

Chapter 12

Role of Galactosylceramide and Sulfatide in Oligodendrocytes and CNS Myelin: Formation of a Glycosynapse

Joan M. Boggs

Abstract The two major glycosphingolipids of myelin, galactosylceramide (GalC) and sulfatide (SGC), interact with each other by *trans* carbohydrate–carbohydrate interactions in vitro. They face each other in the apposed extracellular surfaces of the multilayered myelin sheath produced by oligodendrocytes and could also contact each other between apposed oligodendrocyte processes. Multivalent galactose and sulfated galactose, in the form of GalC/SGC-containing liposomes or silica nanoparticles conjugated to galactose and galactose-3-sulfate, interact with GalC and SGC in the membrane sheets of oligodendrocytes in culture. This interaction causes transmembrane signaling, loss of the cytoskeleton and clustering of membrane domains, similar to the effects of cross-linking by anti-GalC and anti-SGC antibodies. These effects suggest that GalC and SGC could participate in glycosynapses, similar to neural synapses or the immunological synapse, between GSL-enriched membrane domains in apposed oligodendrocyte membranes or extracellular surfaces of mature myelin. Formation of such glycosynapses in vivo would be important for myelination and/or oligodendrocyte/myelin function.

Keywords Carbohydrate–carbohydrate interactions • Glycosphingolipids • Membrane domains • Membrane rafts • Cytoskeleton • Signaling • Actin • Microtubules • Myelin basic protein • Silica nanoparticles • Liposomes

J.M. Boggs (✉)

Molecular Structure and Function Program, Research Institute, Hospital for Sick Children, 686 Bay St., Toronto, ON, Canada M5G 0A4

Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada M5G 1L5

e-mail: jmboggs@sickkids.ca

Abbreviations

Ab	Antibody
CGT	UDP-galactose:ceramide galactosyltransferase
CM-DiI	Cell tracker lipophilic red fluorescent dye
CNP	2',3'-Cyclic nucleotide 3'-phosphodiesterase
CST	Galactosylceramide 3'-sulfotransferase
DIGs	Detergent-insoluble glycosphingolipid-enriched membrane domains
FA2H	Fatty acid 2-hydroxylase
Gal-BSA	Galactose conjugated to bovine serum albumin
GalC	Galactosylceramide
GlcC	Glucosylceramide
Glyco-nanoparticles	Where glyco=Gal, S-Gal, Glc, Man, silica nanoparticles conjugated to galactose, galactose-3-sulfate, glucose, or mannose
GPI	Glycosylphosphatidylinositol
GSLs	Glycosphingolipids
HFA	Hydroxy fatty acid form of GSL
KO	Gene knockout
LacC	Lactosylceramide
MAG	Myelin-associated glycoprotein
MAPK	Mitogen activated protein kinase (p42 and p44, 42 and 44 kDa isoforms)
MBP	Myelin basic protein
MCT	Lactate transporter
MGDG	Monogalactosyldiglyceride
MOG	Myelin/oligodendrocyte glycoprotein
NFA	Nonhydroxy fatty acid form of GSL
NMDA	<i>N</i> -methyl-D-aspartate
OLG	Oligodendrocyte
PLP	Proteolipid protein
SGC	Sulfatide sulfated form of GalC, galactosylceramide I ³ -sulfate
SGG	Seminolipid sulfogalactosyldiglyceride, 3-sulfated form of MGDG
SM	Sphingomyelin

12.1 Requirement for Myelin Glycosphingolipids for Maintenance of the Myelin Sheath

Oligodendrocytes (OLGs), the cells that form myelin in the central nervous system (CNS), contain large amounts of the simple glycosphingolipids (GSLs), galactosylceramide (GalC) and sulfatide (SGC) (the sulfated form of GalC,

galactosylceramide I³-sulfate). Myelin is especially enriched in these two GSLs. GalC is 23 wt% and SGC is 4 wt% of the total lipid of myelin (Norton 1977). Myelin also contains low concentrations of two glyco-glycerolipids with the same sugar head groups, monogalactosyldiglyceride (MGDG) and sulfogalactosyldiglyceride (SGG). Their synthesis peaks at the time of most rapid myelination in the rat, and in cultured OLGs at the time of formation of membrane sheets, suggesting a role in myelin formation (Ishizuka and Inomata 1979; Pieringer et al. 1977; Burgisser et al. 1988; Shimomura and Kishimoto 1984).

The galacto and sulfated galactolipids are not essential for formation of myelin, but are essential for its maintenance and stability, to maintain axonal health, and for survival of the animal. Their absence, due to knocking out the genes encoding the enzymes that are necessary for their synthesis, uridine diphosphate-galactose:ceramide galactosyltransferase (CGT) and galactosylceramide 3'-sulfotransferase (CST), causes a more severe phenotype (Coetzee et al. 1996; Bosio et al. 1996; Honke et al. 2002) than knocking out the genes for several myelin-specific proteins (proteins that are much more abundant in myelin than other tissues) including proteolipid protein (PLP) (Boison and Stoffel 1994; Rosenbluth et al. 2009), which is the most abundant protein of myelin and 15 % of the mass of myelin, myelin-associated glycoprotein (MAG) (Roder 1994; Li et al. 1994), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) (Edgar et al. 2009), and others. The only structural protein known to be even more essential for myelin formation and similarly essential for survival is myelin basic protein (MBP), which is the second most abundant protein in myelin (Readhead et al. 1990).

In the CGT and CST mutants, compact myelin is formed and is almost as thick as normal myelin. However, interaction of the paranodal loops with the axon is abnormal (Marcus et al. 2002, 2006; Hirahara et al. 2004). Delocalization of axolemmal proteins occurs around the node and paranode in these mutants (Marcus et al. 2002). The CGT-null mice have a more severe clinical phenotype than the CST-null mice, with nerve conduction deficits, paralysis, extensive myelin vacuolation and splitting at the intraperiod line, and early death. With age in the CST knock-out (KO), the nodal structure also deteriorates, the amount of cytoplasm in myelin increases, and myelin vacuolar degeneration occurs (Marcus et al. 2006). Terminal differentiation of OLGs from the CST KO is enhanced *in vitro* and *in vivo*, suggesting that SGC is a negative regulator of OLG differentiation (Hirahara et al. 2004; Bansal and Pfeiffer 1989; Bansal et al. 1999). The number of terminally differentiated OLGs is also increased *in vivo* and this increase persists into adulthood in CST-null mice, due to increased proliferation and decreased apoptosis (Shroff et al. 2009; Honke 2013). In addition to changes observed in the CNS of the CST KO mouse, the number of Schmidt-Lanterman incisures was remarkably increased in the peripheral nervous system (PNS) (Hoshi et al. 2007).

Myelin galactosphingolipids contain either hydroxy fatty acids (HFA) or non-hydroxy fatty acids (NFA). The fatty acid 2-hydroxylase (FA2H) gene responsible for synthesis of hydroxy fatty acids is highly enriched in the brain, especially in OLGs (Edvardson et al. 2008). The myelin produced in the CGT mutant contains the hydroxy-fatty acid form of glucosylceramide (HFA-Glc) (normally absent in OLGs) instead of GalC and SGC, and also increased ceramide and sphingomyelin

(SM) levels (Coetzee et al. 1996; Bosio et al. 1996). However, elimination of HFA-GlcC by knockout of the gene for uridine diphosphate-glucose:ceramide glucosyltransferase, targeted to OLGs in the CGT mutant, had no significant effect on phenotype, indicating that the HFA-GlcC produced does not compensate for the loss of GalC and SGC (Saadat et al. 2010).

Indeed, elimination of the HFA forms of myelin GSLs, by knocking out the fatty acid 2-hydroxylase (FA2H) gene, had little effect in young animals, although NFA-GalC was also decreased in amount (Zoller et al. 2008; Potter et al. 2011). Compact myelin formed and appeared normal and there was no significant effect on nerve conduction velocity, although some behavioral changes occurred. However, aged mice (18 months) had scattered axonal and myelin sheath degeneration in the spinal cord and even more pronounced loss of myelin sheaths in sciatic nerve, but not in the brain, indicating impairment of long-term maintenance of myelin. Progressive hind limb paralysis occurred at 22 months. These changes also occurred in mice in which FA2H was knocked out only in OLGs and Schwann cells (Fa2hflox/flox Cnp1-Cre mice) (Potter et al. 2011). Axonal degeneration started before myelin degeneration suggesting that the OLG/myelin HFA-GSLs were required for glial support of axonal function rather than myelin formation. The ratio of HFA to NFA-SGC increases in the brain with age (Shimomura and Kishimoto 1983). Mice over-expressing CGT had unstable myelin with progressive demyelination and a decreased ratio of HFA- to NFA-GalC (Fewou et al. 2005), suggesting that the HFA–NFA ratio may be important for myelin maintenance. However, MGDG was also increased in these mice and might also contribute to the myelin instability.

Several mutations of FA2H have been found in humans that cause decreased synthesis of the HFA-species of myelin GSLs, resulting in leukodystrophy and spastic paraplegia, with severity depending on the site of the mutation (Edvardson et al. 2008; Dick et al. 2010). These mutations affected only the CNS (contrary to the mouse) suggesting an additional FA2H is present in the PNS in humans. Compact myelin was formed initially in the CNS, but with age, thinning of the corpus callosum and the pons, as well as volume depletion of the cerebellum occurred, suggestive of demyelination, as found in the mouse. This is the first identified deficiency in the lipid composition of myelin that results in disease in humans.

If both CGT and FA2H were knocked out in the mouse, the mice lacked Sulf, GalC, HFA-GlcC, and HFA-SM. The amount of NFA-GlcC was also reduced and NFA-SM was significantly increased. However, the mice still formed compact myelin initially and there was no obvious phenotypic difference from the CGT KO (Meixner et al. 2011).

Myelin GSLs have primarily long chain C24:0 and 24:1 fatty acids (both HFA and NFA species). Knockout of ceramide synthase 2, which uses C22–24 acyl chain CoAs to synthesize the long fatty acid chain forms of ceramide, caused reduced levels of GalC, especially long chain NFA and HFA forms, in myelin, resulting in myelin degeneration and detachment at 5 months. The HFA-18:0-GalC species was elevated instead (Ben-David et al. 2011). Thus, fatty acid chain length in addition to the HFA–NFA ratio and carbohydrate moiety of GSLs is important for myelin maintenance.

The importance of glycolipids in formation of compact myelin has also been revealed by phylogenetic studies. Earthworm myelin, with only traces of glycolipids, and shrimp myelin with only GlcC, have a loosely wrapped, noncompacted form of myelin (Okamura et al. 1985; Kishimoto 1986). The space at the extracellular apposition of myelin teleost and higher vertebrates (Inouye and Kirschner 1990), correlates inversely with their proportions of galactolipids (Selivonchick and Roots 1976; Burgisser et al. 1986). In addition, a phylogenetically lower order of deep sea fish, the Gadiformes, whose myelin has an unusual glycolipid composition, including GlcC and its fatty acid ester, and galactoglycerolipids rather than galactosphingolipids, have thin loosely compacted myelin compared to more advanced species of fish (Tamai et al. 1992).

These studies indicate that galactolipids, especially galactosphingolipids, are necessary for maintenance of compact myelin structure with age, although they are not necessary for the process of myelination. They, including their HFA and long chain fatty acid species, are also necessary within compact myelin to sustain the myelinated axon and prevent neurodegeneration, as found also for myelin proteins that are not necessary for formation of compact myelin, such as PLP and MAG, but are necessary to prevent neurodegeneration with age.

12.2 Functions of Myelin Glycosphingolipids

The special properties of GSLs, and sphingolipids in general, could allow them to perform a number of roles in OLGs and myelin (Jackman et al. 2009). GSLs have longer, more saturated fatty acids than phospholipids, and can also participate in an intermolecular hydrogen bonding network (Boggs 1987; Boggs et al. 1984), which causes them to form more ordered membrane domains or rafts that may be involved in protein trafficking and signaling (Gielen et al. 2006). The hydroxyl group on the HFA species can also participate in intermolecular hydrogen bonding interactions (Boggs et al. 1984), and contribute to ordering and membrane domain formation. Interestingly, in the FA2H KO, the ratio of saturated 24:0 to unsaturated 24:1 species of Sulf and GalC was increased (Zoller et al. 2008), which might partially compensate for the loss of HFA species and help maintain ordered membrane domains.

