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On the biogenesis of the myelin sheath: Cognate polarized trafficking pathways in oligodendrocytes

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Oligodendrocytes, the myelinating cells of the central nervous system, are capable of transporting vast quantities of proteins and of lipids, in particular galactosphingolipids, to the myelin sheath. The sheath is continuous with the plasma membrane of the oligodendrocyte, but the composition of both membrane domains differs substantially. Given its high glycosphingolipid and cholesterol content the myelin sheath bears similarity to the lipid composition of the apical domain of a polarized cell. The question thus arises whether myelin components, like typical apical membrane proteins are transported by an apical-like trafficking mechanism to the sheath, involving a 'raft'-mediated mechanism. Indeed, the evidence indicates the presence of cognate apical and basolateral pathways in oligodendrocytes. However, all major myelin proteins do not participate in this pathway, and remarkably apical-like trafficking seems to be restricted to the oligodendrocyte cell body. In this review, we summarize the evidence on the existence of different trafficking pathways in the oligodendrocyte, and discuss possible mechanisms separating the oligodendrocyte's membrane domains.

Keywords: **oligodendrocyte, myelin, glycosphingolipids, membrane domain, rafts, differentiation**

Abbreviations: **OLG, oligodendrocyte; GSL, glycosphingolipid; GPI, glycosylphosphatidyl-inositol; CNS, central nervous system; TGN, trans-Golgi network; CGT, UDP-galactose: ceramide galactosyltransferase; GlcCer, glucosylceramide; GalCer, galactosylceramide.**

Introduction

The efficient and rapid propagation of pulse conduction along the neuronal axons in a saltatory manner requires axonal insulation. This is provided by a multilayered organization of a membrane, called myelin, which surrounds the axon. Abnormalities in myelin development or perturbation and degradation of its structure have severe pathological consequences, causing diseases such as multiple sclerosis and Pelizaeus-Merzbacher disease. The myelin membrane is a specialized but continuous extension of the plasma membrane of oligodendrocytes (OLGs), the myelinating cells of the central nervous system (CNS). During myelination, OLGs express in a coordinated manner large quantities of glycosphingolipids (GSL), in particular galactosylceramide (GalCer) and its sulfated derivative sulfatide, as well as several myelin-specific proteins, which are utilized for biogenesis of the myelin membrane. Thus, oligodendrocytes display a seemingly polarized phenotype in that the composition of the extending myelin membrane is dramatically different from the plasma membrane, bounding the cell body. This implies that during myelin biogenesis molecular sorting occurs, leading to the segregation of molecular entities that are directed towards either the myelin membrane or the plasma membrane of the cell body. Accordingly, interesting fundamental questions can be raised as to whether domain-specific trafficking exists in OLGs and how these cells maintain the physical segregation between both domains, given the apparent continuity that exists between myelin and plasma membrane. In light of these considerations, it is tempting to suggest an analogy with the apical and the basolateral membrane domains, as distinguished in polarized epithelial cells [1–5]. Also in neurons, different membrane domains can be distinguished, and the basolateral- and apical-like properties of dendrite and axon, respectively, have well been established [6]. A major difference in the lipid composition of both domains is that the apical domain displays a two-four fold higher level of GSL and a concomitant proportional decrease in the content of phospholipids. Such a typical apical lipid composition, i.e. a relatively high GSL and cholesterol content, combined with a low phospholipid ratio is also

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observed in CNS myelin [7]. It would seem reasonable, therefore, to consider the possibility that the plasma membrane domains of oligodendrocytes might be structured according to the same molecular and mechanistic principles as those governing the plasma membrane organization in epithelial cells. Typically, in the biosynthetic pathway sorting to these domains is occurring in the *trans*-Golgi network (TGN), involving vesicular transport of apical-specific proteins, clustered in sphingolipid/cholesterol microdomains, known as 'rafts'. Similarly, transport of basolateral proteins, sorted via signals that are primarily localized in their cytoplasmic tails which govern their interaction with adaptor complexes of specific composition at the TGN, is mediated via basolaterally directed vesicles [8,9]. At the level of the membrane domains *per se*, the segregation of their surface-specific components is maintained via tight junctions, preventing the randomization of outer leaflet components between basolateral and apical domains. Extrapolating these findings, to what extent are similar features and mechanisms playing a role in the biogenesis and maintenance of myelin and plasma membrane domains in oligodendrocytes? These issues will be briefly addressed in the present review.

The Myelin Membrane: Composition and Formation

The primary function of the OLG membrane is to provide the electrical isolation for the axons of the CNS. Only when this 'myelin jacket' is functionally present, saltatory conduction of electrical signals along the nerves is possible. Both the lipids and the proteins of the myelin membrane display a variety of important functions, including those in stabilizing the structure of myelin, acting as possible signaling receptors and regulating the differentiation of the OLG, and participating in glia-axon attachment.

