Myelin Proteomics: Molecular Anatomy of an Insulating Sheath

Olaf Jahn · Stefan Tenzer · Hauke B. Werner

Abstract Fast-transmitting vertebrate axons are electrically insulated with multiple layers of nonconductive plasma membrane of glial cell origin, termed myelin. The myelin membrane is dominated by lipids, and its protein composition has historically been viewed to be of very low complexity. In this review, we discuss an updated reference compendium of 342 proteins associated with central nervous system myelin that represents a valuable resource for analyzing myelin biogenesis and white matter homeostasis. Cataloging the myelin proteome has been made possible by technical advances in the separation and mass spectrometric detection of proteins, also referred to as proteomics. This led to the identification of a large number of novel myelin-associated proteins, many of which represent low abundant components involved in catalytic activities, the cytoskeleton, vesicular trafficking, or cell adhesion. By mass spectrometry-based quantification, proteolipid protein and myelin basic protein constitute 17% and 8% of total myelin protein, respectively, suggesting that their abundance was previously overestimated. As the biochemical profile of myelin-associated proteins is highly reproducible, differential proteome analyses can be applied to material isolated from patients or animal models of myelin-related diseases such as multiple sclerosis and leukodystrophies.

Keyword Oligodendrocyte · Leukodystrophy · Myelin · Internode · Proteome · Proteomics · Cytoskeleton · Neurodegeneration · Proteolipid protein · Myelin basic protein

Introduction

Neuronal signal propagation in vertebrates is sped up by the electrical insulation of axons with an ensheathing, specialized glial plasma membrane: myelin. Myelination of axons reduces their transverse capacitance and increases their transverse resistance [1]. Insulation is achieved by the multilayered arrangement of the myelin membrane (Fig. 1) and its special molecular composition, mainly its very high lipid content. In myelinated axons, action potentials are restricted to periodically spaced small segments spared from coverage with myelin, termed the nodes of Ranvier [2]. In the central nervous system (CNS), any individual oligodendrocyte myelinates up to 50 axon segments, termed internodes [3]. Oligodendrocyte precursor cell division, migration, and regular alignment along the axons have been recently visualized in vivo in...
zebrafish [4], which today complement rodents as an important model organism for myelin research [5–8]. Myelin formation proceeds with outgrowth and retraction of glial cell processes, target axon recognition, stabilization of cellular contacts, rapid biosynthesis and trafficking of lipid and protein constituents of the myelin membrane, and its organization as a multilayered structure around the axon [9, 10]. Once myelinated, axons become dependent on glial support [11]. Some of the molecules involved in myelin development and function are known but a detailed molecular picture has not been gained yet.

That CNS myelin is important for normal sensation, cognition, and motor function is obvious considering that myelin-related disorders often affect humans lethally.

Besides the inflammatory demyelinating disease multiple sclerosis [12], there are genetically inherited disorders that affect CNS myelin, collectively termed leukodystrophies [13]. This heterogeneous group of diseases is characterized by the loss of motoric, sensory, and mental capabilities and the susceptibility to seizures. A detailed knowledge of the molecular expression profiles of oligodendrocytes and myelin will be crucial to understand the pathomechanisms of white matter diseases. For example, the mRNAs [14–16] and proteins expressed in cultivated oligodendrocytes [17] and oligodendrogial exosomes [18] have been recently examined. This review focuses on systematic analyses of the molecular composition of mammalian CNS myelin, while no such compendium of
peripheral nervous system (PNS) myelin proteins has been published yet. Proteomics approaches to myelin provide a valuable resource to understand its biogenesis, function, and pathology. Although only a few comparative studies have been reported to date, novel insights into the molecular basis of myelin-related diseases are beginning to emerge.

A Myelin-Enriched Fraction from the Central Nervous System

A comparatively simple method is available for the isolation of a myelin-enriched fraction from the CNS. Biochemically, myelin is defined as the lightweight membranous material accumulating at the interface between 0.32 and 0.85 M sucrose after sequential ultracentrifugation combined with osmotic shocks [19, 20]. The most commonly used protocol starts from brain homogenate contained in 0.32 M sucrose as the top layer, “spinning-down” myelin to accumulate at the interface with the bottom 0.85 M sucrose layer. One valuable modification is “floating-up” of myelin starting from brain homogenate contained in a more concentrated sucrose solution as the bottom layer (0.85, 1.2, 1.44, or 2 M). During ultracentrifugation, myelin also accumulates at the interface between the upper 0.85 and 0.32 M sucrose layers, while other fractions of interest assemble at higher sucrose concentrations. This method allows the simultaneous isolation of other brain fractions such as rough microsomes [21] or axogliosomes [22, 23]. The light-weight fraction from the interphase between 0.32 and 0.85 M sucrose is the most frequently used one for biochemical and proteomic experiments. This fraction is enriched in the most abundant proteins of compact myelin, proteolipid protein (PLP), and myelin basic protein (MBP), and as revealed by electron microscopy, mainly contains multimembranous membranes with a periodicity comparable to that of myelin in native or perfused brains [24, 25]. However, we suggest to term this fraction “myelin-enriched” rather than “compact myelin”, as it also contains proteins from the noncompacted cytosolic channels in myelin (i.e., adaxonal and paranodal myelin) and proteins associated with the axonal membrane. Myelin purification is very reproducible across different laboratories, even when applied to different species (e.g., mouse–rat) or to mutant mice with altered myelin protein or lipid composition, such as Cnp<sup>Cre+·Fdt1<sup>fl<sup>ox/flox</sup></sup></sup> [26], Ugt3a1<sup>null</sup> [27], Arsa<sup>null</sup> [28], and Plp<sup>null</sup> [29] (see below). Thus, the method has proven to be very robust, explaining why the original protocol from the early 1970s is still in common use. It is generally assumed that myelin purification relies on its special lipid content and composition.

Myelin Lipids

The molecular composition of myelin differs from other plasma membranes in that it contains 70–75% of its dry weight as lipid, unusually high compared to other eukaryotic plasma membranes. Also, its molar ratio of lipids with approximately 2:2:1:1 for cholesterol/phospholipid/galactolipid/plasmalogen [30, 31] distinguishes myelin from other cellular membranes. The abundance of cholesterol within a membrane affects its biophysical properties, including fluidity and curving [32]. Cholesterol has earlier been identified as unusually enriched in myelin and constitutes 24–28% of the total myelin lipids [19]. That the cellular cholesterol supply is rate-limiting for myelin membrane biogenesis has been shown in mice lacking squalene synthase (also termed farnesyl diphosphate farnesyl transferase [FDFT]) exclusively in myelinating glia [26]. FDFT mediates a crucial step of cholesterol biosynthesis. CNS myelination is severely delayed in Cnp<sup>Cre+·Fdt1<sup>fl<sup>ox/flox</sup></sup></sup> mice, and that any myelin made in these mice is likely due to compensatory cholesterol uptake from other cells [26].

The biophysical properties of myelin are also influenced by its unusually high concentration of the galactolipids galactosylceramide (GalC), its sulfated form 3-O-sulfogalactosylceramide (SGalC), and their hydroxylated forms GalC-OH and SGalC-OH. Together, they add up to 20–26% of total myelin lipids. Myelination is moderately delayed in mice lacking UDP-galactose:ceramide galactosyltransferase (Ugt3a1), an enzyme required for galactolipid synthesis. Additionally, impaired glia–axonal interactions at the paranodes were observed [27, 33, 34]. Paranodal disruption was at least partly due to the lack of SGalC and hydroxylated galactolipids, since the long-term integrity of the sodium channel domain of the nodes of Ranvier was also impaired in mice lacking galactosylceramide-3-O-sulfotransferase (Gal3st1), the enzyme converting GalC into SGalC [35–37], and late onset myelin degeneration was also reported for mice lacking fatty acid 2-hydroxylase (Fa2h), the enzyme hydroxylating GalC and SGalC [38]. Absence of functional arylsulfatase A (ARSA), the enzyme degrading SGalC, causes metachromatic leukodystrophy (MLD), illustrating that a regulated galactolipid metabolism is required for long-term integrity of the white matter. SGalC accumulation and many pathological features of MLD are modeled in Arsa<sup>null</sup> mice and in transgenic mice overexpressing Ugt3a1 or Gal3st1 in neurons or oligodendrocytes [28, 39, 40]. Sulfatide metabolism with respect to myelin and MLD pathology was recently reviewed [41].

Also, the plasmalogen class of phospholipids is associated with white matter disease. Plasmalogens are ether-linked (as opposed to ester-linked) phospholipids, the main species being ethanolamine–plasmalogen. They are ubiquitous structural components of mammalian cell membranes.
and amount to 12–15% of total myelin lipid [19] and, when processed by plasmalogen-selective phospholipase A2, give rise to the second messengers arachidonic acid and eicosanoids [42]. At low concentrations, these metabolites have trophic effects, but at high levels, they are cytotoxic and may induce inflammation [43]. The reactivity of the alkenyl ether bond makes plasmalogens more susceptible to oxidative reactions than their fatty acid ester analogs. Thus, myelin plasmalogens may act as endogenous antioxidants protecting cells from oxidative stress [44]. Disrupted activity of peroxisomal plasmalogen synthesizing enzymes results in peroxisomal biogenesis disorders such as rhizomelic chondrodysplasia punctata (RCDP) in which hypo-myelination of the optic nerve has been observed. Decreased plasmalogen levels [45, 46] and increased levels of reactive oxygen species [47, 48] may also contribute to the demyelination in X-linked adrenoleukodystrophy caused by the mutated peroxisomal transporter ABCD1, suggesting that a normal plasmalogen metabolism may prevent peroxisomal- and myelin-related disease. Mice lacking flotillin-1 and contactin with plasmalogen-deficient brain resulted in peroxisomal- and myelin-related disease. Mice lack- suggesting that a normal plasmalogen metabolism may prevent peroxisomal- and myelin-related disease. Mice lacking dihydroxyacetonephosphate acyltransferase (DAPAT) prevent peroxisomal- and myelin-related disease. Mice lack- suggesting that a normal plasmalogen metabolism may prevent peroxisomal- and myelin-related disease. Mice lack- suggesting that a normal plasmalogen metabolism may prevent peroxisomal- and myelin-related disease. Mice lacking 

**Association of Myelin Lipids and Proteins**

Cholesterol assembles with galactolipids and plasmalogens within the plane of the membrane, but how they are enriched to the levels found in myelin is unknown. It has been suggested that lipids are targeted to future myelin membrane by their association with myelin-bound proteins [9]. SGalC appears to be an example to the contrary. SGalC is associated with myelin and lymphocyte protein (MAL) [50]. Lack of SGalC and lack of MAL lead to similar paranodal malformation [35, 51]. The subcellular trafficking of MAL, as well as its abundance in myelin, is determined by SgalC [28], whereas SGalC abundance is not altered in Malmut myelin [51]. It is likely that other myelin proteins are also incorporated into the sheath by attachment with future myelin membrane because of its special lipid composition. Thus, whether myelin proteins dictate the fate of lipids or vice versa may not be generalized. It appears likely that the association of both molecule classes results in each other’s control of abundance and trafficking.