The absence of some paranodal proteins in the CGT/CST mutant may be partly due to altered trafficking of these proteins to the plasma membrane resulting in abnormal paranodal loop formation. Indeed, the content of a number of proteins involved in regulation of cytoskeletal dynamics, energy metabolism, vesicular trafficking, or adhesion was altered, with some being increased and others decreased, in the myelin from the CGT and CST mutants (Fewou et al. 2010). Some proteins that were decreased in myelin were increased in the OLG cell body, suggesting transport from the OLG cell bodies into myelin may be differentially dysregulated in the absence of these GSLs. Similar loss of a lactate transporter (MCT) may occur in spermatocytes in the CST mutant due to impaired trafficking, since CST is also

responsible for sulfation of SGG (also called seminolipid) in spermatocytes (Honke 2013). However, the increase in SM in the CGT/CST KO's may partially compensate for the loss of GSLs in raft formation and protein trafficking. The CGT/FA2H double knockout had significantly increased NFA-SM and still formed low density CHAPS-insoluble membrane domains (Meixner et al. 2011).

These GSLs, especially sulfatide, could also interact with various protein ligands. SGC has been described as a multifunctional lipid (Honke 2013). It interacts with a variety of cell adhesion molecules including fibrinogen, von Willebrand factor, P-selectin, thrombospondin and laminin and could interact with extracellular matrix proteins in the CNS. Impaired interaction of SGC with axonal proteins has been suggested to be involved in altered paranodal loop formation in the CGT/CST KO's. SGC, but not GalC, is one of the constituents in myelin responsible for myelin inhibition of CNS axonal outgrowth further suggestive of its interaction with an axonal constituent (Winzler et al. 2011).

GSLs also organize specific proteins into signaling complexes. GalC is concentrated with cholesterol, GM1, the raft marker flotillin, caveolin, GPI-linked proteins, and kinases (Simons et al. 2000; Arvanitis et al. 2005; DeBruin and Harauz 2007; Gielen et al. 2006; Fitzner et al. 2006; Dupree and Pomicter 2010) in low density detergent-insoluble GSL-enriched fractions (DIGs), believed to come from membrane domains or rafts in OLG and myelin membranes. These DIGs from myelin also contain SGC but are not enriched in it compared to myelin (Arvanitis et al. 2005). They also contain some myelin proteins including MBP, which is a peripheral membrane protein bound to the cytoplasmic side of the membrane, and phosphorylated MBP (Arvanitis et al. 2005; DeBruin and Harauz 2007). The GalC and SGC in these membrane domains can also behave as receptors and receive extracellular signals that are then transmitted to the cytosol.

There is much evidence that GSLs and raft proteins are receptors for extracellular signals as indicated by the ability of antibodies to these constituents to trigger signaling. Antibodies, especially IgM antibodies, or IgG antibodies after cross-linking by second antibodies, or ligands such as cholera toxin B (for GM1), cause cross-linking of raft constituents and clustering of rafts, which may bring different signaling molecules in different raft domains into contact with each other and concentrate them into one domain (Sonnino et al. 2009; Harder and Simons 1999; Iwabuchi et al. 1998; Prinetti et al. 1999). These antibodies mimic natural ligands that are multivalent, such as agrin, which cross-links the acetylcholine receptor and is essential for neural synapse formation (Khan et al. 2001). Galectins, which bind Gal-GlcNAc branches of N-glycans of glycoproteins, are another type of multivalent ligand that can cross-link receptors in cells and regulate receptor signaling (Lajoie et al. 2009). Other multivalent interactions with cell receptors are due to *trans* interaction with a membrane domain in another cell, which allows for interactions between a number of constituents in the apposed membrane domains. This creates a multi-molecular complex with a multivalent cross-linking effect, such as the T cell receptor complex interacting with MHC proteins on antigen presenting cells, forming the immunological synapse (Vogt et al. 2002). Interestingly, agrin is also expressed in lymphocytes, and reorganizes lipid rafts in T cells and sets the

threshold for T cell signaling (Khan et al. 2001). The immunological synapse is an example of an intermembrane intercellular interaction that triggers signaling in one or both cells. Similar synaptic interactions may occur in multilayered compact myelin, in which the extracellular surfaces face each other and are separated only by a small distance, and between the paranodal loops and the axon.

12.3 Involvement of OLG/Myelin GSLs in Signaling

Anti-GalC and anti-SGC antibodies (Abs) have long been known to have diverse effects on cultured mouse OLGs and Schwann cells suggesting that they can activate GSL-enriched signaling domains in these cells (Ranscht et al. 1987). The extracellular signals imparted by the Abs have effects on the cytoskeleton (Dyer 1993; Benjamins and Dyer 1990; Dyer et al. 1994) and affect OLG differentiation (Bansal et al. 1999; Bansal and Pfeiffer 1994, 1989), indicating that these signals are transmitted across the membrane to the cytoplasmic side. Anti-GalC and anti-SGC Abs caused redistribution of GalC and SGC on the extracellular surface over domains containing MBP on the cytoplasmic side in OLGs, and depolymerization of a lacy network of microtubules in the membrane sheets (Dyer 1993; Benjamins and Dyer 1990; Dyer et al. 1994). Anti-GalC Ab caused an influx of extracellular Ca^{2+} , rapid cycling of the phospholipid polar head groups, and a decrease in phosphorylation of MBP. Significantly, it did not have these effects on cultured OLGs from the *shiverer* mutant mouse, which lacks MBP, indicating that MBP is required to mediate the effects of anti-GalC Ab on OLGs (Dyer et al. 1994). Anti-SGC Ab inhibited differentiation of progenitor OLGs (Bansal et al. 1999; Bansal and Pfeiffer 1989) and downregulated gene expression in mature OLGs (Bansal and Pfeiffer 1994) consistent with the accelerated developmental time course of cultured OLGs from the CGT and CST mutants. This suggests that SGC is involved in signaling mechanisms that regulate differentiation (Hirahara et al. 2004; Bansal et al. 1999; Shroff et al. 2009).

12.4 Natural Ligands That Interact with GalC and SGC and Transmit Signals Across Apposed Membranes

The effects of anti-GalC and anti-SGC Abs on cultured OLGs suggest that there must be natural ligands that interact with these GSLs in signaling domains to confer signals that are transmitted across the membrane. These ligands may include axonal, OLG, or extracellular matrix proteins, but can also be glycolipids in apposed membranes. *Trans* carbohydrate-carbohydrate interactions between sugars, mediated by divalent cations, occur between simple sugars in crystals (Cook and Bugg 1975), and between multivalent polysaccharides in the presence of water. They mediate processes such as cellulose fibril formation and their subsequent cross-linking by xyloglucan (Gorshkova et al. 2010), as well as proteoglycan-mediated

self-association of sponge cells (Bucior 2004; Bucior and Burger 2004). Interactions between single sugar molecules are disrupted by water, but the interactions between polymeric multivalent glycoconjugates in solution or in membrane lipid domains are stronger and persist in water. Furthermore, the water at the membrane surface is more ordered than bulk water and less likely to hydrate the carbohydrate groups (Vogler 1998). The forces between multivalent glycoconjugates have been measured by atomic force microscopy, surface plasmon resonance and other techniques and can be strong enough to cause specific adhesion of sponge cells into a multicellular organism (reviewed in Boggs et al. 2004, 2008a).

Membrane domains present glycolipids as a multivalent array of carbohydrate head groups. Hakomori's pioneering work on GSL-enriched signaling domains demonstrated that they interact by homotypic or heterotypic *trans* carbohydrate-carbohydrate interactions with each other across apposed cell membranes, similar to the neurosynapse or the immunological synapse, and he has termed these interacting domains a "glycosynapse" (Hakomori 1991, 2002). The interactions between these GSLs in GSL-enriched microdomains in apposed membranes trigger signals, probably due to noncovalent cross-linking or patching of the GSLs by the multivalent array of sugars, similar to the effect of Abs cross-linked by anti-Ig Abs, multivalent ligands, or multi-protein complexes in membrane domains. These signals are transmitted across the membrane to cytosolic signal transduction proteins (Iwabuchi et al. 1998; Prinetti et al. 1999).

Like several other GSLs in various cells, the myelin GSLs, GalC and SGC, can also participate in *trans* carbohydrate-carbohydrate interactions between apposed membranes (Hakomori 1991; Stewart and Boggs 1993). The HFA species interact more strongly than the NFA species (Stewart and Boggs 1993; Koshy et al. 1999). Bilayers of GalC only, or containing up to 15 mol% of a negatively charged phospholipid, are separated by a narrow space less than the diameter of a single water molecule, indicating an attractive interaction between them (Kulkarni et al. 1999). The adhesive forces between GalC and SGC bilayers have not been measured, but must be larger than for GalC alone, because heterotypic interactions between liposomes containing GalC and liposomes containing SGC were greater than their homotypic interactions (Stewart and Boggs 1993). Similar behavior was observed by mass spectrometry for GalC and SGC micelles in methanol/water (Koshy and Boggs 1996). If these GSLs are present only on the extracellular surface of myelin, the extracellular lipid must be about 40 mol% glycolipid, with cholesterol estimated to be also about 40 mol% (Inouye and Kirschner 1988). The extracellular surfaces of myelin, which face each other at the intraperiod line in the multilayered myelin sheath (Fig. 12.1c), would thus be covered with the simple sugars, galactose and sulfated galactose.

GalC and SGC are likely to act as ligands for each other in OLGs and myelin since *trans* interactions between them cause similar signaling effects as the Abs, as we showed by adding multivalent forms of galactose/sulfated galactose to cultured rat OLGs (Boggs et al. 2004, 2008a). Multivalent forms used included phospholipid/cholesterol liposomes containing GalC/SGC (Boggs and Wang 2001, 2004), a polyvalent form of galactose conjugated to bovine serum albumin (Gal-BSA) (Boggs and Wang 2004; Boggs et al. 2008b), and silica nanoparticles bearing

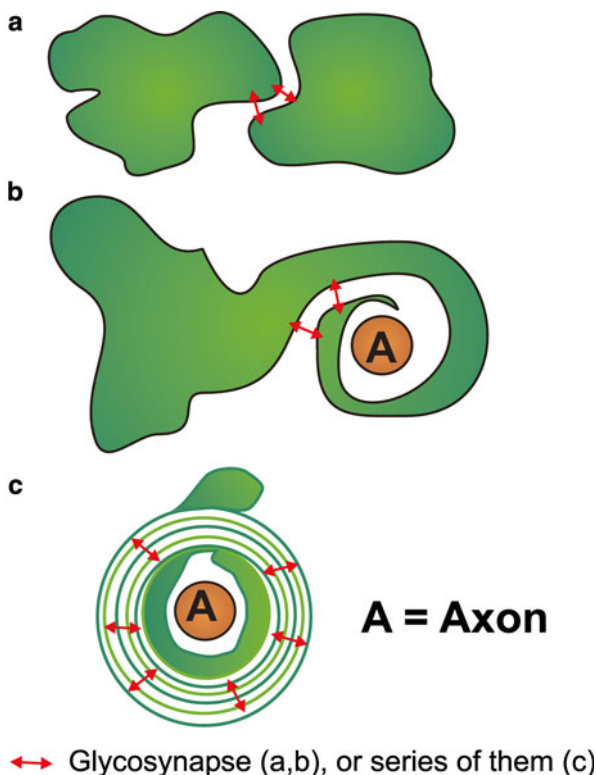


Fig. 12.1 Sites where glycosynapses between GalC/SGC-enriched domains in OL/myelin membranes might occur, resulting in signaling and depolymerization of the cytoskeleton. A *double headed orange arrow* represents glycosynapse formation between two apposed OLG membranes (panels **a**, **b**), or a series of glycosynapses between the multilayers of the myelin sheath (panel **c**). These glycosynapses are postulated to form transiently under certain conditions. (**a**) Glycosynapse between two different OLGs—could be a signal for process retraction or cessation of growth of a membrane sheet. (**b**) Glycosynapse between extracellular surfaces of membranes of a process of the same cell wrapping around an axon (tan, labeled A)—could be a signal for elimination of cytosol and formation of closely packed (compact) myelin layers. (**c**) Series of glycosynapses between the extracellular surfaces (at intraperiod line shown in *light green*) of compact myelin surrounding a nerve axon—could transmit extracellular or axonal signals throughout myelin layers. Major dense line where cytosolic surfaces are apposed is shown in *darker green*. The outer loop and inner loop containing cytoplasm are shown in *green*. Reprinted from Boggs et al. (2008a, 2010) with permission from Elsevier

galactose (Gal) and sulfated-galactose (SGal) (Boggs et al. 2010; Zhao et al. 2012). These glycoconjugates can be used to mimic interactions which are postulated to occur between GalC and SGC in apposed membranes, such as between the facing pairs of extracellular surfaces of myelin, or between OLGs or their processes in contact with each other (Fig. 12.1).