The lipids comprise 70–80% of the dry weight of the myelin, and next to a major fraction of cholesterol, 25–30% of the lipid pool consists of the glycosphingolipids galactosylceramide (GalCer) and its sulfated derivative sulfatide. Specifically, it has been estimated that in the outer leaflet of the myelin membrane the molar ratio of GSL : cholesterol : phospholipid corresponds to 4 : 5 : 1, while in the inner leaflet this ratio is 0 : 3 : 7, respectively [7].

The quantitatively major myelin proteins are PLP (50% of total myelin protein) and MBP (30%), whose primary functions are to stabilize the apposed myelin membranes in compacted myelin, MBP in the intracellular, PLP in the extracellular space (Morell et al., 1994). Myelin-associated glycoprotein (MAG) is a member of the immuno-globulin superfamily, which is mainly localized to the periaxonal region [11]. MAG is thought to be involved in (co-)mediating the adhesion between OLG and axon, and in myelin formation, but its exact function remains elusive [12]. 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNP) is present in both the OLG's plasma membrane and in the non-compacted part of the sheath [13]. CNP appears to play an important role in early differentiation, in particular in the formation of the processes [14]. A host of additional minor proteins can also be detected in myelin, including myelin/oligodendrocyte glycoprotein (MOG), one of the potential antigenic determinants in multiple sclerosis. Furthermore, several GPI-anchored proteins, including the neural-cell adhesion molecule, NCAM, are also synthesized in OLGs [15]. Finally, a protein designated as MAL or VIP17 [16–18] is present in OLGs. Interestingly, in polarized epithelial cells (MDCK) cells, MAL/VIP17 has been shown to play an essential function in apical sorting and transport [19,20].

The biogenesis of the myelin sheath is a carefully regulated process, presumably involving a direct functional role of GalCer and sulfatide, triggered during development of progenitor cells into mature, fully differentiated OLGs

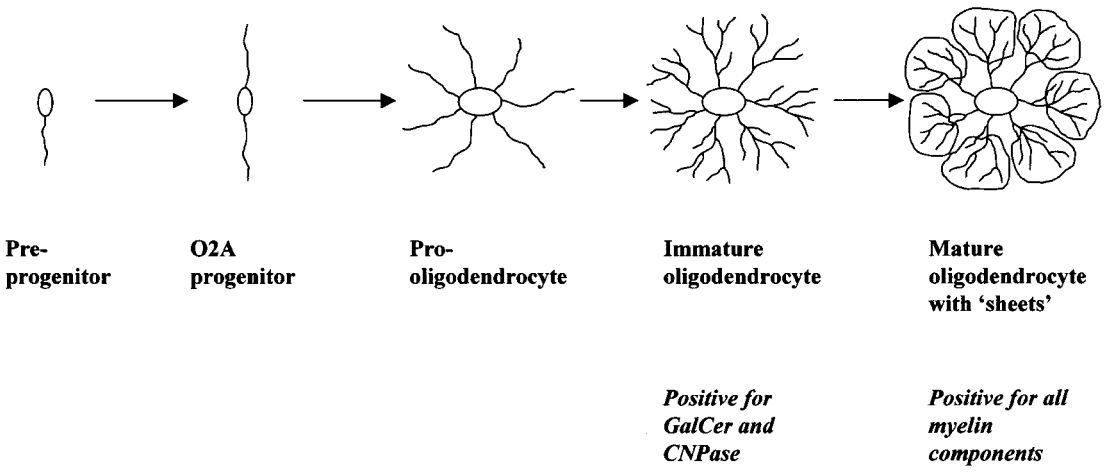

Figure 1. Simplified model for oligodendrocyte development. The typical morphological changes and the concomitant expression of cell-stage specific markers represent four major developmental stages. The model was adapted from Pfeiffer et al [24].

