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10 Functional Dynamics of Myelin Lipids*

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*This book chapter is dedicated to the memory of Prof. Steven E. Pfeiffer (1940–2007). May every reader of this chapter remember him as one of the scientists who largely contributed to the comprehension of the myelin physiology and oligodendrocyte development.

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Abstract: Biological membranes of living organism are composed of two fundamental components: proteins and lipids. Lipids are defined as water-insoluble biomolecules, which have high solubility in nonpolar organic solvents. They account for more than half of the total mass of myelin, which is an extension of oligodendrocyte plasma membrane that spirally enwraps axons and is critical for efficient nerve conduction. Because of the high lipid content of myelin, in particular glycosphingolipids and cholesterol, it was thought to play a central role in myelin/oligodendrocyte physiology. This view has been strongly supported by multiple approaches, most prominently the gene knockout studies that have significantly enhanced our understanding and appreciation of lipids in the overall function and structure of the CNS myelin. This chapter discusses the role of lipids in the regulation of myelin/oligodendrocyte physiology including oligodendrocyte development, myelin biogenesis and maintenance, and sorting and transport of myelin components.

List of Abbreviations: ABC, ATP-binding cassette; CNP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; CNS, central nervous system; CST, cerebroside sulfotransferase; DHAP, dihydroxyacetone-phosphate; GalCer, galactosylceramide; GD1a, Neu5Acα3Galβ3GalNAcβ4-(Neu5Acα3)Galβ4GlcCer; GD1b, Galβ3-GalNAcβ4(Neu5Acα8Neu5Acα3)Galβ4-GlcCer; GD2, GalNAcβ4-(Neu5Acα8Neu5Acα3)Galβ4GlcCer; GD3, Neu5Acα8Neu5Acα3Galβ4GlcCer; GlcCer, glucosylceramide; GM1a, Galβ3GalNAcβ4(Neu5Acα3) Galβ4GlcCer; GM1b, Neu5Acα3Galβ3GalNAcβ4Galβ4GlcCer; GM2, GalNAcβ4(Neu5Acα3)Galβ4GlcCer; GM3, Neu5Acα3Galβ4GlcCer; GM4, *N*-acetylneuraminylgalactosylceramide; GQ1b, Neu5Acα8Neu5Acα3-Galβ3GalNAcβ4(Neu5Acα8Neu5Acα3)Galβ4GlcCer; Gro-3P, glycerol-3-phosphate; GT1a, Neu5Acα8Neu5-Acα3Galβ3GalNAcβ4(Neu5Acα3)Galβ4GlcCer; GT1b, Neu5Acα3-Galβ3GalNAcβ4(Neu5Acα8Neu5Acα3) Galβ4GlcCer; GT1c, Galβ3GalNAcβ4(Neu5Acα8Neu5Acα8Neu5Acα3)Galβ4GlcCer; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MDR, multidrug resistant protein; MGDG, monogalactosyl-diacylglycerol; MOG, myelin oligodendrocyte glycoprotein; NeuAc, *N*-acetylneuraminic acid; PLP, proteolipid protein; PNS, peripheral nervous system; SialT, sialyltransferase; SPTLCB, serine palmitoyltransferase long-chain base

1 Introduction

By means of light microscopy, the pathologist Rudolf Virchow (1854) found that the axon of the nerve fibers was surrounded by a substance to which he gave the name "myelin." A breakthrough in the understanding of myelin was the observation by Ranvier (1878) that myelin forms a covering of the nerve that is periodically interrupted at regular spacings along the nerve. The constrictions in the nerve fiber that separate two internodal regions now carry his name, nodes of Ranvier, and were found in the first half of the twentieth century to allow the saltatory conduction of the nerve impulse (Rosenbluth, 1999). Studies with polarized light, X-ray diffraction, and electron microscopy have shown that, in both the central and peripheral nervous systems (CNS and PNS, respectively), myelin is made up of regular concentric lamellae appearing as alternating dark and less dark lines separated by lipid hydrocarbon chains that appear as unstained zones (*Figure 10-1*). One of the biochemical characteristics that distinguish myelin from other biological membranes is its high lipid-to-protein ratio. Indeed, the lipid/protein ratio of myelin is ~2:1, in contrast to the whole brain, which contains more proteins than lipids (*Table 10-1*). Because of its high lipid content, myelin appears white in the macroscopic view. Therefore, highly myelinated regions of the CNS are called "white matter," in contrast to the poorly myelinated regions which are called "grey matter" (Stegemeyer and Stegemeyer, 2004).

During the last 25 years, important data have been gathered concerning the synthesis and function of lipids in the nervous system, although this field has received little attention compared with that of their protein counterparts. The same period has seen the identification and cloning of cDNAs and genes implicated in the biosynthesis of myelin lipids and proteins, which signaled the beginning of the knockout era: transgenic null-mutant animals have been created for almost every enzyme implicated in the biosynthesis of myelin-enriched lipids. The study of these animals shows that the formation of myelin is considerably less sensitive to the alteration of lipid content than the maintenance of myelin.

