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Rafts in oligodendrocytes: evidence and structure-function relationship

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LIST OF ABBREVIATIONS

Caspr1	contactin-associated protein, also known as paranodin
CGT	ceramide galactosyl transferase
CHAPS	3-[(3-chloramidopropyl)-dimethylammonio]-1-propane-sulfonate
CNP	2',3'-cyclic nucleotide 3'-phosphodiesterase
CNS	central nervous system
Cx32	connexin-32
DRM	detergent-resistant membrane
EAE	experimental allergic encephalitis
F3	contactin
FIIM	fluorescence intensity imaging microscopy
GalC	galactosylceramide
GPI-AP	glycosylphosphatidylinositol-anchored protein
GSL	glycosphingolipid
mAb	monoclonal antibody
MAG	myelin-associated glycoprotein
MAL	myelin and lymphocyte protein
MβCD	methyl-β-cyclodextrin
MBP	myelin basic protein
MDCK	Madin-Darby canine kidney
MOBP	myelin-associated oligodendrocytic basic protein
MOG	myelin/oligodendrocyte glycoprotein
MS	multiple sclerosis
NCAM 120	neuronal cell adhesion molecule of 120 kDa
NF155	155 kDa isoform of neurofascin
NgR	Nogo receptor
OLG	oligodendrocyte
OMgp	oligodendrocyte myelin glycoprotein
OPC	oligodendrocyte progenitor cell
OSP	oligodendrocyte-specific protein/claudin-11
PDGF	platelet-derived growth factor
PLP	proteolipid protein
Siglec	sialic acid-dependent immunoglobulin-like family member lectin
SL	sphingolipid
Src	Rous sarcoma
TX-100	Triton X-100, t-octylphenoxypolyethoxyethanol

ABSTRACT

The plasma membrane of eukaryotic cells exhibits lateral inhomogeneities, mainly containing cholesterol and sphingomyelin, which provide liquid-ordered microdomains (lipid 'rafts') that segregate membrane components. Rafts are thought to modulate the biological functions of molecules that become associated with them, and as such, they appear to be involved in a variety of processes, including signal transduction, membrane sorting, cell adhesion and pathogen entry. Although still a matter of ongoing debate, evidence in favor of the presence of these microdomains is gradually accumulating but a consensus on issues like their size, lifetime, composition and biological significance has yet to be reached. Here, we provide an overview of the evidence supporting the presence of rafts in oligodendrocytes, the myelin-producing cells of the central nervous system, and discuss their functional significance. The myelin membrane differs fundamentally from the plasma membrane, both in lipid and protein composition. Moreover, since myelin membranes are unusually enriched in glycosphingolipids, questions concerning the biogenesis and functional relevance of microdomains thus appear of special interest in oligodendrocytes. The current picture of rafts in oligodendrocytes is mainly based on detergent methods. The robustness of such data is discussed and alternative methods that may provide complementary data are indicated.

INTRODUCTION

Oligodendrocytes (OLGs) are the myelin-producing cells of the central nervous system (CNS). They originate from mitotic and migratory precursors which go through discrete stages of maturation, i.e. from a pre-progenitor (precursor) cell, to a bipolar, migratory cell (O-2A, also called OLG progenitor cell (OPC)), a sulfatide-positive pre-OLG, an immature galactosylceramide (GalC)-positive OLG and finally to the mature, post-mitotic myelin-producing OLG (Figure 1) (Baumann and Pham-Dinh, 2001; Pfeiffer et al., 1993). The sequential steps in the maturation process of OLGs are characterized by the differential expression of developmental markers, which are recognized by specific monoclonal antibodies (mAbs) (Hardy and Reynolds, 1993).

Mature, myelin-producing OLGs extend a complex array of thin processes (Figure 2), which project outward from the cell body. Each process forms a segment of a highly specialized membrane that wraps around an axon, i.e. the myelin sheath. Architecturally, the myelin sheath is a complex membrane structure. While the cytoplasmic compartment is continuous from the OLG cell body to the myelin sheath, distinct membrane domains can be discerned which differ dramatically in lipid and protein composition (Figure 3). In the compact myelin region the cytoplasm has been virtually extruded. However, in the non-compact region (cytoplasmic incisures and abaxonal, periaxonal and paranodal loops) cytoplasm is still present. The protein fraction of purified myelin consists mainly (60 to 80%) of proteolipid protein (PLP), its isoform DM20, and myelin basic protein (MBP) (Table 1). The primary function of these proteins is to stabilise the apposed myelin membranes in compact myelin (Table 2). Myelin and lymphocyte protein (MAL) and myelin-associated oligodendrocytic basic protein (MOBP) are also localized in compact myelin. The non-compact regions

of myelin contain 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), myelin/oligodendrocyte glycoprotein (MOG), the 155 kDa isoform of neurofascin (NF155), oligodendrocyte myelin glycoprotein (OMgp) and connexin-32 (Cx32) (Baumann and Pham-Dinh, 2001). A tight junctional array containing oligodendrocyte-specific protein/claudin-11 (OSP) marks the border between compact and non-compact myelin and may act as a diffusion barrier between these myelin subdomains (Bronstein et al., 2000; Gow et al., 1999; Krämer et al., 2001; Morita et al., 1999).

The ensheathment of axons with myelin is essential for the fast saltatory conduction of action potentials along the nerve cells and thus for the proper functioning of the nervous system. Abnormalities in myelin development or disturbance and destruction of its structure lead to severe neurological symptoms observed in diseases such as multiple sclerosis (MS) (Bartlett and Mackay, 1983; Baumann and Pham-Dinh, 2001; de Vries and Hoekstra, 2000). MS is the most common human demyelinating disease. It affects about 0.1% of the population in temperate climates (Wingerchuk et al., 2001). The disabling nature of MS strongly highlights the importance of OLGs. In addition to the initial elaboration of myelin, these cells are also required for the maintenance of the myelin sheath during adult life. As myelin is a compacted multilamellar membrane structure containing little cytoplasm, it is difficult to imagine how myelin membrane constituents are actively metabolized, recycled and/or reassembled. Nevertheless, during the lifespan of mice, individual myelin components are metabolized at different rates and the evidence suggests that these turnover rates are differently affected by aging (Ando et al., 2003). Maintenance of the functional myelin sheath during adult life would thus require a carefully regulated balance of myelin synthesis and turnover.

Besides the subdivision of the fully mature myelin sheath into compact and non-compact myelin, the overall myelin membrane composition differs dramatically from that of the plasma membrane that surrounds the OLG cell body. Therefore, it is clear that the processes of myelin formation and maintenance require precise sorting and targeting mechanisms, which at least in part originate in the OLG cell body.

The myelin sheath, being strongly enriched in glycosphingolipids (GSLs), bears some resemblance to the apical membrane of polarized epithelial cells, whereas the OLG plasma membrane shows similarity to the basolateral membrane. Particularly in membranes enriched in GSLs and cholesterol, such as in apical membranes, GSL/cholesterol-enriched microdomains or so called 'lipid rafts' may exist and/or arise. These microdomains have been postulated to be involved in signaling and to act as targeting devices in the direct transport of apical proteins from the trans-Golgi network (TGN) to the apical membrane (Brown and Rose, 1992; Hoekstra et al., 2003; Ikonen and Simons, 1998; Ikonen, 2001; Simons and van Meer, 1988; Simons and Wandinger-Ness, 1990; Zurzolo et al., 1994). Given the enrichment of GSLs and cholesterol in myelin, it is tempting to consider that transport and functioning of myelin-specific proteins towards and in the myelin sheath are accomplished in a similar fashion.