That myelin lipids and proteins are closely associated was suggested earlier after the characterization of two types of protein fractions isolated from the white matter based on their resistance to aqueous or organic solvents or to enzymatic proteolysis. One fraction behaved as a lipid with regard to its solubility and was termed PLP [52, 53]. PLP was later identified to be the most abundant protein of mammalian CNS myelin. It has a high affinity to phospholipids and cholesterol [54–56], and impaired interactions of mutant PLP with membrane lipids are a likely key step in the molecular pathogenesis of the leukodystrophy Pelizaeus–Merzbacher disease [57]. The other fraction, termed trypsin-resistant protein residue, was insoluble in organic solvent and attached to the membrane lipid phosphatidylinositol phosphate [58, 59]. The application of extraction methods by Folch became commonly used to categorize myelin proteins according to their biophysical properties.

More recently, the myelin-enriched brain fraction has been chemically subfractionated by differential detergent extraction at low temperatures, resulting in distinct non-identical but overlapping assemblies of myelin-associated proteins and lipids that were suggested to represent myelin subcompartments [60, 61]. Cholesterol- and galactolipid-rich membrane microdomains (also referred to as “lipid rafts”) have been suggested to deliver myelin proteins to the plasma membrane [62–64]. The relevance of applying the analysis of biochemical characteristics established for membrane microdomains to such a large structure as myelin has remained debated. However, it is widely accepted now that lipid-associated cell signaling molecules, such as the protein tyrosine kinase fyn, have central roles in myelina- tion [65, 66].

In oligodendroglial processes, fyn is activated by axonal signals via integrin alpha6beta1 [67]. Among other fyn substrates [68, 69], the protein translation repressor heterogeneous nuclear ribonucleoprotein (hnRNP) A2 upon phosphorylation is released from its binding site in the 3’ UTR of mRNA encoding MBP [70], the second-most abundant myelin protein. hnRNP A2 binding represses translation during the translocation of MBP mRNA to distal sites of the cell [71] where newly translated MBP is directly incorporated into the extending oligodendroglial process [21, 72]. It is generally assumed that MBP mediates the adhesion of the cytoplasmic surfaces between the individual layers of compact myelin [73] via binding of its many basic residues with the negatively charged headgroups of membrane lipids. Indeed, membrane association of MBP is controlled by the membrane lipid phosphatidylinositol-(4,5)-bisphosphate [74–76]. For over 30 years, it has been known that MBP is highly heterogeneous due to alternative splicing and multiple post-translational modifications (PTMs) [77]. More recently, modern mass spectrometric techniques have been used to compare the PTMs of MBP from normal and multiple sclerosis brains with respect to
methylations, phosphorylation, and arginine deamination [78]. PTM alterations affect charge, conformation, and hydrogen bonding of MBP, which may modulate its affinity to the myelin membrane and play a role in myelin compaction and in the pathogenesis of demyelinating diseases. MBP is the only myelin protein that has been shown to be essential for myelin formation, as became obvious with the analysis of the natural mouse mutant *shiverer* and the rat mutant *long evans shaker* [79, 80], which are severely hypomyelinated. Interestingly, mice lacking fyn are also hypomyelinated [81, 82], likely due to affected translational regulation of MBP expression [70, 83]. Together, a multitude of factors affects mRNA transcription and transport, translation at axonal contact sites, or membrane binding of MBP, and we speculate that several myelin proteins with yet unidentified roles affect MBP abundance and function.

**Systematic Analysis of the CNS Myelin Protein Composition**

The relative abundance of myelin proteins has previously been calculated based on their binding to Buffalo black [84], Fast green [85], or Coomassie blue [86] after separation in one-dimensional (1D) sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE). In these measurements, a small number of proteins was determined to be extraordinarily abundant in CNS myelin. PLP and its smaller splice isoform DM20 accounted for 30–45% of total myelin protein, two of the four MBP splice isoforms for 22–35%, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) for 4–15%, and all remaining proteins for 5–25% [19, 85, 87, 88]. Similarly, PNS myelin is also dominated by two proteins, myelin protein zero (MPZ, P0) and MBP, which have been estimated to account for 50–70% and 15%, respectively [89]. In comparison, the most abundant proteins in a brain fraction enriched for synaptic vesicles are synaptobrevin 2 and synaptophysin, which constitute 8% and 10% of the total synaptic vesicle proteins, respectively, as revealed by quantitative immunoblotting [90]. How and why myelin proteins are enriched to their unusual relative abundance is unclear, considering that PLP and CNP are not essential for the formation of normal amounts of CNS myelin [29, 91, 92].

Various proteomic techniques have been applied towards the systematic protein composition analysis of the myelin-enriched fraction. Traditionally, first insights into proteomes of subcellular structures often come from two-dimensional (2D) protein maps generated by utilizing isoelectric focusing (IEF) with immobilized pH gradients in the first and SDS-PAGE in the second dimension (2D-IEF/SDS-PAGE) (Fig. 2a). Proteins of interest are then excised from the gel, proteolytically digested *in situ*, and finally, identified by mass spectrometry (MS) [93]. Due to its high resolving power, 2D-IEF/SDS-PAGE can be routinely applied for profiling of proteins from complex mixtures and, as protein integrity is retained, also leads to information on protein abundance and processing [94]. However, major shortcomings of 2D-IEF/SDS-PAGE concern a limited dynamic range, the display of basic and hydrophobic proteins, and—most importantly—the under-representation of membrane proteins. As myelin is dominated by MBP (a highly basic protein) and PLP (a hydrophobic tetraspan protein), incremental improvements in 2D-IEF/SDS-PAGE technology were required before the first 2D mapping of myelin was presented [95]. By using the zwitterionic detergent amidosulfobetaine-14 (ASB-14) instead of the most commonly used 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate (CHAPS) [96], it was possible to solubilize myelin proteins much more effectively and to identify 98 proteins (91 by MS and seven by immunoblotting) in the myelin-enriched fraction from mouse CNS [95]. This crucial effect of the solubilization conditions is further underscored by two more recent 2D-IEF/SDS-PAGE mapping studies of similar input material. Thirty-eight myelin-associated proteins were identified in one study after CHAPS solubilization [97], but 131 proteins were identified in another study with ASB-14 [25]. Thus, at least in the presence of appropriate detergents, myelin can now be considered as well accessible by 2D-IEF/SDS-PAGE, which not only facilitates protein cataloging but also paves the way for differential myelin proteomics on the basis of the 2D differential fluorescence intensity gel electrophoresis technology (2D-DIGE, see below). It is important to note that all conventional 2D mapping approaches mentioned above failed to appropriately display relatively abundant transmembrane myelin marker proteins such as PLP, myelin-associated glycoprotein (MAG) [98], myelin oligodendrocyte glycoprotein (MOG) [99], tetraspanin 2 [100], M6B [101], or oligodendrocyte-specific protein (OSP/claudin-11) [102–104]. A potential remedy is to perform the first dimension separation as nonequilibrium pH gradient electrophoresis for the 2D mapping of myelin proteins [105]. However, although this method appeared promising particularly for displaying highly basic proteins, it did not get as popular as 2D-IEF/SDS-PAGE with immobilized pH gradients, mainly due to limitations in reproducibility and resolution.

More complete proteome coverage while retaining the benefits of displaying intact proteins can be reached by the additional use of alternative 2D gel systems. Here, the charge-dependent separation in the first dimension (i.e., the IEF) is replaced by a size-dependent separation in the presence of cationic detergents such as 16-benzyldimethyl-\(n\)-hexadecylammonium chloride (16-BAC; Fig. 2b) [106]
or cetyltrimethylammonium bromide (CTAB; Fig. 2c) [107]. Due to the similar separation principle in both dimensions, proteins are typically dispersed along a diagonal rather than distributed over the entire gel area. Accordingly, these gel systems have a lower resolution compared to 2D-IEF/SDS-PAGE, but can resolve highly basic and even membrane-spanning proteins [108]. Application of 2D-16-BAC/SDS-PAGE to mouse CNS myelin resulted in the identification of 62 proteins and readily enabled displaying of the transmembrane myelin proteins PLP, MAG, MOG, and OSP/claudin-11 [25]. Thus, the combination of 2D-IEF/SDS-PAGE and 2D-16-BAC/SDS-PAGE has, so far, yielded the most comprehensive gel-based proteome compendium of mouse CNS myelin, consisting of 162 nonredundant proteins [25]. Further technical refinements of the method were established in a recent systematic evaluation of five different cationic detergents for the 2D gel electrophoresis of myelin proteins. Here, 16-BAC was the most effective agent for the separation of myelin proteins in the first dimension, while CTAB was most effective for their solubilization [109, 110]. As resolution improves, 2D gel electrophoresis with cationic detergents may be combined with the DIGE technology as a future tool for monitoring abundance changes of highly basic and membrane-spanning myelin proteins [111].