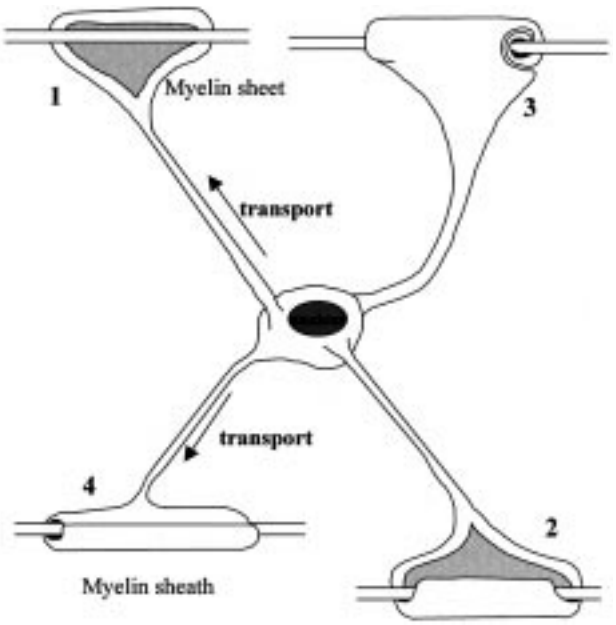

Figure 2. Schematic representation of a myelinating oligodendrocyte. An oligodendrocyte (center) will elaborate multiple processes, which produce large membrane sheets which are wrapped around axons several times. The compacted, multiple insulating myelin layers thus formed are separated by nodes of Ranvier. 1–4: different stages of myelin formation.

[21,22]. OLG lineage progression is typified morphologically by the sequential appearance of several broad processes, followed by the development of smaller, secondary and tertiary processes, while at the same time the expression of the myelin constituents is initiated. When axons are absent a vast, web-like structure is formed around the cell which contains all myelin components (the 'myelin *sheet*,' Figure 1). As soon as an axon is detected the secondary and tertiary processes, and myelin sheet retract [23], while only a few of the processes grow towards the axon and envelope it (Figure 2). All myelin components are delivered to the tip of these processes, which are concomitantly wrapped around the axon, thus leading to the typical stacked multilayered myelin membrane: the myelin *sheath*. In this process, the membrane is compacted, which in essence involves the extrusion of virtually all of the cytosol, resulting in the close apposition of inner and outer surface of the myelin membrane. As noted above, this membrane is stabilized by, among others, MBP and PLP. Although OLGs grown in monoculture obviously do not wrap myelin sheaths around axons, all myelin components are expressed in a coordinated fashion and transported to the sheets [24,25].

Galactosphingolipids are Essential Components of the Myelin Sheath

GalCer and sulfatide are both structural components of myelin, but are also involved in regulating the differentiation of OLGs. In mouse mutants, lacking both lipids due to a deficiency in ceramide : galactosyltransferase (CGT), OLG differentiation is enhanced, as reflected by an increase of late progenitors that differentiate into fully matured OLGs and implying that the presence of GalCer and/or sulfatide act as negative regulators in differentiation [22]. The underlying mechanism remains unclear, but a role of galactosphingolipids in affecting the orientation and/or lateral movement of signaling proteins in the outer leaflet of the plasma membrane, has been suggested. Possibly, for effective signaling, molecules like growth factor receptors and cell adhesion molecules require oligomerization, which could be driven by the clustering capacity of glycosphingolipids. In fact such domains have been proposed to function in the coupling of cell adhesion interactions with signaling [26]. Using CGT-knockout mice, referred to above, evidence has also been provided that the presence of GalCer is not necessary for myelin formation *per se*, but vital for postnatal development of the sheath [21,27–29]. Thus, after an initial phase of seemingly normal development, a few months after birth of the mice, myelin becomes abnormal in the sense that the myelin sheath is easily detached from the axon, in particular at the paranodal loops. This leads to partly demyelinated axons, as well as to overlapping sheaths. It appears that the galactocerebrosides are necessary for the formation of the transverse bands, structures through which the myelin sheath is attached to the axon at the paranodal loops. Thus, GalCer and sulfatide rather than myelin-specific proteins may play a major role in inter-cellular attachment, providing anchorage to the sheath when aligning along the axon. This implies that in galactolipid-deficient mutants, the proper interactions between the approaching membranes of myelin and axolemmal binding partners are not established [29]. The notion that antiGalCer may inhibit myelination both in vitro and in vivo is therefore likely related to indirect effects, possibly relying on a perturbation of the stability of the lipid's interaction with cell adhesion molecules like N-CAM and the cell adhesion molecule F3, i.e., complexes that likely play a role in myelin-axon interaction [15,30].

Interestingly, these observations underscore the vital role that glycosphingolipids play during development and differentiation, and that they are involved in crucial processes that trigger these events. In the CGT-knockout mice, a minor part of the ceramide pool available for GalCer and sulfatide synthesis is used for biosynthesis of GlcCer, normally present in minor amounts in OLGs, and sphingomyelin instead. However, whether and to what extent these lipids overtake functions of the galactolipids with respect to early developments in myelin assembly is unclear, but evidently, for appropriate functioning of myelin, this compensation does not suffice. Glycosphingolipid synthesis is critical for embryonic development and differentiation of certain tissues. The GSL are derived from GlcCer, the core structure of complex higher order GSL structures. In *Ugcg* knockout mice, lacking GlcCer synthase, the enzyme that encodes for glucosylceramide, embryogenesis is abruptly halted, leading

