine 18.5 kDa MBP from Val83 to Thr92 was subsequently studied by Cys-substitution (SDSL) of each residue, followed by EPR of each protein species in turn. The periodicity of the penetration depth was fitted to a sine function. The resulting fit revealed a periodicity of 3.6 residues, and amplitude of 10 Å, indicative of an amphipathic α-helix. Moreover, the helix was tilted by ~9° with respect to the plane of the bilayer, similar to fusogenic peptides. **(B)** Helical wheel representations of the Phe86-Phe87 region of MBP (murine numbering), including 5-6 residues on either side of the Phe-Phe pair. The gray shading represents apolar and hydrophobic residues, and white represents polar residues. **(C)** A schematic representation of the depth of penetration of the α-helical immunodominant epitope with the most exposed (His85) and deepest penetrating residues (Phe87) indicated. The hydrocarbon region starts ~7 Å from the lipid-water interface. Here, Lys88 is shown in a snorkelling orientation interacting with the negatively-charged phosphate group of a phospholipid. **(D)** The average of the 10 lowest overall energy structures of an 18-residue peptide (murine residues Gln78-Thr95, which include the murine residues Pro82-Pro93 corresponding exactly to the immunodominant epitope of MBP, human 18.5 kDa isoform residues Pro85-Pro96) are illustrated as either a ribbon diagram or a space-filling model. The surfaces in the space filling model are coloured with a red-to-white-to-blue gradient indicating the electrostatic partial charge distribution (red = negative, white = neutral, blue = positive) to illustrate the amphipathic nature of the α-helix. Figure adapted from references [89, 99], and used with permission from the American Society for Biochemistry and Molecular Biology, and Elsevier Ltd.

in a membrane-mimetic environment, as shown by the uninterrupted downfield C^{α} and upfield H^{α} shifts for that stretch of amino acids. In an aqueous environment, there was conflicting evidence of secondary structure formation – the H^{α} shifts indicated a tendency towards weak α-helix formation, which was unsubstantiated by the C^{α} chemical shifts. Overall, these experiments underscored the conformational flexibility inherent to MBP, and demonstrated that this segment of MBP would only form a stabilised α-helix in the presence of a membrane or membrane-mimetic environment. We believe that the flexibility of this motif imparts important structural and functional characteristics to this classic MBP isoform.

The presence of ordered secondary structure in this central segment of 18.5 kDa MBP was further confirmed by the pattern and magnitude of the observed NOE (nuclear Overhauser effect) connectivities, which reinforced the conclusion of an α-helical conformation for this region in a membrane environment. In aqueous solution (100 mM KCl, pH 6.5), the observed NOE values suggested that the polypeptide formed a relatively stable core, and hinted at a weakly α-helical conformation in the most highly conserved region (Val83-Phe87 in the murine 18.5 kDa sequence, (Fig. **2**)). These results were consistent with previous CD data (cited in [99]), and with our molecular dynamics simulations that showed this segment to have a propensity to form transient α-helices in aqueous solution [118].

The calculated structures of the polypeptide revealed a partitioning of charges onto opposing faces of the helix, demonstrating clearly the amphipathic nature of this peptide (Figs. **6B** and **6D**). Paramagnetic relaxation effects were evaluated using the reagents 5-doxylstearic acid (5-DSA) and $FeCl_3$, which respectively partitioned inside or outside the hydrophobic interior of the micelles. These molecules acted locally as strong signal-relaxing agents, causing a broadening proportional to the inverse of the average of the distance to the sixth power ($<r^{-6}>$), between the unpaired electron of the paramagnetic agent and the interacting nucleus. Thus, these agents could report the positioning of individual residues, and on the orientation of the whole helix relative to the membrane surface. The results indicated that the polypeptide α-helix formed distinct hydrophobic and electrostatic contacts with the membrane (Fig. **6D**), and were in excellent agreement with the SDSL/EPR mapping and positioning of the α-helical model of this epitope of full-length 18.5 kDa MBP on the surface of a lipid bilayer [88, 89].

STRUCTURE OF THE PROLINE-RICH SEGMENT OF 18.5 KDA MBP

The immunodominant epitope just discussed is highly conserved evolutionarily compared to the rest of the protein (Fig. **2**). In all mammalian species, this central amphiphathic α-helix is followed by a proline-rich TPRTPPPS (murine Thr92-Ser99) segment (Fig. **2**) that comprises a minimal SH3-ligand (XP-x-XP). This region of the protein has been demonstrated *in vitro* to form a polyproline type II helix (PPII), and the 18.5 kDa isoform has been shown to bind to SH3-domain (Src homology 3) containing proteins, such as non-receptor tyrosine kinases [34]. In addition, the two threonyl residues within this domain are MAP-kinase targets (Fig. **4**) [119, 120] which may be sequentially phosphorylated [121], suggesting structural or functional regulation. The SH3-domains are small conserved protein modules about 60 residues in size that interact with proline-rich ligands, and that are found in a large number of intracellular signaling proteins [122]. In spin-labelled recombinant murine MBP (rmMBP, Ser99Cys), EPR spectroscopy demonstrated that this region was exposed to the aqueous phase when the protein was bound to a membrane surface (Fig. **5A**) [88]. This residue's accessibility suggested that the entire Pro-rich region C-terminal to the amphipathic α-helix should be accessible to enzymes and other proteins, including those with SH3-domains, even when MBP is bound to the membrane. (Solid-state NMR studies of membrane-associated 18.5 kDa MBP have also revealed a significant proportion of the protein to be exposed [87].) Indeed, lipid-associated 18.5 kDa MBP has been shown to bind the SH3-domain of Fyn to the lipid bilayer [34]. Thus, in addition to its membrane-associating and immunogenic roles, the central amphipathic α-helical segment of MBP may be critical in the proper positioning of the SH3-ligand, and the known MAP-kinase sites within it, for functional roles beyond membrane adhesion.

Polyproline-type-II helices are characterised by the average backbone (φ,ψ) dihedral angles of (-75^{o},$+145^{o}$), resulting in a left-handed, all *trans*, extended helix of approximately 3.12 Å in length per residue [123]. One complete turn of the helix will span approximately 9 Å, giving the helix a three-fold rotational symmetry. Due to this extended arrangement, the residues that participate in the helix are precluded from forming stabilising inter-residue interactions, *i.e.*, there are no N-H↔O hydrogen bonds. Thus, PPII helices are often confined to short stretches of amino acids, and are prevalent in disordered proteins as one of numerous conformational states [124].

In ideal model systems, the CD spectra of the PPII conformation are characterised by a negative band near 200-205 nm and a weaker, positive transition around 217-225 nm. However, identification of PPII in experimental systems is complicated by the absorbance of aromatic sidechains (F, Y, and W) in the far UV spectral region [125]. Furthermore, a notable characteristic of IDPs is the interconversion of the structural ensemble between other secondary structure types, notably α-helices, which also complicates the interpretation of CD data. Thus, the CD spectra of IDPs displaying PPII character have often been misinterpreted as random coil, or have been described as similar to spectra of denatured polypeptides [126, 127]. Circular dichroic spectra recorded as a function of temperature are an effective method to discern the presence of the PPII structure, since two readily identifiable features of the PPII may be observed [128]. First, an isodichroic point centered at around 210 nm is indicative of a temperature-dependent, two-state equilibrium, where the lower temperature traces are most characteristic of the PPII structure. Secondly, upon increasing the temperature a reduction in the absolute intensity of the positive transition is observed, signifying a disruption of regular (PPII) structure [125]. Subtraction of the low and high temperature traces may further emphasise the PPII character of the spectra.

We demonstrate this approach here for an MBP polypeptide encompassing the proline-rich segment in membrane-mimetic conditions, *viz*., DPC micelles. Fig. (**7**) shows the temperature-dependent (**7A, 7C**) and difference (**7B, 7D**) CD spectra for the unmodified (**7A, 7B**) and phosphorylated (**7C, 7D**) polypeptides reconstituted in 100 mM DPC, 50 mM potassium phosphate at pH 6.5. Both peptides display PPII character evidenced by the isodichroic point at 210 nm and the reduction of intensity of the 220 nm transition at higher temperatures (Figs. **7A** and **7C**). Furthermore, the spectra of the two peptides do not display any significant temperature-induced disorder, likely due to the stabilisation effects of the DPC micelles. The transition at 200 nm does not become appreciatively more positive upon an increase in temperature, in contrast to what has previously been observed for rmMBP in 100 mM KCl [34]. This observation can be rationalised as follows, noting that these polypeptides are a hybrid of half the immunodominant epitope and the entire proline-rich region. First of all, in the case of both the unmodified and phosphorylated peptides, the lack of relative observable disorder suggests that the N-terminal portion of these peptides (F86-T92, Fig. **2**) which encompasses the immunodominant epitope (Figs. **2** and **4**), associates with the DPC micelle, producing a global stabilising effect on the peptides (Fig. **6D**) [99]. The temperature-dependent reduction in the 220 nm transition, however, is suggestive of the local destabilisation of the PPII structure [128], as could be expected if this proline-rich region (adjacent to the membrane-associated amphipathic α-helix) remains solvent-exposed (Fig. **6A**) [88, 89].

Recent CD spectroscopic investigations of full-length 18.5 kDa MBP have revealed the potential for a PPII helix to exist in the highly conserved, proline-rich middle third of the protein [34]. We have shown that this motif indeed forms a PPII helix under aqueous solution conditions, which structure appears to be stabilised by phosphorylation of the threonyl 95 residue (murine 18.5 kDa sequence numbering, corresponding to human Thr98, Fig. **2**) [34] (*cf*., [129]). In the nuclear-localised MBPs (*i.e.*, exon-6/II containing, Figs. **1** and **4**), the proline-rich region has been postulated to function as a flexible hinge or possibly a targeting sequence [130, 131]. Earlier NMR spectroscopic work on MBP-derived peptides had indicated that all prolyl residues were in *trans* configuration, consistent with the requirements for PPII structure [132, 133].