In this review we will first critically discuss some aspects of the current concept of lipid rafts and subsequently provide an overview of the evidence that supports the presence and relevance of such microdomains in OLGs. The two main techniques used to study rafts in OLGs, detergent extraction and co-localization imaging, are discussed with respect to possible pitfalls. Alternative methods that can be used to study rafts in living cells are briefly described.

THE RAFT CONCEPT

Although the cell membrane may be considered as a two-dimensional, liquid-like structure, it is far from being a homogeneous mixture of lipids and proteins (Jain and White, 1977; Karnovsky et al., 1982; Klausner et al., 1980; Lai, 2003). Rather, it is a non-ideal liquid mixture of molecules with variable degrees of mutual miscibility (Kusumi et al., 2004). As a consequence, the cell membrane contains a variety of molecular complexes and domains, characterized by different composition and spatial arrangement of the membrane-constituting lipids, which implies constraints on the diffusion of the membrane components (Kusumi et al., 2004; Lommerse et al., 2004; Mukherjee and Maxfield, 2004; Ritchie and Kusumi, 2004; Simons and Vaz, 2004; Vereb et al., 2003).

Lipid rafts are a particular class of membrane 'inhomogeneities', and they can be seen as relatively small 'liquid-ordered' membrane domains enriched in cholesterol, GSLs and phospholipids with saturated acyl chains. The lipids are ordered as in the gel phase but nevertheless remain mobile in the plane of the membrane, so that this phase has been denoted as the 'liquid-ordered' phase (Ahmed et al., 1997; Brown, 1998; Ipsen et al., 1987; Lentz et al., 1980; London, 2002; Schroeder et al., 1994; Simons and Toomre, 2000). Within these phases, specific lipids (and proteins) may dynamically associate with each other to form functionally relevant platforms that are important in processes as diverse as membrane protein sorting, signaling and (caveolae-mediated) endocytosis. An operational basis for this lateral functional compartmentalization was given by the discovery that a specific set of membrane components was insoluble in cold (4°C) non-ionic detergent, t-octylphenoxypolyethoxyethanol (Triton X-100, TX-100), resulting in a detergent-resistant membrane (DRM) fraction that could be recovered by density gradient

flotation (Brown and Rose, 1992; Brown and London, 2000; London and Brown, 2000; Simons and Ikonen, 1997). Resistance to detergent extraction has since become an operational definition of membrane rafts, and 'raft association' is defined as the partitioning of proteins and lipids into DRMs. Another criterion generally used to assign proteins to 'rafts' is the disruption of this association after cholesterol depletion. Cholesterol is an inherent part of such domains and its depletion by agents such as methyl-β-cyclodextrin (mβCD) ruptures the raft structure and, consequently, results in loss of the raft-associated function.

Compositional analysis of DRMs has provided a list of potential raft-associated molecules (Foster et al., 2003). However, the outcome of the detergent solubilization appears to depend on the cell type and the extraction condition (concentration, temperature) used (Banerjee et al., 1995; Chamberlain, 2004; Schuck et al., 2003). Moreover, detergent-resistance to solubilization also strongly depends on the nature of the detergent, which is thought to reflect in part the presence of microdomains of distinct composition.

The reliability of detergent resistance and cholesterol dependence as raft-supporting criteria has been subject of criticism (see last section). However, studies on living cells, using highly sophisticated approaches, corroborate the lipid raft hypothesis by demonstrating that 'raft' proteins exhibit cholesterol-dependent clustering at the plasma membrane, and display membrane-anchor dependent lateral diffusion rates only after dissociation from the raft structures (Friedrichson and Kurzchalia, 1998; Pralle et al., 2000; Varma and Mayor, 1998). Thus, although size and precise function of rafts may still be a matter of debate (Edidin, 2003), circumstantial evidence favors the presence of microdomains in the lateral plane of membranes. Pralle et al. (2000) estimated the presence per se and the size of rafts in eukaryotic cell membranes from

local diffusion measurements of single GPI-anchored and transmembrane proteins, using a laser trap procedure. When associated with rafts, the diffusion rates were independent of the nature of the protein's association with the membrane. Dissolving rafts by cholesterol depletion accelerated the diffusion to rates as determined for non-raft associated membrane proteins. Moreover, the increase in diffusion rate of the initially raft-associated proteins appeared to be co-determined by the nature of the membrane anchor, GPI-linked proteins diffusing faster than transmembrane proteins (Pralle et al., 2000). Gaus et al. (2003) used two-photon microscopy to monitor the fluorescent membrane probe Laurdan (6-acyl-2-dimethylaminonaphthalene) in living cells. The so-called generalized polarization based on the environmental dependence of the fluorescence spectrum of Laurdan reflects the local organization of the surrounding lipids (gel, liquid-disordered and liquid-ordered phase). The distribution of generalized polarization values from a stack of images indicated the existence of different membrane phases in the living cell. Their relative coverage was sensitive to cholesterol depletion agents (Gaus et al., 2003).

Depending on the time-resolution of the technique used, different properties can be revealed (Kusumi et al., 2004). Rafts are dynamic so that proteins and lipids can move in and out. Resting cells may contain "reserve rafts", which are postulated to be sufficiently small (down to only 3 molecules, detectable by the sphingomyelin-binding protein earthworm toxin, lysenin; Kiyokawa et al., 2005) and short-lived (lifetimes on the order of a few microseconds; Kusumi et al., 2004) to allow for rapid diffusion of its "transient" constituent molecules to quickly reach the site of signal input. Ligand binding and cross-linking of raft-preferring molecules could induce "receptor-clustered rafts". These larger and stabilized rafts are thought to have a sufficient size and lifetime (up to 1-10h) to facilitate the incorporation of various key

signaling molecules which in turn may result in downstream signaling (Brown and London, 1998; Brown and London, 2000; Kusumi et al., 2004; Kusumi et al., 2005; Mukherjee and Maxfield, 2004; Simons and Ikonen, 1997).

RAFTS AND MYELIN FORMATION

As early as in 1989, it was postulated that sorting and trafficking of PLP, the major myelin protein in the CNS, is coupled to that of myelin GSLs (Pasquini et al., 1989). Evidence for a common transport route came from observations in isolated brain slices that the inhibition of GSL synthesis reduced the translocation of PLP into myelin by about 50%, while the incorporation of MBP and overall protein synthesis were unaffected (Pasquini et al., 1989). In subsequent work, sulfatide was identified to be co-transported with PLP (Brown et al., 1993), although this GSL species largely resides in the plasma membrane of the OLG cell body rather than in the myelin membrane, which is strongly enriched in GalC. However, others (Bansal and Pfeiffer, 1994; van der Haar et al., 1998) demonstrated in primary OLG cell cultures that an inhibition of sulfation did not affect PLP transport to processes and sheets in these cells. Moreover, when expressed in GalC- and sulfatide-deficient CHO (Chinese hamster ovary) cells, PLP-delivery to the plasma membrane was unaffected compared to such delivery in cells expressing these SLs, whereas PLP was found to be fully soluble in TX-100. Taken together these data suggest that transport of PLP to the OLG membrane does not involve a raft-mediated sorting system (Kim and Pfeiffer, 1999; Krämer et al., 1997; van der Haar et al., 1998). It is possible however that the apparent controversy may originate from differences in cell systems, the early work having been carried out in tissue, while the latter studies were performed in primary cell cultures. In a culture dish, in the absence of neurons, there are no cell-cell (axon-

glial) or cell-matrix contacts. However, OLGs in monoculture do differentiate and form flat myelin networks, called myelin sheets. They may not fully mature and compaction of the myelin sheath does not occur. By contrast, *in vivo* (and possibly in OLG-neuron co-cultures) the fully mature myelin sheath is further segregated in compact and non-compact myelin. Transport mechanisms used by developing OLGs *in vitro* might therefore differ from those used during the maintenance stage of the fully mature myelin sheath. Thus, *in vivo*, PLP may reside in different myelin fractions and, depending on its localization, may only partly solubilize in TX-100, as is the case in myelin of adult mice where PLP is partly TX-100 resistant (Saravanan et al., 2004). Evidently, further work *in vitro*, using mixed brain cultures as well as careful fractionation of *in vivo* brain slice material, should clarify this issue.