Figure 10-1

The structure of the myelin membrane. (a) Ultrastructure of myelin in the mouse optic nerve. Note that myelin (m) appears dark compared to the axon that appears white. (b) Schematic structure of CNS myelin. Note that the extracellular leaflets of adjacent lamellae become closely apposed to each other to form the intraperiod line (IPL), while the cytoplasmic membrane leaflets fuse to form the major dense line (MDL). The two major proteins (MBP and PLP) of the CNS myelin are depicted. One postulated function for PLP is that it acts like glue to keep the adjacent layers of myelin tightly joined together. The lipid bilayers are shown as plasma membrane. CNP, MAG, MOG, and other minor myelin proteins are not shown. Figure adapted from Quarles et al., 2006 (Courtesy of Dr. Simon Ngamli Fewou)

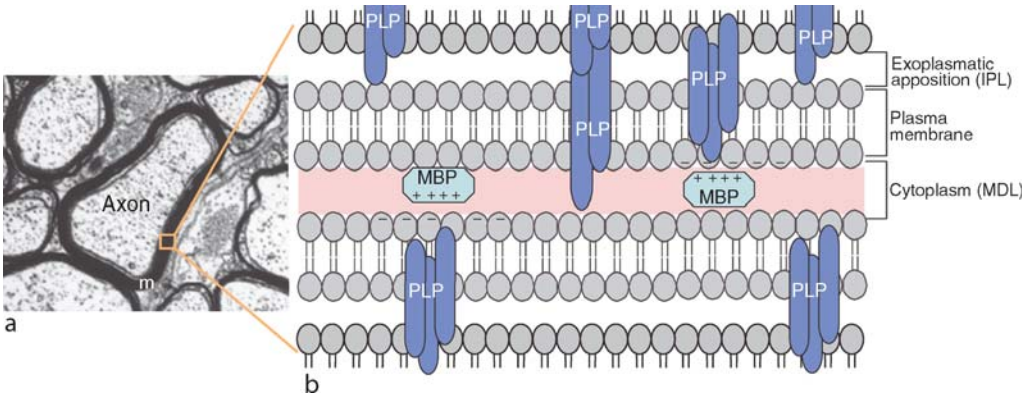


Table 10-1

Lipid composition of the CNS myelin and brain of different species

	Myelin			White matter		Gray matter	Whole Brain
Component[a]	Human	Bovine	Rat	Human	Bovine	(Human)	(Rat)
Total protein	30.0	24.7	29.5	39.0	39.5	55.3	56.9
Total lipid	70.0	75.3	70.5	54.9	55.0	32.7	37.0
Cholesterol	27.7	28.1	27.3	27.7	23.6	22.0	23.0
Cerebroside	22.7	24.0	23.7	19.8	22.5	5.4	14.6
Sulfatide	3.8	3.6	7.1	5.4	5.0	1.7	4.8
Total galactolipids	27.5	29.3	31.5	26.4	28.6	7.3	21.3
Phospholipids	43.1	43.0	44.0	45.9	46.3	69.5	57.6
Sphingomyelin	7.9	7.1	3.2	7.7	6.7	6.9	3.8

[a]Total protein and lipid figure in percentage dry weight. All others are in percentage total lipid weight. For further quantification, we refer the reader to Quarles et al. (2006)

In this chapter, we review current ideas regarding the lipid biosynthesis, its role in the regulation of protein transport, in myelin biogenesis, and its impact on the regulation of oligodendrocyte (OL) physiology. As further background, we refer the reader to our recent chapter on myelin lipids (Taylor et al., 2004).

2 Lipid Biosynthesis

2.1 Sphingolipid Biosynthesis

Sphingolipids are a family of lipids that comprise both structural lipids and a series of highly bioactive compounds that participate in the regulation of cell growth, differentiation, diverse cell functions, and

apoptosis. They are typically found in high amounts in eukaryotic plasma membranes, and their content is particularly high in apical membranes of epithelial cells (Simons and van Meer, 1988) and in CNS and PNS myelin where they constitute about 30% of total lipids (*Table 10-1*). Structurally, they are composed of a sphingoid base, a straight chain amino alcohol of 18–20 carbon atoms, which normally carries a singular long- or very long-chain fatty acid, saturated or unsaturated at C15, bound to the amino group at the C2 carbon (for review see Holthuis et al., 2001) to form ceramide. Sphingolipids are classified as phosphosphingolipids and glycosphingolipids based on the polar head group on the ceramide backbone.

The biosynthesis of sphingolipids starts in the endoplasmic reticulum (ER) with the synthesis of the sphingoid base and ceramide. Serine-palmitoyltransferase (SPT; EC 2.3.1.50) catalyzes the rate-limiting step in de novo synthesis of sphingolipids (Merrill and Jones, 1990), the pyridoxal-5′-phosphate-dependent condensation of L-serine and palmitoyl-CoA to 3-ketosphinganine (Weiss and Stoffel, 1997). Mammalian SPT comprises two homologous proteins, SPT long-chain base 1 (SPTLCB1) and SPT long-chain base 2 (SPTLCB2), which are heterodimers of 53- and 63-kDa subunits, respectively, and both of which are required for its full enzymatic activity (Hanada et al., 1997, 1998, 2000; Yasuda et al., 2003).