Although CD at different temperatures is the spectroscopy of choice for identification of PPII structures in proteins and peptides, it cannot report high-resolution, residue specific conformational information necessary for structure determinations. Aside from X-ray crystallography, NMR spectroscopy is the only direct method available to obtain such information, and has the added advantage of being able to report on the dynamic behaviour of polypeptide chains [134]. There are several NMR experiments available to obtain the required information for structural restraint quantification of the PPII helix. Chemical shifts of the H^{α}, C^{α}, C^{β}, and C' nuclei are correlated with the main chain ϕ and ψ angles, and the H^{N} and N chemical shifts are correlated with the ϕ angles, and would provide valuable structural information for the polypeptides [135]. The chemical shifts of H^{N} and N are temperature- and sequence-dependent, and the relationship of this dependence has been correlated to specific structural elements [115, 116, 136]. Therefore, comparison of the sequence-corrected N chemical shift with random coil values (*i.e.,* $\Delta\delta^{15}N$) can help quantify PPII structure. The temperature-dependence of the N chemical shift has also been demonstrated to correlate with the change in molar ellipticity at 220 nm, providing additional evidence for the PPII conformation [137].

Scalar couplings can be related to the backbone dihedral angles through the Karplus equation [138, 139]. The one-bond $^{1}J_{C\alpha H\alpha}$ coupling is related to ϕ and ψ angles, and can provide additional structural determinants for PPII helices [140]. Of particular use in the case of prolines is the two-bond $^{2}J_{C'H\alpha}$ coupling, which can be readily determined from two-dimensional experiments. There is a statistically significant relationship between this coupling and the ϕ angle, although there may be other factors that influence the magnitude of $^{2}J_{C'H\alpha}$ [141]. In future, a detailed NMR investigation is required, similar to what was performed on the immunodominant epitope alone [99], but this time with fully (or residue-specific) $^{13}C^{15}N$-labelled sample to facilitate resonance assignment and structure elucidation [142, 143]. Determining the structural implications of phosphorylation on the conformation of this region will help us to understand fully the functional consequences of this modification – is it a molecular switch?

DYNAMIC BARCODING – CONTROL BY PHOSPHORYLATION/DEPHOSPHORYLATION

Phosphorylation and dephosphorylation activate or deactivate proteins in cellular signalling events, by modifying their structure, function, subcellular localisation, or interactions [144]. Protein kinases and phosphatases are thus key members of signalling cascades in cellular processes such as proliferation, differentiation, apoptosis, and regulation of gene transcription. These modifications are especially important for cationic proteins such as MBP, where combinatorial phosphorylation/dephosphorylation events may be tuned to regulate their local and global electrostatic properties [74, 145-149]. Furthermore, changes in the phosphorylation patterns of specific proteins have been implicated in a variety of human diseases - Alzheimer's, Niemann-Pick Type C1, and amyotrophic lateral sclerosis are marked by aberrant kinases and hyperphosphorylation of their substrates (*e.g.*, [150]). In oligodendrocytes, the antibody cross-linking of myelin oligodendrocyte glycoprotein leads to its trafficking to a specific myelin microdomain, and subsequently to changes in activity of specific kinases and phosphatases [151, 152]. Several proteins become dephosphorylated whereas many others become hyperphosphorylated, thereby modulating several signalling pathways.

The 18.5 kDa isoform of MBP fits intriguingly into these paradigms. Phosphorylation of MBP (at seryl and threonyl residues, (Fig. **4**)) is effected by several protein kinase (PK) families [32, 153], and yields charge components with altered conformations and protein-lipid and protein-protein interactions [4, 12, 34, 46-48, 154-156]. In particular, charge components of MBP that are phosphorylated have a decreased ability to assemble actin [47, 48], but curiously an enhanced ability to polymerise and bundle tubulin [4]. Thus,

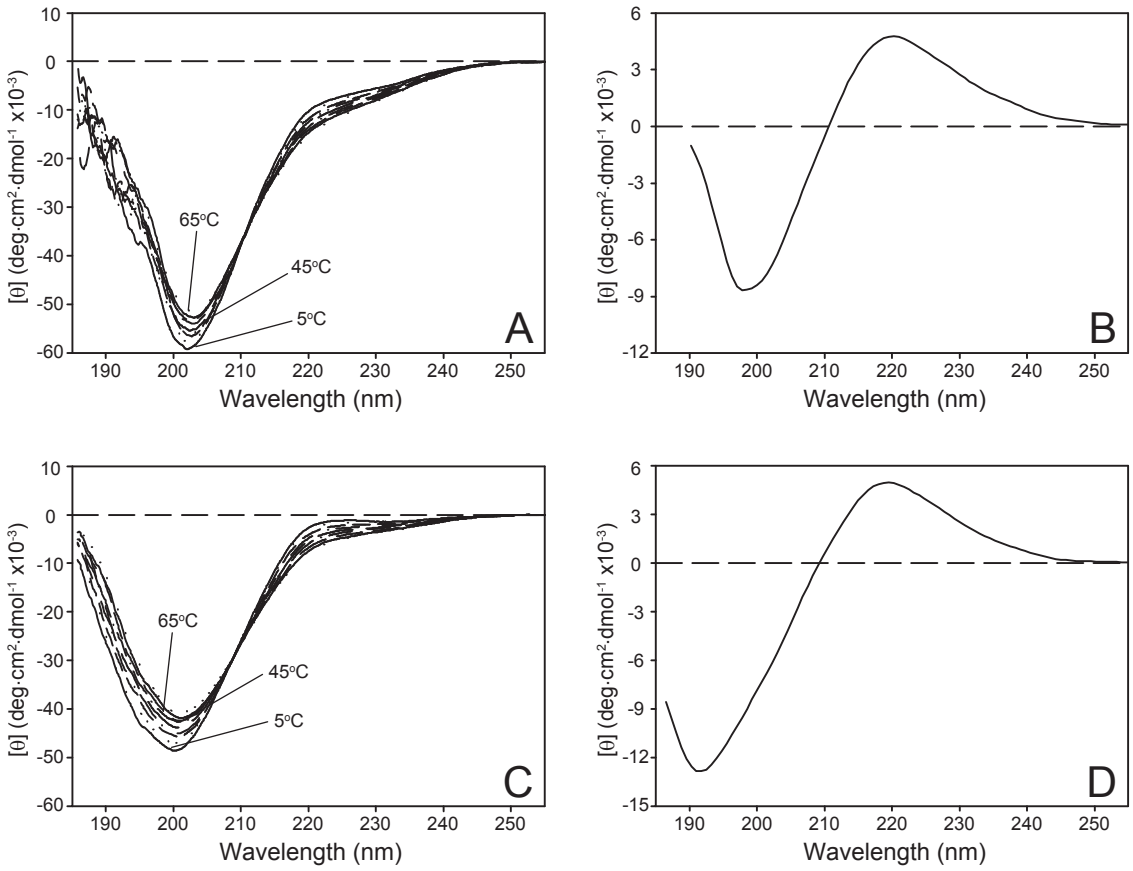

Fig. (7). Circular dichroic spectra for the unmodified **(A** and **B)** and phosphorylated peptides **(C** and **D)** (86-FFKNIVTPRTPPPSQGK-102, murine numbering, (Fig. **2**)) associated with dodecylphosphocholine micelles. The latter peptide was phosphorylated on residue T95. Spectra in panels **A** and **C** were obtained by increasing the temperature in 5°C increments from 5°C to 45°C. Panels **B** and **D** each show the corresponding difference spectrum obtained by subtracting the 45°C spectrum from the 5°C spectrum. The shapes of the difference spectra for both peptides (panels **B** and **D**) are indicative of a polyproline type II helix. The PPII helical character is lost with increasing temperature due to the absence of the stabilisation of inter-residue hydrogen-bonding. An isodichroic (isoelliptic) point at approximately 210 nm (panels **A** and **C**) suggests that there is a two-state equilibrium between the left-handed PPII helix and the disordered chain, consistent with the transient nature of PPII helices. Comparative data on the polypeptide in aqueous solution have been published [34].

this modification does not have the same overall or specific effects as charge reduction by deimination solely [28]. Phosphorylation of MBP is not a spurious event - it is altered during development and ageing, and the overall level is decreased in multiple sclerosis [19, 157-159]. The exon-6/II-containing phosphorylated MBPs have been suggested to be involved in myelinogenesis [158, 160], and the exon-6/II-lacking phosphorylated MBPs in myelin maintenance [160], particularly in the putative adherens junctions and tight junctions of myelin [161].

We particularly note the mitogen-activated protein kinases (MAP-kinases), including the extracellular signal-regulated protein kinases (ERK, *e.g.*, ERK1 and ERK2; also known as p42/44 MAPK), the stress-activated c-Jun N-terminal kinase (JNK), and the 38 kDa high osmolarity glycerol response kinase (p38) [153]. The MAP-kinase family is important in oligodendrocyte proliferation, cell survival, differentiation, and apoptosis. The phosphorylation of murine Thr95 (Fig. **4**; human Thr98, bovine Thr97) within the segment Thr92-Pro93-Arg94-Thr95-Pro96 (the putative SH3-ligand) by MAP-kinases is regulated by action potential generation in axons [162], and we have shown that MBP phosphorylated at this site is developmentally partitioned into myelin microdomains called "lipid rafts", some of which may represent signalling domains in mature myelin [81, 163, 164]. Using an ND4 transgenic mouse line overexpressing the PLP variant DM20, and which exhibit spontaneous demyelination and which represent a model for multiple sclerosis, we have shown that, compared to healthy mice, there are significant changes in the distribution of MBP splice isoforms and their phosphorylated and methylated isomers in myelin rafts [163].

The residue Thr92 is a second MAP-kinase target within the putative SH3-ligand segment [120]. In *in vitro* studies, MAP-kinase phosphorylation of MBP affects its ability to polymerise actin and tubulin, and to bundle microfilaments and microtubules, as just mentioned [4, 47, 48]. We thus consider these two residues to be key post-translationally modified sites that can affect local structure, protein-protein interactions, and microdomain targetting and stability, thereby operating as a "molecular switch" and conferring a "function" on classic MBP isoforms beyond membrane adhesion.