More recently, it has become clear that detergent-resistant microdomains do not necessarily have to be defined exclusively by their insolubility in TX-100. Rather, such fractions can also be isolated by gradient flotation following extraction with other non-ionic detergents such as Lubrol, Brij 98, 3-[(3-chloramidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS), etc. When OLGs are extracted with CHAPS, PLP is recovered in the DRM fraction and indeed behaves as a raft-associated protein (Simons et al., 2000). On the other hand, the glycosylphosphatidylinositol-anchored proteins (GPI-APs) NCAM 120 (neuronal cell adhesion molecule of 120 kDa) and F3 (also known as contactin) are soluble in CHAPS but highly enriched in TX-100-insoluble fractions (Simons et al., 2000). This led to the conclusion that PLP may assemble into a specialized “myelin-raft”, which directs sorting and trafficking of myelin components (Simons et al., 2000). Interestingly, as NCAM 120 resides in the plasma membrane of the cell body whereas F3 localizes to the sheet, these data suggest that, based on detergent-resistence of PLP

versus F3, different DRMs coexist in the myelin sheet. These findings would favor the notion (Brügger et al., 2004; Madore et al., 1999) that the disparate results obtained by different detergents may relate to the co-existence of different domains, characterized by differences in composition. However, claims have been made that differential insolubility of proteins in different detergents is not sufficient to imply their association with distinct lipid rafts (Chamberlain, 2004; Pike, 2004).

From a functional point of view, the specific association of a given molecular compound with a DRM has been correlated with sorting and, in polarized epithelial cells, with apical-directed membrane transport (Hoekstra et al., 2003; Ikonen and Simons, 1998; Ikonen, 2001; Simons and van Meer, 1988; Simons and Wandinger-Ness, 1990; Zurzolo et al., 1994). As noted above and elsewhere (de Vries and Hoekstra, 2000), OLGs can also be considered to be polarized, given the remarkable differences in lipid composition of plasma and myelin membrane, the latter being particularly enriched in GSLs, saturated phospholipids and cholesterol, a composition typical of raft-like domains. Remarkably, when the trafficking of the viral membrane proteins influenza haemagglutinin (HA) and vesicular stomatitis virus G protein (VSVG) were monitored in infected OLGs, HA was expressed at the plasma membrane of the cell body, where it localizes in a TX-100 detergent-resistant fraction, while VSVG is transported to the sheet as a TX-100 soluble protein (de Vries et al., 1998). When expressed in polarized epithelial cells, HA and VSVG are sorted and transported to the apical and basolateral surface, respectively. Using these findings as criteria, it can be suggested that the myelin membrane is target of a basolateral-like mechanism, while the plasma membrane displays apical-like features. Consistent with this notion is the observation that the t-SNARES (target-membrane-associated soluble N-ethylmaleimide fusion protein attachment protein (SNAP) receptor) syntaxin-3 and

syntaxin-4 similarly distribute in a polarized manner, syntaxin-3 being localized at the plasma membrane, while syntaxin-4 is strongly enriched in the sheet (Klunder and Hoekstra, personal communication). Alternatively, Kroepfl and Gardinier (2001) stably transfected (polarized) Madin-Darby canine kidney (MDCK) cells with the myelin proteins MOG, PLP and MAG, and subsequently examined the membrane targeting of these myelin proteins. As in OLGs, MOG and PLP were sorted to mutually exclusive compartments in MDCK cells. MOG, a non-compact myelin protein, was solely found in the basolateral membrane, whereas PLP, a compact myelin protein, was exclusively found within the apical membrane (Kroepfl and Gardinier, 2001). Whether such data can be extrapolated to oligodendrocytes in terms of basolateral versus apical sorting mechanisms remains to be determined. As noted, in the same cells, HA is specifically sorted to the apical membrane, but when expressed in OLGs its localization is largely restricted to the cell body plasma membrane. MAL, present in both epithelial cells and OLGs, is sorted to the apical membrane and into the sheath in TX-100 detergent-insoluble microdomains, in contrast to PLP, which is soluble in this detergent. Together, these data emphasize the complexity in directly comparing the role of detergent-resistence in apical versus basolateral sorting, and extrapolation of polarized sorting in epithelial cells to that in OLGs.

Indeed in several recent studies (Paladino et al., 2004; Slimane et al., 2003) it has become clear that microdomain or raft-mediated transport, as defined by detergent-resistence, is not restricted to apical membrane directed trafficking, but is similarly operational in basolateral transport pathways. Accordingly, formation of different raft domains might be a mechanism of membrane subdomain organization, important for compartmentalization of signaling molecules as well as for the sorting of myelin

components. Whether the distinction that many of the major myelin-specific proteins display different preferences for localization in microdomains, as reflected by differences in detergent solubility (Table 3), coincides with differences in sorting/transport mechanisms, remains to be elucidated. For this, further analyses including pulse-chase experiments and OLG development-dependent transport studies rather than determination of a detergent-resistent localization at steady state are necessary. This notion also relates to the potentially distinct distribution of a myelin protein in fully mature OLGs, where it may partition in both compact and non-compact myelin, thus possibly displaying distinct detergent-solubility properties which, moreover, do not necessarily have to correlate with those in its sheet-directed transport. Thus the dynamics and transient association of proteins in distinct rafts should be particularly taken into account when investigating their association with membrane microdomains.

Thus far, the picture of molecular parameters involved in DRM-mediated sorting is far from complete. In apical sorting pathways, the formation of high-molecular-weight complexes as part of the mechanism of DRM recruitment seems to be required (Helms and Zurzolo, 2004; Paladino et al., 2004; Schuck and Simons, 2004; Zurzolo et al., 2003). Oligomerization or association with a high-molecular-weight complex might lead to an exponential increase in raft affinity with increasing oligomer size (Simons and Vaz, 2004), thereby stabilizing raft association. In addition, oligomerization might cause the coalescence of small rafts into a stable functional sorting domain or signal. Are such mechanisms operational in OLGs? One protein that might promote raft clustering is MAL, which is also known as MVP17 (myelin vesicular protein 17; Kim et al., 1995) and VIP17 (vesicular integral protein 17; Zacchetti et al., 1995). MAL is a 17 kDa non-glycosylated integral membrane protein

that localizes in the Golgi (Puertollano et al., 1999) and is required for apical targeting in epithial cells (Cheong et al., 1999; Martin-Belmonte et al., 2000; Puertollano et al., 1999). In OLGs, MAL has been shown to be a component of GSL-rafts (Frank et al., 1998; Frank, 2000; Kim et al., 1995; Kim and Pfeiffer, 2002; Schaeren-Wiemers et al., 1995) and to end up in compact myelin (Frank et al., 1998). MAL is upregulated during active myelination (Kim et al., 1995; Schaeren-Wiemers et al., 1995). In addition, MAL can form oligomers, which might function to cluster rafts at the sites at which sorting takes place (Schuck and Simons, 2004). However, MAL is relatively late expressed in myelinogenesis, i.e., after expression of most major myelin proteins, including PLP. Whether the role of MAL is thus restricted to the reorganization of myelin proteins in compact myelin or is involved in sorting and transport during maintenance of the myelin sheath, remains to be determined. In this context it is worthwhile to mention that, based on observations in MAL-deficient mice, MAL has recently been implicated in the maintenance of axon-glial interactions at the CNS paranodes, presumably by playing a role in the trafficking and/or sorting of paranodal proteins NF155 and MAG (Schaeren-Wiemers et al., 2004).