to apoptosis and embryonic death [31]. However, in vitro, a less strict dependence of cell differentiation on GSL synthesis was observed, as neuronal and erythroid differentiation can proceed in a seemingly unperturbed manner. In this case, however, the ceramide pool can become available for sphingomyelin biosynthesis, which largely overtakes at least some of the functions of glycosphingolipids, for example in raft-like intracellular transport [32; see below]. It is evident though, that GSL play crucial roles in a number of cellular events, which *in vivo* are vital for functional tissue differentiation and development, and embryonic/postnatal survival. In vitro, differentiation in the simpler culture systems requires less critical functions of GSL, allowing a wider spectrum for compensation of functional loss.

Interestingly, in myelination, the lethal consequences of a perturbation in the bio-synthesis of even such 'simple' GSL as GalCer and sulfatide, further highlights the crucial role of these particular sphingolipid species in myelin sheath stability.

Trafficking Pathways in Oligodendrocytes

GalCer is synthesized in the endoplasmic reticulum by UDP-galactose:ceramide galactosyltransferase (CGT) [33,34], and its expression becomes apparent during development of progenitor cells to immature OLGs, i.e., at an early stage of myelin biogenesis. A significant fraction of the newly synthesized glycosphingolipid is subsequently transported to the Golgi lumen, where it is sulfated by cerebroside sulfotransferase [35]. Given the onset of their appearance relative to cell and process development of the OLG lineage, the lipids are subsequently transported to the plasma membrane. Since both lipids have been found associated with (isolated) vesicles [36], it is reasonable to suggest that at least part of this transport step is accomplished by means of a vesicular mechanism. In fact, a priori it is not unlikely that the sulfatide is transported via vesicular transport, as sulfation occurs in the lumen of the Golgi. Whether a fraction of the GalCer pool may also reach the cell surface by monomeric transport, as observed for GlcCer [37,38], is unclear.

Given the typical similarity between the lipid composition of the myelin sheath and OLG cell body PM on the one hand, and the apical and basolateral membrane domains, respectively, on the other, it is tempting to consider intracellular OLG transport in terms of apical and basolateral transport, as defined in epithelial cells. In the latter cell type, the delivery of a major part of the apical proteins seems to rely upon their partitioning into lipid microdomains [1,4], the assembly mechanism of which is still largely unknown. These microdomains consist of sphingolipids (GSL and sphingomyelin) and cholesterol, that are insoluble in detergents at low temperature, which can be isolated by sucrose density gradient flotation, and are often referred to as DIGs (detergent-insoluble glycolipid domains), DRMs (detergent resistant membranes) or GSL-'rafts' [2,39]. They appear to be ubiquitously present within cellular membranes, likely coexisting with less ordered fluid domains. These DRM-located lipids show a higher degree of acyl chain saturation than total cell lipids and, in conjunction with cholesterol, favor partitioning in a liquid-ordered state, i.e., a condition in which the lipids are fluid, like in the liquid crystalline state, but their acyl chains display a substantial degree of lateral ordering, like in the gel state, thereby generating microdomains. Such patches may form platforms for numerous cellular events including membrane trafficking, signaling and cell adhesion. Cholesterol seems essential for microdomain formation as its depletion (60–70%) by cyclodextrin usually severely affects apical transport and abolishes detergent resistence [39]. Although glycosphingolipids can participate in the formation of such domains, these lipids, in spite of their natural strong tendency, dictated by their unique richness in hydrogen bond donors and acceptors, to engage in hydrogen bonding and thus cluster formation, neither act as driving force for domain formation, nor is their presence essential for maintaining domain stability. This was demonstrated in the MEB-4 melanoma cell line and its GSL-deficient derivative, GM-95 [32]. In both cell types raft-mediated trafficking of distinct raft-associated proteins was kinetically indistinguishable. In fact, it turned out that SM, by biosynthetic upregulation in the mutant cell line, essentially overtakes the role of the glycosphingolipids. As will be further discussed below, also in neurons, a similar phenomenon has been demonstrated, as the kinetics of the biogenesis of rafts is concomitant with that of SM biosynthesis, while the levels of GlcCer, the precursor of complex GSL, and cholesterol remain largely unchanged, i.e., prior to and after (the developmentally regulated) formation of the rafts [40]. The data suggest that a critical cholesterol/SM ratio may suffice for assembly into rafts.