Regarding structure and protein-protein interactions, the potential isomerisation of prolyl residues in MBP *in vivo* has been mentioned as a possible signalling or functional control mechanism [34]. The spontaneous rate of proline isomerisation is very slow and almost negligible, and has been observed to increase to about 5% in membrane-associated MBP [133]. Phosphorylation of the threonyl residues within the TP-R-TP motif has the potential to stabilise the PPII conformation, and thus slow the rate of spontaneous isomerisation [129, 165]. Phosphorylation of these threonyl residues may concomitantly result in a localised *de*stabilisation of the preceding α-helix [166], and/or disruption of the C-terminus of the central amphipathic α-helix from the phospholipid membrane, a phenomenon similar to what has been observed for the deiminated protein [90]. Alternatively, there are novel prolyl isomerases (*e.g.*, Pin1) that efficiently isomerise phosphorylated Ser/Thr-Pro bonds in certain proteins, as a novel control mechanism in signalling pathways [167]. These isomerases have been identified in neurons, but their presence in myelin remains to be determined [168, 169]. They may participate, in conjunction with kinases and phosphatases, on modifying this proline-rich segment of the putative central molecular switch domain.

CALMODULIN-BINDING BY CLASSIC 18.5 KDA MBP

The 18.5 kDa isoform of MBP has previously been demonstrated to interact with calmodulin (CaM) in a Ca^{2+}-dependent manner using a variety of techniques including intrinsic single Trp fluorescence spectroscopy [170-173], chemical cross-linking and gel-shift assays [171], and dynamic light scattering [173]. The *in vitro* interaction was shown to be specific (*i.e.*, Ca^{2+}-dependent), and occur at a 1:1 (MBP:CaM) ratio under near physiological conditions (250 mM NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$) with a dissociation constant of 144±76 nM [173]. Subsequent proteomics analyses have revealed MBP to be one of many CaM-binding proteins in the brain [174, 175], and MBP has recently been shown to be able to function as an adaptor protein linking calmodulin to the BK_{Ca} channel in neurons, although the physiological significance of this observation is still unclear [33].

Our first prediction for the CaM-target was of a C-terminal segment on MBP (Lys132-Arg167, murine numbering, (see Fig. (**2**)), which was consistent with the previous experimental data [170, 171]. However, a C-terminal deletion mutant lacking this region also bound CaM specifically [172], and deiminated MBP (*i.e.*, the C8 isoform) bound CaM in a manner also suggestive of a second binding site [173]. We postulated that this putative second CaM-target could be the central immunodominant epitope (Pro82-Pro93, murine numbering), which we showed by molecular modelling also to be a plausible CaM-target (when modelled as an α-helix), just as the C-terminal segment (Thr147-Asp158, murine numbering) of MBP was [176].

We have recently undertaken solution NMR studies of CaM bound to full-length 18.5 kDa MBP, with the goal of identifying definitively the primary CaM interaction site on MBP [37]. The studies revealed three major segments of the protein with a propensity towards α-helicity that were stabilised by membrane-mimetic conditions: Thr33-Asp46, Val83-Thr92, and Tyr142-Leu154 (murine 18.5 kDa sequence numbering). All of these regions corresponded with bioinformatics predictions of ordered secondary structure (Fig. **5B**). The Val83-Thr92 (murine numbering) region has already been discussed – it comprises a primary immunodominant epitope that had previously been shown by EPR and NMR spectroscopies to be α-helical in membrane-reconstituted systems (Fig. **6**). The Tyr142-Leu154 segment overlapped with our predicted CaM-binding site [170, 171].

Chemical shift perturbation experiments using ^{15}N-labelled MBP and unlabelled calmodulin demonstrated a dramatic conformational change in MBP upon association of the two proteins, and were consistent with the C-terminal segment of MBP being the primary binding site for calmodulin. Approximately 40 resonances were observed shifted upfield in the ^{1}H dimension (7.0-8.2 ppm), and downfield in the ^{15}N dimension (123-133 ppm), relative to unbound MBP. Furthermore, the spectrum of MBP bound with CaM displayed an increase in the absolute dispersion of peaks, indicating a more structured state for MBP. The same extreme chemical shift perturbations were not observed in the complementary experiment (*i.e.*, ^{15}N-labelled CaM interacting with unlabelled MBP), indicating that the structure of CaM was not significantly perturbed upon the proteins' association. These data were consistent with our previous results using SDSL/EPR, which indicated that the entire MBP protein was involved in the interaction with CaM [176]. Many of the perturbed peaks of MBP mapped to bulky hydrophobic residues (*e.g.*, F, Y, and L), which may have indicated exclusion of bulk solvent from the hydrophobic face, and/or a specific interaction. Again, these results were consistent with the SDSL/EPR data showing greatest immobilisation of the spin label at sites closest to small hydrophobic clusters on the protein [176].

Taken together, these observations reflected a general phenomenon characteristic of IDPs, *i.e.*, that they gain ordered structure upon interaction with a binding partner. Although IDPs bind targets with high specificity, often involving multiple electrostatic interactions, the unbound portion of the polypeptide chain often retains considerable plasticity; thus, multiple hydrophobic interactions could be expected [62, 74, 177, 178]. There are other CaM-binding proteins that appear to have a similar, extended conformation of the binding site when complexed with CaM, *e.g.*, MARCKS and MARCKS-related peptides, and caldesmon [179-181]. Recently, the hypothesis of coupled binding and folding has been explicitly stated for CaM and binding targets on IDPs [182], with which our NMR spectroscopic observations are fully compatible.

Based on chemical shift index scoring of secondary structure, the central immunodominant epitope (Pro82-Pro93, murine numbering) was destabilised in the CaM-bound form of MBP relative to the free form. Since our previous molecular docking simulations indicated that an extended local conformation of this epitope would be unfavourable for CaM-binding (as opposed to an α-helical conformation), this region was excluded from consideration as being the main CaM-binding site [176]. Furthermore, the C-terminal region displayed a redistribution of secondary struc-

ture within the PONDR®-predicted ordered region (Fig. **5B**). These observations suggested that the stabilisation was coming from a different source (*i.e.*, CaM) instead of the solvent or intrachain interactions. The residues from Ala144-Ser159 showed an increased propensity to form α-helical structure in the CaM-bound MBP, compared to the unbound form. Although large chemical shift deviations occurred throughout the entire polypeptide chain, they were clustered in the C-terminal region. Therefore, taken collectively, these observations are consistent with the premise that the C-terminus of post-translationally unmodified 18.5 kDa MBP is the primary interaction site with CaM, as previously posited [170-173]. A contemporaneous, independent biophysical study using a peptide fragment of MBP has shown it to interact with CaM in a manner consistent with our results [36].

Conformational adaptability is an important property of IDPs, and underscores the importance of comparative structural studies of members of this class of proteins. The studies demonstrating that MBP can act as an adapter linking the BK_{Ca} channel with CaM suggest that there are multiple, functional interaction sites on MBP (although it is not clear which isoform of MBP may be operative in this particular interaction) [33]. Myelin basic protein can and does adopt multiple unique conformations based on the local environment, and combinatorial post-translational modifications (*i.e.*, the dynamic molecular barcode) could themselves induce or stabilise protein-binding targets [70, 154, 183]. Currently, the data support the conjecture that MBP interacts with CaM primarily through an interaction of the C-terminal residues A144-S159 [36, 37]. However, these observations are not sufficient to provide the atomic details of MBP-CaM binding. Thus, future studies (*e.g.*, ^{15}N-^{13}C edited NOE spectroscopy experiments) will be necessary to delineate further the details of MBP binding to CaM. Moreover, the effects of post-translational modifications in the C-terminal region (Fig. **4**), such as phosphorylation by the protein kinase C family, remain to be explored further experimentally [173, 176].

IS THE CARBOXY-TERMINUS OF 18.5 KDA MBP ANOTHER FUNCTIONAL MOTIF?

So far, we have primarily discussed the idea of the regions of 18.5 kDa MBP encoded by exon-IV/8 and exon-V/9 constituting a molecular switch. We have also indicated that the primary CaM-target appears to be a region encoded by exon-VI/10 and exon-VII/11. In fact, we had previously compared this C-terminal segment of 18.5 kDa MBP (human 18.5 kDa residues L150-S165, corresponding to murine 18.5 kDa residues L148-S163) with the lysine/arginine rich regions of phosphoinositide- and actin-binding proteins such as *Listeria monocytogenes* ActA, MARCKS, and vinculin, and showed that it also was amphipathic when portrayed on a helical wheel projection [84]. At the time, this C-terminal 18.5 kDa domain was discussed in terms of its interactions with phosphoinositide-containing membranes (*cf.*, [184]), but it has subsequently turned out to be interesting from the viewpoints of exposing a secondary immunodominant epitope upon deimination of the whole protein [88, 98], and representing a primary CaM-target [37, 172, 173]. Moreover, it is highly conserved in sequence (Fig. **2**), is first structurally intrinsically ordered and then suddenly becomes highly intrinsically disordered (Fig. **4**), and is highly post-translationally modified by deimination, and by phosphorylation of seryl residues by the protein kinase C family (Fig. **5**). All these collected considerations lead us to suggest that the C-terminus of 18.5 kDa MBP may be another important molecular switch.

FUNCTIONAL DIVERSITY OF CLASSIC MBP ISOFORMS AND CHARGE COMPONENTS

While various novel functions are currently being discovered for the Golli-MBP isoforms *in vivo* [5-10], the primarily *in vitro* physicochemical and phenomenological properties of the classic MBP isoforms described here suggest that they, too, must possess a diverse multifunctionality that is yet to be elucidated fully. The classically accepted biological function of 18.5 kDa myelin basic protein is the maintenance of myelin sheath compaction – a membrane "Velcro™". Owing to its extreme net positive charge, MBP serves as an adhesive molecule in central nervous system myelin sheaths, bringing together the two apposing faces of the cytoplasmic leaflets of the cell membrane processes of myelinating cells, oligodendrocytes, to effect myelin compaction around axons. The MBP-lipid interactions rely on a balance of interactions between the basic residues of MBP and the acidic headgroups of the lipid bilayer, in order to assemble the proper multilamellar structure seen in myelin sheaths [185, 186].

In non-compact regions of myelin, MBP may also tether the cytoskeleton to the membrane, and bundle microfilaments and microtubules together and perhaps to each other [12]. In a sense, MBP would represent a scaffold underlying the cytoplasmic leaflet of the membrane, connecting it to the canonical actin-tubulin cytoskeleton underneath. Again, these associations could be modulated by post-translational modifications, and further by interactions with proteins such as calmodulin or SH3-domains. So it appears that MBP may serve as another point of intersection in phosphorylation- and CaM-mediated signal transduction pathways controlling cytoskeletal architecture in oligodendrocytes.