Although liposomes have the advantage that they can be made to resemble the extracellular surface of myelin or its GSL-enriched membrane domains, and

therefore, the putative natural multivalent ligand, they have the disadvantage that they can affect cells not just by adhering to them, but possibly by exchange of lipids between the liposomes and the cell membranes or by internalization into the cell. The use of Gal-BSA or glyco-nanoparticles eliminates this possibility.

12.5 Binding and Effect of Multivalent Glyco-nanoparticles on OLGs

Dendrimers or silica nanoparticles conjugated with saccharides are multivalent glycoconjugates that have been used to mimic *trans* interactions between cell surface glycans (Seah et al. 2009). Azide-functionalized silica nanoparticles were conjugated with propargyl derivatives of sugars or fluorescent FITC-labeled sugars by copper-promoted azide–alkyne cycloaddition. We examined the binding and effect of these silica nanoparticles bearing galactose (Gal-nanoparticles), galactose-3-sulfate (SGal-nanoparticles), or a combination of galactose and galactose-3-sulfate (Gal/SGal-nanoparticles) on OLGs, as well as control glyco-nanoparticles bearing glucose (Glc-nanoparticles) or mannose (Man-nanoparticles) (Boggs et al. 2010; Zhao et al. 2012). Unglycosylated control particles were capped with propargyl alcohol to provide a hydroxyl-terminated nanoparticle (OH-nanoparticles). In each case, the nanoparticles carried about 78,000 carbohydrate groups per particle, and thus were highly multivalent. They bound specifically to microtiter plates coated with galactolipid, but not glucolipid, and bound to each other in a heterotypic fashion (Zhao et al. 2012). The binding did not require divalent cations. *Trans* interactions between many sugars require divalent cations (Hakomori 1991) as did the interactions between GalC and SGC in phosphatidylcholine/cholesterol liposomes (Stewart and Boggs 1993), but those between pure GalC bilayers and pure SGC bilayers did not (Boggs et al. 2000), similar to the glyco-nanoparticles where galactose and sulfated galactose are highly concentrated. The requirement for divalent cations for a lipid or lipid/protein composition resembling that of the extracellular surface of OLGs or myelin is not known. *Trans* interactions between the galactoglycerolipids of myelin, MGDG and SGG, have not been investigated but would probably occur since the interaction between Gal-nanoparticles and SGal-nanoparticles does not depend on a lipidic moiety. Furthermore, divalent cations have been shown to complex digalactosyldiglyceride membranes to each other (Webb et al. 1988).

The Gal- and SGal-nanoparticles also bound specifically to live or fixed OLGs (Fig. 12.2e, f) and did not bind to astrocytes present in the culture, which lack GalC

Fig. 12.2 (continued) fixation and staining with anti-MAG Ab (red); yellow indicates overlap. Similar results were obtained by addition of nanoparticles to fixed OLGs; (e) Gal/SGal-nanoparticles; (f) Gal-nanoparticles; (g) Cells were preincubated with anti-GalC O1 Ab prior to binding of fluorescent Gal/SGal-nanoparticles; binding was greatly diminished; (h) Cells were preincubated with anti-GalC O1 Ab prior to binding of Gal-nanoparticles; binding was not affected as expected for a heterotypic interaction of galactose with SGC. Scale bars = 20 μ m. Panels a–d, e, f, and h are reprinted with permission from Zhao et al. (2012). Copyright (2012) American Chemical Society

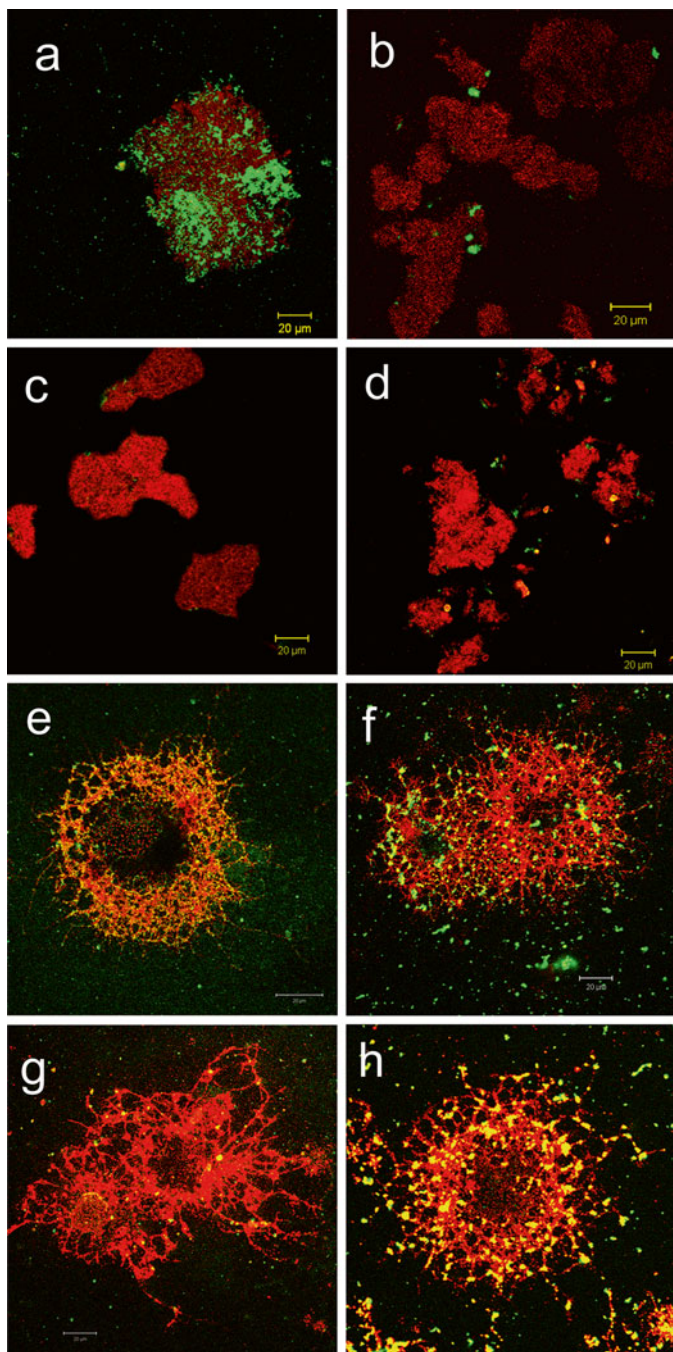


Fig. 12.2 (a–d) Fluorescence microscope images of fluorescein-labeled nanoparticles (*green*) bound to myelin fragments labeled with CD-DiI (*red*); *yellow* indicates overlap. (a) Gal/SGal-nanoparticles; (b) control OH-nanoparticles; (c) 4× excess nonfluorescent Gal/SGal nano particles followed by fluorescent Gal/SGal-nanoparticles; (d) myelin was preincubated with anti-GalC IgG Fab fragments followed by fluorescent Gal/SGal-nanoparticles. Bar= 20 μm. (e–h) Fluorescence microscope images of fluorescein-labeled nanoparticles (*green*) bound to live OLGs followed by

and SGC (Zhao et al. 2012). Pre-incubation of OLGs with the O1 anti-GalC monoclonal antibody greatly reduced binding of the Gal/SGal (Fig. 12.2g) or SGal-bearing particles to the OLGs, but had no effect on the binding of Gal-particles (Fig. 12.2h), as expected for a heterotypic interaction. The Gal/SGal nanoparticles also bound specifically to purified myelin fragments (Fig. 12.2a). Control nanoparticles bound much less to OLGs and myelin (shown for OH-nanoparticles and myelin in Fig. 12.2b). The binding was inhibited by an excess of nonfluorescent versions of Gal/SGal nanoparticles (Fig. 12.2c) and also by preincubation of myelin with monovalent anti-GalC IgG Fab fragments (Fig. 12.2d).

The Gal/SGal nanoparticles, GalC/SGC-containing liposomes and Gal-BSA all had sugar-specific and relatively similar effects on the distribution of GalC and MBP in cultured OLGs. They caused redistribution and clustering of GalC on the extracellular side, and MBP on the cytosolic side, into clusters of varying size such that the GalC domains usually overlaid the MBP domains (Boggs and Wang 2001, 2004; Boggs et al. 2010) (MBP clustering is shown for Gal-nanoparticles in panels d–f of Fig. 12.3, compared to untreated cells in panels a–c, and MBP or GalC clustering is shown for GalC/SGC-containing liposomes in Fig. 12.4c, g, compared to control liposome-treated cells in Fig. 12.4a, e). Pre-incubation of the OLGs with anti-GalC IgG Fab fragments prevented the effect of the Gal/SGal-nanoparticles on GalC and MBP distribution and OLG morphology (Boggs et al. 2010). Untreated mature cells have a complex cytoskeletal network with major veins and a lacy network of microtubules (Dyer 1993) (Fig. 12.4f). The Gal/SGal nanoparticles and GalC/SGC-containing liposomes also caused loss of the microtubular network (Fig. 12.4h) (Boggs and Wang 2001, 2004; Boggs et al. 2010).

The glyco-nanoparticles had a quantitatively greater effect on the OLGs than GalC/SGC-containing liposomes or Gal-BSA, with some effect seen after 6 h and a greater effect after overnight culture, whereas overnight culture was required in order to detect an effect of GalC/SGC-containing liposomes or Gal-BSA. At 8 days in culture, when the glyco-nanoparticles are added, the cell population typically consists of about 30 % mature cells with flat membrane sheets (resembling Fig. 12.3a–c) and the cells are almost completely GalC and MBP positive, even when less mature, with only thin processes instead of membrane sheets. However, after treatment with glyco-nanoparticles, more cells appeared less mature than in the control cell population, with many narrow processes, and only about 5 % appearing mature with membrane sheets. In many of these less mature GalC⁺ cells, MBP staining was very low or absent, in contrast to untreated OLGs (Boggs et al. 2010). An example of this type of cell after treatment with Gal/SGal-nanoparticles, is shown in Fig. 12.3g–i. These effects, particularly the almost complete absence of MBP staining, were not commonly observed after liposome treatment (e.g., Fig. 12.4c). Since MBP appears relatively late in OLG differentiation (Bansal and Pfeiffer 1994), its loss in the treated cells suggests that dedifferentiation may have occurred. The Gal, SGal, or mixed Gal/SGal-nanoparticles had significantly more effect than Glc-, Man-, or unglycosylated-nanoparticles. Similarly, control liposomes with no GSL, or with GlcC or lactosylceramide (LacC) instead of GalC, had much less effect on GalC redistribution than those containing GalC and/or