To overcome the limitations of gel-based proteomic methods, in particular those of 2D-IEF/SDS-PAGE, gel-free techniques, commonly referred to as shotgun approaches, have emerged in recent years [93, 112]. Here, separation at the level of intact proteins is omitted and the protein preparation is proteolytically digested at the expense of information related to protein integrity, such as protein size and charge. Separation takes place at the level of proteolytic

![Fig. 2 Gel-based myelin proteome maps. Purified mouse brain myelin was two-dimensionally separated in different gel systems. Proteins were visualized by colloidal Coomassie staining, and spots constituted by selected myelin proteins are indicated. a 2D-IEF/SDS-PAGE with IEF in a nonlinear pH gradient (pH 3–10) as the first and gradient SDS-PAGE (8–16% acrylamide) as the second dimension. To improve resolution, myelin was delipidated and precipitated by a methanol/chloroform treatment prior to IEF [25]. b 2D-16-BAC/SDS-PAGE with separation in a 16-BAC gel (10% acrylamide) as the first and gradient SDS-PAGE (8–16% acrylamide) as the second dimension. c 2D-CTAB/SDS-PAGE with separation in a CTAB gel (10% acrylamide) as the first and gradient SDS-PAGE (8–16% acrylamide) as the second dimension. To deplete soluble and membrane-associated proteins, myelin was subjected to a multistep wash procedure before separation [25]. 16-BAC and CTAB resulted in similar spot patterns. 2D-IEF/SDS-PAGE provides good resolution but basic, hydrophobic, and transmembrane proteins are under-represented. 2D-16-BAC/SDS-PAGE and 2D-CTAB/SDS-PAGE lead to efficient representation of basic, hydrophobic, and transmembrane proteins but have a lower resolution since separation occurs by protein size in both dimensions.]

![Image of gel-based myelin proteome maps.](image-url)
peptides before interfacing with MS. The tremendous complexity of such peptide mixtures requires a high resolving power and is, therefore, often addressed by the application of 2D liquid chromatography (2D-LC), usually consisting of strong cation exchange in the first and reversed-phase chromatography in the second dimension. In the first application of shotgun proteomics to the myelin-enriched fraction from the mouse CNS [97], 93 proteins were identified resulting—by combination with 2D-IEF/SDS-PAGE (see above)—in a myelin proteome compendium consisting of 103 proteins. The application of a similar shotgun approach to a myelin-enriched fraction from rat CNS led to the identification of 97 myelin proteins [23]. Both shotgun approaches yielded quite a high overlap of approximately 50% with the so far most comprehensive gel-based library [25] and contained transmembrane myelin proteins such as PLP, MAG, and MOG.

Relative Abundance of Myelin Proteins

To understand myelin biogenesis and pathology, a comprehensive knowledge of the proteins associated with myelin is a prerequisite. We have confirmed and expanded the previous myelin protein compendia by applying nanoscale 1D ultra performance liquid chromatography (1D-UP-LC) separation coupled to detection with a quadrupole time-of-flight (QTOF) mass spectrometer (Tenzer et al., unpublished). Data were acquired by LC-MS using an alternating low (MS) and elevated (MSE) collision energy mode of acquisition (LC-MS\textsuperscript{E}), which allows simultaneous identification and label-free relative quantification of the proteins in the sample [113–115]. The identified peptides were annotated to a total of 294 myelin-associated proteins (Table 1) based on a minimum of two peptides per protein with an effective false-positive rate of <0.2%. They showed a very good overlap of 141 proteins that were also detected in previous myelin proteome analyses and included several established myelin markers (Table 1 and Fig. 3). We have calculated the relative abundance of the myelin-associated proteins based on the average intensity of the three most abundant peptides per protein. In the few cases where only two peptides were identified, their average intensity was used. Strikingly, PLP, MBP, and CNP constituted only 17%, 8%, and 4% of the total myelin-associated proteins, respectively (Fig. 4). All previously known myelin proteins together constituted 35%, while newly identified myelin-associated proteins accounted for 65%. These quantifications take into question previous estimates based on conventional techniques (Fig. 4b and see above). We suggest that the complexity of myelin protein composition has been overlooked because low abundant proteins did not constitute significant bands on gels when compared to the highly abundant PLP and MBP due to limitations concerning gel separation and/or protein staining.

We conclude that modern LC-MS-based approaches—though technically more demanding than gel-based studies—appear to be appropriate for tackling the myelin proteome as they cover several orders of magnitude of protein abundance and detect highly basic, hydrophobic, and membrane-spanning proteins. This tackles the bias towards certain protein classes, which is the major shortcoming particularly of 2D-IEF/SDS-PAGE (Fig. 4c). Moreover, LC-MS-based approaches enable the gel- and label-free quantification of proteins from complex mixtures, which allowed for the systematic reassignment of protein abundance in CNS myelin (see above). Finally, they require only low amounts of sample, which is of special relevance for the proteome analysis of myelin purified from hypomyelinated model animals or human brain autopsy material.

Technical Limitations

How pure is the myelin-enriched fraction? Myelin-associated proteins are defined as proteins in the myelin-enriched fraction since all studies have operationally defined the term “myelin protein” without systematic experimental verification. Although the identification of new myelin proteins by more than one study and the detection of established myelin markers increase confidence, some of these proteins may only have copurified with myelin. The high dynamic range of LC-MS\textsuperscript{E} leads to the new identification of proteins as myelin-associated, but also to the false-positive identification of contaminants. These mainly stem from copurifying mitochondria and synaptic vesicles. In reverse, proteomic compendia of mammalian brain mitochondria [116] or synaptic vesicles [90] include classical myelin proteins such as PLP, MBP, MOBP, and MAG. Notwithstanding that some of these proteins may have a dual localization, cross-contamination occurs likely due to similar floatation properties in sucrose or Percoll gradients and can only be excluded once improved separation protocols become available. Proteins of the axonal plasma membrane, such as potassium channels or Na\textsuperscript{+}K\textsuperscript{+}-ATPases, have also been detected in the myelin fraction, which can be explained by the tight linkage of the membranes via adhesion proteins, sometimes referred to as the myelin–axolemma complex [24]. Indeed, some adhesion complexes are present in the myelin-enriched fraction, such as the glial neurofascin (NF155) and contactin and their axonal partner contactin associated protein 1 (Caspr) [117–120] and the glial necl-like protein Necl4 and its axonal counterpart Necl1 [121–124]. Importantly, myelin proteome analysis also revealed novel candidate proteins to mediate intracellular or intercellular
### Table 1 The CNS myelin proteome

| Protein name | ID     | Gene | Reference |
|--------------|--------|------|-----------|
| **A: Known myelin proteins** |        |      |           |
| CD81         | P35762 | Cd81 | E         |
| CD9          | P40240 | Cd9  | ND        |
| Claudin 11, OSP | Q60771 | Cldn11 | B,S,T,E  |
| CNP          | P16330 | Cnp  | W,B,S,R,TE|
| Contactin 1  | P12960 | Cntn1| B,S,R,TE  |
| Ermin        | Q5EBJ4 | Ermn | E         |
| Ezrin        | P26040 | Ezr  | W,T,E     |
| Glycoprotein M6B | P35803 | Gpm6b | E         |
| Myelin and lymphocyte protein | O09198 | Mal  | ND, T (6x4) |
| Myelin-associated glycoprotein | P20917 | Mag  | B,S,R,E   |
| Myelin basic protein | P04370 | Mbp  | W,B,S,R,E |
| Myelin oligodendrocyte glycoprotein | Q61885 | Mog  | B,S,R,TE  |
| Myelin protein zero, P0 | P27573 | Mpz  | R         |
| Myelin proteolipid protein | P60202 | Plp1 | B,S,R,TE  |
| Myelin/oligodendrocyte basic protein | Q9D2P8 | M0bp | E         |
| Nectin, Ig superfamily member 4b | Q99288 | Cadr3 | S         |
| Nectin, Ig superfamily member 4c | Q8R464 | Cadr4 | S,E       |
| Neural cell adhesion molecule 1 | P13595 | Ncam1 | W,S,R,TE  |
| Neurofascin | Q810U3 | Nfasc| B,R,E     |
| Oligodendrocyte myelin glycoprotein | Q63912 | Omg  | ND        |
| Opalin, TMP10 | Q7M750 | Opalin | R,E      |
| Plasminogen | Q9DCU2 | Pllp | E         |
| Ras-related protein Rab 3A | P63011 | Rab3a | E         |
| Ras-related protein Rab 3C | P62823 | Rab3c | E         |
| Sirtuin 2    | Q9VDQ8 | Sirt2| W,S,V,R,TE|
| Tetraspanin 2 | Q92236 | Tspan2 | E        |
| **B: Newly identified myelin-associated proteins** |        |      |           |
| 14-3-3 protein beta | Q9CQV8 | Ywhab | E         |
| 14-3-3 protein epsilon | P62259 | Ywhae | S,R,E     |
| 14-3-3 protein eta | P68510 | Ywhah | E         |
| 14-3-3 protein gamma | P61982 | Ywhag | W,V,R,TE  |
| 14-3-3 protein sigma, stratifin | O70456 | Sfn  | E         |
| 14-3-3 protein theta | P68254 | Ywhaq | E         |
| Actin α cardiac muscle 1 | P68033 | Actc1 | E         |
| Actin α1     | P61318 | Acta1| E         |
| Actin α      | P62737 | Acta2| R,E       |
| Actin β      | P60710 | Actb | W,S,V,R,TE|
| Actin γ1     | P63260 | Actg1| B,E       |
| Actin γ2     | P63268 | Actg2| E         |
| Acyl-CoA thioesterase 7 | Q911V12 | Acot7 | R,E       |
| ADAM 23      | Q9R1V7 | Adam23| E         |
| Adenylate cyclase associated 1 | P40124 | Cap1 | T         |
| ADP ribosylation factor 1 | P84078 | Arfl  | S,T,E     |
| ADP ribosylation factor 2 | Q8BSL7 | Arfl2 | E         |
| ADP ribosylation factor 3 | P61205 | Arfl3 | E         |