Small changes to the overall balance of the various post-translational modifications of MBP could result in significant changes in myelin adhesion or stability, and in the interactions with the underlying cytoskeleton in some regions of the myelin sheath. Alteration of the cationicity of MBP (*i.e.*, dynamic molecular barcoding) may represent a regulatory mechanism for normal myelin assembly, defining microdomains of altered microstructure (*e.g.*, rigidity or corrugation) or protein-lipid composition (Fig. **8**) [40, 84, 164, 187, 188]. Overall then, the post-translational modifications serve to modulate MBP's interactions with membranes and other proteins, and in the neurodegenerative human disease multiple sclerosis, the equilibrium of modified forms is shifted towards hyperdeimination and hypophosphorylation, precipitating physical degeneration of the myelin sheath, autoimmune responses, and frustrating inherent attempts at repair [163, 189].

We have presented in this review a variety of spectroscopic evidence probing important structural domains (encoded by exon-III/7 and exon-IV/8) that are common to all of the classic MBP isoforms, and that may represent a mo-

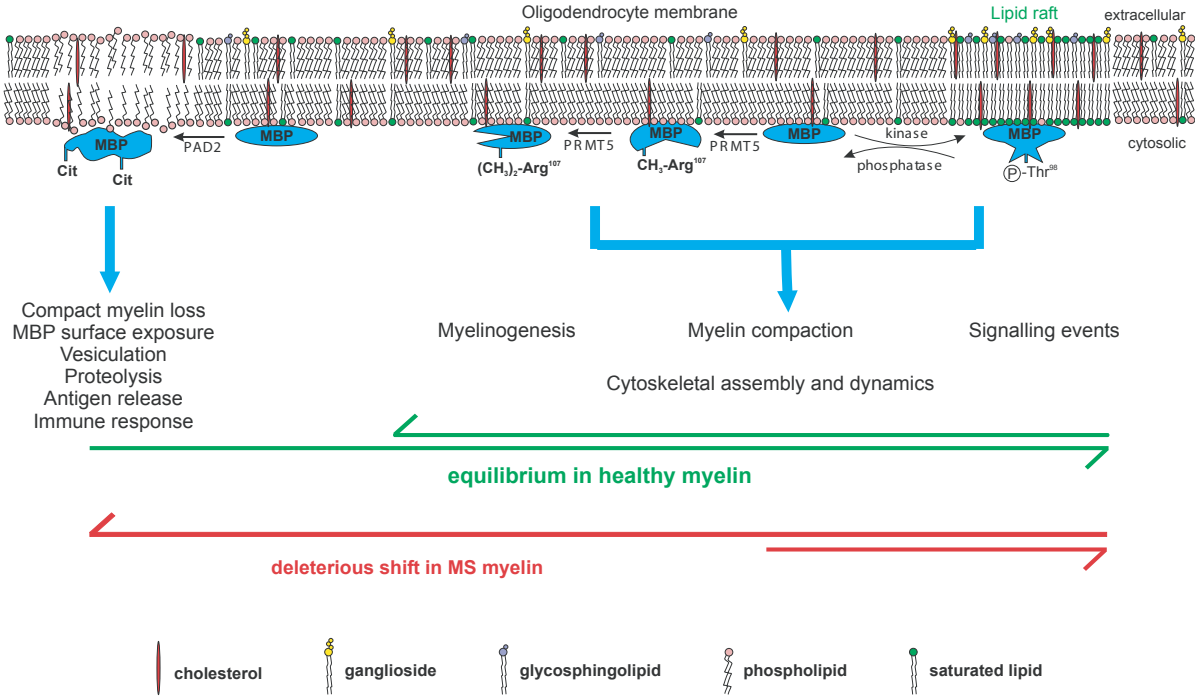

Fig. (8). Simplified overview of the roles and localisation of modified MBP in the myelin membrane. MBP can be mono- or symmetrically dimethylated at Arg104 (murine 18.5 kDa sequence; bovine Arg106, human Arg107), can be phosphorylated at various Ser and Thr residues by a range of kinases, and can be deiminated at specific Arg residues by PAD2. Whereas methylation and phosphorylation of MBP are temporally correlated with the process of myelinogenesis, deimination of this protein is generally linked to the pathology of demyelination. Methylation and phosphorylation of MBP enhance myelin compaction and endow this protein with resistance to proteolysis. Myelin basic protein that is phosphorylated by MAP-kinase (on human Thr98, bovine Thr97, murine Thr95) is localised in a lipid raft, and has a putative role in signal transduction. Deiminated MBP is susceptible to proteolysis and represents a pathway for protein turnover. Overall, methylated, phosphorylated, and deiminated forms of MBP are necessary for the maintenance and integrity of the myelin sheath. In a demyelinated state, the equilibrium of the various methylated, phosphorylated, and deiminated forms cannot be maintained. As a result, the higher level of deiminated MBP precipitates disruption of the myelin sheath, and a cascade of deleterious effects. Figure reproduced with permission from reference [28].

lecular switch for this protein. Another important structural and functional motif may be the C-terminus of the protein (exon-VI/10 and exon-VII/11). In general, though, the entire protein appears to behave as an entropic spring, and it is difficult to define a specific domain with a precise role in either membrane- or protein-association. Further structural studies, and complementary *in vivo* interaction studies, will serve to define what classic MBP's various roles may be within myelin. There are numerous other examples in the current literature of important intrinsically unstructured domains of proteins such as the cystic fibrosis transmembrane conductance regulator or α-synuclein, whose conformational and interaction properties are modulated by phosphorylation [150, 183, 190], and we posit that this modification of both Golli and classic MBP isoforms is pivotal.

PARALLELISM AND SYNERGY IN THE CENTRAL NERVOUS SYSTEM

In the central nervous system, there are other intrinsically disordered proteins (*e.g.*, myristoylated alanine-rich C kinase substrate (MARCKS), myelin-oligodendrocyte basic protein, and microtubule-associated proteins such as tau and stathmin) with analogous properties to MBP, particularly in terms of membrane and cytoskeletal association modulated by multiple phosphorylation events (*e.g.*, [4, 47, 83, 191]). The other major protein of central nervous system myelin, proteolipid protein, has also been suggested to have multiple functions [80, 192, 193]. These various proteins may behave synergistically, predominating in certain cell types or developmental stages, or in response to specific stimuli. The idea of global molecular networks in the central nervous system has recently been described elsewhere [194], and MBP may be operative in several of them, perhaps in specific microdomains of myelin. Therein lies a great challenge – mature myelin has a tremendous amount of MBP within it, and compact myelin architecture is also difficult to replicate using cell culture. The high redundancy achieved by multiple families of intrinsically disordered proteins that seem to do similar things confounds attempts to prove that MBP also participates in more than membrane compaction.

CONCLUDING REMARKS

The classic 18.5 kDa isoform of MBP is essential to maintaining the compact arrangement of the myelin sheath of the adult central nervous system. It contains several highly-conserved segments, of which we have discussed in detail a central region comprising an amphipathic α-helix followed by a PPII helix. Phosphorylation of two threonyl

residues within the region may be a means of modulating the local structure and interactions with the membrane and other proteins, as well as targeting the protein to myelin membrane microdomains. We are thus beginning to understand a part of the dynamic molecular barcode of this protein. It has been suggested that the body's failure to remyelinate in multiple sclerosis is due to perturbation of proper signalling pathways [189] – a shifted molecular barcode of 18.5 kDa post-translational modifications may thus be part of the degradative mechanism. Intrinsically disordered proteins are, in general, multifunctional, and have different structure-function relationships than do the classically-folded proteins such as enzymes. Perturbations of these proteins' interaction networks have been correlated with human illnesses such as Parkinson's and cardiovascular diseases [59, 195, 196], and we hypothesise this to be the case also in multiple sclerosis, mediated in large degree by MBP [28, 163, 164, 197]. Many neurodegenerative disorders (Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, transmissible spongiform encephalopathies) are considered to be "protein mis-folding" diseases [198, 199]. Multiple sclerosis does not fall into this category – nor does it appear to be directly a "protein modification" disease such as rheumatoid arthritis, another autoimmune disorder [200]; multiple sclerosis may instead represent a "protein mis-interacting and mis-signalling" syndrome.

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ABBREVIATIONS

C1-C8 = Charge components ("charge isomers") 1 to 8

CaM = Calmodulin

CD = Circular dichroism

DPC = Dodecylphosphocholine

5-DSA = 5-Doxylstearic acid

EPR = Electron paramagnetic resonance

Golli = Genes of the oligodendrocyte lineage

IDP = Intrinsically disordered protein

MAP = Mitogen-activated protein

MARCKS = Myristoylated alanine-rich C kinase substrate

MBP = Myelin basic protein

mMBP = Murine myelin basic protein

NMR = Nuclear magnetic resonance

NOE = Nuclear Overhauser effect

PAD = Peptidylarginine deiminase, EC 3.5.3.15

$PI(4,5)P_2$ = Phosphatidylinositol-4,5-bis-phosphate

PONDR® = Predictor of Natural Disordered Regions

PPII = Polyproline type II helix

ppm = Parts per million

rmMBP = Recombinant murine MBP

SDSL = Site-directed spin-labelling

SH3 = Src homology domain 3

TEMPO = 2,2,6,6-Tetramethyl-1-piperidinyloxyl

REFERENCES

[1] Lazzarini, R. A. (**2004**) in *Myelin Biology and Disorders,* Elsevier, Academic Press, San Diego.

[2] Campagnoni, A. T. and Campagnoni, C. (**2004**) in *Myelin Biology and Disorders*, (Lazzarini, R. A., Griffin, J. W., Lassman, H., Nave, K.-A., Miller, R. H., & Trapp, B. D., Eds.). pp. 387-400. Elsevier, Academic Press, San Diego.

[3] Givogri, M.I., Bongarzone, E.R. and Campagnoni, A.T. (**2000**) New insights on the biology of myelin basic protein gene: the neural-immune connection. *J. Neurosci. Res.*, *59*, 153-159.