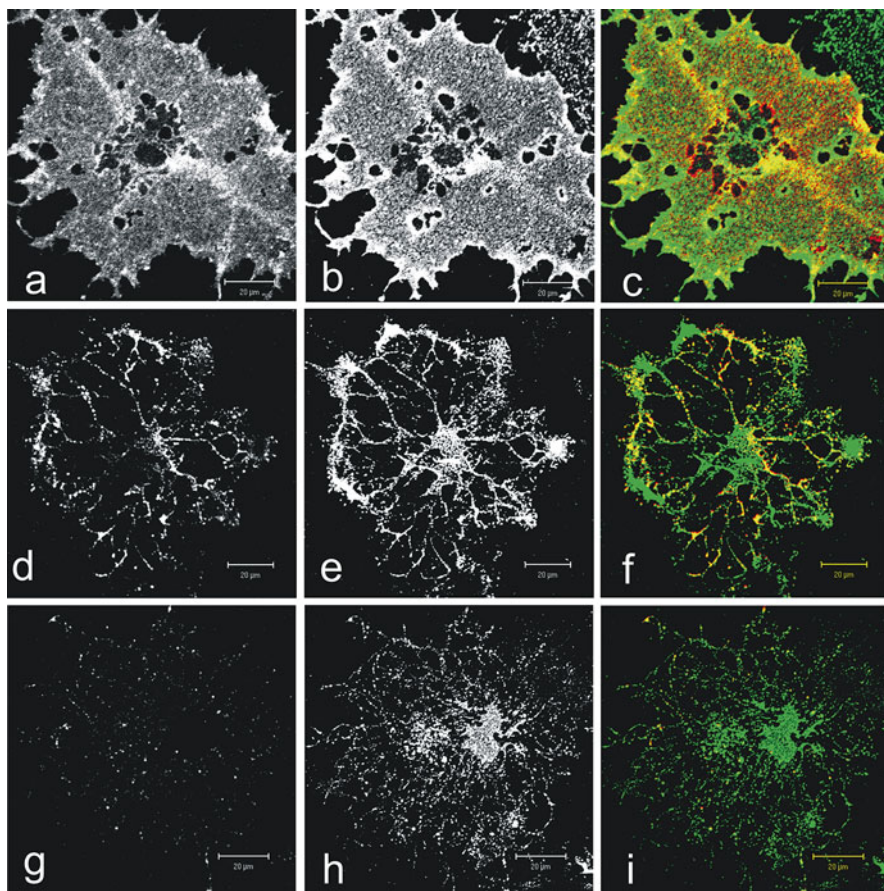


Fig. 12.3 Confocal microscope images of OLGs fixed and stained externally with monoclonal anti-GalC Ab (O1) (**b, e, h**); then stained internally with anti-MBP Ab (**a, d, g**). Merge is shown in (**c, f, i**) (MBP, *red*; GalC, *green*). Untreated OLGs (**a–c**); OLGs treated overnight with 2 µg/ml Gal-nanoparticles (**d–i**). Panels (**d–f**) show MBP and GalC redistribution/clustering in a more mature cell typical of that caused by GalC/SGC-containing liposomes. Panels (**g–i**) represent a cell which looks less mature and has lost most of its MBP; this occurs frequently after nanoparticle treatment but is much less frequent with liposome treatment. Bar=20 µm. Reprinted from Boggs et al. (2010), with permission from Elsevier

SGC. BSA conjugated to glucose and mannose had significantly less effect than Gal-BSA (Boggs and Wang 2004). The nanoparticles bearing both Gal and SGC had a significantly greater effect on GalC redistribution than those with only Gal or SGC (Boggs et al. 2010). Similarly, liposomes containing both myelin GSLs had a greater effect than those with only SGC or GalC (Boggs et al. 2004).

Using liposomes, effects on clustering of other proteins and on the cytoskeleton were also determined. Similar clustering of GPI-linked proteins and of two trans-membrane proteins, proteolipid protein (PLP) and myelin/OLG glycoprotein

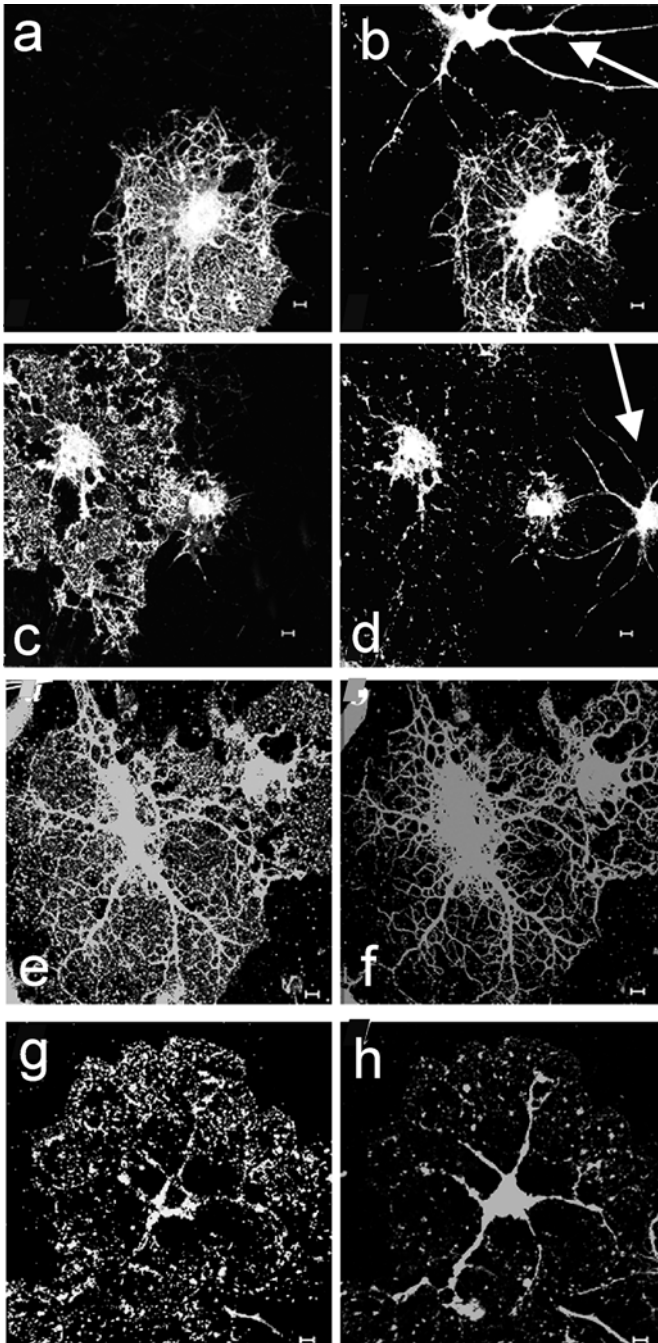


Fig. 12.4 Confocal microscope images of cultured OLGs treated overnight with control phospholipid/cholesterol liposomes (a, b, e, f) or GalC/SGC-containing phospholipid/cholesterol liposomes (c, d, g, h). (a, c) OLGs fixed, permeabilized, and stained with monoclonal anti-MBP and FITC-labeled second Ab and then (b, d) stained with Texas Red-phalloidin. The MBP-negative astrocytes present in the field in b above the OLG and in d to the far right of the OLG are stained only with phalloidin (white arrows in b, d). The GalC/SGC-containing liposomes caused

(MOG), occurred, suggesting that the domains which cluster are membrane rafts (Boggs and Wang 2004; Boggs et al. 2004, 2008a). Several proteins or phosphorylated proteins involved in signal transduction, MAPK, phosphorylated MBP, and some phospho-tyrosine-containing proteins also clustered with MBP and GalC (Boggs and Wang 2004), suggesting that these rafts are membrane signaling domains. Indeed, the GalC/SGC-containing liposomes also caused depolymerization of microtubules (Fig. 12.4h, compare to Fig. 12.4f), and actin filaments (Fig. 12.4d, compare to Fig. 12.4b) that form a lacy cytoskeletal network in the membrane sheets, indicating that the interaction of GalC/SGC-containing liposomes with the extracellular surface of the OLG caused transmission of a signal across the membrane (Boggs and Wang 2001, 2004). Note that the GalC/SGC-containing liposomes had no effect on the actin cytoskeleton of an astrocyte (MBP negative, white arrow) also present in the culture (Fig. 12.4d, compare to Fig. 12.4b). The Gal/SGal nanoparticles also caused loss of the microtubular network (Boggs et al. 2010), but their effect on actin has not been examined. Inhibition, using various reagents, of a number of kinases, such as Rho kinase, and phosphatases that are involved in regulation of the cytoskeleton prevented the liposome-mediated effects on the cytoskeleton (Boggs et al. 2008a, b).

These results show that a lipidic form of the sugar is not necessary for the effect; rather a multivalent form of the sugar, as found on the surface of GSL-containing liposomes, or bound to a polymer or nanoparticle, is sufficient. Finally, the effects are specific for Gal and SGal.

12.6 Receptors in OLGs Which Interact with Multivalent Gal/SGal by *Trans* Interactions

The receptor(s) in the OLG membrane which interact with the multivalent Gal/SGal presented by liposomes or polymers could be protein(s), but are likely to be GalC and SGC (and possibly also MGDG and SGG) for the following reasons: (1) GalC and SGC bind to each other by *trans* interactions across apposed surfaces and SGal-bearing nanoparticles bind to Gal-nanoparticles and to galactolipid in vitro (Hakomori 1991; Stewart and Boggs 1993; Zhao et al. 2012); (2) The effects of multivalent presentations of Gal/SGal on OLGs resemble the effects of anti-GalC/SGC Abs on

←

Fig. 12.4 (continued) depolymerization of the actin filaments only in the MBP-positive OLG and not in the astrocyte. (e, g) OLGs fixed, stained externally with polyclonal anti-GalC Ab and FITC-labeled second Ab and then (f, h) permeabilized and stained internally with monoclonal anti- α , β -tubulin Abs. Cell has large veins of microtubules and a lacy network of smaller microtubules in the membrane sheets (f). The GalC/SGC-containing liposomes caused loss of the lacy network of microtubules but the major veins remain (h). Scale bar=20 μ m. Panels a–d reprinted from Boggs and Wang (2001) with permission from John Wiley and Sons. Panels e–h reprinted from Boggs et al. (2004) with permission from Springer

OLGs reported by Dyer and Benjamins (Benjamins and Dyer 1990; Dyer 1993); (3) Fab fragments of anti-GalC IgG Ab, which had no effects on OLGs themselves, prevented the binding and effects of the Gal/SGal-nanoparticles on GalC and MBP distribution and OLG morphology (Boggs et al. 2010; Zhao et al. 2012); (4) The multivalent Gal/SGal did not bind to and had no effect on the cytoskeleton of astrocytes that were also present in the culture, and that lack these two GSLs, indicating a specific effect on GalC/SGC-containing OLGs (Boggs and Wang 2001; Boggs et al. 2004; Zhao et al. 2012); (5) Inhibition of GSL synthesis by treatment of OLGs with fumonisin B1 prevented the effect of liposomal GalC/SGC on MBP redistribution in the GalC/SGC-negative OLGs (Boggs et al. 2008a, b). These results support our suggestion that the natural ligand(s) for GalC and SGC in OLGs that are mimicked by anti-GalC/SGC Abs or multivalent Gal/SGal are, or include, a multivalent array of GalC and SGC (and possibly also MGDG and SGG) in apposed OL/myelin membranes. However, proteins with terminal galactose and galactose-3-sulfate moieties might also be able to serve as ligands for GalC and SGC; this has not been investigated.

We suggest that *trans* interactions between these GSLs clustered in apposed membrane domains can occur transiently under certain conditions between extracellular OLG membranes or the extracellular surfaces of compact myelin membranes. They cannot cause strong adhesion of these surfaces, but might contribute weak adhesive forces. Interestingly, in the PLP knockout mouse, compact myelin is formed with a smaller separation between the extracellular surfaces than in wild type mouse, although the myelin is more unstable and separates more easily under fixation conditions used for electron microscopy (Rosenbluth et al. 2009). Myelin particles have been shown to bind to cultured OLGs, and myelin particles bind to each other (Bakhti et al. 2013). The interaction between them is reduced, but still significant, if PLP is absent, indicating that although PLP contributes to adhesive interactions, other constituents, such as GalC and SGC, also contribute.

Loss of sialic acid on the OLG surface and downregulation of proteins with large extracellular domains, such as chondroitin sulfate proteoglycan 4 (NG2) and CD44, occur with maturation of OLGs and their production of myelin membranes. This prevents the electrostatic repulsion which occurs between other cell surfaces (Bakhti et al. 2013). Nevertheless, X-ray diffraction and electron microscopy indicate that the static separation of the extracellular surfaces of compact myelin is too great for the carbohydrate head groups of these GSLs to be in constant contact (Kirschner et al. 1989). However, they may come into transient contact under some conditions that cause protein clustering away from GSL-enriched domains, such as increased extracellular Ca^{2+} concentration (Hollingshead et al. 1981; Boggs et al. 2004, 2008a). Furthermore, membrane surfaces in multibilayers undulate, allowing the intermembrane separation to fluctuate (Niles et al. 1996) so that transient contact between GalC and SGC could occur. Transient, weak interactions between myelin GSLs, in addition to PLP, would allow myelin layers to slip by each other as the axon is ensheathed by growing OLG processes.