The conversion of 3-ketosphinganine to the sphingoid base, sphinganine, is catalyzed by the 3-ketosphinganine reductase, an enzyme encoded by the follicular lymphoma variant translocation-1 gene (Kihara and Igarashi, 2004). Sphinganine is then N-acylated by ceramide synthase to form dihydroceramide, which is finally desaturated to form ceramide (*Figure 10-2*). In mammals, ceramide synthase protein is encoded by six members of the ceramide synthase (CerS) gene family also called longevity-assurance homologue (Lass) gene family (Pewzner-Jung et al., 2006). Overexpression of any CerS protein in cultured cells results in an increase in cellular ceramide, but the ceramide species produced varies. Overproduction of CerS1 protein increased $C_{18:0}$-ceramide levels preferentially, and overproduction of CerS2 and CerS4 increased levels of $C_{22:0}$- and $C_{24:0}$-ceramides. CerS5 and CerS6 produced shorter ceramide species ($C_{14:0}$- and $C_{16:0}$-ceramides); however, only CerS-5 was able to incorporate $C_{18:1}$-CoA (Mizutani et al., 2005, 2006; Pewzner-Jung et al., 2006). In addition to being implicated in the synthesis of very long chain fatty acid ceramide, CerS2 is specifically expressed in OLs and Schwann cells. Moreover the level of CerS2 in the mouse brain is developmentally upregulated, with a maximum expression level at postnatal day 21 (Becker et al., 2007), which correlate with the peak of myelination. These results might suggest a close relationship between CerS2 expression and myelination. In contrast, CerS1 is specifically expressed in brain, in the cortical region (Becker et al., 2007), while CerS5 and -6 are expressed in brain but also in other tissues (Mizutani et al., 2005).

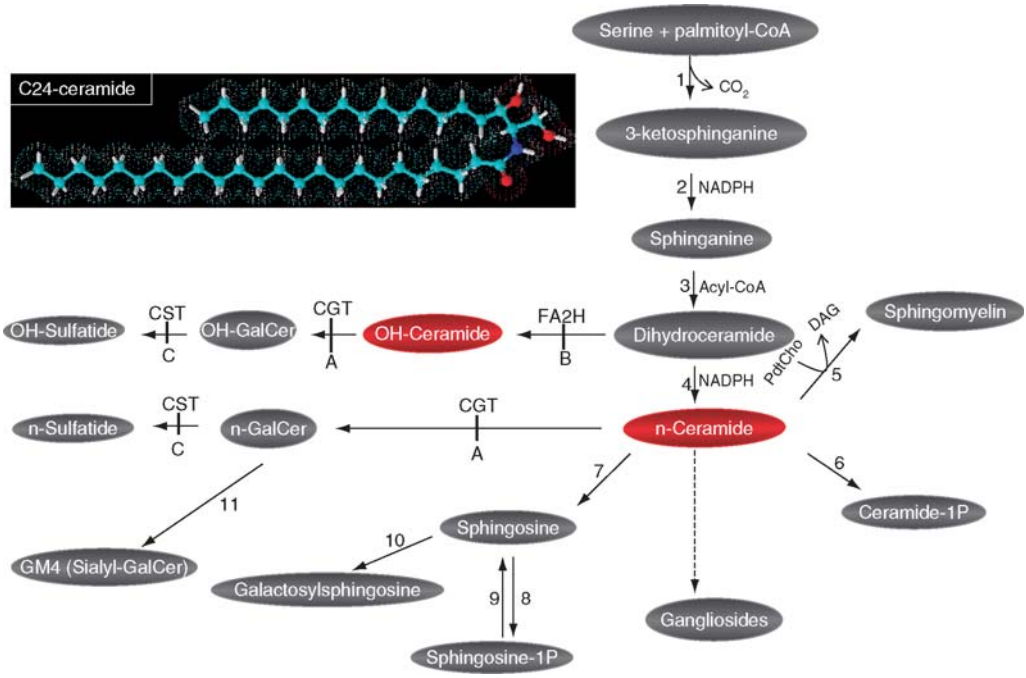
The galactosylation of ceramide in the ER lumen produces galactosylceramide (GalCer) and is catalyzed by UDP-galactose:ceramide galactosyltransferase (CGT), a type 1 integral membrane protein (Sprong et al., 1998). Following its biosynthesis, a fraction of GalCer reaches the lumen of Golgi and is sialylated by the action of sialyltransferase to form *N*-acetylneuraminyl-galactosylceramide (GM4), or used as a substrate by cerebroside sulfotransferase (CST) with 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to synthesize sulfatide (*Figure 10-2*). CST is a Golgi type II membrane protein that also catalyzes the synthesis of seminolipid and sulfated lactosylceramide (Honke et al., 1996, 1997). GalCer and sulfatide comprise 23% and 4% of the total mass of myelin lipids, respectively, and together account for one-third of the lipid content in the myelin sheath (Norton and Cammer, 1984), and more than half of the GalC in myelin exists as a 2-hydroxy fatty acid containing isoform that is unique to myelin (Schaeren-Wiemers et al., 1995).

2.1.1 Ganglioside Synthesis

Gangliosides are sialic acid-containing glycosphingolipids that are known to modulate the activity of a number of receptor tyrosine kinases, including the insulin receptor (Allende and Proia, 2002). Our present knowledge on the mechanism of ganglioside biosynthesis comes mainly from the pioneering studies of Roseman, Brady, and coworkers (Kaufman et al., 1966; Roseman, 1970; Fishman et al., 1972; Basu et al., 1973). These investigators demonstrated that the glycosyl chains of gangliosides are formed in a stepwise manner by the sequential addition of individual sugar and sialyl groups to the growing glycolipids. Gangliosides are synthesized in the lumen of Golgi from lactosylceramide (LacCer) by specific glycosyltransferases and sialyltransferases (*Figure 10-3*) (Kaufman et al., 1968; Basu et al., 1973; Keenan et al., 1974;