[4] Hill, C.M.D., 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.

[5] Feng, J.M., Fernandes, A.O., Campagnoni, C.W., Hu, Y.H. and Campagnoni, A.T. (**2004**) The golli-myelin basic protein negatively regulates signal transduction in T lymphocytes. *J. Neuroimmunol.*, *152*, 57-66.

[6] Fernandes, A.O., Campagnoni, C.W., Kampf, K., Feng, J.M., Handley, V.W., Schonmann, V., Bongarzone, E.R., Reyes, S. and Campagnoni, A.T. (**2004**) Identification of a protein that interacts with the golli-myelin basic protein and with nuclear LIM interactor in the nervous system. *J. Neurosci. Res.*, *75*, 461-471.

[7] 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.

[8] Feng, J.M., Hu, Y.K., Xie, L.H., Colwell, C.S., Shao, X.M., Sun, X.P., Chen, B., Tang, H. and Campagnoni, A.T. (**2006**) Golli protein negatively regulates store depletion-induced calcium influx in T cells. *Immunity*, *24*, 717-727.

[9] Campagnoni, C., Campagnoni, A.T., Bassilian, S., Ryan, C., Whitelegge, J. and Faull, K. (**2008**) A myristoylated fragment of the Golli-MBP protein in mouse brain. *J. Neurochem.*, *104 (Suppl. 1)*, 43-44.

[10] Paez, P.M., Fulton, D.J., Spreur, V., Handley, V., Campagnoni, C.W. and Campagnoni, A.T. (**2008**) Regulation of Ca^{2+} entry into oligodendrocytes by Golli products of the *Mbp* gene. *J. Neurochem.*, *104 (Suppl. 1)*, 43.

[11] Harauz, G., Ishiyama, N., Hill, C.M.D., 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.

[12] Boggs, J.M. (**2006**) Myelin basic protein: a multifunctional protein. *Cell. Mol. Life Sci.*, *63*, 1945-1961.

[13] 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.

[14] Inouye, H. and Kirschner, D.A. (**1991**) Folding and function of the myelin proteins from primary sequence data. *J. Neurosci. Res.*, *28*, 1-17.

[15] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (**1994**) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, *22*, 4673-4680.

[16] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (**1997**) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, *25*, 3389-3402.

[17] Mitchell, C. and Sokal, R. (**1957**) A quantitative approach to a problem in classification. *Evolution*, *11*, 130-162.

[18] Sneath, P. & Sokal, R. (**1973**) *Numerical Taxonomy* (Freeman, San Francisco).

[19] 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. Proteomics*, *2*, 453-462.

[20] 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.

[21] 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.

[22] Whitaker, J.N. and Mitchell, G.W. (**1996**) A possible role for altered myelin basic protein in multiple sclerosis. *Ann. Neurol.*, *40*, 3-4.

[23] Yang, X.J. (**2005**) Multisite protein modification and intramolecular signaling. *Oncogene*, *24*, 1653-1662.

[24] Minagar, A. (**2008**) in *The neurobiology of multiple sclerosis,* in Elsevier, Academic Press, San Diego.

[25] Kouzarides, T. (**2007**) Chromatin modifications and their function. *Cell*, *128*, 693-705.

[26] Verhey, K.J. and Gaertig, J. (**2007**) The tubulin code. *Cell Cycle*, *6*, 2152-2160.

[27] Vossenaar, E.R., Zendman, A.J., Van Venrooij, W.J. and Pruijn, G.J. (**2003**) PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays*, *25*, 1106-1118.

[28] Harauz, G. and Musse, A.A. (**2007**) A tale of two citrullines - structural and functional aspects of myelin basic protein deimination in health and disease. *Neurochem. Res.*, *32*, 137-158.

[29] Moscarello, M.A., Mastronardi, F.G. and Wood, D.D. (**2007**) The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple sclerosis. *Neurochem. Res.*, *32*, 251-256.

[30] Kim, S., Chanderkar, L.P., Ghosh, S.K., Park, J.O. and Paik, W.K. (**1988**) Enzymatic methylation of arginine residue in myelin basic protein. *Adv. Exp. Med. Biol.*, *231*, 327-340.

[31] Raijmakers, R., Zendman, A.J., Egberts, W.V., Vossenaar, E.R., Raats, J., Soede-Huijbregts, C., Rutjes, F.P., van Veelen, P.A., Drijfhout, J.W. and Pruijn, G.J. (**2007**) Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases *in vitro*. *J. Mol. Biol.*, *367*, 1118-1129.

[32] Eichberg, J. and Iyer, S. (**1996**) Phosphorylation of myelin protein: recent advances. *Neurochem. Res.*, *21*, 527-535.

[33] Kim, H., Jo, S., Song, H.J., Park, Z.Y. and Park, C.S. (**2007**) Myelin basic protein as a binding partner and calmodulin adaptor for the BK(Ca) channel. *Proteomics*, *7*, 2591-2602.

[34] Polverini, E., Rangaraj, G., Libich, D.S., Boggs, J.M. and Harauz, G. (**2008**) Binding of the proline-rich segment of myelin basic protein to SH3-domains - Spectroscopic, microarray, and modelling studies of ligand conformation and effects of post-translational modifications. *Biochemistry*, *47*, 267-282.

[35] Stapulionis, R., Oliveira, C.L.P., Gjelstrup, M.C., Pedersen, J.S., Hokland, M.A., Hoffmann, S.V., Poulsen, K., Jacobsen, C. and Vorup-Jensen, T. (**2008**) Structural insight into the function of myelin basic protein as a ligand for integrin αMβ2. *J. Immunol.*, *180*, 3946-3956.

[36] Majava, V., Petoukhov, M.V., Hayashi, N., Pirila, P., Svergun, D.I. and Kursula, P. (**2008**) Interaction between the C-terminal region of human myelin basic protein and calmodulin: analysis of complex formation and solution structure. *BMC Struct. Biol.*, *8*, 10.

[37] Libich, D.S. and Harauz, G. (**2008**) Backbone dynamics of the 18.5 kDa isoform of myelin basic protein reveals transient α-helices and a calmodulin-binding site. *Biophys. J.*, *94*, 4847-4866.

[38] Krämer, E.M., Schardt, A. and Nave, K.A. (**2001**) Membrane traffic in myelinating oligodendrocytes. *Microsc. Res. Tech.*, *52*, 656-671.

[39] Sherman, D.L. and Brophy, P.J. (**2005**) Mechanisms of axon ensheathment and myelin growth. *Nat. Rev. Neurosci.*, *6*, 683-690.

[40] Fitzner, D., Schneider, A., Kippert, A., Mobius, W., Willig, K.I., Hell, S.W., Bunt, G., Gaus, K. and Simons, M. (**2006**) Myelin basic protein-dependent plasma membrane reorganization in the formation of myelin. *EMBO J.*, *25*, 5037-5048.

[41] Simons, M. and Trotter, J. (**2007**) Wrapping it up: the cell biology of myelination. *Curr. Opin. Neurobiol.*, *17*, 533-540.

[42] Song, J., Goetz, B.D., Baas, P.W. and Duncan, I.D. (**2001**) Cytoskeletal reorganization during the formation of oligodendrocyte processes and branches. *Mol. Cell Neurosci.*, *17*, 624-636.

[43] Richter-Landsberg, C. (**2001**) Organization and functional roles of the cytoskeleton in oligodendrocytes. *Microsc. Res. Tech.*, *52*, 628-636.

[44] Richter-Landsberg, C. (**2008**) The cytoskeleton in oligodendrocytes: microtubule dynamics in health and disease. *J. Mol. Neurosci.*, *35*, 55-63.

[45] Boggs, J.M. and Rangaraj, G. (**2000**) Interaction of lipid-bound myelin basic protein with actin filaments and calmodulin. *Biochemistry*, *39*, 7799-7806.

[46] 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.

[47] 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.

[48] 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.

[49] Galiano, M.R., Andrieux, A., Deloulme, J.C., Bosc, C., Schweitzer, A., Job, D. and Hallak, M.E. (**2006**) Myelin basic protein functions as a microtubule stabilizing protein in differentiated oligodendrocytes. *J. Neurosci. Res.*, *84*, 534-541.

[50] Perkins, G.A., Sosinsky, G.E., Ghassemzadeh, S., Perez, A., Jones, Y. and Ellisman, M.H. (**2008**) Electron tomographic analysis of cytoskeletal cross-bridges in the paranodal region of the node of Ranvier in peripheral nerves. *J. Struct. Biol.*, *161*, 469-480.

[51] Martenson, R. E. (**1980**) in *Biochemistry of Brain*, (Kumar, S., Ed.). pp. 49-79. Pergamon Press, Oxford.

[52] Benmerah, A., Scott, M., Poupon, V. and Marullo, S. (**2003**) Nuclear functions for plasma membrane-associated proteins? *Traffic*, *4*, 503-511.

[53] Sedzik, J. and Kirschner, D.A. (**1992**) Is myelin basic protein crystallizable? *Neurochem. Res.*, *17*, 157-166.

[54] Hill, C.M.D., 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.

[55] Receveur-Bréchot, V., Bourhis, J.M., Uversky, V.N., Canard, B. and Longhi, S. (**2006**) Assessing protein disorder and induced folding. *Proteins*, *62*, 24-45.

[56] Radivojac, P., Iakoucheva, L.M., Oldfield, C.J., Obradovic, Z., Uversky, V.N. and Dunker, A.K. (**2007**) Intrinsic disorder and functional proteomics. *Biophys. J.*, *92*, 1439-1456.

[57] Xie, H., Vucetic, S., Iakoucheva, L.M., Oldfield, C.J., Dunker, A.K., Uversky, V.N. and Obradovic, Z. (**2007**) Functional anthology of intrinsic disorder. 1. Biological processes and functions of proteins with long disordered regions. *J. Proteome Res.*, *6*, 1882-1898.

[58] Vucetic, S., Xie, H., Iakoucheva, L.M., Oldfield, C.J., Dunker, A.K., Obradovic, Z. and Uversky, V.N. (**2007**) Functional anthology of intrinsic disorder. 2. Cellular components, domains, technical terms, developmental processes, and coding sequence diversities correlated with long disordered regions. *J. Proteome Res.*, *6*, 1899-1916.