12.7 GalC/SGC Signaling Releases Cytoskeletal Restriction of Membrane Domains

Jasplakinolide, a reagent that stabilizes actin filaments, inhibited liposome-induced redistribution of all the membrane constituents that were clustered in its absence, including GalC, on the extracellular surface (Boggs and Wang 2004). It also prevented depolymerization of the microtubules. This result indicates that depolymerization of the actin filaments is required both for redistribution of the membrane constituents and for depolymerization of the microtubules. Thus, it is an early event in the transmembrane signaling mediated by multivalent Gal/SGal. The fact that jasplakinolide also prevented clustering of GalC on the extracellular surface suggests that it is not just individual GalC molecules that redistribute, but rather entire membrane domains/rafts that redistribute and coalesce (Fig. 12.5).

Since actin depolymerization is an early event following interaction of multivalent Gal/SGal with the OLG membrane, this interaction must first cause transmission of a signal across the membrane that affects actin. This initial signal may be Ca^{2+} entry, as found when anti-GalC Ab was added to OLGs (Dyer 1993; Benjamins and Dyer 1990; Paz Soldan et al. 2003), or a mechanical signal due to GalC/SGC cross-linking. Depolymerization of the cytoskeleton then allows for redistribution and coalescence of microdomains enriched in GalC, MBP, and the other membrane constituents examined. This sequence of events suggests that the cytoskeleton restricts lateral diffusion of these membrane constituents, either by binding to them or by binding to other transmembrane proteins. This is consistent with the membrane skeleton fence or picket fence model (Kusumi et al. 1999, 2012) as well as with studies indicating that lipids and transmembrane proteins undergo hop diffusion in compartmentalized membrane domains of 50–200 nm (Fujiwara et al. 2002; Marone et al. 2006). Upon depolymerization of the cytoskeleton, these domains are able to redistribute and coalesce into large clusters (Fig. 12.5). Multivalent Gal/SGal cross-linking or patching of the small membrane domains/rafts restrained within these compartments would facilitate this redistribution.

In some cells, such as T cells, receptor clustering causes actin polymerization or requires the actin cytoskeleton for clustering to occur (Harder and Simons 1999; Mitchell et al. 2009; Rodgers and Zavzavadjian 2001; Gomez-Mouton et al. 2001; Baumgartner et al. 2003). However, in other cells, receptor clustering increased on depolymerization of the cytoskeleton (Wang et al. 2001; Treanor et al. 2010; Hao and August 2005). Coclustering of separate GM1-containing rafts with GM3-containing rafts in fibroblasts increased significantly after actin depolymerization (Fujita et al. 2009). In B cells, activation by cross-linking of the B cell receptor caused rapid (15–30 s) global actin depolymerization, although actin repolymerization later recurred (Hao and August 2005), resulting in “corrals” around individual microclusters of B cell receptors that had formed (Treanor et al. 2011). Cross-linking of glycan moieties on retinal pigment epithelial cells with galectin-3 also caused depolymerization of the cytoskeleton, resulting in their failure to form pseudopodia and to attach and spread on a fibronectin-coated surface (Alge-Priglinger

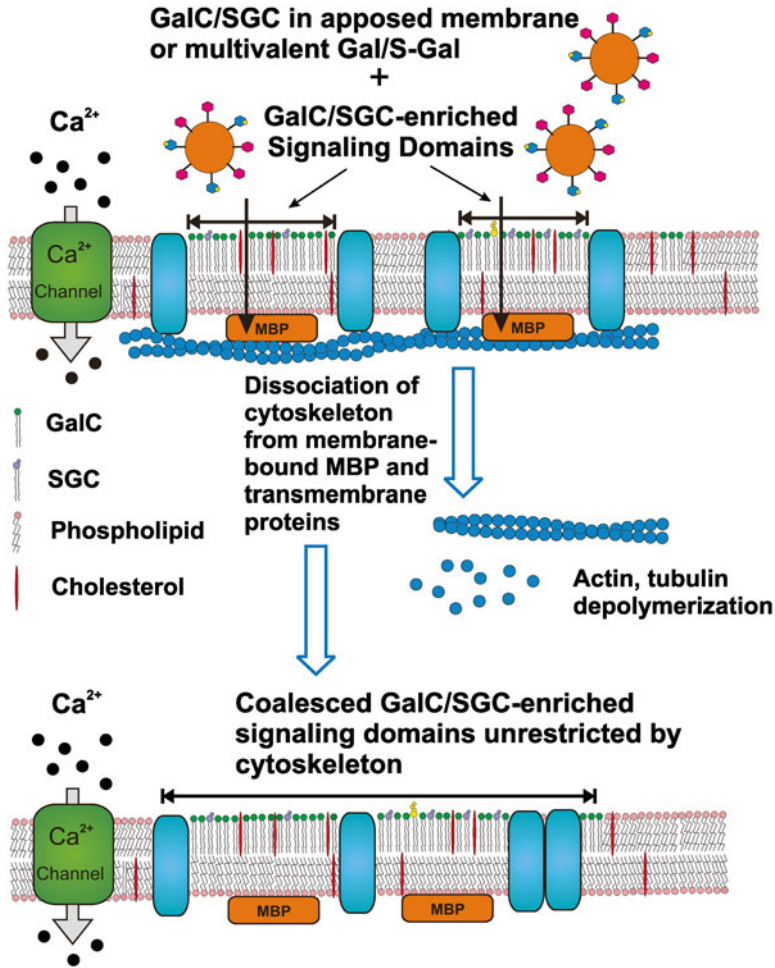


Fig. 12.5 Schematic of effects of multivalent Gal/SGal on OLG membrane sheets. GalC and SGC-enriched membrane domains (rafts) in OLG membrane sheets also contain MBP, a peripheral membrane protein on the cytoplasmic side, and are linked to the membrane skeleton (made up of linked *blue spheres*) via MBP (Boggs 2006; Harauz and Boggs 2013), transmembrane proteins and/or other membrane-actin binding proteins. Some transmembrane proteins (*blue oblongs*) bound to the membrane skeleton serve as picket fences, according to the hypothesis of Kusumi and colleagues (Kusumi et al. 1999, 2012; Fujiwara et al. 2002; Marone et al. 2006), which restrict lateral diffusion of both lipids and proteins in the membrane domains. *Upper panel*—binding of multivalent Gal/SGal (GalC/SGC-containing liposomes or glyco-nanoparticles conjugated to Gal and SGal, depicted by large *orange spheres* bearing Gal (*magenta hexagon*) and SGal (*blue hexagon*)), cross-links GalC and SGC molecules in the membrane domains, and triggers an initial signal, possibly Ca^{2+} entry, which causes dissociation of actin filaments and microtubules and their depolymerization. *Lower panel*—loss of the membrane skeleton permits lateral diffusion of the membrane domains so that they coalesce into larger clusters. The membrane domains may contain a number of other transmembrane proteins such as PLP and MOG, and signaling proteins such as MAPK (not shown), since they redistribute together with GalC, SGC, and MBP (Boggs and Wang 2004). MBP on the cytoplasmic side may be linked to GalC/SGC on the extracellular side via one

et al. 2011). An IgM that reacted with a raft constituent of neurons caused raft clustering and signaling, and coupled the rafts to microtubules, but caused F-actin networks to recede from the growth cone periphery (Xu et al. 2011). Thus, receptor cross-linking and clustering is coupled to dynamic reorganization of the cytoskeleton in many cells.

12.8 Role of MBP in Transmission of GalC/SGC-Mediated Signal

MBP on the cytosolic side may play an important role in transmission of the GalC/SGC-mediated signal to the cytoskeleton. Earlier studies by Dyer et al. (1994) showed that anti-GalC Ab did not cause effects in OLGs from the *shiverer* mutant mouse, which lacks MBP. Suppression of MBP synthesis in normal rat OLGs using MBP siRNA significantly inhibited the effect of GalC/SGC-containing liposomes on GalC redistribution in the MBP-negative OLGs (Boggs et al. 2008b) and on the cytoskeleton (Boggs et al. 2010). Coculture of OLGs with neurons induced dramatic lipid condensation or ordering, as detected by laurdan fluorescence, in OLG membranes and clustering of GalC-containing domains (Fitzner et al. 2006), which may be related to the GalC clustering induced by anti-GalC liposomes or multivalent Gal/SGal arrays. This effect of neurons was not observed in OLGs from *shiverer* mice indicating that MBP was required for this phenomenon also. CHAPS-insoluble membrane domains prepared from the OLGs contained more PLP and MBP after coculture with neurons, indicating protein redistribution in the membrane had also occurred.

MBP binds to and assembles actin filaments and microtubules and binds actin and microtubules to a lipid bilayer, and it may tether the cytoskeleton to the plasma membrane in OLGs (Boggs 2006; Harauz and Boggs 2013). Dynamic changes in co-localization of MBP with actin and tubulin, occurred in transfected N19-oligodendroglial cells during membrane ruffling stimulated by PMA, with enrichment of these proteins in the membrane ruffles, and in membrane domains resembling focal adhesion contacts induced by IGF-1 (Smith et al. 2012). MBP was coimmunoprecipitated with actin, tubulin, and signaling molecules from detergent extracts of primary OLGs (Boggs et al. 2014). Low density DIGs isolated from OLGs and myelin also contained actin and tubulin (Arvanitis et al. 2005; Taguchi et al. 2005; Marta et al. 2003) in addition to MBP. These studies indicate that OLG

←
Fig. 12.5 (continued) of these transmembrane proteins, since it is influenced by the GalC/SGC cross-linking and is necessary for transmission of the extracellular signal to the cytoskeleton (Boggs et al. 2008b; Dyer et al. 1994). The head groups of the lipids are depicted as GalC (*green*) SGC (*purple*), gangliosides (*yellow*), phospholipids (PL) (*pink*); cholesterol (*red rod*). Similar *trans* interactions between GalC/SGC-enriched domains in apposed OLG or myelin membranes are postulated to create a glycosynapse and have a similar signaling effect

membrane domains/rafts may be linked to the membrane skeleton via MBP, in addition to other proteins (Fig. 12.5). They also suggest that MBP may be linked to cytoskeletal proteins even in myelin. DIGs from myelin also contain the radial component, a junctional specialization within intermodal CNS myelin that passes through many layers of compact myelin (Karthigasan et al. 1994). It appears as a radial array by electron microscopy and has a similar appearance in isolated DIGs. It is made up of tight junctions that may control the ionic content of the extracellular space in myelin (Dyer 2002; Boggs et al. 2008a).

12.9 Role of Glycosynapses in OLGs or Myelin

GalC/SGC-enriched microdomains in the OL/myelin membrane may form glycosynapses at different sites and under certain conditions in OLG or myelin membranes. Glycosynapses could occur between apposed membranes of OLG processes in contact with each other, or between the extracellular surfaces of compact myelin (Fig. 12.1). If OLGs are in contact at high densities, or if OLG processes contact an already myelinated axon, this contact might cause process retraction requiring disruption of the cytoskeleton (Fig. 12.1a). Contact inhibition of oligodendrocyte progenitor cells occurs for cells grown at high density (Zhang and Miller 1996), but it is not known if this also occurs for more mature SGC and GalC-containing OLGs. However, *in vivo* and *in vitro* time lapse imaging shows that OLG processes often seem to withdraw after contact with a nearby process or myelinated axon, suggesting that they influence one another (Kirby et al. 2006; Ioannidou et al. 2012). This may be a mechanism to ensure uniform myelination. A signal applied to SGC via a glycosynapse between two OLG processes might be expected to inhibit differentiation and process extension (Hirahara et al. 2004) as observed with anti-SGC Ab (Bansal et al. 1999). The increased number and decreased apoptosis of OLGs in the CST-null mouse (Shroff et al. 2009) may be partly due to a failure of contact inhibition normally mediated by GalC–SGC interactions.