### Table 1 (continued)

| Protein name | ID     | Gene | Reference |
|--------------|--------|------|-----------|
| ADP ribosylation factor 4 | P61750 | Arf4  | E         |
| ADP ribosylation factor 5 | P84084 | Arf5  | E         |
| ADP ribosylation factor 6 | P62331 | Arf6  | E         |
| Aldehyde dehydrogenase 1A1 | P23549 | Aldh1a1 | E         |
| Aldolase A, fructose-bisphosphate | P05064 | Aldoa | W,S,V,R,TE|
| Aldolase C, fructose bisphosphate | P05063 | Aldoc | R,T,E     |
| Amphilphysin 2, bridging integrator 1 | O08539 | Bin1  | E         |
| Anillin      | Q8K289 | Anln | R,E       |
| Annexin A2   | P07356 | Anxa2| E         |
| Annexin A6   | P14824 | Anxa6| R,T       |
| Argininosuccinate synthase 1 | P16460 | Ass1  | B,E       |
| α-Synuclein  | O55042 | Snc1 | E         |
| Band 4.1 like protein 3 | Q9WYV9 | Epba3 | E         |
| Brain acid soluble protein 1, NAP22 | Q9J1V3 | Basp1 | E         |
| Breast carcinoma amplified seq 1 | Q80YN3 | Bcas1 | E         |
| β-Synuclein  | Q9IZZ3 | Sncb | E         |
| Ca++ ATPase 1 | Q3TSK3 | Atp1  | B         |
| Ca++ ATPase 2 | Q9R0K7 | Atp2  | B          |
| Ca++ ATPase 3 | Q9VF55 | Atp3  | B         |
| Ca++ ATPase 4 | Q60476 | Atp4  | B         |
| Calmodulin CaM | P62204 | Calm3 | S,V,E     |
| Calnexin     | P35564 | Canx | B,R       |
| Calpain 5    | O08688 | Capn5| T         |
| Calpain 1/2  | P11798 | Camk2a| E         |
| Calpain 2    | P28652 | Camk2b| E         |
| Calpain 6    | Q6PH2 | Camk2d| E         |
| CaM kinase IIγ | Q92379 | Camk2g| E         |
| Cannabinoid receptor interacting 1 | Q5MN0 | Cnrip1| E         |
| Carbonic anhydrase 2 | P09020 | Car2 | W,S,T,E  |
| CD47, integrin signal transducer | Q61735 | Cd47 | E         |
| CD82         | P40237 | Cds2 | E         |
| CDGSH iron sulfur domain 1 | Q91WS0 | Csd1 | E         |
| Cell cycle exit and neuronal diff. | Q9JKC6 | Cnd1 | E         |
| Cell division control protein 42 | P60766 | Cdc42| E         |
| Centracin α  | P61164 | Actr1a| W         |
| Choline transporter CD92 | Q6X893 | Slc44a1| E         |
| Clathrin heavy chain | Q68F5D | Ctlc | B,R,E     |
| Cofilin 1    | P18760 | Cofil1| S,V,T,E   |
| Cofilin 2    | P45591 | Cofil2| E         |
| Contactin associated protein 1 | O54991 | Cntnap1| E         |
| Corin 1C     | Q9WUM4 | Cor1c| E         |
| Creatine kinase brain | Q04447 | Cknb | W,S,V,TE  |
| Crystallin α2 | P23927 | Cryab| W,S,T,E   |
| Cyclophilin A | P17742 | Ppia | W,S,V,E   |
| Cysteine and glycine rich protein 1 | P97315 | Crsp1| E         |
| Cytokeratin 1 | P04104 | Krt1  | E         |
| Cytokeratin 1B | Q6FZ6 | Krt77| E         |
| Protein name | ID     | Gene   | Reference |
|--------------|--------|--------|-----------|
| Cytokeratin 5| Q922U2 | Krt5   | E         |
| Cytokeratin 6A| P50446 | Krt6a  | E         |
| Cytokeratin 6G| Q9R0H5 | Krt7l  | E         |
| Cytokeratin 10| P02535 | Krt10  | R,E       |
| Cytokeratin 16| Q9Z2K1 | Krt16  | E         |
| Desmin      | P31001 | Des    | E         |
| Destrin     | Q9R0P5 | Dstn   | E         |
| Dihydropyrimidinase-like 1, CRMP1| P97427 | Crmp1 | E         |
| Dihydropyrimidinase-like 2, CRMP2| O08553 | Dpysl2 | W,B,S,R,T,E |
| Dihydropyrimidinase-like 3, CRMP4| Q62188 | Dpysl3 | E         |
| Dihydropyrimidinase-like 4, CRMP3| O35098 | Dpysl4 | E         |
| Dipeptidylpeptidase 6| Q9Z218 | Dpp6  | T         |
| Down syndrome cell adhesion like 1| Q8R4B4 | Dscaml1 | E         |
| Dynactin 2  | Q99KJ8 | Dctn2  | V         |
| Dynamin 1   | P39053 | Dnm1   | W,B,R,E   |
| Dynamin 2   | P39054 | Dnm2   | E         |
| Dynamin 3   | Q8BZ98 | Dnm3   | R         |
| Dynein heavy chain| Q9JH4 | Dynch1 | R         |
| Ectonucleotide pyrophosphatase 6| Q8BGN3 | Enpp6 | E         |
| EH domain containing protein 1| Q9WWK4 | Ehd1  | B,S,R,E   |
| EH domain containing protein 3| Q9QXY6 | Ehd3  | B,E       |
| EH domain containing protein 4| Q9EQP2 | Ehd4  | E         |
| Elongation factor 1α1| P10126 | Eef1a1 | W,B,S,R,E |
| Elongation factor 1α2| P62631 | Eef1a2 | W,B,R,E   |
| Elongation factor 1β| O70251 | Eef1b2 | T         |
| Elongation factor 2| P58252 | Eef2   | T         |
| Endonuclease domain containing 1| Q8C522 | Endod1 | E         |
| Enolase 1, non-neuronal| P17182 | Enol1  | W,B,S,R,E |
| Enolase 2, neuronal| P17183 | Enol2  | W,B,S,R,E |
| Enolase 3, muscle| P21550 | Enol3  | E         |
| Fascin      | Q61553 | Fscn1  | W,E       |
| Fatty acid synthase| P19096 | Fasn   | R         |
| FK506 binding protein 1a| P26883 | Fkbp1a | S,E       |
| Flotillin 1  | O08917 | Flot1  | ND, T (640)|
| G protein α transducing 1| P20612 | Gnat1  | E         |
| G protein α transducing 2| P50149 | Gnat2  | E         |
| G protein α transducing 3| Q3V312 | Gnat3  | E         |
| G protein α11| P21278 | Gna1   | E         |
| G protein α14| P30677 | Gna14  | E         |
| G protein α12| B2RSH2 | Gna12  | E         |
| G protein α12| P08752 | Gna12  | E         |
| G protein α13| Q9DC51 | Gna13  | E         |
| G protein αO1| P18872 | Gna1   | S,T,E     |
| G protein αO2| P18873 | Gna0   | B,T,E     |
| G protein αq| P21279 | Gnaq   | T,E       |

| Protein name | ID     | Gene   | Reference |
|--------------|--------|--------|-----------|
| G protein αS| P63094 | Gnas   | S,E       |
| G protein αS olfactory| P8CGK7 | Gna1   | E         |
| G protein β1| P62874 | Gnb1   | W,S,R,E   |
| G protein β2| P62880 | Gnb2   | W,V,R,E   |
| G protein β3| Q61011 | Gnb3   | E         |
| G protein β4| P29387 | Gnb4   | W,E       |
| G protein β5| P62881 | Gnb5   | W         |
| G protein γ12| Q9DAS9 | Gng12  | E         |
| GAPDH       | P16858 | Gapdh  | W,S,R,E   |
| GAPDH sperm| Q64467 | Gapdhs | E         |
| Gelsoin     | P13020 | Gsn    | V,R,T     |
| G protein α11| P21279 | Gna11  | E         |
| G protein α14| P30677 | Gna14  | E         |
| G protein αI| P21278 | Gna1   | E         |
| G protein α2| B2RSH2 | Gna2   | E         |
| G protein α3| Q9DC51 | Gna3   | E         |
| G protein αO1| P18872 | Gna1   | S,T,E     |
| G protein αO2| P18873 | Gna0   | B,T,E     |
| G protein αq| P21279 | Gnaq   | T,E       |