Figure 10-2

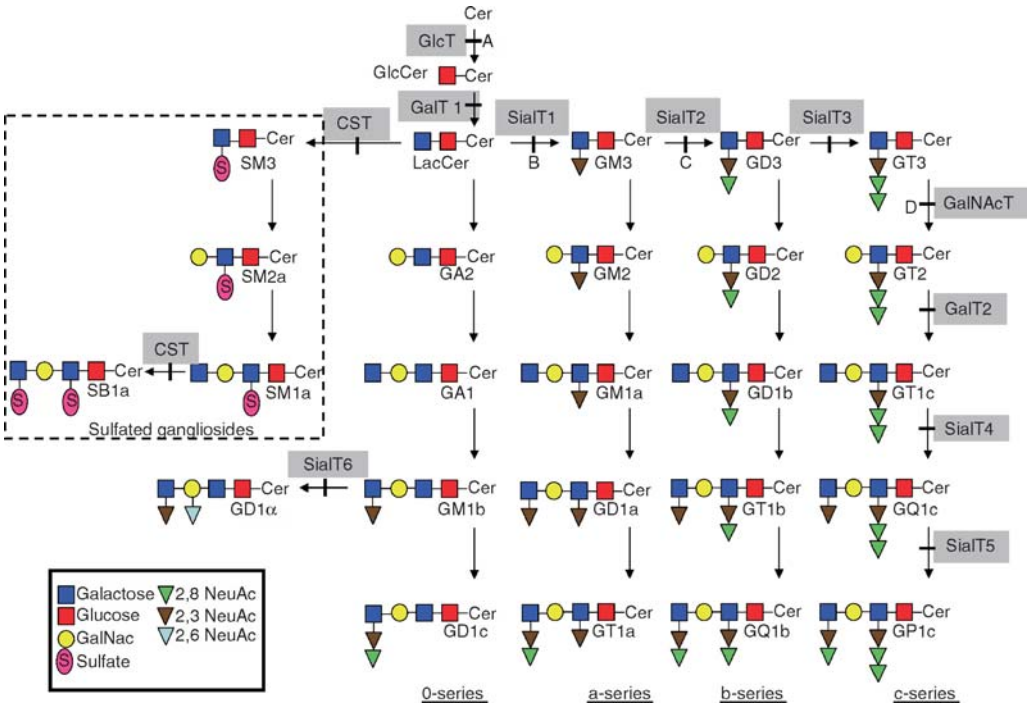
Pathways of sphingolipid biosynthesis. The biosynthesis of sphingolipids starts in the ER with the condensation of palmitoyl CoA and serine. The enzymes involved in the biosynthesis of ceramide are listed in order as follows: (1) serine palmitoyltransferase, (2) 3-ketosphinganine reductase, (3) dihydroceramide synthase, (4) dihydroceramide desaturase. Ceramide can be converted to sphingomyelin upon the action of (5) sphingomyelin synthase. In addition, conversion of ceramide to ceramide 1-phosphate is catalyzed by ceramide kinase (6). The hydrolysis of ceramide by ceramidase (7) yields sphingosine, which can be either transformed to sphingosine 1-phosphate by the action of sphingosine kinase (8) or galactosylated by the UDP-Galactose:ceramide galactosyltransferase (CGT: 10) to form galactosylsphingosine, a highly toxic molecule in oligodendrocytes. Sphingosine can be also synthesized from sphingosine-1P by the action of sphingosine-1P phosphatase (9). Ceramide can be converted to galactosylceramide (GalCer) by the action of CGT (A), which utilizes both hydroxylated (OH^-) and nonhydroxylated (n-) fatty acid-containing ceramide. For the synthesis of hydroxyl fatty acid GalCer, the hydroxy fatty acid ceramide is first synthesized by the action of fatty acid 2-hydroxylase (B), an ER resident membrane protein (Alderson et al., 2004; Eckhardt et al., 2005). GalCer can be also sialylated in the Golgi by sialyltransferase (11) to form sialylgalactosylceramide or GM4. Finally, sulfatide is synthesized by using GalCer as substrate, a reaction catalyzed by cerebroside sulfotransferase (CST) (C) (Adapted from Taylor et al., 2004)



Lloyd et al., 1998; Maccioni et al., 1999). The biosynthesis of LacCer starts on the cytosolic face of the Golgi by the transfer of glucose from UDP-glucose to ceramide to form glucosylceramide (GlcCer). This reaction is catalyzed by UDP-glucose:ceramide glucosyltransferase, a type-III transmembrane protein (Futerman and Pagano, 1991; Jeckel et al., 1992; Ichikawa et al., 1996; Paul et al., 1996). Part of the GlcCer then reaches the lumen of Golgi via FAPP2, a GlcCer binding protein associated with the trans Golgi via phosphatidylinositol-4-phosphate and ARF (D'Angelo et al., 2007; Halter et al., 2007). Although previous studies on fluorescent GlcCer analogues had suggested that GlcCer is translocated across the Golgi membrane by the multidrug transporter ABCB1 (van Helvoort et al., 1996; Nicholson et al., 1999; Lala et al., 2000; Veldman et al., 2002; Eckford and Sharom, 2005), it was later shown that translocation by ABCB1 was specific for the fluorescent analog and that natural GlcCer flips by an independent mechanism possibly in the ER after retrograde

Figure 10-3

Ganglioside biosynthesis: The entry point in the biosynthetic pathway of gangliosides of all series is the conversion of glucosylceramide to lactosylceramide (precursor of gangliosides of 0-series) by GalT1. LacCer can then be converted to GM3, GD3, and GT3, the precursor of gangliosides of a-, b-, and c-series, respectively. The respective reactions are catalyzed by SialT1, -2, and -3; SialT2 and 3 are possibly the same enzyme (see text). CST catalyzes the conversion of LacCer to SM3, the precursor of all sulfated gangliosides. Knockout mice have been generated for the following enzymes: GlcT (A), SialT1 (B), SialT2 (C), and GalNAcT (D) (Adapted from van Echten and Sandhoff, 1993, Kolter et al. (2002) and Nagai et al. (2005))



transport via FAPP2 (Halter et al., 2007). After the FAPP2-dependent transport to the Golgi lumen, a galactose is transferred onto GlcCer, leading to the synthesis of LacCer by galactosyltransferase-1 (GalT1).