Dynamic regulation of the cytoskeleton is necessary for various stages of myelination (Bauer et al. 2009; Boggs et al. 2008a; Harauz and Boggs 2013). When mature myelinating OLGs initially ensheath axons, the first few layers of membrane around the axon contain cytosol (Remahl and Hildebrand 1990). Disruption of the cytoskeleton in these layers is necessary for the cytoplasmic surfaces to adhere and create compact myelin. GalC–SGC interactions could occur as the membrane sheets wrap around the nerve axon allowing GSLs in apposed surfaces to come into contact at least transiently and/or in localized domains and confer a signal for compaction (Fig. 12.1b). In CST-null mice, the processes which myelinate are thicker and retain more cytoplasm than in wild-type mice (Shroff et al. 2009), perhaps due to retention of the cytoskeleton in the absence of GalC–Sulf interactions. Formation of compact myelin requires close apposition between each pair of facing extracellular surfaces, and between each pair of facing cytoplasmic surfaces, which may be promoted by protein and GSL clustering. Fitzner et al. (2006) have shown that coculture of OLGs

with neurons induced GalC ordering in OLG membranes, which was probably due to GalC clustering, and increased MBP distribution into DIGs. Thus, a neuronal signal inducing OLG processes to ensheath the axon could be followed by GalC–SGC contact between the apposed membranes, depolymerization of the cytoskeleton, and protein and GSL clustering, leading to elimination of cytosol and adhesion of the cytosolic surfaces.

Signaling resulting from *trans* GalC–SGC interactions may also occur in the mature myelin sheath (Fig. 12.1c). These signals may in turn be transmitted across the membrane to MBP and the cytoskeletal elements and junctions in the radial component in myelin, as found in cultured OLGs for anti-GalC antibody and GalC/sulfatide-containing liposomes, possibly by causing Ca^{2+} entry into the cytosolic domains. The accumulation of Ca^{2+} into the cytosolic space of compact myelin, mediated by *N*-methyl-D-aspartate (NMDA) receptors, has been detected within compact myelin (Micu et al. 2005). Subsequent effects on tight junctions or gap junctions between myelin layers may regulate their permeability (Morita et al. 1999; Dyer 2002) as occurs for intestinal epithelial cells (van Itallie and Anderson 2004). This process may allow for transmission of signals from the axon throughout compact myelin.

Communication between the myelin sheath and the axon may regulate both axonal and myelin function and is necessary to prevent neurodegeneration (Witt and Brady 2000; Edgar and Garbern 2004). Myelination affects the axon caliber, phosphorylation of axonal neurofilaments, the axonal cytoskeleton, and ion channel organization (Baba et al. 1999). Phosphorylation of MBP occurs in myelin in response to the nerve action potential (Murray and Steck 1984; Atkins and Sweatt 1999), and lipids and metabolic precursors are transferred from the axon to the myelin sheath (Chakraborty et al. 1999). The myelin sheath provides trophic support to the axon, which may compensate for its shielding of the axon from extracellular metabolic support (Nave and Trapp 2008; Simons and Lyons 2013). Lactate is one substance taken up by OLGs and myelin that may allow for such trophic support. MCT1, a lactate and pyruvate transporter, has been localized to OLGs and also shown to be in compact myelin by immunogold labeling, whereas a different lactate transporter, MCT2, is in axons. Lactate uptake causing a pH change was detected in both OLG cell bodies and processes aligned with axons (Rinholm, et al. 2011). Downregulation of MCT1 selectively in OLGs caused axonal damage and neuronal loss in animal and cell culture models, indicating a new mechanism by which OLGs support neurons and axons (Lee et al. 2012). It would be interesting to determine whether MCT1 is expressed normally in OLGs and myelin from the CGT/CST KO's since a lactate transporter in spermatocytes in the CST KO is suspected to be absent due to altered trafficking (Honke 2013).

The myelin sheath also produces ATP through generation of a proton gradient across the lamellae, and has been postulated to be a site of oxygen absorption and aerobic metabolism for the axons (Ravera et al. 2009). Neuronal activity also results in release of glutamate and ATP causing shifts in extracellular pH (Ro and Carson 2004; Butt et al. 2004). A Na^+/H^+ exchanger, $\text{Na}^+/\text{HCO}_3^-$ cotransporter and carbonic anhydrase II are present in OLGs that can respond to shifts in pH of the extracellular

space during neuronal activity (Ro and Carson 2004). pH microdomains in OLGs occur which differ in pH by over 0.1 pH unit. Similar pH fluctuations may be able to occur in the cytosolic spaces of myelin if the permeability of tight and gap junctions can be influenced by GSL-mediated signaling.

It appears to be necessary to have compact myelin containing all of its normal constituents surrounding an axon in order to provide trophic support to neurons, since neurodegeneration occurs in mutant mice in which one of several myelin proteins, such as PLP, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), or myelin-associated glycoprotein (MAG), is eliminated (Edgar and Garbern; 2004; Edgar et al. 2009). Neurodegeneration also occurs in the CST, CGT, and FA2H KO's, even though a compact myelin sheath is formed initially in all cases. Participation of transient GalC and SGC interactions between the apposed extracellular surfaces of mature myelin might allow for transmission of signals throughout the myelin sheath regulating its metabolic activity, and thus facilitate myelin-axonal communication and trophic support of the axon. In support of this conclusion, deletion of SGC in the CST-null mouse decreased the caliber and shape of the axon with age (Marcus et al. 2006), suggesting that the lack of SGC decreased signaling from myelin to the axon.

12.10 Treatment of Demyelinating Disease by Stimulation of OLGs by GSL Cross-Linking

Interestingly, IgM antibody to GalC or SGC, produced by hybridoma cells implanted in the spinal cord of neonatal pups *in vivo*, or in an *in vitro* myelinating culture, resulted in formation of myelin with a large space between the extracellular surfaces and paranodal loops, without tight junctions between them (Rosenbluth et al. 1996; Rosenbluth and Moon 2003). Thus, the multivalent IgM Ab replaced normal interactions between the extracellular surfaces. The abnormal myelin was stable enough to persist at least 18 days; the effect at longer times was not examined. A similar IgG Ab, however, which would not be able to link the apposed extracellular surfaces, prevented myelin formation. These results indicate that some type of interaction between the extracellular surfaces is necessary for myelin to form and function, but that the spacing can be wider than normal as long as the surfaces are linked via a molecular interaction. IgM Abs produced in peripheral neuropathies and in multiple sclerosis, including anti-GalC and anti-SGC Abs (Ilyas et al. 2003), cause formation of myelin with similar wide-spaced lamellae (Jacobs and Scadding 1990). The Ab linking of apposed myelin lamellae could mediate signals that could have both positive and negative effects on myelin maintenance and function.

Anti-GalC and anti-SGC Abs produced in these diseases may also have signaling effects on OLGs *in vivo*, as found in the *in vitro* studies. Indeed, IgM antibodies to these and other myelin constituents have been found to increase Ca^{2+} entry into OLGs, inhibit apoptotic signaling and OLG differentiation, and ameliorate demyelinating disease in animal models (Warrington et al. 2007; Asakura et al. 1998;

Paz Soldan et al. 2003; Watzlawik et al. 2010). IgM monoclonal antibodies O1 and O4 bind to GalC and SGalC (in lipid bilayers of phosphatidylcholine with 6 % SGC and 16 % GalC) with unusually small apparent dissociation constants ($K_D=0.9$ nM) for natural antibodies, although they have similar properties to natural antibodies (Wittenberg et al. 2012). Such IgM antibodies for OLG/myelin constituents are now in clinical trials to treat multiple sclerosis (Wootla et al. 2013). Similarly, multivalent arrays of Gal and SGal, such as the Gal/SGal-nanoparticles used here, could have potential beneficial effects on myelination or myelin function, a possibility that warrants further investigation.

Acknowledgements Studies from the Boggs laboratory were supported by operating grants from the Multiple Sclerosis Society of Canada. My collaborator for the glyco-nanoparticle studies, Dr. Amit Basu, Brown University, Providence, Rhode Island, is gratefully acknowledged. Ms. Huimin Wang, Dr. Wen Gao, Dr. Yuanfang Liu, Mr. Jingsha Zhao, Dr. Yukie Hirahara, and Dr. Hyun-Joo Park are thanked for their excellent assistance with these studies.

Compliance with Ethics Requirements Joan Boggs declares that she has no conflict of interest. This article made use of cultured oligodendrocytes obtained from rats. All institutional and national guidelines for the care and use of laboratory animals were followed. This article does not contain any studies with human subjects.

References

- Alge-Priglinger CS, Andre S, Schoeffl H, Kampik A, Strauss RW, Kernt M, et al. Negative regulation of RPE cell attachment by carbohydrate-dependent cell surface binding of galectin-3 and inhibition of the ERK-MAPK pathway. *Biochimie*. 2011;93:477–88.
- Arvanitis DN, Min W, Gong Y, Meng YM, Boggs JM. Two types of detergent-insoluble, glycosphingolipid/cholesterol-rich membrane domains in isolated myelin. *J Neurochem*. 2005;94:1696–710.
- Asakura K, Miller DJ, Pease LR, Rodriguez M. Targeting of IgM kappa antibodies to oligodendrocytes promotes CNS remyelination. *J Neurosci*. 1998;18:7700–8.
- Atkins CM, Sweatt JD. Reactive oxygen species mediate activity-dependent neuron-glia signaling in output fibers of the hippocampus. *J Neurosci*. 1999;19:7241–8.
- Baba H, Akita H, Ishibashi T, Inoue Y, Nakahira K, Ikenaka K. Completion of myelin compaction, but not the attachment of oligodendroglial processes triggers K channel clustering. *J Neurosci Res*. 1999;58:752–64.
- Bakhti M, Snaidero N, Schneider D, Aggarwal S, Mobius W, Janshoff A, et al. Loss of electrostatic cell-surface repulsion mediates myelin membrane adhesion and compaction in the central nervous system. *Proc Natl Acad Sci U S A*. 2013;110:3143–8.
- Bansal R, Pfeiffer SE. Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. *Proc Natl Acad Sci U S A*. 1989;86:6181–5.
- Bansal R, Pfeiffer SE. Regulation of gene expression in mature oligodendrocytes by the specialized myelin-like membrane environment: antibody perturbation in culture with the monoclonal antibody R-mAb. *Glia*. 1994;12:173–9.
- Bansal R, Winkler S, Bheddah S. Negative regulation of oligodendrocyte differentiation by galactosphingolipids. *J Neurosci*. 1999;19:7913–24.
- Bauer NG, Richter-Landsberg C, Ffrench-Constant C. Role of the oligodendroglial cytoskeleton in differentiation and myelination. *Glia*. 2009;57:1691–705.