[59] Xie, H., Vucetic, S., Iakoucheva, L.M., Oldfield, C.J., Dunker, A.K., Obradovic, Z. and Uversky, V.N. (**2007**) Functional anthology of intrinsic disorder. 3. Ligands, post-translational

modifications, and diseases associated with intrinsically disordered proteins. *J. Proteome Res.*, *6*, 1917-1932.

[60] Tompa, P., Szasz, C. and Buday, L. (**2005**) Structural disorder throws new light on moonlighting. *Trends Biochem. Sci.*, *30*, 484-489.

[61] Tompa, P. (**2005**) The interplay between structure and function in intrinsically unstructured proteins. *FEBS Lett.*, *579*, 3346-3354.

[62] Fuxreiter, M., Tompa, P. and Simon, I. (**2007**) Local structural disorder imparts plasticity on linear motifs. *Bioinformatics*, *23*, 950-956.

[63] Mohan, A., Oldfield, C.J., Radivojac, P., Vacic, V., Cortese, M.S., Dunker, A.K. and Uversky, V.N. (**2006**) Analysis of molecular recognition features (MoRFs). *J. Mol. Biol.*, *362*, 1043-1059.

[64] Vacic, V., Oldfield, C.J., Mohan, A., Radivojac, P., Cortese, M.S., Uversky, V.N. and Dunker, A.K. (**2007**) Characterization of molecular recognition features, MoRFs, and their binding partners. *J. Proteome Res.*, *6*, 2351-2366.

[65] Demarest, S.J., Martinez-Yamout, M., Chung, J., Chen, H., Xu, W., Dyson, H.J., Evans, R.M. and Wright, P.E. (**2002**) Mutual synergistic folding in recruitment of CBP/p300 by p160 nuclear receptor coactivators. *Nature*, *415*, 549-553.

[66] Dosztanyi, Z., Chen, J., Dunker, A.K., Simon, I. and Tompa, P. (**2006**) Disorder and sequence repeats in hub proteins and their implications for network evolution. *J. Proteome Res.*, *5*, 2985-2995.

[67] Patil, A. and Nakamura, H. (**2006**) Disordered domains and high surface charge confer hubs with the ability to interact with multiple proteins in interaction networks. *FEBS Lett.*, *580*, 2041-2045.

[68] Hershey, P.E., McWhirter, S.M., Gross, J.D., Wagner, G., Alber, T. and Sachs, A.B. (**1999**) The Cap-binding protein eIF4E promotes folding of a functional domain of yeast translation initiation factor eIF4G1. *J. Biol. Chem.*, *274*, 21297-21304.

[69] Chi, S.W., Lee, S.H., Kim, D.H., Ahn, M.J., Kim, J.S., Woo, J.Y., Torizawa, T., Kainosho, M. and Han, K.H. (**2005**) Structural details on mdm2-p53 interaction. *J. Biol. Chem.*, *280*, 38795-38802.

[70] Solt, I., Magyar, C., Simon, I., Tompa, P. and Fuxreiter, M. (**2006**) Phosphorylation-induced transient intrinsic structure in the kinase-inducible domain of CREB facilitates its recognition by the KIX domain of CBP. *Proteins*, *64*, 749-757.

[71] Takeuchi, K. and Wagner, G. (**2006**) NMR studies of protein interactions. *Curr. Opin. Struct. Biol.*, *16*, 109-117.

[72] Iakoucheva, L.M., Radivojac, P., Brown, C.J., O'Connor, T.R., Sikes, J.G., Obradovic, Z. and Dunker, A.K. (**2004**) The importance of intrinsic disorder for protein phosphorylation. *Nucleic Acids Res.*, *32*, 1037-1049.

[73] Finerty, P.J., Jr., Mittermaier, A.K., Muhandiram, R., Kay, L.E. and Forman-Kay, J.D. (**2005**) NMR dynamics-derived insights into the binding properties of a peptide interacting with an SH2 domain. *Biochemistry*, *44*, 694-703.

[74] Borg, M., Mittag, T., Pawson, T., Tyers, M., Forman-Kay, J.D. and Chan, H.S. (**2007**) Polyelectrostatic interactions of disordered ligands suggest a physical basis for ultrasensitivity. *Proc. Natl. Acad. Sci. U. S. A.*, *104*, 9650-9655.

[75] Bamm, V.V., Ahmed, M.A., Ladizhansky, V. and Harauz, G. (**2007**) Purification and spectroscopic characterization of the recombinant BG21 isoform of murine golli myelin basic protein. *J. Neurosci. Res.*, *85*, 272-284.

[76] Ahmed, M.A., Bamm, V.V., Harauz, G. and Ladizhansky, V. (**2007**) The BG21 isoform of Golli myelin basic protein is intrinsically disordered with a highly flexible amino-terminal domain. *Biochemistry*, *46*, 9700-9712.

[77] Moscarello, M.A., Gagnon, J., Wood, D.D., Anthony, J. and Epand, R. (**1973**) Conformational flexibility of a myelin protein. *Biochemistry*, *12*, 3402-3406.

[78] 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.

[79] Dyer, C.A. (**1997**) 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. 69-74. Plenum Press, New York.

[80] Campagnoni, A.T. and Skoff, R.P. (**2001**) The pathobiology of myelin mutants reveal novel biological functions of the MBP and PLP genes. *Brain Pathol.*, *11*, 74-91.

[81] 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.

[82] Harauz, G., Musse, A.A., Gao, W. and Boggs, J.M. (**2008**) MBP as a "PIP2-modulin: New biological functions for an old CNS protein. *J. Neurochem.*, *104 (Suppl. 1)*, 19.

[83] 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.

[84] Ishiyama, N., Bates, I.R., Hill, C.M.D., Wood, D.D., Matharu, P., Viner, N.J., Moscarello, M.A. and Harauz, G. (**2001**) The effects of deimination of myelin basic protein on structures formed by its interaction with phosphoinositide-containing lipid monolayers. *J. Struct. Biol.*, *136*, 30-45.

[85] Boggs, J.M. and Moscarello, M.A. (**1978**) Effect of basic protein from human central nervous system myelin on lipid bilayer structure. *J. Membr. Biol.*, *39*, 75-96.

[86] Sedzik, J., Blaurock, A.E. and Höchli, M. (**1984**) Lipid/myelin basic protein multilayers: a model for the cytoplasmic space in central nervous system myelin. *J. Mol. Biol.*, *174*, 385-409.

[87] Zhong, L., Bamm, V.V., Ahmed, M.A., Harauz, G. and Ladizhansky, V. (**2007**) Solid-state NMR spectroscopy of 18.5 kDa myelin basic protein reconstituted with lipid vesicles: spectroscopic characterisation and spectral assignments of solvent-exposed protein fragments. *Biochim. Biophys. Acta (Biomembranes)*, *1768*, 3193-3205.

[88] 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.

[89] Bates, I.R., Feix, J.B., Boggs, J.M. and Harauz, G. (**2004**) An immunodominant epitope of myelin basic protein is an amphipathic alpha-helix. *J. Biol. Chem.*, *279*, 5757-5764.

[90] 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.

[91] Feix, J. B. & Klug, C. S. (**1998**) in *Spin Labeling: The Next Millenium*, (Berliner, X., Ed.). pp. 251-281. Plenum, New York.

[92] Fanucci, G.E. and Cafiso, D.S. (**2006**) Recent advances and applications of site-directed spin labeling. *Curr. Opin. Struct. Biol.*, *16*, 644-653.

[93] Biswas, R., Kuhne, H., Brudvig, G.W. and Gopalan, V. (**2001**) Use of EPR spectroscopy to study macromolecular structure and function. *Sci. Prog.*, *84*, 45-67.

[94] Li, X., Romero, P., Rani, M., Dunker, A.K. and Obradovic, Z. (**1999**) Predicting protein disorder for N-, C-, and internal regions. *Genome Inform. Ser. Workshop Genome Inform.*, *10*, 30-40.

[95] Romero, P., Obradovic, Z., Li, X., Garner, E.C., Brown, C.J. and Dunker, A.K. (**2001**) Sequence complexity of disordered protein. *Proteins*, *42*, 38-48.

[96] Gaboriaud, C., Bissery, V., Benchetrit, T. and Mornon, J.P. (**1987**) Hydrophobic cluster analysis: an efficient new way to compare and analyse amino acid sequences. *FEBS Lett.*, *224*, 149-155.

[97] Collins, M.O., Yu, L., Campuzano, I., Grant, S.G. and Choudhary, J.S. (**2008**) Phosphoproteomic analysis of the mouse brain cytosol reveals a predominance of protein phosphorylation in regions of intrinsic sequence disorder. *Mol. Cell Proteomics*, *7*, 1331-1348.

[98] Sospedra, M. and Martin, R. (**2005**) Immunology of multiple sclerosis. *Annu. Rev. Immunol.*, *23*, 683-747.

[99] Farès, C., Libich, D.S. and Harauz, G. (**2006**) Solution NMR structure of an immunodominant epitope of myelin basic protein. Conformational dependence on environment of an intrinsically unstructured protein. *FEBS J.*, *273*, 601-614.

[100] Tselios, T., Probert, L., Kollias, G., Matsoukas, E., Roumelioti, P., Alexopoulos, K., Moore, G.J. and Matsoukas, J. (**1998**) Design and synthesis of small semi-mimetic peptides with immunomodulatory activity based on myelin basic protein (MBP). *Amino Acids*, *14*, 333-341.

[101] Tselios, T., Probert, L., Daliani, I., Matsoukas, E., Troganis, A., Gerothanassis, I.P., Mavromoustakos, T., Moore, G.J. and Matsoukas, J.M. (**1999**) Design and synthesis of a potent cyclic analogue of the myelin basic protein epitope MBP72-85: importance of the Ala81 carboxyl group and of a cyclic

conformation for induction of experimental allergic encephalomyelitis. *J. Med. Chem.*, *42*, 1170-1177.

[102] 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*, 300-311.