RAFTS IN AXON-GLIAL INTERACTION

Myelination of axons by OLGs involves recognition of the axonal surface, subsequent interaction between the OLG and the axon, spiral enwrapment of the axonal segment and finally compaction of the multilamellar OLG membrane into a functional myelin sheath (Sherman and Brophy, 2005, and references therein). There is substantial evidence that microdomain assembly is a prerequisite for proper recruitment of the molecular machinery involved in axon-glial interaction.

Initial axon-glial contact

Fyn is a key molecule in OLG differentiation and myelination (Osterhout et al., 1999; Sperber and McMorris, 2001; Umemori et al., 1994). This molecule belongs to the Src (Rous sarcoma) family of kinases and is associated with cell migration, proliferation, differentiation, adhesion, apoptosis and cytoskeletal rearrangements (Osterhout et al., 1999). Fyn is associated with the F3/contactin adhesion protein in OLGs and this interaction occurs within rafts (Krämer et al., 1999). Since antibody-mediated cross-linking of F3/contactin in the oligodendroglial cell line Oli-neu results in activation of Fyn kinase (Krämer et al., 1999), the functional advantage of being localized in a microdomain thus becomes readily apparent, i.e., providing an environment for molecular clustering which triggers an efficient signal transduction between axons and OLGs in the early phases of myelination. Activation of this pathway causes Fyn to bind to the cytoskeletal proteins Tau, which further strengthens initial axon-glial contact, and α-tubulin in OLGs. It is thought that the local reorganization of the cytoskeleton, accomplished in this manner, might subsequently facilitate directed transport of myelin-specific lipids and proteins to the expanding myelin sheath (Klein et al., 2002).

Myelin integrity

The functional role that raft domains may play in establishing axon-glial interactions is further supported by observations that NF155, an ankyrin-binding cell adhesion molecule, also localizes in such microdomains, which seems necessary for carrying out its function. NF155 is localized within the paranodal loops where it interacts with the Caspr1 (contactin-associated protein, also known as paranodin)-F3/contactin protein complex on the axon (Charles et al., 2002; Tait et al., 2000), an interaction that is required for myelination in co-cultures of OLGs and neurons (Charles et al.,

2002). The finding that NF155 is completely absent from paranodes of CGT (ceramide galactosyl transferase) knockout mice (Menon et al., 2003) indicates that raft assembly might be critical for the accumulation of NF155 in paranodes. CGT is the key enzyme of the galactolipid biosynthesis pathway and myelin of mice missing the CGT enzyme completely lacks GalC and sulfatide (Bosio et al., 1996; Lee, 2001). Recently, it has been shown that NF155 associates with DRMs (rafts) in the CNS (Schaeren-Wiemers et al., 2004) and that this association is commensurate with the timing of paranode formation (Schafer et al., 2004). Given the abnormal structure of the paranodal loops in CGT knockout mice (Bosio et al., 1998), it is likely that the correct membrane association of NF155 is important for the stability of the paranodal structure and thus for the integrity of the myelin sheath. During myelination, close contact between the axon and the OLG will lead to interaction between NF155 and Caspr1-F3/contactin, which in turn will stabilize these proteins and the lipid environment in which they reside, providing a "nucleation" site for the formation of a lipid raft protein adhesion complex. Additional NF155, Caspr and F3/contactin might be recruited because of an increased affinity for the raft environment, which in turn leads to further stabilization through the axon-glial interaction. The sum of these protein-protein interactions within the raft environment would provide the basis for the strong adhesion complex at the paranode and thus for myelination and myelin integrity. Of relevance to potential causes of a demyelinating disease could thus be that an interference with raft stability/association might result in destabilization of the axon-glial interaction and eventually lead to demyelination. Culturing OLGs on fibronectin, which mimics changes in the extracellular matrix (ECM) as occurs in MS due to the perturbation of the blood brain barrier (BBB), results in dissociation of NF155 from the DRM fraction as well as in inhibition of the morphological

differentiation of OLGs (Maier et al., 2005). Consistent with *in vitro* data, raft association of NF155 is substantially reduced in spinal cord of experimental allergic encephalitis (EAE) rats, an animal model for the demyelinating disease MS. Hence, the association of NF155 to microdomains in the myelin membrane is required for its participation in intermolecular interactions, which are important for myelination and myelin integrity.

Raft-mediated adverse effects

Interestingly, the association of MOG with microdomains seems to induce process retraction rather then stabilizing myelin integrity. MOG is an integral myelin-specific protein, which is localized in the outer lamella of the myelin sheath and therefore exposed to the extracellular environment. Although MOG is only a minor component of the myelin membrane (0.01-0.05 % of the total myelin protein content), it induces severe EAE after administration to both rodents and primates (Iglesias et al., 2001; Johns and Bernard, 1999). Furthermore, injection of mAbs against MOG into rodents causes extensive myelin destruction *in situ* (Linington et al., 1988). In addition, anti-MOG antibodies are found in the cerebrospinal fluid and in lesions of acute MS patients (Linington and Lassmann, 1987; Reindl et al., 1999). Therefore, it appears that MOG/anti-MOG interactions could be mediators in the process of demyelination in EAE and MS. The role of MOG in this process is closely related to its dynamic association with DRMs, which causes activation of distinct signal transduction pathways. Following Ab binding, the non-raft associated fraction causes the activation of mitogen-activated protein kinase (MAPK) and Akt pathways. When present in TX-100 insoluble rafts, signaling pathways related to stress response and cytoskeletal instability are activated, which result in the retraction of OLG processes (Marta et al., 2003; Marta et al., 2005). Worth noting is that MOG-mediated retraction of OLG

processes requires a secondary cross-linking antibody. The role of this secondary cross-linking antibody might be to increase raft affinity and to promote stabilization of MOG in rafts with a lifetime that suffices to recruit the signaling molecules necessary for process retraction. In MS, macrophages and complement could mimic the effect of the secondary cross-linking antibody since they are able to bind IgG (immunoglobulin G) molecules. In fact, a secondary cross-linking antibody might not be necessary when polyclonal anti-MOG antibodies with different epitope specificity are present in MS patients.

Dual functions mediated through rafts

Like the NF155-Caspr1-F3/contactin interaction, the interplay of MAG with its axonal receptors might also occur by raft-mediated intercellular interactions between OLGs and neurons. MAG, a quantitatively minor protein of myelin (< 1%), serves both as a myelin-stabilizing factor and as inhibitor of nerve regeneration (Vyas and Schnaar, 2001; Weiss et al., 2000). It is a sialic acid-binding protein of the siglec (sialic acid-dependent immunoglobulin-like family member lectin) family. MAG is restricted to the periaxonal membrane of the myelin sheath where it interacts with molecules on the axonal membrane, including the gangliosides GD1a and GT1b (Vinson et al., 2001; Vyas et al., 2002) and the GPI-linked Nogo receptor (NgR) (Domeniconi et al., 2002; Fournier et al., 2001; Liu et al., 2002). As the affinity of a monomeric siglec molecule (e.g. MAG) for its sialic acid ligand is thought to be relatively low, the rationale for localization of MAG and its receptors in lipid rafts might be the creation of discrete areas of high local molecular density necessary for the activation of signaling pathways in both cell types. In neurons, this interaction would result in the inhibition of neurite outgrowth, whereas in OLGs it would be necessary for the maintenance of myelin integrity (Vinson et al., 2003). MAG was

found to interact with lipid rafts on the surface of neurons, which contain known binding partners of MAG, namely GT1b and NgR, as well as p75 and Rho. The latter are required for transmitting MAG-mediated signals into neurons. Interestingly, in primary OLGs, antibody-induced cross-linking of MAG causes the (re-)partitioning of the protein from a soluble into a DRM fraction (Marta et al., 2004). Cross-linking apparently seems to induce recruitment into rafts and the observed clustering thus results from the cross-linking as such rather than from the coalescence of individual, MAG containing rafts.