- Baumgartner W, Schutz GJ, Wiegand J, Golenhofen N, Drenckhahn D. Cadherin function probed by laser tweezer and single molecule fluorescence in vascular endothelial cells. *J Cell Sci.* 2003;116:1001–11.
- Ben-David O, Pewzner-Jung Y, Brenner O, Laviad EL, Kogot-Levin A, Weissberg I, et al. Encephalopathy caused by ablation of very long acyl chain ceramide synthesis may be largely due to reduced galactosylceramide levels. *J Biol Chem.* 2011;286:30022–33.
- Benjamins JA, Dyer CA. Glycolipids and transmembrane signaling in oligodendroglia. *Ann N Y Acad Sci.* 1990;605:90–100.
- Boggs JM. Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. *Biochim Biophys Acta.* 1987;906:353–404.
- Boggs JM. Myelin basic protein: a multifunctional protein. *Cell Mol Life Sci.* 2006;63:1945–61.
- Boggs JM, Wang H. Effect of liposomes containing cerebroside and cerebroside sulfate on cytoskeleton of cultured oligodendrocytes. *J Neurosci Res.* 2001;66:242–53.
- Boggs JM, Wang H. Co-clustering of galactosylceramide and membrane proteins in oligodendrocyte membranes on interaction with polyvalent carbohydrate and prevention by an intact cytoskeleton. *J Neurosci Res.* 2004;76:342–55.
- Boggs JM, Koshy KM, Rangaraj G. Effect of fatty acid chain length, fatty acid hydroxylation, and various cations on the phase behavior of synthetic forms of cerebroside sulfate. *Chem Phys Lipids.* 1984;36:65–89.
- Boggs JM, Menikh A, Rangaraj G. Trans interaction between galactosylceramide and cerebroside sulfate across apposed bilayers. *Biophys J.* 2000;78:874–85.
- Boggs JM, Wang H, Gao W, Arvanitis D, Gong Y, Min W. A glycosynapse in myelin? *Glycoconj J.* 2004;21:97–110.
- Boggs JM, Gao W, Hirahara Y. Myelin glycosphingolipids, galactosylceramide and sulfatide, participate in carbohydrate-carbohydrate interactions between apposed membranes and may form glycosynapses between oligodendrocyte or myelin membranes. *Biochim Biophys Acta.* 2008a;1780:445–55.
- Boggs JM, Gao W, Hirahara Y. Signal transduction pathways involved on interaction of galactosylceramide/sulfatide-containing liposomes with cultured oligodendrocytes and requirement for myelin basic protein and glycosphingolipids. *J Neurosci Res.* 2008b;86:48–1458.
- Boggs JM, Homchaudhuri L, Rangaraj G, Liu YF, Smith GST, Harauz G. Interaction of myelin basic protein with cytoskeletal and signaling proteins in cultured primary oligodendrocytes and N19 oligodendroglial cells. *BioMed Central*, in press, 2004.
- Boggs JM, Gao W, Zhao J, Park H-J, Liu Y, Basu A. Participation of galactosylceramide and sulfatide in glycosynapses between oligodendrocyte or myelin membranes. *FEBS Lett.* 2010;584:1771–8.
- Boison D, Stoffel W. Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. *Proc Natl Acad Sci U S A.* 1994;91:11709–13.
- Bosio A, Binczek E, Stoffel W. Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. *Proc Natl Acad Sci U S A.* 1996;93:13280–5.
- Bucior I. Carbohydrate-carbohydrate interaction provides adhesion force and specificity for cellular recognition. *J Cell Biol.* 2004;165:529–37.
- Bucior I, Burger MM. Carbohydrate-carbohydrate interaction as a major force initiating cell-cell recognition. *Glycoconj J.* 2004;21:111–23.
- Burgisser P, Matthieu J-M, Waehnelndt TV. Myelin lipids: a phylogenetic study. *Neurochem Res.* 1986;11:1261–72.
- Burgisser P, Althaus H-H, Rohmann A, Neuhoff V. Lipid synthesis by oligodendrocytes from adult pig brain maintained in long-term culture. *Neurochem Int.* 1988;13:111–8.
- Butt AM, Pugh M, Hubbard P, James G. Functions of optic nerve glia: axoglial signalling in physiology and pathology. *Eye.* 2004;18:1110–21.
- Chakraborty G, Drivas A, Ledeen R. The phosphoinositide signaling cycle in myelin requires cooperative interaction with the axon. *Neurochem Res.* 1999;24:249–54.

- Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, et al. Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. *Cell*. 1996;86:209–19.
- Cook WJ, Bugg CE. Calcium-carbohydrate bridges composed of uncharged sugars. Structure of a hydrated calcium bromide complex of α -fucose. *Biochim Biophys Acta*. 1975;389:428–35.
- DeBruin LS, Harauz G. White matter rafting—membrane microdomains in myelin. *Neurochem Res*. 2007;32:213–28.
- Dick KJ, Eckhardt M, Paisan-Ruiz C, Alshehhi AA, Proukakis C, Sibtain NA, et al. Mutation of FA2H underlies a complicated form of hereditary spastic paraplegia (SPG35). *Hum Mutat*. 2010;31:E1251–60.
- Dupree JL, Pomicter AD. Myelin, DIGs, and membrane rafts in the central nervous system. *Prostaglandins Other Lipid Mediat*. 2010;91:118–29.
- Dyer CA. Novel oligodendrocyte transmembrane signaling systems. *Mol Neurobiol*. 1993;7:1–22.
- Dyer CA. The structure and function of myelin: from inert membrane to perfusion pump. *Neurochem Res*. 2002;27:1279–92.
- Dyer CA, Philibotte TM, Wolf MK, Billings-Gagliardi S. MBP mediates extracellular signals that regulate microtubule stability in oligodendrocyte membrane sheets. *J Neurosci Res*. 1994;39:97–107.
- Edgar JM, Garbern J. The myelinated axon is dependent on the myelinating cell for support and maintenance: molecules involved. *J Neurosci Res*. 2004;76:593–8.
- Edgar JM, McLaughlin M, Werner HB, McCulloch MC, Barrie JA, Brown A, et al. Early ultrastructural defects of axons and axon-glia junctions in mice lacking expression of *Cnp1*. *Glia*. 2009;57:1815–24.
- Edvardson S, Hama H, Shaag A, Gomori JM, Berger I, Soffer D, et al. Mutations in the fatty acid 2-hydroxylase gene are associated with leukodystrophy with spastic paraparesis and dystonia. *Am J Hum Genet*. 2008;83:643–8.
- Fewou SN, Bussow H, Schaeren-Wiemers N, Vanier MT, Macklin WB, Gieselmann V, et al. Reversal of non-hydroxy; α -hydroxy galactosylceramide ratio and unstable myelin in transgenic mice overexpressing UDP-galactose:ceramide galactosyltransferase. *J Neurochem*. 2005;94:469–81.
- Fewou SN, Fernandes A, Stockdale K, Francone VP, Dupree JL, Rosenbluth JR, et al. Myelin protein composition is altered in mice lacking either sulfated or both sulfated and non-sulfated galactolipids. *J Neurochem*. 2010;112:599–610.
- Fitzner D, Schneider A, Kippert A, Mobius W, Willig KI, Hell SW, et al. Myelin basic protein-dependent plasma membrane reorganization in the formation of myelin. *EMBO J*. 2006;25:1–12.
- Fujita A, Cheng J, Fujimoto T. Segregation of GM1 and GM3 clusters in the cell membrane depends on the intact actin cytoskeleton. *Biochim Biophys Acta*. 2009;1791:388–96.
- Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A. Phospholipids undergo hop diffusion in compartmentalized cell membrane. *J Cell Biol*. 2002;157:1071–81.
- Gielen E, Baron W, Vandeven M, Steels P, Hoekstra D, Ameloot M. Rafts in oligodendrocytes: evidence and structure-function relationship. *Glia*. 2006;54:499–512.
- Gomez-Mouton C, Abad JL, Mira E, Lacalle RA, Gallardo E, Jimenez-Baranda S, et al. Segregation of leading edge and uropod components into specific lipid rafts during T cell polarization. *Proc Natl Acad Sci U S A*. 2001;98:9642–7.
- Gorshkova TA, Mikshina PV, Gurjanov OP, Chemikosova SB. Formation of plant cell wall supra-molecular structure. *Biochemistry (Mosc)*. 2010;75:159–72.
- Hakomori S. Carbohydrate-carbohydrate interaction as an initial step in cell recognition. *Pure Appl Chem*. 1991;63:473–82.
- Hakomori S. The glycosynapse. *Proc Natl Acad Sci U S A*. 2002;99:225–32.
- Hao S, August A. Actin depolymerization transduces the strength of B-cell receptor stimulation. *Mol Biol Cell*. 2005;16:2275–84.
- Harauz G, Boggs JM. Myelin management by the 18.5-kDa and 21.5-kDa myelin basic protein isoforms. *J Neurochem*. 2013;125:334–61.

- Harder T, Simons K. Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. *Eur J Immunol.* 1999;29:556–62.
- Hirahara Y, Bansal R, Honke K, Ikenaka K, Wada Y. Sulfatide is a negative regulator of oligodendrocyte differentiation: development in sulfatide-null mice. *Glia.* 2004;45:269–77.
- Hollingshead CJ, Caspar DLD, Melchior V, Kirschner DA. Compaction and particle segregation in myelin membrane arrays. *J Cell Biol.* 1981;89:631–44.
- Honke K. Biosynthesis and biological function of sulfoglycolipids. *Proc Jpn Acad Ser B Phys Biol Sci.* 2013;89:129–38.
- Honke K, Hirahara Y, Dupree J, Suzuki K, Popko B, Fukushima K, et al. Paranodal junction formation and spermatogenesis require sulfoglycolipids. *Proc Natl Acad Sci U S A.* 2002;99:4227–32.
- Hoshi T, Suzuki A, Hayashi S, Tohyama K, Hayashi A, Yamaguchi Y, et al. Nodal protrusions, increased Schmidt-Lanterman incisures, and paranodal disorganization are characteristic features of sulfatide-deficient peripheral nerves. *Glia.* 2007;55:584–94.
- Ilyas AA, Chen ZW, Cook SD. Antibodies to sulfatide in cerebrospinal fluid of patients with multiple sclerosis. *J Neuroimmunol.* 2003;139:76–80.
- Inouye H, Kirschner DA. Membrane interactions in nerve myelin. II. Determination of surface charge from biochemical data. *Biophys J.* 1988;53:247–60.
- Inouye H, Kirschner DA. Phylogenetic aspects of myelin structure. In: Jeserich G, Althaus HH, Waehnel TV, editors. *Cellular and molecular biology of myelination.* Berlin: Springer; 1990. p. 376–87.
- Ioannidou K, Anderson KI, Strachan D, Edgar JM, Barnett SC. Time-lapse imaging of the dynamics of CNS glial-axonal interactions in vitro and ex vivo. *PLoS One.* 2012;7:e30775.
- Ishizuka I, Inomata M. Sulphated glycolipids in rat brain: decrease and disappearance after developmental age. *J Neurochem.* 1979;33:387–8.
- Iwabuchi K, Handa K, Hakomori S. Separation of “glycosphingolipid signaling domain” from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling. *J Biol Chem.* 1998;273:33766–73.
- Jackman N, Ishii A, Bansal R. Oligodendrocyte development and myelin biogenesis: parsing out the roles of glycosphingolipids. *Physiology.* 2009;24:290–7.
- Jacobs JM, Scadding JW. Morphological changes in IgM paraproteinemic neuropathy. *Acta Neuropathol (Berl).* 1990;80:77–84.
- Karthigasan J, Kosaras B, Nguyen J, Kirschner DA. Protein and lipid composition of the radial component-enriched CNS myelin. *J Neurochem.* 1994;62:1203–13.
- Khan AA, Bose C, Yam LS, Soloski MJ, Rupp F. Physiological regulation of the immunological synapse by agrin. *Science.* 2001;292:1681–6.
- Kirby BB, Takada N, Latimer AJ, Shin J, Carney TJ, Kelsh RN, et al. In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. *Nat Neurosci.* 2006;9:1506–11.
- Kirschner DA, Inouye H, Ganser AL, Mann V. Myelin membrane structure and composition correlated: a phylogenetic study. *J Neurochem.* 1989;53:1599–609.
- Kishimoto Y. Phylogenetic development of myelin glycosphingolipids. *Chem Phys Lipids.* 1986;42:117–28.
- Koshy KM, Boggs JM. Investigation of the calcium-mediated interaction between the carbohydrate head groups of galactosylceramide and galactosylceramide I³ sulfate by electrospray ionization mass spectrometry. *J Biol Chem.* 1996;271:3496–9.
- Koshy KM, Wang J, Boggs JM. Divalent-cation mediated interaction between cerebroside sulfate and cerebroside: an investigation of the effect of structural variations of lipids by electrospray ionization mass spectrometry. *Biophys J.* 1999;77:306–18.
- Kulkarni K, Snyder DS, McIntosh TJ. Adhesion between cerebroside bilayers. *Biochemistry.* 1999;38:15264–71.
- Kusumi A, Suzuki K, Koyasako K. Mobility and cytoskeletal interactions of cell adhesion receptors. *Curr Opin Cell Biol.* 1999;11:582–90.