Sialyltransferase-1 (SialT1) catalyzes the addition of sialic acid (*N*-acetylneuraminic acid; NeuAc) on the galactose residue of LacCer to generate the monosialoganglioside (GM3), which is the precursor of complex gangliosides. Further sialylation of GM3 gives rise to the disialoganglioside GD3 and the trisialoganglioside GT3. GM3, GD3, and GT3 represent the entry substrates for the biosynthesis of gangliosides of the a-, b-, and c-series pathways, respectively, while direct conversion of LacCer to GA2 followed by subsequent addition of sugar and sialic acid gives rise to GD1c, the end product of gangliosides of the 0-series (*Figure 10-3*). The synthesis of GD3 and GT3 might be performed by a single enzyme since cDNA alignment indicates that SialT2 and SialT3 have identical nucleotide sequence (Nakayama et al., 1996), and transfection with SialT2 gave rise to GT3 synthesis (Daniotti et al., 2002). In contrast to these observations, lipidomic analysis of the brain of mice lacking β1,4-*N*-acetylgalactosaminyl-transferase (GalNacT), the enzyme that converts LacCer, GM3, GD3, and GT3 into their respective products, shows no accumulation of GT3 (Takamiya et al., 1996). GT3 accumulation should have occurred, because GD3 accumulation in the brain of that mutant mouse should have been converted to GT3 as the only downstream metabolite from GD3 in that mutant animal. Thus, it has been concluded that a sialyltransferase different from SialT2 is required for GT3 synthesis in vivo (Kolter et al., 2002). The glycosylation of GM3, GD3, and GT3 is performed by a few glycosyltransferases of limited specificity. It is known that these glycosyltransferases physically associate as a multiprotein and that the N-terminal domain of each enzyme participates in this

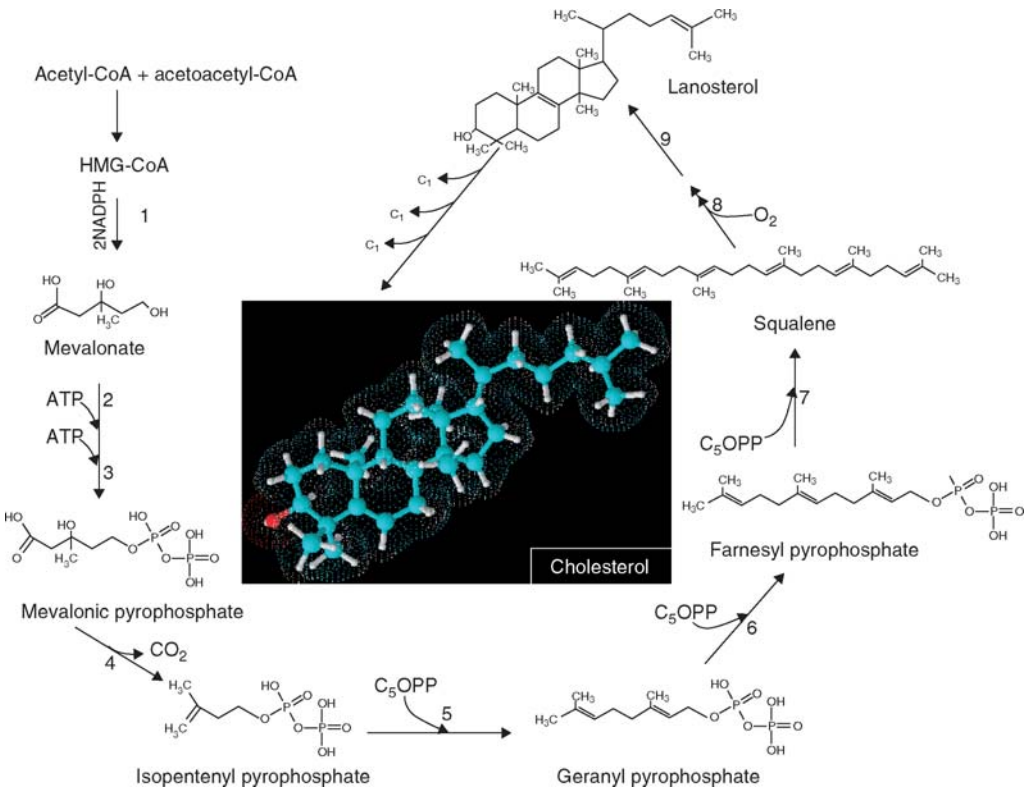
interaction (Giraudo and Maccioni, 2003) and, therefore, dictates the Golgi compartmentalization of the multienzyme complex (Uliana et al., 2006a). For example, the complex formed by GalT1/SialT1/SialT2 is located in the Golgi stack (Uliana et al., 2006b; Halter et al., 2007), while the GalNacT and GalT2 complex is mostly located in the trans-Golgi network (TGN) (Giraudo et al., 1999, 2001). The glycosylation of LacCer, GM3, GD3, and GT3 leads to the generation of asialo-GM2, GM2, GD2, and GT2 by the action of GalNacT. Subsequently, GalT2 converts the previous products to GA1 (asialo-GM1a), GM1a, GD1b, and GT1c, which are further sialylated by the consecutive action of SialT4 and 5 to generate the complex final products of the ganglioside synthesis pathway (Kaufman et al., 1968; Sandhoff and van Echten, 1993; Yamashiro et al., 1995; Taylor et al., 2004).