ON A FUNCTIONAL ROLE OF RAFTS IN OLIGODENDROCYTE BEHAVIOR

Raft recruitment and coalescence of rafts may provide OLGs with a means for differential regulation of growth factor responses during development. Platelet-derived growth factor (PDGF), for example, does not only regulate OPC proliferation but also survival (Calver et al., 1998). The nature of the response to PDGF, proliferation or survival, is determined by the identity of the integrin associated with the PDGFα receptor (PDGFαR). αvβ3, which stimulates proliferation, interacts with the PDGFαR in OLG precursors (Baron et al., 2002), while α6β1, which is involved in cell survival (Colognato et al., 2002) and myelination (Relvas et al., 2001), is found to be the interaction partner in immature Galc-positive OLGs (Baron et al., 2003). Rafts could contribute to integrin signaling by facilitating growth factor receptor-integrin interactions necessary to trigger integrin activation by concentrating the necessary complex of signaling molecules required for activation and/or down-stream signaling. Alternatively, the conformational equilibrium between inactive and active integrins might be displaced in favor of the activated integrin by the altered membrane

structure of the lipid raft (ffrench-Constant and Colognato, 2004). Activation of integrins is associated with a change from a bent to an extended conformation, with associated intramolecular interactions altering the conformation of the ligand-binding pocket, thereby increasing ligand affinity (Baron et al., 2005, and references therein). The OLG PDGFαR becomes associated with lipid rafts at a stage when it no longer promotes proliferation but instead is required for survival (Baron et al., 2003). A pool of integrin α6β1 is also present in membrane rafts, but these are different from the PDGFαR-containing rafts. Laminin-2, which is expressed on axons of the CNS (Colognato et al., 2002), induces coalescence of the integrin $\alpha6\beta1^{+}$ rafts with the $PDGF\alpha R^{+}$ lipid rafts, resulting in receptor co-association, integrin activation, affinity modulation and signal amplification at physiological PDGF levels, respectively, ultimately leading to survival of the OLGs (Baron et al., 2003; Decker and ffrench-Constant, 2004; Decker et al., 2004). Merging of PDGFαR-containing microdomains with those that contain the integrin α6β1 could thus provide a distinct signalling microenvironment that allows the PDGF signaling response during OLG development to switch from proliferation to survival. Fyn and Lyn (another member of the Src kinase family) were recently shown to be key effector molecules within the integrin-growth factor receptor complexes that selectively promote either proliferation or differentiation/survival (Colognato et al., 2004). As discussed earlier, Fyn has been postulated to play a role in OLG differentiation and myelin formation (Klein et al., 2002; Krämer et al., 1999). Lyn, on the other hand, is associated with the PDGFαR-αvβ3 integrin complex and contributes to proliferation signaling (Colognato et al., 2004). After axonal contact, Lyn dissociates from the integrin-growth factor complex whereas Fyn is activated thereby promoting OLG survival, differentiation and myelin formation (Colognato et al., 2004).

RAFT ANALYSIS: CRITICAL HINDSIGHT AND FUTURE PERSPECTIVES

The formation of lipid rafts in OLGs seems to be a mechanism of membrane subdomain organization, important for compartmentalization of signaling molecules as well as for the sorting of myelin components. However, all the information available at present about the existence and possible function of lipid rafts in OLGs is mainly based on detergent extraction. Different detergents, extraction procedures and cell/tissue sources have been used (Table 3). As already indicated by others (Banerjee et al., 1995; Chamberlain, 2004; Edidin, 2001a; London and Brown, 2000; Schuck et al., 2003), the detergent insolubility of proteins depends highly on the detergent and the extraction conditions used. DeBruin et al. (2005) used three different detergents (TX-100, CHAPS and Brij 96V) to characterize membrane microdomains in developing and mature bovine myelin. Taylor et al. (2002) screened four different detergents (under four extraction conditions) to determine which supported the retention of four integral (MAG, OSP/claudin-11, MOG and PLP) and three peripheral (NCAM-120, CNP and MBP) proteins of the myelin membrane in a low-density DRM fraction. Both groups reported that the amount of proteins and lipids, as well as the sucrose gradient buoyancy of the DRM-complexes, varied substantially among the detergents and extraction procedure used.

In addition to the detergent dependence of the results, it is not clear whether proteins found to be present in DRMs were associated with lipid rafts in the living cell as detergent extraction seems to dramatically alter the lipid composition of preexisting domains (de Almeida et al., 2003; Heerklotz, 2002; Heerklotz et al., 2003; Skwarek, 2004). However, others provide evidence that isolation of DRM from biological membranes by detergent-induced extraction is not an artefact (Staneva et al., 2005).

Different detergents differ in structure, which results in differential partitioning into the plasma membrane and the disruption of specific protein-lipid and protein-protein interactions by individual detergents. In addition, individual detergents have a different ability to deplete inner leaflet lipids. TX-100, for example, selectively extracts inner leaflet lipids giving rise to membrane preparations with a preponderance of outer leaflet lipids, whereas Brij 98-resistant rafts have a normal balance of inner and outer leaflet lipids (Pike et al., 2005). It is unlikely that different detergents reflect the same aspects of membrane organization. In a complex environment as the cell membrane, DRM association may at best serve to define a circumstantial steady-state biochemical characteristic. It cannot provide reliable information regarding the preexisting molecular organization on the multicomponent cell surface. This underscores both the structural complexity of cell membranes and the need for additional approaches to understand their architecture and in particular the dynamics of that architecture. Detergent extraction only reflects information concerning the steady-state but does not yield any information about dynamic events, which may influence the recruitment of molecules into lipid rafts. As mentioned before, cross-linking induces the relocalization of certain proteins, e.g. MOG, into DRM domains (Marta et al., 2003, 2005). The relocalization of MOG within microdomains, however, is a reversible event, and is abolished after removal of the cross-linking antibody. The effective partitioning of a given protein in such domains could vary, reflected by differences in the fraction recovered, using a given detergent, or by differences in detergent-dependent solubilization. Hence, extraction per se may also not properly reflect the functional relevance of localization of a protein in a particular microdomain, since at steady-state the protein is likely not restricted to one domain only. Knowledge about the dynamic behaviour of membrane architecture,

however, may in turn determine the molecular composition of a microdomain and hence its biophysical properties, including the lifetime.

The method of cholesterol depletion seems to be rather controversial as well. Acute cholesterol depletion blocks both endo- and exocytosis, alters the actin cytoskeleton and inhibits lateral diffusion of membrane proteins. A number of pleiotropic effects may thus result, including the dissociation of various proteins from lipid rafts (Edidin, 2003; Lai, 2003). Biochemical approaches should be combined with other methods that could identify lipid rafts in intact cell membranes and provide information about the size, lifetime, dynamics and functional role of rafts in living cells.