Galactocerebrosides and Raft-mediated Transport

The question thus arises as to whether GalCer and sulfatide, as major sphingolipids in myelin may participate in raft(-like) assembly. Using GalCer, tagged with a fluorophore that shifts its emission maximum in a lipid-concentration dependent manner (see below), it has been shown that also this lipid can be captured into cholesterol-enriched microdomains, its flow being regulated in a cholesterol-dependent manner [41]. Moreover, prevailing evidence, based among others upon detergent-extractions at low temperature, indeed reveals that GalCer and sulfatide are present in (raft-like) patches in the OLG's myelin sheet [15,22,25]. Obviously, if proteins partition in these rafts, it is also possible that they can be co-transported with the lipids. Therefore, assuming that GalCer and sulfatide as major GSL would constitute the core of rafts in OLG, it would be anticipated that inhibition of CGT would perturb the transport of myelin proteins in cultured OLGs, and, consequently, lead to malfunctioning of myelin biogenesis and/or assembly. Neither appears to be the case. Thus, it has been

shown that PLP transport to the sheet does not require synthesis of sulfatide or GalCer [42]. Furthermore, neither were PLP and GSL, at any stage during OLG development, colocalizing in the Triton-X100-insoluble cell fraction. Moreover, entirely consistent with these observations is the recent demonstration that the galactolipids are in principle not necessary for formation of a myelin sheet [22] or sheath [21,43]. Although it is possible that GlcCer and SM could have partly overtaken the role of the galactolipids, compensating for their diminished biosynthesis, the absence of PLP in detergent-resistant fractions is still indicative of a raft(-like) independent trafficking pathway of this protein into the myelin sheet. Next to PLP, MAG, CNP and MBP are also directed to the myelin sheet. For MBP it is well known that its traffic is an exception in that instead of the protein, it is the mRNA that is transported as part of a ribosomal complex along the cytoskeleton into the processes and the sheet [44]. In this way, MBP is translated near its site of destination, and becomes locally inserted into the myelin membrane. However, like PLP, CNP, MAG and MOG are synthesized in the cell body and subsequently transported to the myelin membrane, and as observed for PLP [42], we (de Vries et al., in preparation) and others [15] found no evidence for a 'raft-like' transport of either protein, based upon criteria of detergent-solubility resistence. Hence, these results demonstrate that these myelin proteins are neither transported to nor present in a detergent-insoluble GSL-containing microenvironment at any time during myelin biogenesis. Yet, by exploiting the observation that distinct enveloped viruses preferentially bud from either the apical or basolateral domain in polarized cells, typical apical- and basolateral-like pathways have been revealed in OLGs. Thus, in MDCK cells influenza virus (marked by its major spike protein, hemagglutinin HA) buds from the apical surface, whereas vesicular stomatitus virus (major envelope protein G) acquires its envelope by budding through the basolateral plasma membrane [1]. When expressed in OLGs, the plasma membrane is reached by HA, while the sheet appears to be the exclusive target of VSV G protein [Figure 3; 45]. Consistently, detergent extraction revealed that, in OLGs, VSVG is soluble at low temperature, whereas HA is detergent-insoluble at low temperature. These data demonstrate that in the OLG a cognate basolateral-like pathway, rather than an apical-like one is used to transport proteins to the myelin sheet. This is entirely in line with the data that the major myelin specific proteins, including PLP, CNP and MAG, do not exploit a GSL-dependent i.e., raft-like route towards the myelin sheet. Nevertheless, evidence has been presented which demonstrates the presence of various GPI-linked proteins, including NCAM and F3, in detergent-insoluble structures, isolated from mature OLGs and isolated myelin [15]. However, the ratio of the DIG-localized GPI-proteins found in common in either fraction differed, as well as their density and morphological appearance. One of the GPI-linked molecules found in these fractions is NCAM, an adhesion molecule that is also present in neural cells. The 120-kDa isoform is GPI-linked, as opposed to the 140 and 180-kDa forms. As expected, the latter forms are soluble in detergent, whereas the 120-kDa isoform is not. Nevertheless, both forms apparently end up in the myelin sheath in vivo, and also in mature OLGs in vitro.

Taken together, in the context of the delivery of most major myelin proteins according to a largely non-raft type mechanisms, it is not clear therefore (i) to what extent the myelin-associated GPI-linked proteins reach the myelin sheath by raft-like trafficking, originating from the cell body, (ii) whether their assembly into rafts is at all a prerequisite for myelin-directed sorting, and (iii) whether raft or microdomain formation into detergent-insoluble complexes may have occurred after delivery to the sheath. However, if both raft-mediated and raft-independent trafficking to the sheath may take place, then the intriguing question remains why HA, which like GPI-linked proteins partitions into rafts, does not