[103] Tselios, T., Daliani, I., Deraos, S., Thymianou, S., Matsoukas, E., Troganis, A., Gerothanassis, I., Mouzaki, A., Mavromoustakos, T., Probert, L. and Matsoukas, J. (**2000**) Treatment of experimental allergic encephalomyelitis (EAE) by a rationally designed cyclic analogue of myelin basic protein (MBP) epitope 72-85. *Bioorg. Med. Chem. Lett.*, *10*, 2713-2717.

[104] Tselios, T., Daliani, I., Probert, L., Deraos, S., Matsoukas, E., Roy, S., Pires, J., Moore, G. and Matsoukas, J. (**2000**) Treatment of experimental allergic encephalomyelitis (EAE) induced by guinea pig myelin basic protein epitope 72-85 with a human MBP(87-99) analogue and effects of cyclic peptides. *Bioorg. Med. Chem.*, *8*, 1903-1909.

[105] Tselios, T., Apostolopoulos, V., Daliani, I., Deraos, S., Grdadolnik, S., Mavromoustakos, T., Melachrinou, M., Thymianou, S., Probert, L., Mouzaki, A. and Matsoukas, J. (**2002**) Antagonistic effects of human cyclic MBP(87-99) altered peptide ligands in experimental allergic encephalomyelitis and human T-cell proliferation. *J. Med. Chem.*, *45*, 275-283.

[106] Mouzaki, A., Tselios, T., Papathanassopoulos, P., Matsoukas, I. and Chatzantoni, K. (**2004**) Immunotherapy for multiple sclerosis: basic insights for new clinical strategies. *Curr. Neurovasc. Res.*, *1*, 325-340.

[107] Tzakos, A.G., Fuchs, P., Van Nuland, N.A., Troganis, A., Tselios, T., Deraos, S., Matsoukas, J., Gerothanassis, I.P. and Bonvin, A.M. (**2004**) NMR and molecular dynamics studies of an autoimmune myelin basic protein peptide and its antagonist: structural implications for the MHC II (I-Au)-peptide complex from docking calculations. *Eur. J. Biochem.*, *271*, 3399-3413.

[108] Matsoukas, J., Apostolopoulos, V., Kalbacher, H., Papini, A.M., Tselios, T., Chatzantoni, K., Biagioli, T., Lolli, F., Deraos, S., Papathanassopoulos, P., Troganis, A., Mantzourani, E., Mavromoustakos, T. and Mouzaki, A. (**2005**) Design and synthesis of a novel potent myelin basic protein epitope 87-99 cyclic analogue: enhanced stability and biological properties of mimics render them a potentially new class of immunomodulators. *J. Med. Chem.*, *48*, 1470-1480.

[109] Warren, K.G., Catz, I., Ferenczi, L.Z. and Krantz, M.J. (**2006**) Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment. *Eur. J. Neurol.*, *13*, 887-895.

[110] Darlington, C. (**2007**) MBP-8298, a synthetic peptide analog of myelin basic protein for the treatment of multiple sclerosis. *Curr. Opin. Mol. Ther.*, *9*, 398-402.

[111] Macosko, J.C., Kim, C.H. and Shin, Y.K. (**1997**) The membrane topology of the fusion peptide region of influenza hemagglutinin determined by spin-labeling EPR. *J. Mol. Biol.*, *267*, 1139-1148.

[112] 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.

[113] Fernandez-Vidal, M., Jayasinghe, S., Ladokhin, A.S. and White, S.H. (**2007**) Folding amphipathic helices into membranes: amphiphilicity trumps hydrophobicity. *J. Mol. Biol.*, *370*, 459-470.

[114] Strandberg, E. and Killian, J.A. (**2003**) Snorkeling of lysine side chains in transmembrane helices: how easy can it get? *FEBS Lett.*, *544*, 69-73.

[115] Wishart, D.S., Bigam, C.G., Holm, A., Hodges, R.S. and Sykes, B.D. (**1995**) 1H, 13C and 15N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects. *J. Biomol. NMR*, *5*, 67-81.

[116] Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (**1995**) 1H, 13C and 15N chemical shift referencing in biomolecular NMR. *J. Biomol. NMR*, *6*, 135-140.

[117] Wishart, D.S. and Nip, A.M. (**1998**) Protein chemical shift analysis: a practical guide. *Biochem. Cell Biol.*, *76*, 153-163.

[118] Bates, I.R. and Harauz, G. (**2003**) Molecular dynamics exposes α-helices in myelin basic protein. *J. Mol. Model*, *9*, 290-297.

[119] 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.

[120] 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). *Journal of Protein Chemistry*, *22*, 177-181.

[121] Persaud, R., Fraser, P., Wood, D.D. and Moscarello, M.A. (**1988**) The glycosylation of human myelin basic protein at threonines 95 and 98 occurs sequentially. *Biochim. Biophys. Acta*, *966*, 357-361.

[122] Li, S.S. (**2005**) Specificity and versatility of SH3 and other proline-recognition domains: structural basis and implications for cellular signal transduction. *Biochem. J.*, *390*, 641-653.

[123] Rath, A., Davidson, A.R. and Deber, C.M. (**2005**) The structure of "unstructured" regions in peptides and proteins: role of the polyproline II helix in protein folding and recognition. *Biopolymers*, *80*, 179-185.

[124] Chellgren, B.W. and Creamer, T.P. (**2004**) Short sequences of non-proline residues can adopt the polyproline II helical conformation. *Biochemistry*, *43*, 5864-5869.

[125] Bochicchio, B. and Tamburro, A.M. (**2002**) Polyproline II structure in proteins: identification by chiroptical spectroscopies, stability, and functions. *Chirality*, *14*, 782-792.

[126] Tiffany, M.L. and Krimm, S. (**1968**) Circular dichroism of poly-L-proline in an unordered conformation. *Biopolymers*, *6*, 1767-1770.

[127] Rucker, A.L. and Creamer, T.P. (**2002**) Polyproline II helical structure in protein unfolded states: lysine peptides revisited. *Protein Sci.*, *11*, 980-985.

[128] Ruzza, P., Calderan, A., Guiotto, A., Osler, A. and Borin, G. (**2004**) Tat cell-penetrating peptide has the characteristics of a poly(proline) II helix in aqueous solution and in SDS micelles. *J. Pept. Sci.*, *10*, 423-426.

[129] 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.

[130] Yang, S.T., Jeon, J.H., Kim, Y., Shin, S.Y., Hahm, K.S. and Kim, J.I. (**2006**) Possible role of a PXXP central hinge in the antibacterial activity and membrane interaction of PMAP-23, a member of cathelicidin family. *Biochemistry*, *45*, 1775-1784.

[131] Yang, S.T., Lee, J.Y., Kim, H.J., Eu, Y.J., Shin, S.Y., Hahm, K.S. and Kim, J.I. (**2006**) Contribution of a central proline in model amphipathic alpha-helical peptides to self-association, interaction with phospholipids, and antimicrobial mode of action. *FEBS J.*, *273*, 4040-4054.

[132] Nygaard, E., Mendz, G.L., Moore, W.J. and Martenson, R.E. (**1984**) NMR of a peptic peptide spanning the triprolyl sequence in myelin basic protein. *Biochemistry*, *23*, 4003-4010.

[133] Fraser, P.E. and Deber, C.M. (**1985**) Structure and function of the proline-rich region of myelin basic protein. *Biochemistry*, *24*, 4593-4598.

[134] Dyson, H.J. and Wright, P.E. (**2004**) Unfolded proteins and protein folding studied by NMR. *Chem. Rev.*, *104*, 3607-3622.

[135] Wishart, D.S. and Sykes, B.D. (**1994**) Chemical shifts as a tool for structure determination. *Meth. Enzymol.*, *239*, 363-392.

[136] Schwarzinger, S., Kroon, G.J., Foss, T.R., Chung, J., Wright, P.E. and Dyson, H.J. (**2001**) Sequence-dependent correction of random coil NMR chemical shifts. *J. Am. Chem. Soc.*, *123*, 2970-2978.

[137] Lam, S.L. and Hsu, V.L. (**2003**) NMR identification of left-handed polyproline type II helices. *Biopolymers*, *69*, 270-281.

[138] Karplus, S. and Karplus, M. (**1972**) Nuclear magnetic resonance determination of the angle psi in peptides. *Proc. Natl. Acad. Sci. USA*, *69*, 3204-3206.

[139] Rule, G. S. and Hitchens, T. K. (**2006**) in *Fundamentals of protein NMR spectroscopy,* Springer, Dordrecht, The Netherlands.

[140] Vuister, G.W., Delaglio, F. and Bax, A. (**1993**) The use of 1JC alpha H alpha coupling constants as a probe for protein backbone conformation. *J. Biomol. NMR*, *3*, 67-80.

[141] Vuister, G.W. and Bax, A. (**1992**) Measurement of two-bond JCOH alpha coupling constants in proteins uniformly enriched with 13C. *J. Biomol. NMR*, *2*, 401-405.

[142] 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.

[143] Libich, D.S., Monette, M.M., Robertson, V.J. and Harauz, G. (**2007**) NMR assignment of an intrinsically disordered protein under physiological conditions: the 18.5 kDa isoform of myelin basic protein. *Biomol. NMR Assign.*, *1*, 61-63.

[144] Pawson, T. and Scott, J.D. (**2005**) Protein phosphorylation in signaling - 50 years and counting. *Trends Biochem. Sci.*, *30*, 286-290.

[145] Jones, L.S., Yazzie, B. and Middaugh, C.R. (**2004**) Polyanions and the proteome. *Mol. Cell. Proteomics*, *3*, 746-769.

[146] Woods, A.S. (**2004**) The mighty arginine, the stable quaternary amines, the powerful aromatics, and the aggressive phosphate: their role in the noncovalent minuet. *J. Proteome Res.*, *3*, 478-484.

[147] Jackson, S.N., Wang, H.Y., Yergey, A. and Woods, A.S. (**2006**) Phosphate stabilization of intermolecular interactions. *J. Proteome Res.*, *5*, 122-126.

[148] Salazar, C. and Höfer, T. (**2007**) Versatile regulation of multisite protein phosphorylation by the order of phosphate processing and protein-protein interactions. *FEBS J.*, *274*, 1046-1061.

[149] Serber, Z. and Ferrell, J.E., Jr. (**2007**) Tuning bulk electrostatics to regulate protein function. *Cell*, *128*, 441-444.