Fluorescence intensity imaging microscopy (FIIM) is commonly used to determine the distribution and possible co-localization of putative raft markers labelled with different fluorescent conjugated antibodies. A physical limitation of FIIM methods is the optical resolution (airy disk diameter) of ~250 nm, which makes the resolvable detail related to the pixel size larger than the size of the rafts (Kusumi and Suzuki, 2005; Pralle et al., 2000). The observation of co-localization, although required, is therefore insufficient to conclude that the labeled components are within the same raft. The co-localization imaging approach has a limited time resolution so that restricted information, if any, about the kinetic properties of the domains can be obtained. The problem of the restricted spatial resolution can be circumvented by the application of Förster resonance energy transfer (FRET) (Acasandrei et al., 2006; Kenworthy and Edidin, 1998; Kenworthy and Edidin, 1999; Rao and Mayor, 2005). In order to obtain information about the kinetic properties of molecules, methods such as single particle tracking (SPT) and photonic force microscopy (Lommerse et al., 2004; Pralle et al., 2000) can be used. Diffusional mobility can also be measured at single-molecule sensitivity by fluorescence correlation spectroscopy (FCS). It was

recently demonstrated that FCS promises to be a valuable tool to elucidate lipid raft associations both in domain-exhibiting model membranes and in cell membranes (Bacia et al., 2004; Wawrezinieck et al., 2005). Moreover, a first attempt has been made to study the membrane heterogeneity of living OLN-93 oligodendroglial cells by means of one-photon FCS (Gielen et al., 2005; Humpolíčková et al., 2006). Fluorescence recovery after photobleaching (FRAP) is another technique that can be used to determine the translational mobility of fluorescent molecules in the cell membrane (Kenworthy et al., 2004; Lippincott-Schwartz et al., 2003; Phair and Misteli, 2001).

A major advantage of the described microfluorimetric techniques in comparison to detergent extraction is that they can be applied to single cells so that only tiny amounts of valuable brain tissue are required for these experiments. Other techniques that can be used to study rafts in intact cell membranes are electron microscopy (EM: Prior et al., 2003; Wilson et al., 2000), atomic force microscopy (AFM: Giocondi et al., 2004; Henderson et al., 2004; Rinia et al., 2001; Yuan et al., 2002) and near-field scanning optical microscopy (NSOM: Dunn, 1999; Edidin, 2001b).

The different techniques described above characterize rafts on different time and spatial scales (Edidin, 1997; Kenworthy 2005; Lagerholm et al., 2005; Lommerse et al., 2004; Mátko and Szöllõsi, 2002), yielding a wide range of characteristic parameter values for lipid rafts in terms of size, stability and abundance (Lommerse et al., 2004). This clearly emphasizes the need to combine several techniques to assess detailed information about the size, structure and function of lipid rafts. Techniques, such as FRAP, FCS, FRET and SPT allow the investigation of membrane heterogeneity in living cells under physiological conditions. The combination of biochemical studies with these photophysical, microfluorimetric methods will yield a

better insight in the biological relevance of rafts in OLGs and help to come to a consensus on lipid rafts concerning their existence, size, lifetime and molecular organization.

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Table 1 Lipid and protein composition of human central nervous system myelin[3]

	% Total dry weight
Lipid	70.0
Protein	30.0

Protein	% Total dry weight
Myelin basic protein	22.5
Proteolipid proteins	30.0
Other LH-20 components[1]	17.5
Myelin-associated glycoprotein	<1.0
2',3'-cyclic nucleotide 3'-phosphodiesterase	4.0
Others (Wolfgram, glycoproteins, etc.)	25.0

Lipid	% Total dry weight
Cholesterol	27.7
Galactosylceramide	22.7
Sulfatide	3.8
Ethanolamine phosphatides	15.6
Phosphatidylcholine	11.2
Phosphatidylserine	4.8
Phosphatidylinositol	0.6
Sphingomyelin	7.9
Plasmalogens[2]	12.3
Gangliosides (primarily GM1 and GM4)	<1.0

[1]The proteolipid fraction is a family of proteins that can be isolated on Sephadex LH-20 in acidified chloroform-methanol. The major homogeneous protein is PLP
[2]Plasmalogens are ether-linked lipids composed primarily of ethanolamine phosphatides
[3]Adapted from Deber and Reynolds (1991)

Table 2 Function of myelin-specific proteins

Myelin component	Function
PLP/DM20	Myelin compaction[1]
MBP	Myelin compaction[1]
MAL	Might function to form and maintain stable protein-lipid microdomains in myelin effecting reorganization of myelin proteins in compact myelin and sorting and transport during maintenance of the myelin sheath[2, 3] Maintenance of proper axon-glia interactions[2]
MAG	Myelin stabilization/maintenance of myelin integrity[4] Inhibition of neurite outgrowth, i.e. axon regeneration in the CNS after lesion[4]
OSP/claudin-11	Mediator of parallel-array tight junction strands in CNS myelin[5] May act as a diffusion barrier between compact and non-compact myelin[5, 6]
MOG	May function in transmitting extracellular information to the interior of oligodendrocytes[7] Target antigen in the process of demyelination in EAE and MS[8]
CNP	Microtubule assembly myelin protein[9] Process outgrowth[9]
NF155	Establishment of the paranodal septate junction required for tight interaction between myelin and axon[10]
MOBP	Myelin compaction[1]

[1]Baumann and Pham-Dinh, 2001; [2]Schaeren-Wiemers et al., 2004; [3]Frank et al., 1998; Frank, 2000; [4]Vyas and Schnaar, 2001; Weiss et al., 2000; [5]Gow et al., 1999; [6]Bronstein et al., 2000; Morita et al., 1999; [7]Johns and Bernard, 1999; [8]Iglesias et al., 2001; [9]Lee et al., 2005; [10]Bhat, 2003

Table 3 Overview of extraction conditions in which myelin proteins resist solubilization

Protein	Cell/tissue source	Species	Detergent	Extraction condition			Reference
				Conc	Incubation time	Temp	
PLP/DM20	Purified myelin	35-days-old mice	CHAPS	1%	30 min	4°C/37°C	Taylor et al. 2002
			TX-102	1%	30 min	4°C	
		4-months-old mice: WT and MAL KO	CHAPS	na	30 min	4°C	Schaeren-Wiemers et al. 2004
		Adult mouse brain	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
		2-year-old mice	TX-100	2%	30 min	4°C	Saravanan et al. 2004
		Bovine brain E20 - E40	CHAPS	1.5%	30 min	4°C	Debruin et al. 2005
	Primary OLGs	Mouse OLG 5 DIV	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
MBP	Whole brain	Adult rat	TX-100	1%	na	4°C	Boyanapalli et al. 2005
			CHAPS	30 mM	na	4°C	
		Rabbit	TX-100	1%	16h + 24h	4°C	Pereyra et al. 1988
	Purified myelin	4 to 6-weeks-old rat	TX-100	0.5%	3 min	RT	Gillespie et al. 1989
		35-days-old mice	TX-102	1%	30 min	4°C	Taylor et al. 2002
		4-months-old mice: WT and MAL KO	CHAPS	na	30 min	4°C	Schaeren-Wiemers et al. 2004
		Bovine brain E29 - E40	CHAPS	1.5%	30 min	4°C	Debruin et al. 2005
	Primary OLGs	1 to 2-days-old rat brain OLGs 12 DIV	TX-100	0.5%	3 min	RT	Wilson and Brophy 1989
MAL	Purified myelin	30-days-old rat brain	TX-100	1%	30 min	4°C	Kim and Pfeiffer 2002
		Adult rat brain	CHAPS	20 mM	2h	4°C	Kim et al. 1995
		2-year-old mice	TX-100	2%	30 min	4°C	Saravanan et al. 2004
	Primary OLGs	Mature rat OLGs	CHAPS	40 mM	30 min	4°C	Kim et al. 1995
	Spinal cord	14-days-old Rat	CHAPS	1%	30 min	4°C	Frank et al. 1998
MAG	Whole brain	Mouse	Lubrol WX	1%	na	4°C	Vinson et al. 2003
	Purified myelin	Adult mouse brain	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
		35-days-old mice	CHAPS/Brij 96V	1%	30 min	4°C/37°C	Taylor et al. 2002
		Bovine brain E29 - E40	CHAPS	1.5%	30 min	4°C	Debruin et al. 2005
	Primary OLGs	1 to 2-days-old rat brain OLGs 6 DIV	Lubrol WX	1%	na	4°C	Vinson et al. 2003