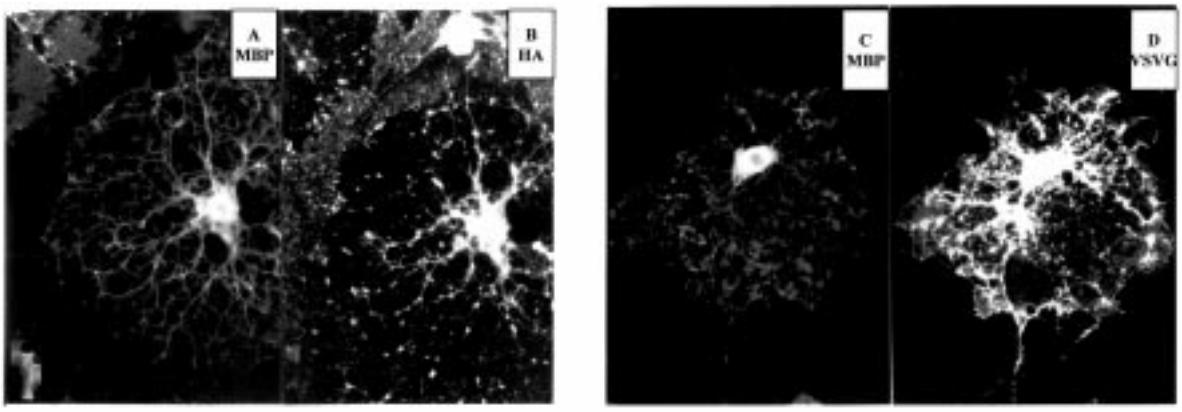

Figure 3. Influenza HA and VSV G protein are localized differentially in infected mature, sheet-forming oligodendrocytes. The respective viral proteins were co-stained with MBP, as an indicator of the myelin sheet. HA (panel B) is excluded from the sheet, whereas VSV G (panel D) is present in the sheet in high concentration (Figure adapted from [45]).

reach the myelin sheath. Evidently, further work is required to investigate these issues which are of major importance to fundamental cell biology.

Proteins Involved in Polarized Trafficking

Lafont et al. [19], using MDCK cells, have shown the involvement of syntaxin-3 and its v-SNARE, VAMP7, in apical transport. Similarly, OLG express SNARE proteins, including the SNARE complex VAMP-2/syntaxin-4 [46], which is involved in basolateral trafficking in epithelial cells [4]. In the same study, the apical tSNARE, syntaxin-3, could not be detected, neither at the protein nor at the mRNA level. However, in immunocytochemical studies of the syntaxins 2, 3 and 4 in cultured OLGs, we obtained unambiguous evidence of the presence of these tSNAREs (De Vries et al, manuscr. in prep.). Moreover, their localization appears site-specific: the basolateral-specific syntaxin-4 is localized to the myelin sheet, whereas syntaxin-3—although present at a minor concentration—remains in the cell body. Syntaxin-2, which does not appear to express a polarity-dependent preference in epithelial cells [4], is found in both the cell body and the sheet. In the context of polarity-dependent trafficking, these data, in conjunction with the characteristics of trafficking described in the foregoing, would suggest that the myelin sheath represents a cellular membrane compartment that is not principally served by the apical cognate pathway, but rather by a pathway that displays basolateral-like features, the apical-like characteristics being restricted to trafficking directed toward the plasma membrane of the cell body. Furthermore, in spite of the compactness of myelin, the presence of syntaxins suggests that vesicular transport may occur within the sheath.

As mentioned above, VIP17 or MAL protein is another important protein, found in OLGs as well as in many other cell types. It is a small hydrophobic tetraspan protein, which has recently been shown to be indispensable for apical transport [20]. It has been proposed that this protein is a component of the protein machinery responsible for the sorting and transport of TGN-derived apical proteins [20,47]. Thus, down-regulation of its expression by means of an antisense technique impairs the targeting of proteins to the apicial membrane, whereas overexpression increases apical delivery and seemingly expands the apical surface. No effect was seen on basolateral trafficking. Interestingly, the protein is expressed during differentiation of OLGs, concomitant with an increase in myelin production [17,18]. However, since the MAL protein is localized in DIGs, while (i) rafts do not appear to mediate myelin-directed trafficking of the major myelin-specific proteins, and (ii) modulation of MAL expression does not affect basolateral trafficking, a direct involvement of MAL in myelin biogenesis can be excluded. Rather, the data support the view that the protein supports a function in the assembly of the OLG plasma membrane, which is consistent with the cognate apical-like pathway directed to this membrane domain. It would be of interest, therefore, to study the fate of this protein in cultured OLGs.