2.2 Cholesterol Biosynthesis

In addition to GalCer and sulfatide that together account for 30% of myelin lipid, the other most abundant lipid in myelin is cholesterol. *Figure 10-4* schematically outlines the cholesterol biosynthesis pathway.

Figure 10-4

Cholesterol biosynthesis: The condensation of acetyl-CoA and acetoacetyl-CoA to form HMG-CoA is the biosynthetic pathway of cholesterol in animal cells, but the rate-limiting step is the reaction catalyzed by HMG-CoA reductase. Additional enzymes in the cholesterol biosynthetic pathway are as follows: (2) mevalonate-5-phosphotransferase, (3) phosphomevalonate kinase, (4) pyrophosphomevalonate decarboxylase, (5) prenyl transferase, (6) prenyl transferase, (7) squalene synthase, (8) squalene epoxidase, (9) squalene oxidocyclase



Briefly, cholesterol synthesis is initiated in the cytosol by the condensation of acetyl-CoA and acetoacetyl-CoA to yield 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), a reaction catalyzed by HMG-CoA synthase. HMG-CoA is then reduced to mevalonate by the action of HMG-CoA reductase (HMGR), a tetrameric protein consisting of two dimers that localize to the ER and peroxisomes in the mouse brain stem and cerebellum (Reinhart et al., 1987; Istvan et al., 2000; Kovacs et al., 2001). HMGR is among the most highly regulated enzymes (Goldstein and Brown, 1990). Transcription and translation of HMGR increase when the concentration of products of the mevalonate pathway is low. Conversely, when sterol concentrations are high, the intracellular HMGR concentration decreases rapidly (Nakanishi et al., 1988). A third level of regulation is achieved by phosphorylation of S872 (human enzyme) by AMP-activated protein kinase, which decreases HMGR activity (Omkumar et al., 1994). The synthesized mevalonate is then transformed to cholesterol after subsequent reactions that include pyrophosphorylation, farnesylation, oxidization, and thereafter cyclization (Taylor et al., 2004).