	Spinal cord	Rat (WT or EAE)	Lubrol WX	0.5%	30 min	4°C	Maier et al. 2005
Cx-32	Purified myelin	30-days-old rat brain	TX-100	1%	30 min	4°C/37°C	Kim and Pfeiffer 1999
OSP	Purified myelin	30-days-old rat brain	TX-100	1%	30 min	4°C	Kim and Pfeiffer 1999
		35-days-old mice	TX-100 / CHAPS Brij 96V / TX-102	1%	30 min	4°C	Taylor et al. 2002
MOG	Purified myelin	30-days-old rat brain	TX-100	1%	30 min	4°C	Kim and Pfeiffer 1999
		35-days-old mice	TX-100/CHAPS Brij 96V/TX-102	1%	30 min	4°C	Taylor et al. 2002
		4-months-old mice: WT and MAL KO	CHAPS	na	30 min	4°C	Schaeren-Wiemers et al. 2004
		Adult mouse brain	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
		2-year-old mice	TX-100	2%	30 min	4°C	Saravanan et al. 2004
		Bovine brain E20 – E40	CHAPS	1.5%	30 min	4°C	Debruin et al. 2005
	Primary OLGs	Mouse OLG 5 DIV	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
CNP	Purified myelin	30-days-old rat	TX-100	1%	30 min	4°C	Kim and Pfeiffer 1999
		4-6 weeks old rat	TX-100	0.5%	3 min	RT	Gillespie et al. 1989
		35-days-old mice	TX-100 / CHAPS / Brij 96V / TX-102	1%	30 min	4°C/37°C	Taylor et al. 2002
		2-year-old mice	TX-100	2%	30 min	4°C	Saravanan et al. 2004
		Mouse	TX-100	1%	na	4°C	Boyanapalli et al. 2005
			CHAPS	30 mM	na	4°C	
		Rabbit	TX-100	1%	16h + 24h	4°C	Pereyra et al. 1988
		Bovine brain E11 – E40	CHAPS	1.5%	30 min	4°C	Debruin et al. 2005
	Primary OLGs	1-2 day old rat brain OLGs 12 DIV	TX-100	0.5%	3 min	RT	Wilson and Brophy 1989
	Optic nerve	p13, p18, p24 and 2 months old rat	TX-100	1%	1h	4°C	Schafer et al. 2004
OMgp	Whole brain	Adult rat	TX100	1%	na	4°C	Boyanapalli et al. 2005
			CHAPS	30 mM	na	4°C	
	Purified myelin	Mouse	TX100	1%	na	4°C	
			CHAPS	30 mM	na	4°C	
NF155	Purified myelin	4-months-old mice	CHAPS	na	30 min	4°C	Schaeren-Wiemers et al. 2004

	Primary OLGs	Rat OLGs 6 DIV	TX-100	1%	30 min	4°C	Maier et al. 2005
			Lubrol WX	0.5%	30 min	4°C	
		premyelinating mouse OLGs	TX-100	1%	1h	4°C	Schafer et al. 2004
	Spinal cord	Rat (WT or EAE)	TX-100	1%	30 min	4°C	Maier et al. 2005
			Lubrol WX	0.5%	30 min	4°C	
	Optic nerve	p13, p18, p24 and 2 months old rat	TX-100	1%	1h	4°C	Schafer et al. 2004
NCAM 120	Purified myelin	35-days-old mice	TX-100 / CHAPS / Brij 96V / TX-102	1%	30 min	4°C	Taylor et al. 2002
		Adult mouse brain	TX-100	2%	30 min	4°C	Krämer et al. 1997, 1999
		2-year-old mice	TX-100	2%	30 min	4°C	Saravanan et al. 2004
	Primary OLGs	Mouse OLGs 5/8 DIV	TX-100	2%	30 min	4°C	Krämer et al. 1997, 1999
		Mouse OLG 5 DIV	CHAPS	20 mM	30 min	4°C	Simons et al. 2000
	Cell line	Oli-neu (mouse)	TX-100	2%	30 min	4°C	Krämer et al. 1997
F3/contactin	Purified myelin	4-months-old mice	CHAPS	na	30 min	4°C	Schaeren-Wiemers et al. 2004
		Adult mouse brain	TX-100	2%	30 min	4°C	Krämer et al. 1997, 1999
	Primary OLGs	Mouse OLGs 5/8 DIV	TX-100	2%	30 min	4°C	Krämer et al. 1997, 1999
		Mouse OLG 5 DIV	CHAPS	20 mM	30 min	4°C	Simons et al. 2000

Conc: concentration; temp: temperature; na: not available; RT: room temperature; E20: embryonic stage week 20; p13: postnatal day 13; DIV: days *in vitro*; WT: wildtype; KO: knockout

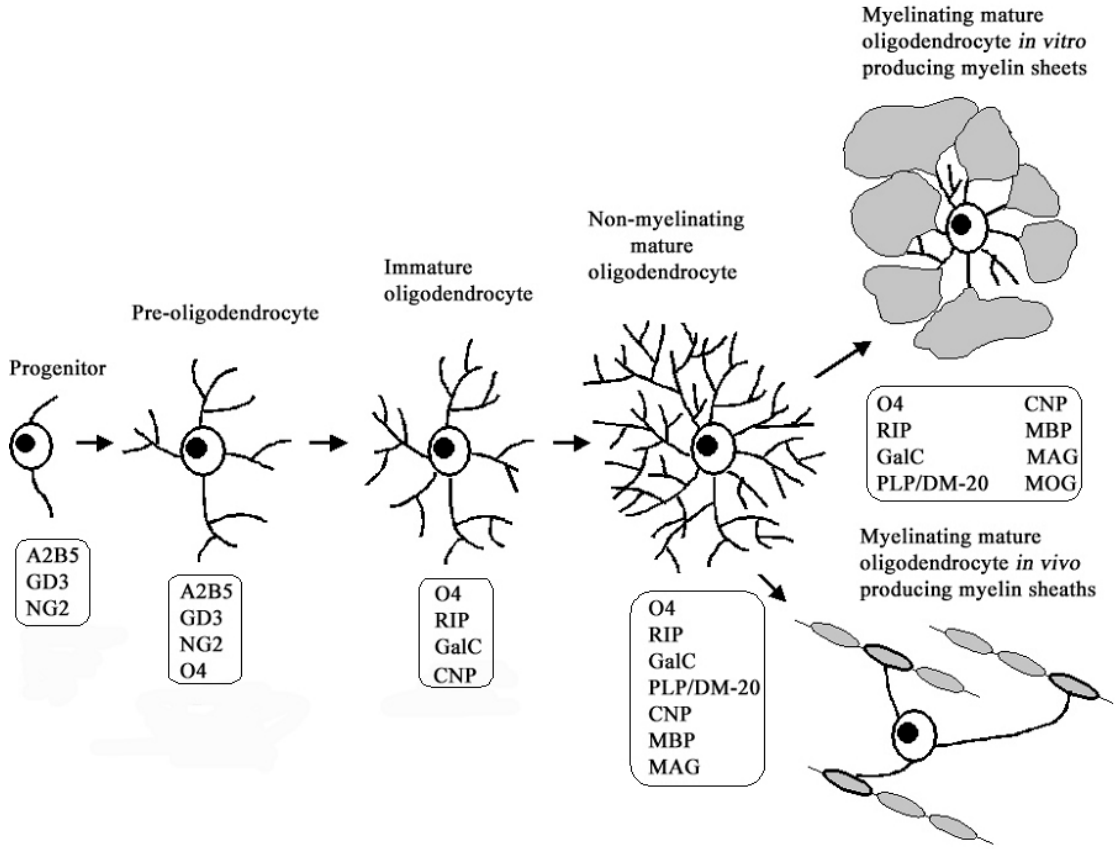
Myelinating mature oligodendrocyte *in vitro* producing myelin sheets
Non-myelinating mature oligodendrocyte
Immature oligodendrocyte
Pre-oligodendrocyte
Progenitor
A2B5 GD3 NG2
A2B5 GD3 NG2 O4
O4 RIP GalC CNP
O4 RIP GalC PLP/DM-20 CNP MBP MAG
O4 RIP GalC PLP/DM-20 CNP MBP MAG MOG
Myelinating mature oligodendrocyte *in vivo* producing myelin sheaths