Transport During Oligodendrocyte Differentiation

The OLG is the final stage of a developmental lineage progression, starting at a pre-progenitor stage, through the 'O2A' progenitor, the pro-OLG and immature OLG (see Fig. 1). These stages have been characterized by several criteria, such as antigenic markers and morphology. The earliest stage at which a myelin protein, CNP, is synthesized is that of the immature OLG, which, morphologically, only shows the appearance of primary and secondary processes. Only in mature OLGs the full complement of myelin proteins is synthesized, which requires a coordinated transport of proteins and lipids to the sheet. It is therefore logical to anticipate a tight regulation of the expression of the pathways involved in myelin transport. Indeed, several studies indicate that transport is developmentally regulated, as revealed by a specific upregulation of several small GTPases, rabs 3a and 8a, which are intimately involved in the regulation of intracellular trafficking [46,48]. Rab3a is probably involved in docking and fusion in secretory events, and may be involved in docking at the myelin sheet in OLGs. Rab8a is participating in Golgi-to-basolateral membrane transport in epithelial and neuronal cells, and therefore the up-regulation in OLGs seems to perfectly match the involvement in sheet-directed transport.

Not surprisingly, the various NCAM isoforms that may play a role in neuron-OLG interaction are also developmentally regulated [15], as indicated above. In precursor cells, GPI-anchored proteins, among which NCAM-120, are not incorporated into raft-like complexes, since raft assembly only becomes apparent upon maturation of the cells. Also, upon maturation of OLGs, there is a marked increase in the synthesis of GalCer and sulfatide [24,49]. Using fluorescent analogues of the glycosphingolipids lactosylceramide and sulfatide, (*N*-{5-(5,7-dimethylborondipyrromethenedifluoride)-1-pentanoyl}-D-lactosylsphingosine (BODIPY-LacCer) and BODIPY-sulfatide, the sorting capacity of the OLG membrane was demonstrated in that during differentiation LacCer is largely retained at the cell surface, while sulfatide is internalized by endocytosis. In the same study it was shown that partitioning of the fluorescent analogues in the plasma membrane as accomplished by exogenous addition, dramatically decreased when the cells matured, implying that the physicochemical properties of the OLG membranes changed remarkably during development, although the nature of these changes remains unknown. Since BODIPY shifts its fluorescence emission maximum from green to red with increasing concentration, a preferential accumulation of sulfatide but not LacCer could be demonstrated to occur in the endosomal population. Although the amount of internalized sulfatide remained fairly constant during differentiation, that of LacCer decreased, implying a relative enrichment of sulfatide when differentiation progressed. Whether these differences reflect

selective internalization of specific domains remains to be determined. However, changes in the composition of local domains, internalized by endocytosis during differentiation seems likely. Nevertheless, it is unclear how these events relate to the biogenesis of the sheet.

Interestingly, as in developing neurons [40], the pool of GlcCer remains relatively constant during differentiation [49]. Similarly, in both developing neurons [40] and developing OLGs [15], rafts are virtually absent, and remarkably, also in both cell types, the level of glucosylceramide is more or less constant during development. The same also holds for the cholesterol level in neurons. However, during neuron development the amount of SM dramatically increased with the concomitant appearance of rafts and raft-mediated sorting of axonally located lipids and proteins. Interestingly, when the pool of SM is artificially increased in neurons in early development, the cells adopt the sorting machinery seen in matured neurons, implying that an enhancement in availability of SM suffices to trigger the apical, i.e. axon-directed traffic machinery. These data imply that a critical amount of SM and presumably, a correct ratio of SM and cholesterol are essential for functional raft assembly, since, in general, extraction of cholesterol with cyclodextrin eliminates raft stability. Thus sphingomyelin and cholesterol homeostasis may well be regulating factors in raft assembly and stability and hence govern a number of functions in which rafts are thought to participate, including sorting, traffic, signaling and attachment. It will be of interest to determine whether developmentally regulated changes in SM biosynthesis may analogously trigger functional raft assembly and accompanying sorting principles in OLG differentiation. In this respect it is finally of interest to note that in influenza-infected O2A pro-OLGs HA accumulates just underneath the plasma membrane [50], whereas in mature OLGs HA reaches the plasma membrane [45]. However, in the progenitor cells, VSV G is uniformly present at the cell surface, implying that the cognate basolateral pathway operates in early OLG development, while in case of the cognate apical pathway, docking seems impaired. Whether HA was transported in 'rafts' was not determined, but seems unlikely [50]. However, the pathway is clearly different from that of VSV G, since the former was strongly stimulated by protein kinase C activation, while VSV G trafficking was not affected.