2.3 Phospholipid Biosynthesis

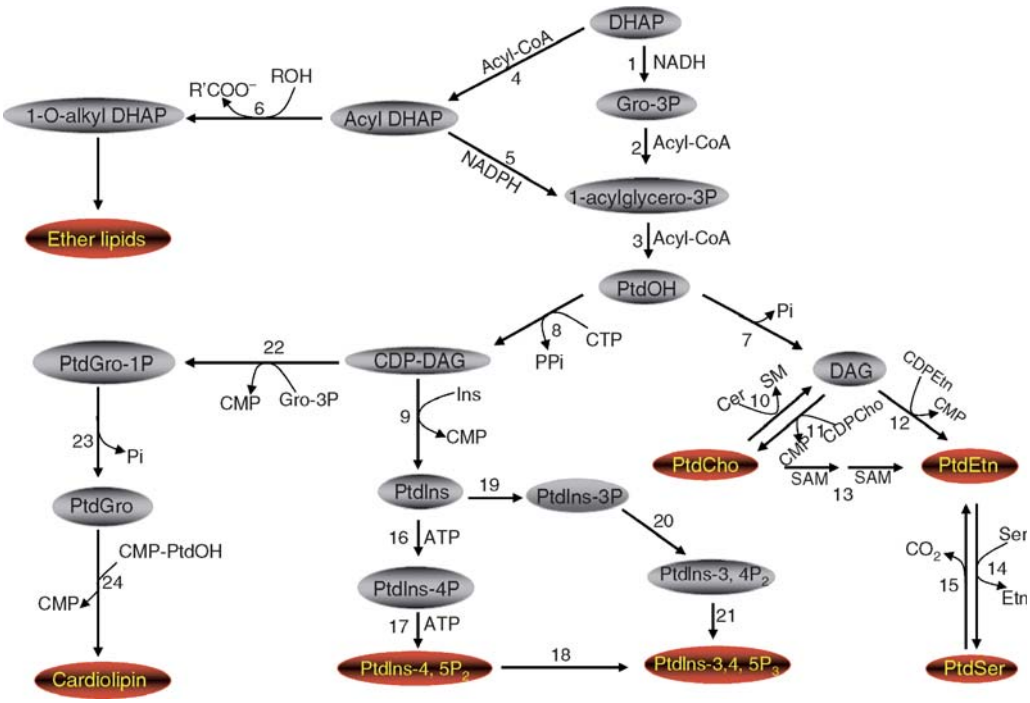
2.3.1 Phosphosphingolipid Biosynthesis

Phosphosphingolipids are sphingolipids that contain a phosphate group attached to the primary hydroxyl of ceramide. Among the phosphosphingolipids, sphingomyelin (SM) is a key membrane component of higher eukaryotes. It provides a reservoir of messenger signals that mediate cellular processes such as programmed cell death, cellular stress, mitogenesis, and senescence (Andrieu-Abadie and Levade, 2002; Kolesnick, 2002; Bektas and Spiegel, 2004; Futerman and Hannun, 2004). The synthesis of SM is mediated by a phosphatidylcholine:ceramide cholinephosphotransferase (SM synthase), a membrane-associated enzyme that transfers the phosphocholine moiety from phosphatidylcholine (PdtCho) onto the primary hydroxyl group of ceramide and generates SM and diacylglycerol (DAG: Ullman and Radin, 1974; Voelker and Kennedy, 1982) (*Figures 10-2* and *5-5*). In OLs, most of the phosphocholine used for the biosynthesis of SM is provided by PdtCho (Vos et al., 1997). In addition, SM synthases can act in the reverse pathway, generating PdtCho from DAG and SM (van Helvoort et al., 1994), indicating that SM synthases might regulate the pool of cellular ceramide and DAG, two highly active biomolecules that are implicated in the regulation of membrane trafficking and apoptosis (Scurlock and Dawson, 1999; Brose and Rosenmund, 2002; Lee et al., 2004). Hence, the physiological significance of the expression of SM synthases in eukaryotic cells might be beyond the regulation of ceramide and DAG pool. Recent investigations have demonstrated that human, mouse, pig, and C. elegans genomes contain at least two SM synthase genes: SM synthase-1 and -2 (Huitema et al., 2004; Yamaoka et al., 2004). Whereas SM synthase-1 was localized at the *cis*/medial Golgi by cell fractionation (Futerman et al., 1990; Jeckel et al., 1990), a tagged SM synthase-1 has now been located in the trans-Golgi by immunoelectron microscopy (Halter et al., 2007). SM synthesis was found to occur in the TGN of neuronal cells (Sadeghlar et al., 2000). SM synthase-2 is mostly detected at the plasma membrane (Futerman et al., 1990; van Helvoort et al., 1994; Huitema et al., 2004). This finding might indicate that SM synthase-2 is transported at the myelin assembly site where it catalyzes the synthesis of SM necessary for myelin biogenesis. Moreover, the most important role of SM is its capacity to participate in the formation of lipid rafts, the sorting platform that is involved in the transport of cell membrane components and signal transduction (Simons and van Meer, 1988; Verkade and Simons, 1997). In this point of view, SM synthase-1 located in the lumen of Golgi in OLs might be responsible for the synthesis of SM necessary for the formation of the lipid raft. Therefore, SM might play a critical role in the sorting and transport of myelin components necessary for the biogenesis of the myelin sheath, since the raft is the sorting platform for apical membrane trafficking (Hoekstra et al., 2003; Fullekrug and Simons, 2004).

The other phosphosphingolipid present in myelin is ceramide 1-phosphate (C1P), which is synthesized by the transfer of a phosphate group to the primary hydroxyl group of ceramide. This reaction is catalyzed by ceramide kinase (*Figure 10-2*; CERK). CERK activity is detected in almost all mammalian tissues, but the level of activity differs from tissue to tissue. In mouse, the highest CERK activity was found in testis and brain (van Overloop et al., 2006). Moreover, by separating the subcellular organelles using differential

Figure 10-5

Biosynthetic pathway of phospholipids and ether lipids: phospholipids and ether lipids are derived from the enzymatic transformation of dihydroxyacetone phosphate (DHAP). The following enzymes play a role in the transformation of DHAP: (1) glycerophosphate dehydrogenase, (2) sn-glycerol-3-phosphate acyltransferase, (3) 1-acyl glycerol-3-phosphate acyltransferase, (4) dihydroxyacetone phosphate acyltransferase, (5) acyl/alkyl dihydroxyacetone phosphate reductase, (6) alkyl dihydroxyacetone phosphate synthase, (7) phosphatidate phosphohydrolase, (8) phosphatidate cytidyltransferase, (9) phosphatidylinositol synthase, (10) phosphatidylcholine: ceramide choline phosphotransferase, (11) diacylglycerol cholinephosphotransferase, (12) diacylglycerol ethanolaminephosphotransferase, (13) phosphatidylethanolamine *N*-methyl transferase and phosphatidyl-*N*-methylethanolamine *N*-methyl transferase, (14) phosphatidylethanolamine:serine transferase, (15) phosphatidylserine decarboxylase, (16) phosphatidylinositol 4-kinase, (17) phosphatidylinositol-4-phosphate 5-kinase, (18) phosphatidylinositol-4,5-phosphate 3-kinase, (19) phosphatidylinositol-3-kinase, (20) phosphatidylinositol-3-phosphate 4-kinase, (21) phosphatidylinositol-3,4 phosphate 5-kinase, (22) CDP-DAG:glycerol-3-phosphate phosphatidyltransferase, (23) phosphatidylglycerol phosphatase, (24) cardiolipin synthase (Adapted from Farooqui et al. (2000), Cooke (2004), and Taylor et al. (2004))



centrifugation techniques, van Overloop et al. (2006) found that the CERK was concentrated in the microsomal fraction. In addition, immunofluorescence analysis using different cell lines has demonstrated that CERK associates with the Golgi and plasma membrane (Carre et al., 2004; van Overloop et al., 2006). Therefore, C1P might be synthesized both at the plasma membrane and in the Golgi. C1P is a highly bioreactive molecule that regulates many cellular processes including cell survival and proliferation and stimulation of DNA synthesis (Gomez-Munoz et al., 1995, 2004, 2005).