Figure 1

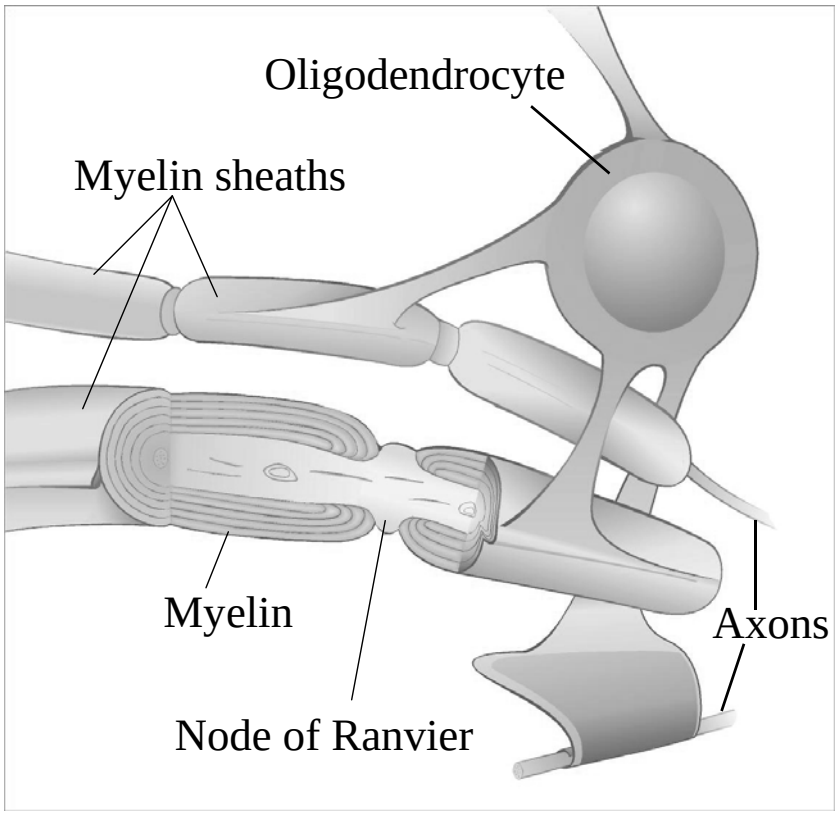
Oligodendrocyte
Myelin sheaths
Myelin
Node of Ranvier
Axons

Figure 2

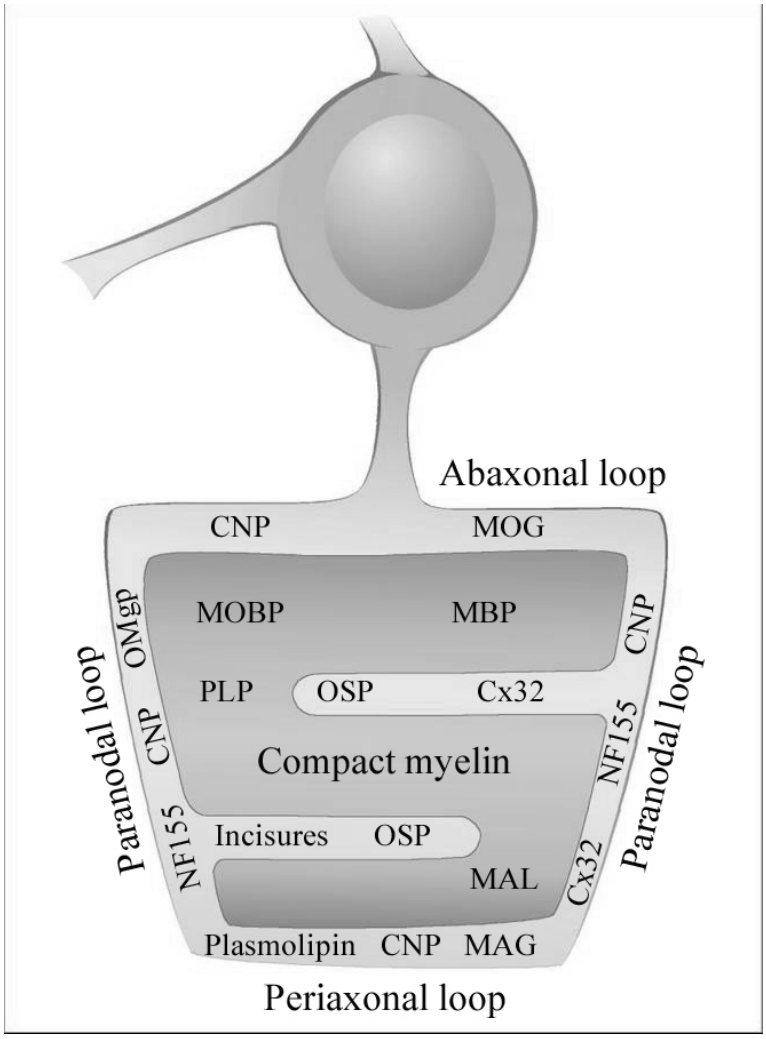
Abaxonal loop
CNP
MOG
MOBP
MBP
OMgp
CNP
Paranodal loop
PLP
OSP
Cx32
CNP
NF155
Compact myelin
NF155
Paranodal loop
Incisures
OSP
MAL
Cx32
Plasmolipin
CNP
MAG
Periaxonal loop

Figure 3

FIGURE LEGENDS

Figure 1: Schematic representation of the morphological and antigenic characteristics of cells of the oligodendroglial lineage differentiating from mitogenic progenitor cells to mature myelinating OLGs. Stage-specific markers are boxed. [Adapted from Baumann and Pham-Dinh, 2001; Maier et al., 2005]
GD3: ganglioside GD3; NG2: NG2 chondroitin sulfate proteoglycan with a core protein of 260 kDa; A2B5: A2B5 mAb recognizes several gangliosides such as GT3; O4: O4 mAb reacts with sulfatides and still unidentified glycolipids; RIP: receptor-interacting protein.

Figure 2: Schematic representation of a myelinating OLG.

Figure 3: The myelin sheath is segregated in different subdomains with unequal protein and lipid distribution. [Adapted from Kim and Pfeiffer, 1999; Krämer et al., 2001]