- Kusumi A, Fujiwara TK, Morone N, Yoshida KJ, Chadda R, Xie M, et al. Membrane mechanisms for signal transduction: the coupling of the meso-scale raft domains to membrane-skeleton-induced compartments and dynamic protein complexes. *Stem Cell Dev Biol*. 2012;23:126–44.
- Lajoie P, Goetz JG, Dennis JW, Nabi IR. Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane. *J Cell Biol*. 2009;185:381–5.
- Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature*. 2012;487:443–8.
- Li C, Tropak MB, Gerlai R, Clapoff S, Abramow-Newerly W, Trapp B, et al. Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. *Proc Natl Acad Sci U S A*. 1994;91:11709–13.
- Marcus J, Dupree JL, Popko B. Myelin-associated glycoprotein and myelin galactolipids stabilize developing axo-glial interactions. *J Cell Biol*. 2002;156:567–77.
- Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, Dupree JL. Sulfatide is essential for the maintenance of CNS myelin and axon structure. *Glia*. 2006;53:372–81.
- Marone N, Fujiwara T, Murase K, Kasai RS, Ike H, Yuasa S, et al. Three-dimensional reconstruction of the membrane skeleton at the plasma membrane interface by electron tomography. *J Cell Biol*. 2006;174:851–62.
- Marta CB, Taylor CM, Coetzee T, Kim T, Winkler S, Bansal R, et al. Antibody cross-linking of myelin oligodendrocyte glycoprotein leads to its rapid repartitioning into detergent-insoluble fractions, and altered protein phosphorylation and cell morphology. *J Neurosci*. 2003;23:5461–71.
- Meixner M, Jungnickel J, Grothe C, Gieselmann V, Eckhardt M. Myelination in the absence of UDP-galactose:ceramide galactosyl-transferase and fatty acid 2-hydroxylase. *BMC Neurosci*. 2011;12:22.
- Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, et al. NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature*. 2005;439:988–92.
- Mitchell JS, Brown WS, Woodside DG, Vanderslice P, McIntyre BW. Clustering T cell GM1 lipid rafts increases cellular resistance to shear on fibronectin through changes in integrin affinity and cytoskeletal dynamics. *Immunol Cell Biol*. 2009;87:324–36.
- Morita K, Sasaki H, Fujimoto K, Furuse M, Tsukita S. Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *J Cell Biol*. 1999;145:579–88.
- Murray N, Steck AJ. Impulse conduction regulates myelin basic protein phosphorylation in rat optic nerve. *J Neurochem*. 1984;43:243–8.
- Nave K-A, Trapp BD. Axon-glial signaling and the glial support of axon function. *Annu Rev Neurosci*. 2008;31:535–61.
- Niles WD, Silvius JR, Cohen FS. Resonance energy transfer imaging of phospholipid vesicle interaction with a planar phospholipid membrane. *J Gen Physiol*. 1996;107:329–51.
- Norton WT. Isolation and characterization of myelin. In: Morell P, editor. *Myelin*. New York: Plenum; 1977. p. 161–99.
- Okamura N, Stoskopf M, Yamaguchi H, Kishimoto Y. Lipid composition of the nervous system of earthworms (*Lumbricus terrestris*). *J Neurochem*. 1985;45:1875–9.
- Paz Soldan MM, Warrington AE, Bieber AJ, Ciric B, van Keulen V, Pease LR, et al. Remyelination-promoting antibodies activate distinct Ca²⁺ influx pathways in astrocytes and oligodendrocytes: relationship to the mechanism of myelin repair. *Mol Cell Neurosci*. 2003;22:14–24.
- Pieringer J, Rao GS, Mandel P, Pieringer RA. The association of the sulphogalactosylglycerolipid of rat brain with myelination. *Biochem J*. 1977;166:421–8.
- Potter KA, Kern MJ, Fullbright G, Bielawski J, Scherer SS, Yum SW, et al. Central nervous system dysfunction in a mouse model of FA2H deficiency. *Glia*. 2011;59:1009–21.
- Prinetti A, Iwabuchi K, Hakomori S. Glycosphingolipid-enriched signaling domain in mouse neuroblastoma neuro2a cells. *J Biol Chem*. 1999;274:20916–24.
- Ranscht B, Wood PM, Bates M, Bunge RP. Role of galactocerebroside in the formation of peripheral myelin. In: Althaus HH, Seifert W, editors. *Glial-neuronal communication in development and regeneration*. New York: Springer; 1987. p. 666–81.

- Ravera S, Panfoli I, Calzia D, Aluigi MG, Bianchini P, Diaspro A, et al. Evidence for aerobic ATP synthesis in isolated myelin vesicles. *Int J Biochem Cell Biol.* 2009;41:1581–91.
- Readhead C, Takasashi N, Shine HD, Saavedra R, Sidman R, Hood L. Role of myelin basic protein in the formation of central nervous system myelin. *Ann N Y Acad Sci.* 1990;605:280–5.
- Remahl S, Hildebrand C. Relations between axons and oligodendroglial cells during initial myelination. II. The individual axon. *J Neurocytol.* 1990;19:883–98.
- Rinholm JE, Hamilton NB, Kessaris N, Richardson WD, Bergersen LH, Attwell D. Regulation of oligodendrocyte development and myelination by glucose and lactate. *J Neurosci.* 2011;31:538–48.
- Ro H, Carson JH. pH microdomains in oligodendrocytes. *J Biol Chem.* 2004;279:37115–23.
- Roder J. Myelination in the absence of myelin-associated glycoprotein. *Nature.* 1994;369:747–50.
- Rodgers W, Zavzavadjian J. Glycolipid-enriched membrane domains are assembled into membrane patches by associating with the actin cytoskeleton. *Exp Cell Res.* 2001;267:173–83.
- Rosenbluth J, Moon D. Dysmyelination induced in vitro by IgM antisulfatide and antigalactocerebroside monoclonal antibodies. *J Neurosci Res.* 2003;71:104–9.
- Rosenbluth J, Liang W-L, Liu Z, Guo D, Schiff R. Expanded CNS myelin sheaths formed *in situ* in the presence of an IgM antigalactocerebroside-producing hybridoma. *J Neurosci.* 1996;16:2635–41.
- Rosenbluth J, Schiff R, Lam P. Effects of osmolality on PLP-null myelin structure: implications re axon damage. *Brain Res.* 2009;1253:191–7.
- Saadat L, Dupree JL, Kilkus J, Han X, Traka M, Proia RL, et al. Absence of oligodendroglial glucosylceramide synthesis does not result in CNS myelin abnormalities or alter the dysmyelinating phenotype of CGT-deficient mice. *Glia.* 2010;58:391–8.
- Seah N, Santacrose PV, Basu A. Probing the lactose-GM3 carbohydrate-carbohydrate interaction with glycodendrimers. *Org Lett.* 2009;11:559–62.
- Selivonchick DP, Roots BI. Variation in myelin lipid composition induced by change in environmental temperature of goldfish (*Carassius auratus* L.). *J Therm Biol.* 1976;1:131–5.
- Shimomura K, Kishimoto Y. An improved procedure for the quantitative determination and characterization of sulfatides in rat kidney and brain by high performance liquid chromatography. *Biochim Biophys Acta.* 1983;754:93–100.
- Shimomura K, Kishimoto Y. Changes in monogalactosyl diacylglycerols, alkylgalactolipids, and cerebroside fatty acid esters in maturing brain measured by high-performance liquid chromatography. *Biochim Biophys Acta.* 1984;794:162–4.
- Shroff SM, Pomicter AD, Chow WN, Fox MA, Colello RJ, Henderson SC, et al. Adult CST-null mice maintain an increased number of oligodendrocytes. *J Neurosci Res.* 2009;87:3403–14.
- Simons M, Lyons DA. Axonal selection and myelin sheath generation in the central nervous system. *Curr Opin Cell Biol.* 2013;25:1–8.
- Simons M, Kramer EM, Thiele C, Stoffel W, Trotter J. Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. *J Cell Biol.* 2000;151:143–54.
- Smith GST, Homchaudhuri L, Boggs JM, Harauz G. Classic 18.5- and 21.5-kDa myelin basic protein isoforms associate with cytoskeletal and SH3-domain proteins in the immortalized N19-oligodendroglial cell line stimulated by phorbol ester and IGF-1. *Neurochem Res.* 2012;37:77–1295.
- Sonnino S, Prinetti A, Nakayama H, Yangida M, Ogawa H, Iwabuchi K. Role of very long fatty acid-containing glycosphingolipids in membrane organization and cell signaling: the model of lactosylceramide in neutrophils. *Glycoconj J.* 2009;26:615–21.
- Stewart RJ, Boggs JM. The carbohydrate-carbohydrate interaction between galactosylceramide-containing liposomes and cerebroside sulfate-containing liposomes: dependence on the glycolipid ceramide composition. *Biochemistry.* 1993;32:10666–74.
- Taguchi K, Yoshinaka K, Yoshino K, Yonezawa K, Maekawa S. Biochemical and morphologic evidence of the interaction of oligodendrocyte membrane rafts with actin filaments. *J Neurosci Res.* 2005;81:218–25.

- Tamai Y, Kojima H, Saito S, Takayama-Abe K, Horichi H. Characteristic distribution of glycolipids in gadoid fish nerve tissues and its bearing on phylogeny. *J Lipid Res.* 1992;33:1351–9.
- Treanor B, Depoil D, Gonzalez-Granja A, Barral P, Weber M, Dushek O, et al. The membrane skeleton controls diffusion dynamics and signaling through the B cell receptor. *Immunity.* 2010;32:187–99.
- Treanor B, Depoil D, Bruckbauer A, Batista FD. Dynamic cortical actin remodeling by ERM proteins controls BCR microcluster organization and integrity. *J Exp Med.* 2011;208:1055–68.
- van Itallie CM, Anderson JM. The molecular physiology of tight junction pores. *Physiology.* 2004;19:331–8.
- Vogler EA. Structure and reactivity of water at biomaterial surfaces. *Adv Colloid Interface Sci.* 1998;74:69–117.
- Vogt AB, Spindeldreher S, Kropshofer H. Clustering of MHC-peptide complexes prior to their engagement in the immunological synapse: lipid raft and tetraspan microdomains. *Immunol Rev.* 2002;189:135–51.
- Wang J, Chen H, Brown EJ. L-plastin peptide activation of $\alpha\text{v}\beta\text{3}$ mediated adhesion requires integrin conformational change and actin filament disassembly. *J Biol Chem.* 2001;276:14474–81.
- Warrington AE, Bieber AJ, Ciric B, Pease LR, Van Keulen V, Rodriguez M. A recombinant human IgM promotes myelin repair after a single, very low dose. *J Neurosci Res.* 2007;85:967–76.
- Watzlawik J, Holicky E, Edberg DD, Marks DL, Warrington AE, Wright BR, et al. Human remyelination promoting antibody inhibits apoptotic signaling and differentiation through Lyn kinase in primary rat oligodendrocytes. *Glia.* 2010;58:1782–93.
- Webb MS, Tilcock CPS, Green BR. Salt-mediated interactions between vesicles of the thylakoid lipid digalactosyldiacylglycerol. *Biochim Biophys Acta.* 1988;938:323–33.
- Winzeler AM, Mandemakers WJ, Sun MZ, Stafford M, Phillips CT, Barres BA. The lipid sulfatide is a novel myelin-associated inhibitor of CNS axon outgrowth. *J Neurosci.* 2011;31:6481–92.
- Witt A, Brady ST. Unwrapping new layers of complexity in axon/glia relationships. *Glia.* 2000;29:112–7.
- Wittenberg NJ, Im H, Xu X, Wootla B, Watzlawik J, Warrington AE, et al. High affinity binding of remyelinating natural autoantibodies to myelin-mimicking lipid bilayers revealed by nanohole surface Plasmon resonance. *Anal Chem.* 2012;84:6031–9.
- Wootla B, Watzlawik JO, Denic A, Rodriguez M. The road to remyelination in demyelinating diseases: current status and prospects for clinical treatment. *Expert Rev Clin Immunol.* 2013;9:535–49.
- Xu X, Warrington AE, Wright BR, Bieber AJ, van Keulen V, Pease LR, et al. A human IgM signals axon outgrowth: coupling lipid raft to microtubules. *J Neurochem.* 2011;119:100–12.
- Zhang H, Miller RH. Density-dependent feedback inhibition of oligodendrocyte precursor expansion. *J Neurosci.* 1996;16:6886–95.
- Zhao J, Liu Y, Park H-J, Boggs JM, Basu A. Carbohydrate-coated fluorescent silica nanoparticles as probes for the galactose/3-sulfogalactose carbohydrate-carbohydrate interaction using model systems and cellular binding studies. *Bioconjug Chem.* 2012;23:1166–73.
- Zoller I, Meixner M, Hartmann D, Bussow H, Meyer R, Gieselmann V, et al. Absence of 2-hydroxylated sphingolipids is compatible with normal neural development but causes late-onset axon and myelin sheath degeneration. *J Neurosci.* 2008;28:9741–54.