**Table 1 (continued)**

| Protein name | ID     | Gene   | Reference |
|--------------|--------|--------|-----------|
| G protein αS| P63094 | Gnas   | S,E       |
| G protein αS olfactory| P8CGK7 | Gna1   | E         |
| G protein β1| P62874 | Gnb1   | W,S,R,E   |
| G protein β2| P62880 | Gnb2   | W,V,R,E   |
| G protein β3| Q61011 | Gnb3   | E         |
| G protein β4| P29387 | Gnb4   | W,E       |
| G protein β5| P62881 | Gnb5   | W         |
| G protein γ12| Q9DAS9 | Gng12  | E         |
| GAPDH       | P16858 | Gapdh  | W,S,R,E   |
| GAPDH sperm| Q64467 | Gapdhs | E         |
| Gelsoin     | P13020 | Gsn    | V,R,T     |
| G protein α11| P21279 | Gna11  | E         |
| G protein α14| P30677 | Gna14  | E         |
| G protein αI| P21278 | Gna1   | E         |
| G protein α2| B2RSH2 | Gna2   | E         |
| G protein α3| Q9DC51 | Gna3   | E         |
| G protein αO1| P18872 | Gna1   | S,T,E     |
| G protein αO2| P18873 | Gna0   | B,T,E     |
| G protein αq| P21279 | Gnaq   | T,E       |
| Protein name | ID | Gene | Reference |
|--------------|----|------|-----------|
| Lactate dehydrogenase A | P06151 | Ldha | T,E |
| Lactate dehydrogenase B | P16125 | Ldhb | W,T,E |
| Lactate dehydrogenase C | P00342 | Ldhe | E |
| Leucine rich repeat containing 57 | Q9D1G5 | Lrrc57 | E |
| Leucine rich repeat LGI 3 | Q8K406 | Lgi3 | E |
| Limbic system associated membrane | Q8BLK3 | Lsamp | S,E |
| Lymphocyte antigen 6H | Q9WUC3 | Ly6h | E |
| Macrophage migration inhibitory factor | P34884 | Mif | W,S,E |
| Malate dehydrogenase | P14152 | Mdh1 | W,S,V,T,E |
| MARCKS related protein | P28667 | Marcksl1 | S,E |
| Microtubule associated protein 1B | P35278 | Mtap1b | E |
| Microtubule associated protein 1B | P14873 | Mtap6 | E |
| Microtubule associated protein tau | P14974 | Ptau | E |
| Mitogen activated protein kinase 1 | P35278 | Pdk | E |
| Moezin | P26041 | Msn | W,E |
| Munc 18, syntaxin binding protein 1 | O08599 | Sxp1 | B,R,T,E |
| N-myristoylated and summed fatty acid 
phosphatidylethanolamine binding 1 | P46460 | Nsf | WBR,T,E |
| Neurocalcin δ | Q91X97 | Ncald | S |
| Neurofilament H | P19246 | Nefh | W,B,E |
| Neurofilament L | P08551 | Nefl | WBR,T,E |
| Neurofilament M | P08553 | Nefm | B,R,E |
| Neurologin 1 | Q99K10 | Nlgn1 | T |
| Neurotomin | Q99P30 | Nnt | E |
| N-mycol downstream regulated | Q62433 | Ndr1 | WSN,T,E |
| Nucleoside diphosphate kinase A | P15532 | Nme1 | W,S,T,E |
| Nucleoside diphosphate kinase B | Q01768 | Nme2 | W,S,T,E |
| Parkinson disease protein 7 | Q99LX0 | Park7 | E |
| Peroxiredoxin 1 | P35700 | Prdx1 | WOR,T,E |
| Peroxiredoxin 2 | Q61171 | Prdx2 | W,V,E |
| Peroxiredoxin 3 | P99029 | Prdx3 | S,E |
| Phosphatidylethanolamine binding 1 | P70296 | Pep1 | W,V,E |
| Phosphatidylinositol transfer | P53810 | Pitpa | W |
| Phosphofructokinase 1 | P47857 | Pfkm | E |
| Phosphoglycerate dehydrogenase | Q61753 | Phgdh | W |
| Phosphoglycerate kinase 1 | P09411 | Pgtk1 | S,V,T,E |
| Phosphoglycerate kinase 2 | P09041 | Pgtk2 | E |
| Phosphoglycerate mutase 1 | Q9DBJ1 | Pgam1 | W,S,T,E |

| Protein name | ID | Gene | Reference |
|--------------|----|------|-----------|
| Phospholipase Cβ1 | Q9Z1B3 | Pkcb1 | W,T,E |
| Phosphoserine aminotransferase | Q9K95X | Psat1 | R |
| Prion protein | P04925 | Prnp | E |
| Prion protein duberl | Q9QY79 | Prmd | E |
| Programmed cell death 6 interacting | Q9WU78 | Pdcd6ip | W |
| Prohibitin | P67778 | Phb | W,R,E |
| Prohibitin 2 | O35129 | Phb2 | E |
| Protein arginine deiminase 2 | Q8O624 | Padi2 | E |
| Protein disulfide isomerase A3 | P27773 | Pdia3 | W,T,E |
| Protein kinase Cy | P63318 | Prkce | E |
| Pyruvate kinase isozyme M2 | P52480 | Pkm2 | WSN,T,E |
| Quinoid dihydropteridine reductase | Q8BV14 | Qdpr | E |
| Rab 1A | P62821 | Rab1 | E |
| Rab 1B | Q9D1G1 | Rab1b | E |
| Rab 2A | P53994 | Rab2a | R,E |
| Rab 2B | P59279 | Rab2b | E |
| Rab 3B | Q9CZT8 | Rab3b | E |
| Rab 3D | P35276 | Rab3d | E |
| Rab 4A | P56371 | Rab4a | E |
| Rab 4B | Q91ZK1 | Rab4b | E |
| Rab 5C | P35278 | Rab5c | E |
| Rab 7A | P51150 | Rab7 | R |
| Rab 8A | P55258 | Rab8a | E |
| Rab 8B | P61028 | Rab8b | E |
| Rab 10 | P61027 | Rab10 | S,E |
| Rab 12 | P35283 | Rab12 | E |
| Rab 13 | Q9DD03 | Rab13 | E |
| Rab 14 | Q91V41 | Rab14 | E |
| Rab 15 | Q8K368 | Rab15 | E |
| Rab 18 | P35293 | Rab18 | E |
| Rab 26 | Q504M8 | Rab26 | E |
| Rab 30 | Q92359 | Rab30 | E |
| Rab 35 | Q6PHN9 | Rab35 | E |
| Rab 37 | Q9JKM7 | Rab37 | E |
| Rab 39B | Q8BH3C | Rab39B | E |
| Rab 43 | Q8CG50 | Rab43 | E |
| Rab GDP dissociation inhibitor α | P50396 | Gdi1 | WSR,T,E |
| Rab GDP dissociation inhibitor β | Q61598 | Gdi2 | W,T,E |
| Rac1 | P63001 | Rac1 | S,R,E |
| Rac2 | Q51424 | Rac2 | E |
| Rac3 | P60764 | Rac3 | E |
| Radixin | P26043 | Rdx | W,E |
| Ras-related protein Ral A | P63321 | Rala | B,E |
| Ras-related protein Ral B | Q91JW9 | Rab | E |
| Ras-related protein Rap 1A | P62835 | Rap1a | WSR,T,E |
| Ras-related protein Rap 1B | Q99J16 | Rap1b | E |
| Ras-related protein Rap 2a | Q80ZJ1 | Rap2a | E |
| Reticulon 3 | Q9ES97 | Rtn3 | E |
adhesion, such as the immunoglobulin domain superfamily protein Igsf8, also termed EWI-2 [23]. Igsf8 is associated with the myelin tetraspanins CD9 and CD81 and regulates integrin function, at least in vitro [125, 126], but its function in vivo remains to be shown. The experimental validation or falsification of newly identified myelin-associated proteins will be a matter of the systematic application of histological techniques, provided that reliable antibodies are available.

How many proteins can be considered true myelin proteins? Though proteomic compendia aim at completeness, the number can only be guessed at this time. As the dynamic range of current MS-based protein identification schemes is in the range of three to five orders of magnitude, detection of infrequent proteins remains a challenge. Additionally, some technical impediments remain. The myelin proteins CD9 [127, 128], oligodendrocyte myelin

| Protein name                          | ID   | Gene    | Reference     |
|---------------------------------------|------|---------|---------------|
| Rho GDP dissociation inhibitor 1      | Q99PT1 | Arhgdia | VT            |
| RhoA                                  | Q9QU0 | Rhoa    | E             |
| RhoB                                  | P62746 | Rhob    | T.E           |
| RhoC                                  | Q62159 | Rhoc    | E             |
| RhoG                                  | P84096 | Rhog    | E             |
| S-100β                                | P50114 | S100b   | R             |
| Septin 2                              | P42208 | Sept2   | W,B,S,TE      |
| Septin 4                              | P28661 | Sept4   | W.E           |
| Septin 7                              | O55131 | Sept7   | W,B,S,R,TE    |
| Septin 8                              | Q8CHH9 | Sept8   | W,R,S,V,TE    |
| Septin 11                             | Q8C1B7  | Sept11  | E             |
| Sideroflexin 3                        | Q91V61 | Sfxn3   | E             |
| Soluble NSF attachment protein α      | Q9DB05 | Napa    | W             |
| Soluble NSF attachment protein β      | P28663 | Napb    | W.E           |
| Soluble NSF attachment protein γ      | Q9CWZ7 | Napg    | W             |
| Spectrin α2                           | P16546 | Spna2   | B,T,E         |
| Spectrin β2                           | Q62266 | Spnb2   | R.E           |
| Stress induced phosphoprotein 1       | Q60864 | Stip1   | W,T           |
| Superoxide dismutase                  | P08228 | Sod1    | W,S           |
| Synapsin 1                            | O88935 | Syn1    | W,E           |
| Synapsin 2                            | Q64332 | Syn2    | W.E           |
| Synaptic vesicle membrane protein     | Q62465 | Vat1    | R,T           |
| Synaptobrevin 2                       | P63044 | Vamp2   | E             |
| Synaptobrevin 3                       | P63024 | Vamp3   | E             |
| Synaptophysin                         | Q62277 | Syp     | E             |
| Synaptosomal associated protein 23    | O09044 | Snap23  | E             |
| Synaptosomal associated protein 25    | P60879 | Snap25  | W,S,R,E       |
| Synaptotagmin 1                       | P46096 | Syt1    | E             |
| Synaptotagmin 5                       | Q9R0N5  | Syt5    | E             |
| Syndapin 1                            | Q61644 | Pasc1   | W,E           |
| Syntaxin 1A                           | O35525 | Stx1a   | E             |
| Syntaxin 1B                           | P61264 | Stx1b   | S,R,E         |
| T-complex 1α                          | P11983 | Tcpl1   | W             |
| T-complex 1β                          | P80314 | Cct2    | W             |
| T-complex 1δ                          | P80315 | Cct4    | R             |
| T-complex 1ε                          | P80316 | Cct5    | W             |
| T-complex 1γ                          | P80318 | Cct3    | W             |
| Thy 1 membrane glycoprotein           | P01831 | Thy1    | W,S,R,E       |
| Transgelin 3                          | Q9R1Q8  | Tagln3  | E             |
| Transitional ER ATPase                | Q01853 | Vcp     | W,T,E         |
| Transketolase                         | P40142 | Tkt     | W,B,S,TE      |
| Triosephosphate isomerase             | P17751 | Tpi1    | S,E           |
| Tubulin α1A                           | P68369 | Tub1a   | W,B,R,E       |
| Tubulin α1B                           | P05213 | Tub1b   | W,S,N,TE      |
| Tubulin α1C                           | P68373 | Tub1c   | E             |
| Tubulin α3A                           | P05214 | Tub3a   | E             |
| Tubulin α4A                           | P68368 | Tub4a   | E             |