[150] Muntane, G., Dalfo, E., Martinez, A. and Ferrer, I. (**2008**) Phosphorylation of tau and alpha-synuclein in synaptic-enriched fractions of the frontal cortex in Alzheimer's disease, and in Parkinson's disease and related alpha-synucleinopathies. *Neuroscience*, *152*, 913-923.

[151] Marta, C.B., Taylor, C.M., Coetzee, T., Kim, T., Winkler, S., Bansal, R. and Pfeiffer, S.E. (**2003**) Antibody cross-linking of myelin oligodendrocyte glycoprotein leads to its rapid repartitioning into detergent-insoluble fractions, and altered protein phosphorylation and cell morphology. *J. Neurosci.*, *23*, 5461-5471.

[152] Marta, C.B., Montano, M.B., Taylor, C.M., Taylor, A.L., Bansal, R. and Pfeiffer, S.E. (**2005**) Signaling cascades activated upon antibody cross-linking of myelin oligodendrocyte glycoprotein: potential implications for multiple sclerosis. *J. Biol. Chem.*, *280*, 8985-8993.

[153] Stariha, R.L. and Kim, S.U. (**2001**) Protein kinase C and mitogen-activated protein kinase signalling in oligodendrocytes. *Microsc. Res. Tech.*, *52*, 680-688.

[154] 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.

[155] Ramwani, J. and Moscarello, M.A. (**1990**) Phosphorylation of charge isomers (components) of human myelin basic protein: identification of phosphorylated sites. *J. Neurochem.*, *55*, 1703-1710.

[156] 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.

[157] Ulmer, J.B. and Braun, P.E. (**1983**) *In vivo* phosphorylation of myelin basic proteins in developing mouse brain: evidence that phosphorylation is an early event in myelin formation. *Dev. Neurosci.*, *6*, 345-355.

[158] 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.

[159] Stariha, R.L., Kikuchi, S., Siow, Y.L., Pelech, S.L., Kim, M. and Kim, S.U. (**1997**) Role of extracellular signal-regulated protein kinases 1 and 2 in oligodendroglial process extension. *J. Neurochem.*, *68*, 945-953.

[160] Campagnoni, A.T. (**1988**) Molecular biology of myelin proteins from the central nervous system. *J. Neurochem.*, *51*, 1-14.

[161] Dyer, C.A. (**2002**) The structure and function of myelin: from inert membrane to perfusion pump. *Neurochem. Res.*, *27*, 1279-1292.

[162] 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.

[163] DeBruin, L.S., Haines, J.D., Bienzle, D. and Harauz, G. (**2006**) Partitioning of myelin basic protein into membrane microdomains in a spontaneously demyelinating mouse model for multiple sclerosis. *Biochem. Cell Biol.*, *84*, 993-1005.

[164] DeBruin, L.S. and Harauz, G. (**2007**) White matter rafting - membrane microdomains in myelin. *Neurochem. Res.*, *32*, 213-228.

[165] Tholey, A., Lindemann, A., Kinzel, V. and Reed, J. (**1999**) Direct effects of phosphorylation on the preferred backbone conformation of peptides: a nuclear magnetic resonance study. *Biophys. J.*, *76*, 76-87.

[166] Andrew, C.D., Warwicker, J., Jones, G.R. and Doig, A.J. (**2002**) Effect of phosphorylation on alpha-helix stability as a function of position. *Biochemistry*, *41*, 1897-1905.

[167] Wulf, G., Finn, G., Suizu, F. and Lu, K.P. (**2005**) Phosphorylation-specific prolyl isomerization: is there an underlying theme? *Nat. Cell Biol.*, *7*, 435-441.

[168] Liou, Y.C., Sun, A., Ryo, A., Zhou, X.Z., Yu, Z.X., Huang, H.K., Uchida, T., Bronson, R., Bing, G., Li, X., Hunter, T. and Lu, K.P. (**2003**) Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature*, *424*, 556-561.

[169] Lu, K.P., Liou, Y.C. and Vincent, I. (**2003**) Proline-directed phosphorylation and isomerization in mitotic regulation and in Alzheimer's Disease. *Bioessays*, *25*, 174-181.

[170] Libich, D.S. and Harauz, G. (**2002**) Interactions of the 18.5 kDa isoform of myelin basic protein with Ca2+-calmodulin: *in vitro* studies using fluorescence microscopy and spectroscopy. *Biochem. Cell Biol.*, *80*, 395-406.

[171] Libich, D.S. and Harauz, G. (**2002**) Interactions of the 18.5 kDa isoform of myelin basic protein with Ca^{2+}-calmodulin - *in vitro* studies using gel shift assays. *Mol. Cell. Biochem.*, *241*, 45-52.

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

[173] 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-kD 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.

[174] Berggard, T., Arrigoni, G., Olsson, O., Fex, M., Linse, S. and James, P. (**2006**) 140 mouse brain proteins identified by Ca^{2+}-calmodulin affinity chromatography and tandem mass spectrometry. *J. Proteome Res.*, *5*, 669-687.

[175] Zhang, Z., Ottens, A.K., Golden, E.C., Hayes, R.L. and Wang, K.K.W. (**2006**) Using calmodulin-affinity capture to study the rat brain calmodulin proteome and its vulnerability to calpain and caspase proteolysis. *Calcium Binding Proteins*, *1*, 125-129.

[176] 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.

[177] Fuxreiter, M., Simon, I., Friedrich, P. and Tompa, P. (**2004**) Preformed structural elements feature in partner recognition by intrinsically unstructured proteins. *J. Mol. Biol.*, *338*, 1015-1026.

[178] Mészáros, B., Tompa, P., Simon, I. and Dosztányi, Z. (**2007**) Molecular principles of the interactions of disordered proteins. *J. Mol. Biol.*, *372*, 549-561.

[179] Porumb, T., Crivici, A., Blackshear, P.J. and Ikura, M. (**1997**) Calcium binding and conformational properties of calmodulin complexed with peptides derived from myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein (MRP). *Eur. Biophys. J.*, *25*, 239-247.

[180] Hoeflich, K.P. and Ikura, M. (**2002**) Calmodulin in action: diversity in target recognition and activation mechanisms. *Cell*, *108*, 739-742.

[181] Permyakov, S.E., Millett, I.S., Doniach, S., Permyakov, E.A. and Uversky, V.N. (**2003**) Natively unfolded C-terminal domain of caldesmon remains substantially unstructured after the effective binding to calmodulin. *Proteins*, *53*, 855-862.

[182] Radivojac, P., Vucetic, S., O'Connor, T.R., Uversky, V.N., Obradovic, Z. and Dunker, A.K. (**2006**) Calmodulin signaling: analysis and prediction of a disorder-dependent molecular recognition. *Proteins*, *63*, 398-410.

[183] Baker, J.M., Hudson, R.P., Kanelis, V., Choy, W.Y., Thibodeau, P.H., Thomas, P.J. and Forman-Kay, J.D. (**2007**) CFTR regulatory region interacts with NBD1 predominantly via multiple transient helices. *Nat. Struct. Mol. Biol.*, *14*, 738-745.

[184] 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.

[185] 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.

[186] Rispoli, P., Carzino, R., Svaldo-Lanero, T., Relini, A., Cavalleri, O., Fasano, A., Liuzzi, G.M., Carlone, G., Riccio, P., Gliozzi, A. and Rolandi, R. (**2007**) A thermodynamic and structural study of myelin basic protein in lipid membrane models. *Biophys. J.*, *93*, 1999-2010.

[187] Rosetti, C.M. and Maggio, B. (**2007**) Protein-induced surface structuring in myelin membrane monolayers. *Biophys. J.*, *93*, 4254-4267.

[188] Hu, Y. and Israelachvili, J. (**2007**) Lateral reorganization of myelin lipid domains by myelin basic protein studied at the air-water interface. *Colloids Surf. B Biointerfaces*, *62*, 22-30.

[189] Franklin, R.J. (**2002**) Why does remyelination fail in multiple sclerosis? *Nat. Rev. Neurosci.*, *3*, 705-714.

[190] Hegedus, T., Serohijos, A.W., Dokholyan, N.V., He, L. and Riordan, J.R. (**2008**) Computational studies reveal phosphorylation-dependent changes in the unstructured R domain of CFTR. *J. Mol. Biol.*, *378*, 1052-1063.

[191] Karthigasan, J., Inouye, H. and Kirschner, D.A. (**1995**) Implications of the sequence similarities between tau and myelin basic protein. *Med. Hypotheses*, *45*, 235-240.

[192] Greer, J.M. and Lees, M.B. (**2002**) Myelin proteolipid protein - the first 50 years. *Int. J. Biochem. Cell Biol.*, *34*, 211-215.

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[193] Duncan, I.D. (**2005**) The PLP mutants from mouse to man. *J. Neurol. Sci.*, *228*, 204-205.

[194] Agnati, L.F., Zunarelli, E., Genedani, S. and Fuxe, K. (**2006**) On the existence of a global molecular network enmeshing the whole central nervous system: physiological and pathological implications. *Curr. Protein Pept. Sci.*, *7*, 3-15.

[195] Uversky, V.N. (**2007**) Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. *J. Neurochem.*, *103*, 17-37.

[196] Cheng, Y., LeGall, T., Oldfield, C.J., Dunker, A.K. and Uversky, V.N. (**2006**) Abundance of intrinsic disorder in protein associated with cardiovascular disease. *Biochemistry*, *45*, 10448-10460.

[197] Musse, A.A. and Harauz, G. (**2007**) Molecular "negativity" may underlie multiple sclerosis: role of the myelin basic protein family in the pathogenesis of MS. *Int. Rev. Neurobiol.*, *79*, 149-172.

[198] Dohm, C.P., Kermer, P. and Bahr, M. (**2008**) Aggregopathy in neurodegenerative diseases: Mechanisms and therapeutic implication. *Neurodegener. Dis.*, *5*, 321-338.

[199] Soto, C. and Estrada, L.D. (**2008**) Protein misfolding and neurodegeneration. *Arch. Neurol.*, *65*, 184-189.

[200] Doyle, H.A. and Mamula, M.J. (**2002**) Posttranslational protein modifications: new flavors in the menu of autoantigens. *Curr. Opin. Rheumatol.*, *14*, 244-249.