Searching for Tight Junctions

In polarized cells, physical separation is achieved by barrier-like structures between the apical and basolateral membranes, called tight junctions (for a review see Stevenson and Keon [51]). In neurons, the proteins with axonal destination are served by an apical pathway, in which case the axonal hillock seems to function as such a barrier [3]. However, in OLGs tight junctions have not been demonstrated thus far, although a tight junction protein, claudin-11/oligodendrocyte-specific protein has been detected in the myelin sheath [52,53]. Also ZO-1, another well-established tight junction protein can be demonstrated in cultured OLGs (De Vries et al., manuscript in prep.). However, the distribution of this protein over the plasma membrane was not clusterlike but homogeneous, indicating that, although present in OLG, tight junctions *per se* are not. The question thus arises which kind of mechanism(s) is operating in OLG to act as a fence in separating the plasma membrane and myelin membrane so as to prevent randomization of the specific proteins and lipids present in either membrane domain.

Besides the presence of an actual molecular barrier, it is also possible that the physical nature of the membrane domains that interconnect plasma and myelin membrane may provide a 'device' for maintaining distinct membrane compartments. Thus local domains of different membrane fluidity and ordering could provide such a function, and distinct proteins or lipids could be selectively excluded or preferentially included in such domains. Such a mechanism would bear analogy to a mechanism that has been proposed for the sorting into either a recycling or a degradation pathway during receptor-mediated endocytosis [54]. This concept was based upon the notion that various diI derivatives, containing either saturated and unsaturated acyl chains, whose preferential partioning into either fluid or solid membrane domains had been determined from model studies, may enter distinct pathways. Entry into either a recycling or an endosomal/lysosomal pathway was proposed to be dictated by fluidity and shape dependent preferences of the lipid probes, governing their partitioning into more or less ordered domains. It has been suggested that access to these domains is determined by different fluidity barriers, provided by the strongly curved protrusion of this compartment. Such a fluidity barrier could exist between plasma membrane and the sphingolipid/cholesterol enriched (outer leaflet) myelin.

Interestingly, such a barrier could potentially be directly involved in sorting as well, during biogenesis of the sheet, implying a selective retention of non-myelin constituents to cross the barrier. Yet, sorting can be similarly accomplished by vesicular trafficking departing from the TGN and subsequently targeted to either the sheet or the plasma membrane. In principle, however, it would appear that lipid sorting may take place at any cellular site where vesicular trafficking might originate. In fact, also at the plasma membrane such steps may occur as revealed by the preferential endocytic processing of sulfatide but not LacCer, implying the existence of domains and preferential partioning of lipids in such domains, also on the surface of OLGs [49]. This partitioning is not exclusively determined by acyl-chain dependent factors. Rather, also headgroup-dependent characteristics are relevant, since sphingolipid analogs differing only in headgroup structure, i.e., GlcCer versus GalCer, are sorted in HepG2 cells from the subapical compartment to the apical and basolateral membrane, respectively [55]. In this context, it is finally interesting to note the similarity in cognate basolateral trafficking of

GalCer to the myelin sheath in OLGs and the basolateral directed trafficking of a major fraction of the pool of GalCer in both HepG2 [55] and polarized MDCK cells [56].

Conclusions and Future Prospects

From a biochemical and morphological point of view, the oligodendrocyte meets a variety of criteria that would designate it as a cell that displays membrane polarity. However, it is equally clear that this 'polarity' does not match and/or reflect the typical features observed in polarized cells like epithelial cells and hepatocytes. Typical tight junctions or a microvilli-enriched apical membrane domain are not apparent. Moreover, essentially all of the major myelin proteins reach the galactolipid/cholesterol enriched sheet according to a raft-independent mechanism, a feature typically associated with apical membrane-directed trafficking in epithelial cells. In fact, in oligodendrocytes such characteristics appear associated with trafficking towards the plasma membrane of the cell body, although the fact that GPI-linked proteins, present in detergent-resistant fractions from myelin, merit further investigation as to the role of raft-like, sheath-directed trafficking in OLGs. In this context it will also be of relevance to carefully investigate the kinetics and significance of distinct lipids, such as glucosylceramide, sphingomyelin and cholesterol, in OLG raft assembly. By the same token, the presence, location and functionality of membrane domain specific SNARES like the syntaxins, requires further investigation.

In several respects, the similarity between polarity and raft-mediated trafficking as well as the genesis of these events in oligodendrocytes and neurons is striking. In that regard, it is anticipated that knowledge and insight obtained in either cell system will be of fundamental significance for the advancement of our understanding of polarity development and polarity-related trafficking.

Finally, an accurate insight in and description of the transport processes used by the oligodendrocyte, as well as of their control by inter- and extracellular signaling compounds is of great importance for the understanding of the myelination process. Ultimately, this knowledge may contribute to the development of therapies that promote remyelination in CNS tissue that has suffered from demyelination, as is the case in multiple sclerosis.

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