Proteins identified in purified CNS myelin by MS

*ID* Swissprot or Trembl accession, *Gene* official NCBI Entrez gene name, *Reference* and method of detection, *T* 2D-IEF/SDS-PAGE or immunoblotting [95], *V* 2D-IEF/SDS-PAGE [97], *W* 2D-IEF/SDS-PAGE [25], *B* 2D-16-BAC/SDS-PAGE [25], *R* shotgun [23], *S* shotgun [97], *E* LC-MS² (Tenzer et al., unpublished), *ND* not detected by MS

adhesion, such as the immunoglobulin domain superfamily protein Igsf8, also termed EWI-2 [23]. Igsf8 is associated with the myelin tetraspanins CD9 and CD81 and regulates integrin function, at least in vitro [125, 126], but its function in vivo remains to be shown. The experimental validation or falsification of newly identified myelin-associated proteins will be a matter of the systematic application of histological techniques, provided that reliable antibodies are available.

How many proteins can be considered true myelin proteins? Though proteomic compendia aim at completeness, the number can only be guessed at this time. As the dynamic range of current MS-based protein identification schemes is in the range of three to five orders of magnitude, detection of infrequent proteins remains a challenge. Additionally, some technical impediments remain. The myelin proteins CD9 [127, 128], oligodendrocyte myelin

| Protein name                          | ID   | Gene    | Reference     |
|---------------------------------------|------|---------|---------------|
| Tubulin α8                            | Q9JJZ2 | Tub8a   | E             |
| Tubulin β2A                           | Q7TM9  | Tub2a   | T.E           |
| Tubulin β2B                           | Q9CWF2  | Tub2b   | E             |
| Tubulin β2C                           | P68372 | Tub2c   | W,R,S,E       |
| Tubulin β3                            | Q9ERP7  | Tub3    | E             |
| Tubulin β4                            | Q9DF69  | Tub4    | W,R,S,V,R,E   |
| Tubulin β5                            | P99024 | Tub5    | E             |
| Tubulin β6                            | Q9224F  | Tub6    | R,E           |
| Tubulin polymerization promoting      | Q7TQD2  | Tppp    | W,E           |
| Tubulin polymerization promoting 3    | Q9CRB6  | Tppp3   | S,E           |
| Ubiquitin                             | P62991 | Ub      | W,S,E         |
| Ubiquitin activating enzyme E1         | Q02053 | Uba1    | T             |
| Ubiquitin C-terminal hydrolase L1      | Q9R0P9  | Uch1    | W,T,E         |
| Vacular ATP synthase A                | P50516 | Atp6v1a | W,E           |
| Vacular ATP synthase B, brain         | P62814 | Atp6v1b | W,E           |
| Vacular ATP synthase C                | Q9ZLG3  | Atp6v1c | T.E           |
| Vacular ATP synthase E1               | P50518 | Atp6v1e | T.E           |
| Vimentin                              | P20152 | Vim     | E             |
| Visinin like protein 1                | P62761 | Vsn1    | S,R,E         |
| Visinin like protein 3                | P62748 | Hpcal1  | S             |
| WD repeat protein 1                    | O88342 | Wdr1    | W             |
glycoprotein [22, 129], and MAL [51] have not yet been detected by proteomic approaches, and the appearance of MAL in one catalog [95] is due to the additional use of immunoblotting. Its nondetectability illustrates the limitations of proteome analysis. MAL is a very hydrophobic protein with four transmembrane domains and very small cytoplasmic and extracellular domains and is, therefore, hardly accessible by MS-based identification. Apart from the membrane-spanning peptides not visible in proteomic approaches, complete tryptic digest of MAL results in only four theoretically detectable peptides: one of 120 amino acids (which is too long for identification by MS), two of two amino acids each (too short to provide useful sequence information), and one of 29 amino acids, which is, in principle, appropriate for identification. However, to obtain a reasonable level of confidence for protein identification, the detection of two peptides per protein is usually set as a prerequisite in the algorithms. This suggests that all proteome approaches requiring protease cleavage have an inherent bias against very small polypeptides or proteins with an unusual cleavage site pattern. In future experiments, the lack of suitable trypsin cleavage sites may be circumvented by the use of endopeptidases with different specificities (e.g., GluC or AspN), although they create proteolytic peptides lacking a basic C-terminal amino acid and are difficult to sequence [130]. This suggests that the detection of more myelin-associated proteins is not just a matter of higher resolving power but also of other technical refinements.

Newly Identified Myelin-Associated Proteins

The compendium of proteins identified in the myelin-enriched brain fraction represents a valuable reference for myelin research. The proteins are candidates for performing important functions in myelin biogenesis and integrity, molecular interactions between myelinating glia and neighboring cells, and white matter homeostasis. By gene ontology terms (http://david.abcc.ncifcrf.gov), many myelin-associated proteins are implicated in catalytic activities (48%), the cytoskeleton (20%), protein transport (21%), vesicular trafficking (6.8%), cell adhesion (6.3%), phospholipid binding (4.2%), or glycolysis/gluconeogenesis (5.1%). Among the recently identified myelin proteins, some were first and others subsequently detected using proteomic approaches. They include proteins of quite various anticipated functions, such as the NAD+-dependent deacetylase sirtuin 2 (SIRT2, see below), cytoskeletal proteins of the septin family [23, 25, 131], and ermin [132], regulators of intracellular vesicle transport in the secretory pathway, such as cdc42 and Rac1 [133], Rab3A, and other Rab-GTPases [134, 135], the paranodal transmembrane glycoprotein Opalin/TMEM10 with a suggested signaling or adhesive function [136–138], the nucleoside diphosphate kinases NM23A and NM23B [95], and a protein particularly abundant in the CNS myelin of teleost fish, the 36K protein, also termed short-chain dehydrogenase/reductase (SDR family) member 12 (DHRS12) [139]. Some of these are quite abundant myelin proteins as judged both by the spots
constituted on 2D gels and LC-based quantification, and the challenge to establish their functions in vivo promises a deepened understanding of myelin. Besides, novel myelin proteins are candidates to cause (when mutated), enhance, or ameliorate white matter disease, such as leukodystrophies.

**Differential Myelin Proteome Analysis in Myelin-Related Disease**

The proteomic comparison of myelin from human patients or animal models with that of respective controls is a powerful approach towards the identification of secondary molecular changes that may contribute to the pathogenesis of myelin-related disease. Such a differential approach has first been applied to myelin purified from PLP\textsuperscript{null} mice [25], which provide a genuine model for spastic paraplegia (SPG-2) in humans, a comparatively mild variant of the leukodystrophy Pelizaeus–Merzbacher Disease with progressive axonal degeneration in the presence of normal amounts of CNS myelin [29, 140]. In that study, 2D-DIGE [141] was used to screen for candidate proteins that could be involved in the oligodendroglial failure to support the long-term integrity of myelinated axons. Three distinct proteins of the cytoskeletal

**Fig. 4** Relative abundance of myelin proteins. a The abundance of known myelin proteins was determined by LC-MS\textsuperscript{5}. Note that known myelin proteins constitute less than 50% of the total myelin protein. Mitochondrial proteins were not considered. b Comparison of myelin protein abundance as quantified by LC-MS\textsuperscript{5} with previous estimates based on band intensity after 1D-PAGE and various protein staining techniques [19, 85, 87, 88]. Note that the abundance of PLP and MBP was previously overestimated because low abundant proteins did not constitute significant bands due to limitations in the resolving power of the 1D gels and in the dynamic range of protein staining.

b Relative abundance of myelin proteins (%)

| Protein | Literature | LC-MS\textsuperscript{5} |
|---------|------------|-------------------------|
| PLP     | 30-45      | 17                      |
| MBP     | 22-35      | 8                       |
| CNP     | 4-15       | 4                       |
| MOG     | ND         | 1                       |
| MAG     | 1.4        | 1                       |
| OSP     | ND         | 1                       |
| Others  | 5-25       | 67                      |

500
200
100
50
20
10
5
3
4
5
6
7
8

Relative abundance of myelin proteins (%)

| Protein | Literature | LC-MS\textsuperscript{5} |
|---------|------------|-------------------------|
| PLP     | 30-45      | 17                      |
| MBP     | 22-35      | 8                       |
| CNP     | 4-15       | 4                       |
| MOG     | ND         | 1                       |
| MAG     | 1.4        | 1                       |
| OSP     | ND         | 1                       |
| Others  | 5-25       | 67                      |

Relative abundance of myelin proteins.

345678

Relative abundance of myelin proteins (%).

c Simulated 2D map of myelin-associated proteins identified by LC-MS\textsuperscript{5}. Proteins are indicated as dots at their molecular weight and isoelectric point as predicted from the amino acid sequence. The size of each dot reflects the relative abundance as determined by LC-MS\textsuperscript{5}. Myelin-associated proteins without transmembrane domains are shown in blue and transmembrane proteins in green, the latter being usually under-represented or absent from conventional 2D gels. Mitochondrial proteins are shown in gray. The red frame indicates the portion of proteins that can be reproducibly displayed by 2D-IEF/SDS-PAGE (see Fig. 2a).
protein Par5 and bind to the tight junction-associated Par3 threonine kinase AKT [149], which is a central signaling dendrocytes to dynamically react to NAD+ level changes in investigation. We speculate that the competence of oligo-myelinating glia will be an important topic of future protein kinases, and tight junctions indeed interact in To determine whether SIRT2, 14-3-3 proteins, Par-proteins, to myelination, at least by Schwann cells in the PNS [155]. 14-3-3 proteins have C. elegans homologs of the myelin has not yet been investigated. 14-3-3 proteins are implicated in membrane protein transport, exocytosis [151], and stress response [152], but their function in partitioning-defective polarity protein Par5 and bind to the tight junction-associated Par3 [153, 154], which is required for establishing polarity prior to myelination, at least by Schwann cells in the PNS [155]. To determine whether SIRT2, 14-3-3 proteins, Par-proteins, protein kinases, and tight junctions indeed interact in myelinating glia will be an important topic of future investigation. We speculate that the competence of oligodendrocytes to dynamically react to NAD+ level changes in white matter tracts is required for their role in maintaining long-term axonal integrity. With the objective to identify novel therapeutic targets for the treatment of multiple sclerosis, a systematic proteomic profiling of tissue samples from three brain lesions affected to various degrees (acute plaque, chronic active plaque, and chronic plaque) has recently been performed [156]. Material from the respective lesion type was collected by laser-capture microdissection and extracted proteins were separated by 1D gel electrophoresis followed by mass spectrometric protein identification. Unexpectedly, five coagulation proteins, including tissue factor and protein C inhibitor, were only present in chronic active plaque characterized by concomitant inflammation and degeneration, a finding that provided new insights in the relationship between the coagulation cascade and inflammation. Most importantly, administration of inhibitors to tissue factor (i.e., hirudin) and protein C inhibitor (i.e., activated protein C [aPC]) indeed ameliorated the disease phenotype in experimental autoimmune encephalomyelitis, a model of multiple sclerosis. The anti-inflammatory treatment with engineered aPC variants may develop into an alternative route to a therapy of multiple sclerosis. Together, differential proteome analysis has identified secondary molecular changes that contribute to understanding the pathogenesis of myelin-related disease and support the design of rational treatment strategies.

Acknowledgements We thank S. Wichert, W. Möbius, J. Patzig, I. Ionescu, and K.-A. Nave for the discussions. ST is supported by the Deutsche Forschungsgemeinschaft (SFB 490 Z3) and the Forschungszentrum Immunologie (FZI) at the University of Mainz, and HW is supported by the BMBF (DLR-Leukonet).

References

1. Hartline DK, Colman DR (2007) Rapid conduction and the evolution of giant axons and myelinated fibers. Curr Biol 17:R29–R35
2. Poliak S, Peles E (2003) The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 4:968–980
3. Hildebrand C, Remahi S, Persson H, Bjartmar C (1993) Myelinated nerve fibres in the CNS. Prog Neurobiol 40:319–384
4. Kirby BB, Takada N, Latimer AJ, Shin J, Carney TJ, Kelsh RN, Appel B (2006) In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. Nat Neurosci 9:1506–1511
5. Brosamle C, Halpern ME (2002) Characterization of myelination in the developing zebrafish. Glia 39:47–57
6. Pogoda HM, Sternheim N, Lyons DA, Diamond B, Hawkins TA, Woods IG, Bhatt DH, Franzini-Armstrong C, Dominguez C, Arana N, Jacobs J, Nix R, Fetto JR, Talbot WS (2006) A generic screen identifies genes essential for development of myelinated axons in zebrafish. Dev Biol 298:118–131
7. Schweitzer J, Becker T, Schachner M, Nave KA, Werner H (2006) Evolution of myelin proteolipid proteins: gene duplication in teleosts and expression pattern divergence. Mol Cell Neurosci 31:161–177
8. Avila RL, Tevlin BR, Lees JP, Inouye H, Kirschner DA (2007) Myelin structure and composition in zebrafish. Neurochem Res 32:197–209
9. Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. Nat Rev Neurosci 6:683–690
10. Simons M, Trotter J (2007) Wrapping it up: the cell biology of myelination. Curr Opin Neurobiol 17:533–540
11. Nave KA, Trapp BD (2008) Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31:535–561
12. Lassmann H, Lucchinetti CF (2008) Cortical demyelination in CNS inflammatory demyelinating diseases. Neurology 70:332–333
13. Boespflug-Tanguy O, Labauge P, Fogli A, Vaurs-Barriere C (2008) Genes involved in leukodystrophies: a glance at glial functions. Curr Neurol Neurosci Rep 8:217–229
14. Dugas JC, Tai YC, Speed TP, Ngai J, Barres BA (2006) Functional genomic analysis of oligodendrocyte differentiation. J Neurosci 26:10967–10983
15. Nielsen JA, Marie D, Lau P, Barker JL, Hudson LD (2006) Identification of a novel oligodendrocyte cell adhesion protein using gene expression profiling. J Neurosci 26:9881–9891
16. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28:264–278
17. Dumont D, Noben JP, Moreels M, Vanderlocht J, Hellings N, Vandenabeele F, Lamberchts I, Stinissen P, Robben J (2007) Characterization of mature rat oligodendrocytes: a proteomic approach. J Neurochem 102:562–576
18. Krämer-Albers E-M, Bretz N, Tenzer S, Winterstein C, Möbius W, Berger H, Nave K-A, Schild H, Trotter J (2007) Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: trophic support for axons? Proteomics Clin Appl 1:1446–1461
19. Norton WT, Poduslo SE (1973) Myelination in rat brain: method of myelin isolation. J Neurochem 21:749–757
20. Larocca JN, Norton WT (2007) Isolation of myelin. Curr Protoc Cell Biol Chapter 3:Unit3.25
21. Colman DR, Kreibich G, Frey AB, Sabatini DD (1982) Synthesis and incorporation of myelin polypeptides into CNS myelin. J Cell Biol 95:598–608
22. Huang JK, Phillips GR, Roth AD, Pedraza L, Shan W, Belkaid W, Mi S, Fex-Svenningsen A, Florens L, Yates JR 3rd, Colman DR (2005) Gliarial membranes at the node of Ranvier prevent neurite outgrowth. Science 310:1813–1817
23. Roth AD, Ivanova A, Colman DR (2006) New observations on the compact myelin proteome. Neuron Glia Biol 2:15–21
24. Menon K, Rasband MN, Taylor CM, Brophy P, Bansal R, Pfeiffer SE (2003) The myelin-axo-膜ment complex: biochemical dissection and the role of galactosphingolipids. J Neurochem 87:995–1009
25. Werner HB, Kuhlmann K, Shen S, Uecker M, Schardt A, Dimova K, Orfanitou F, Dhaunachak A, Brinkmann BG, Mobius W, Guarente L, Casaccia-Bonnefil P, Jahn O, Nave KA (2007) Proteolipid protein is required for transport of sirtuin 2 into CNS myelin. J Cell Biol 177:1717–1730
26. Saher G, Brugger B, Lappe-Siefke C, Mobius W, Tozawa R, Wehr MC, Wieland F, Ishibashi S, Nave KA (2005) High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8:468–475
27. Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, Popko B (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell 86:209–219
28. Saravanan K, Scharen-Wiemers N, Klein D, Sandhoff R, Schwarz A, Yaghoobtian A, Gieselmann V, Franken S (2004) Specific downregulation and mislocalization of the lipid raft-associated protein MAL in a glycolipid storage disorder. Neurobiol Dis 16:396–406
29. Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths JR, Nave KA (1997) Assembly of CNS myelin in the absence of proteolipid protein. Neuron 18:59–70
30. Norton WT, Poduslo SE (1973) Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 21:759–773
31. Morell P, Jurevics H (1996) Origin of cholesterol in myelin. Neurochem Res 21:463–470
32. Huttner WB, Zimmerberg J (2001) Implications of lipid microdomains for membrane curvature, budding and fission. Curr Opin Cell Biol 13:478–484
33. Bosio A, Binczek E, Haupt WF, Stoffel W (1998) Composition and biophysical properties of myelin lipid define the neurological defects in galactocerebroside- and sulfatide-deficient mice. J Neurochem 70:308–315
34. Dupree JL, Coetzee T, Blight A, Suzuki K, Popko B (1998) Myelin galactolipids are essential for proper node of Ranvier formation in the CNS. J Neurosci 18:1642–1649
35. Honke K, Hirahara Y, Dupree J, Suzuki K, Popko B, Fukushima K, Fujikuma K, Nagasawa T, Yoshida N, Wada Y, Taniguchi N (2002) Paranodal junction formation and spematogenesis require sulfoglycolipids. Proc Natl Acad Sci U S A 99:4227–4232
36. Ishibashi T, Dupree JL, Ikenaka K, Hirahara Y, Honke K, Peles E, Popko B, Suzuki K, Nishino H, Baba H (2002) A myelin galactolipid, sulfatide, is essential for maintenance of ion channels on myelinated axon but not essential for initial cluster formation. J Neurosci 22:6507–6514
37. Hirahara Y, Bansal R, Honke K, Ikenaka K, Wada Y (2004) Sulfatide is a negative regulator of oligodendrocyte differentiation: development in sulfatide-null mice. Glia 45:269–277
38. Zoller I, Meixner M, Hartmann D, Bussow H, Meyer R, Gieselmann V, Eckhardt M (2008) Absence of 2-hydroxylated sphingolipids is compatible with normal neural development but causes late-onset axon and myelin sheath degeneration. J Neurosci 28:9741–9754
39. Eckhardt M, Hedayati KK, Pittsch J, Lüllmann-Rauch R, Beck H, Fewou SN, Gieselmann V (2007) Sulfate storage in neurons causes hyperexcitability and axonal degeneration in a mouse model of metachromatic leukodystrophy. J Neurosci 27:9009–9021
40. Ramakrishnan H, Hedayati KK, Lüllmann-Rauch R, Wessig C, Fewou SN, Maier H, Goebel HH, Gieselmann V, Eckhardt M (2007) Increasing sulfatide synthesis in myelin-forming cells of arysulfatase A-deficient mice causes demyelination and neurological symptoms reminiscent of human metachromatic leukodystrophy. J Neurosci 27:9482–9490
41. Eckhardt M (2008) The role and metabolism of sulfatide in the nervous system. Mol Neurobiol 37:93–103
42. Farooqui AA, Horrocks LA (2006) Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12:245–260
43. Kassmann CM, Nave KA (2008) Oligodendroglial impact on axonal function and survival—a hypothesis. Curr Opin Neurobiol 18:235–241
44. Brossche T, Platt D (1998) The biological significance of plasmalogens in defense against oxidative damage. Exp Gerontol 33:363–369
45. Brites P, Mooyer PA, El Mrabet L, Waterham HR, Wanders RJ (2008) Plasmalogens participate in very-long-chain fatty acid-induced pathology. Brain 132:482–492
46. Khan M, Singh J, Singh I (2008) Plasmalogen deficiency in cerebral adrenoleukodystrophy and its modulation by lovastatin. J Neurochem 106:1766–1779
47. Fourcade S, Lopez-Erauskin J, Galino J, Duval C, Naudi A, Jove M, Kemp S, Villarroya F, Ferrer I, Pampolona R, Portero-Otin M, Pujol A (2008) Early oxidative damage underlying neurodegeneration in X-adrenoleukodystrophy. Hum Mol Genet 17:1649–1657
48. Hein S, Schonfeld P, Kahler S, Reiser G (2008) Toxic effects of X-linked adrenoleukodystrophy-associated, very long chain fatty acids on glial cells and neurons from rat hippocampus in culture. Hum Mol Genet 17:1649–1657
49. Rodemer C, Thai TP, Brugger B, Kaercher T, Werner H, Nave KA, Wieland F, Gorgas K, Just WW (2003) Inactivation of ether lipid biosynthesis causes male infertility, defects in eye development and optic nerve hypoplasia in mice. Hum Mol Genet 12:1881–1895
122. Maurel P, Einheber S, Galinska J, Thaker P, Lam I, Rubin MB, Scherer SS, Murakami Y, Gutmann DH, Salzer JL (2007) Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination. J Cell Biol 178:861–874

123. Spiegel I, Adamsky K, Eshel Y, Milo R, Sabanay H, Sarig- Nadir O, Horresh I, Scherer SS, Rasband MN, Peles E (2007) A central role for Nectin4 (SynCAM4) in Schwann cell-axon interaction and myelination. Nat Neurosci 10:861–869

124. Park J, Liu B, Chen T, Li H, Hu X, Gao J, Zhu Y, Zhu Q, Qiang B, Yuan J, Peng X, Qiu M (2008) Disruption of Nectin-like 1 cell adhesion molecule leads to delayed axonal myelination in the CNS. J Neurosci 28:12815–12819

125. Stipp CS, Kolesnikova TV, Hemler ME (2001) EWI-2 is a major CD9 and CD81 partner and member of the novel Ig protein subfamily. J Biol Chem 276:40545–40554

126. Stipp CS, Kolesnikova TV, Hemler ME (2003) EWI-2 regulates alpha3beta1 integrin-dependent cell functions on laminin-5. J Cell Biol 163:1167–1177

127. Terada N, Baracksky K, Kinter M, Melrose S, Steen H, Mann M (2004) The ABC

128. Ishibashi T, Ding L, Ikenaka K, Inoue Y, Miyado K, Mekada E, Terada N, Baracskay K, Kinter M, Melrose S, Brophy PJ, Stipp CS, Kolesnikova TV, Hemler ME (2003) EWI-2 regulates cell morphology. J Neurosci 26:757

129. Ji B, Case LC, Liu K, Shao Z, Lee X, Yang Z, Wang J, Tian T, Terada N, Baracskay K, Kinter M, Melrose S, Brophy PJ, Stipp CS, Kolesnikova TV, Hemler ME (2001) EWI-2 is a major CD9 and CD81 partner and member of the novel Ig protein subfamily. J Biol Chem 276:40545–40554

130. Steen H, Mann M (2004) The ABC’s (and XYZ’s) of peptide sequencing. Nat Rev Mol Cell Biol 5:258–267

131. Buser AM, Erme B, Werner HB, Nave KA, Schraen-Wiemers N (2008) The septin cytoskeleton in myelinating glia. Mol Cell Neurosci 40:156–166

132. Brockschneider D, Sabanay H, Riethmacher D, Peles E (2006) Ermin, a myelinating oligodendrocyte-specific protein that regulates cell morphology. J Neurosci 26:757–762

133. Thurnherr T, Benninger Y, Wu X, Chrostek A, Krause SM, Nave KA, Franklin RJ, Brakebusch C, Suter U, Relvas JB (2006) Cdc42 and Rac1 signaling are both required for and act synergistically in the correct formation of myelin sheaths in the CNS. J Neurosci 26:10110–10119

134. Anitei M, Cowan AE, Pfeiffer SE, Bansal R (2008) Role for Rab3a in oligodendrocyte morphological differentiation. J Neurosci Res 87:342–352

135. Schardt A, Brinkmann BG, Mitkovski M, Sereda MW, Werner HB, Nave K-A (2009) The SNARE protein SNAP-29 interacts with the GTPase Rab3A: implications for membrane trafficking in myelinating glia. J Neurosci Res doi:10.1002/jnr.22005

136. Golan N, Adamsky K, Kartvelishvily E, Brockschneider D, Mobius W, Spiegel I, Roth AD, Thomson CE, Rechavi G, Peles E (2008) Identification of Tmem10/Opalin as an oligodendrocyte enriched gene using expression profiling combined with genetic cell ablation. Glia 56:1176–1186

137. Kippert A, Trajkovic K, Fitzner D, Optiz L, Simons M (2008) Identification of Tmem10/Opalin as a novel marker for oligodendrocytes using gene expression profiling. BMC Neurosci 9:40

138. Yoshikawa F, Sato Y, Tohyama K, Akagi T, Hashikawa T, Nagakura-Takagi Y, Sekine Y, Morita N, Baba H, Suzuki Y, Sugano S, Sato A, Furuchi T (2008) Opalin, a transmembrane sialylglycoprotein located in the central nervous system myelin paranodal loop membrane. J Biol Chem 283:20830–20840

139. Morris JK, Willard BB, Yin X, Jeserich G, Kinter M, Trapp BD (2004) The 36K protein of zebrafish CNS myelin is a short-chain dehydrogenase. Glia 45:378–391

140. Garbern JY (2007) Pelizaeus–Merzbacher disease: genetic and cellular pathogenesis. Cell Mol Life Sci 64:50–65

141. Swatton JE, Prabakaran S, Karp NA, Lillie KS, Bahn S (2004) Protein profiling of human postmortem brain using 2-dimensional fluorescence difference gel electrophoresis (2-D DIGE). Mol Psychiatry 9:128–143

142. Southwood CM, Peppi M, Dryden S, Tainsky MA, Gow A (2007) Microtubule decetylasates, Sirt2 and HDAC6, in the nervous system. Neurochem Res 32:187–195

143. Li W, Zhang B, Tang J, Cao Q, Wu Y, Wu C, Guo J, Ling EA, Liang F (2007) Sirtuin 2, a mammalian homolog of yeast silent information regulator-2 longevity regulator, is an oligodendrogial protein that decelerates cell differentiation through deacetylating alpha-tubulin. J Neurosci 27:2606–2616

144. Rasband MN, Taylor J, Kaga Y, Yang Y, Lappe-Siefke C, Nave KA, Bansal R (2005) CNP is required for maintenance of axon-glia interactions at nodes of Ranvier in the CNS. Glia 50:86–90

145. Bitulco M, Laezza C, Stingo S, Wolff J (2002) 2',3'-Cyclic nucleotide 3'-phosphodiesterase: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin. Proc Natl Acad Sci U S A 99:1807–1812

146. Lee J, Gravel M, Zhang R, Thibault P, Braun PE (2005) Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein. J Cell Biol 170:661–673

147. Polevoda B, Sherman F (2002) The diversity of acetylated proteins. Genome Biol 3(5):reviews0006

148. Shimazu T, Horinouchi S, Yoshida M (2007) Multiple histone decetylasates and the CREB-binding protein regulate pre-mRNA 3'-end processing. J Biol Chem 282:4470–4478

149. Jin YH, Kim YJ, Kim DW, Baek KH, Kang BY, Yeo CY, Lee KY (2008) Sirt2 interacts with 14-3-3 beta/gamma and downregulates the activity of p53. Biochem Biophys Res Commun 368:690–695

150. Flores AI, Narayanan SP, Morse EN, Shick HE, Kidd G, Avila RL, Kirschner DA, Macklin WB (2008) Constitutively active Akt induces enhanced myelination in the CNS. J Neurosci 28:7174–7183

151. Mrowiec T, Schwappach B (2006) 14-3-3 proteins in membrane protein transport. Biol Chem 387:1227–1236

152. Berdichevsky A, Guarente L (2006) A stress response pathway involving sirtuins, forkheads and 14-3-3 proteins. Cell Cycle 5:2588–2591

153. Hurd TW, Fan S, Liu CJ, Kweon HK, Hakansson K, Margolis B (2003) Phosphorylation-dependent binding of 14-3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia. Curr Biol 13:2082–2090

154. Hurd TW, Gao L, Roh MH, Macara IG, Margolis B (2003) Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. Nat Cell Biol 5:137–142

155. Chan JR, Jolicoeur C, Yamauchi J, Elliott J, Fawcett JP, Ng BK, Cayouette M (2006) The polarity protein Par-3 directly interacts with p75NTR to regulate myelination. Science 314:832–836

156. Han MH, Hwang SI, Roy DB, Lundgren DH, Price JV, Osman SS, Fernald GH, Gerlitz B, Robinson WH, Baranzini SE, Grinnell BW, Raine CS, Sobel RA, Han DK, Steinman L (2008) Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 451:1076–1081