2.3.2 Glycerophospholipid Biosynthesis

Glycerophospholipids are compounds similar to triglycerides. However, they have a phosphate group and a simple organic molecule in the place of one of the fatty acids. Brain tissue contains high amounts of

phospholipids. In the adult brain, glycerophospholipids, glycolipids, and cholesterol account for 50–60% of the total membrane mass with proteins accounting for most of the remainder. Within the brain, myelin contains the highest amount of glycerophospholipid (Farooqui et al., 2000). The most abundant glycerophospholipids of the mammalian tissue are phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylserine (PtdSer), and phosphatidylinositol (PtdIns). Besides the above phospholipids, cellular membranes contain plasmalogens, a phospholipid containing a vinyl ether linkage. *Figure 10-5* outlines the biosynthetic pathway of PtdCho, PtdEtn, and PtdSer that have been described in eukaryotic organisms (van Golde et al., 1974; Vance, 1990; Saito et al., 1996; Stone and Vance, 2000). First, dihydroxyacetone-phosphate (DHAP) is reduced to glycerol-3-phosphate (Gro-3P), which is successively acylated to produce phosphatidic acid (PtdOH). Alternatively, DHAP can be directly acylated followed by alkylation to produce the alkyl-DHAP, which is the precursor of ether lipids. Subsequently, PtdOH can be converted to DAG and CDP-DAG. Once formed, DAG is used for the synthesis of PtdCho and PtdEtn via the CDP-choline and CDP-ethanolamine pathway, also known as the Kennedy pathway (Yavin and Zeigler, 1977; Arthur and Page, 1991; Bakovic et al., 2007). In this pathway, choline or ethanolamine is converted in the cytosol into phosphocholine or phosphoethanolamine by choline kinase (CKI) or ethanolamine kinase (EKI), respectively (Kent, 1995). In the second reaction, the phosphocholine or phosphoethanolamine is transferred to a nucleotide diphosphate by the action of phosphocholine or ethanolamine cytidylyltransferase to form CDP-choline or CDP-ethanolamine, respectively. Finally, the phosphocholine or phosphoethanolamine is transferred to the 1,2-diacylglycerol by the action of CDP-choline or CDP-ethanolamine:1,2-diacylglycerol choline or ethanolamine phosphotransferase (Vermeulen et al., 1997). These enzymes are integral membrane proteins that are predominantly located in the ER (Vance, 1996; Ross et al., 1997). PtdCho is also synthesized by successive methylation of PtdEtn by PtdEtn *N*-methyltransferases. An alternative route to synthesize PtdEtn is the decarboxylation of PtdSer by PtdSer decarboxylase, an enzyme located on the outer surface of the mitochondrial inner membrane (Percy et al., 1983; Zborowski et al., 1983). In cultured Chinese hamster ovary cells (Miller and Kent, 1986; Nishijima et al., 1986) and baby hamster kidney cells (Voelker, 1985), the decarboxylation of PtdSer produces more than 80% of the PtdEtn, even when the culture medium is supplemented with ethanolamine, an obligatory substrate of the CDP-ethanolamine pathway. This suggests that the decarboxylation of PtdSer is the primary source of PtdEtn biosynthesis. In the CNS, PtdSer is synthesized exclusively by base exchange. In general, the base-exchange reaction is catalyzed by PtdSer synthase I and II, ER enzymes that are activated by Ca^{2+} (Kuge and Nishijima, 1997). The difference between the two PtdSer synthases is at the level of substrate specificity. While PtdSer synthase I can synthesize PtdSer from PtdCho, the synthase II uses PtdEtn as a substrate (Voelker and Frazier, 1986; Kuge et al., 1997). The structural analysis of PtdSer synthase I has demonstrated that the enzyme lacks the typical N-terminal signal for ER targeting, but contains a C-terminal Lys-Lys motif that was proposed to be an ER-retention sequence (Stone et al., 1998). Biochemical investigation of the subcellular localization of these synthases has demonstrated that the activity of both synthases is associated exclusively with the mitochondrial-associated membranes and the ER membrane (Saito et al., 1996; Stone and Vance, 2000).

On the other hand, CDP-DAG is directly converted to PtdIns by PtdIns synthase and phosphatidylglycerol phosphate (PtdGroP) by PtdGroP synthase. PtdGroP is subsequently dephosphorylated to phosphatidylglycerol (PtdGro) by PtdGroP phosphatase. PtdGro is finally converted to cardiolipin by cardiolipin synthase.

3 Sorting and Transport of Lipids During Myelin Assembly

Myelin formation during development and myelin maintenance throughout adult life depends not only on a tight regulation of the expression of genes implicated in the synthesis of myelin components but also on unique membrane trafficking machinery for the proper sorting and targeting of specific components to the myelin sheath. Individual myelin components are synthesized in several compartments, sorted, and transported to the sites of myelin synthesis by different mechanisms (Benjamins and Smith, 1984; Morell et al., 1994; van Meer and Holthuis, 2000; Anitei and Pfeiffer, 2006). The difference in lipid composition

between cellular organelles and between organelles and plasma membrane can not be explained solely by local metabolism, but can be attributed to the sorting and transport mechanism. How cells decide which lipids need to be moved and in which direction is still a mystery. However, it is known that the selectivity in lipid transport is the main mechanism for lipid sorting (Sprong et al., 2001). In eukaryotic cells, the transport of lipids to the plasma membrane is made possible by monomeric and vesicular transport (van Meer and Holthuis, 2000). A monomeric exchange happens when a lipid desorbs from the membrane into the aqueous phase, diffuses across it, and inserts itself into the opposite membrane (Sprong et al., 2001). Proteins may stimulate lipid transport between membranes by bringing membranes together (Ladinsky et al., 1999). Alternatively, lipid transfer proteins might provide a hydrophobic binding site and act as a carrier. On the other hand, the vesicular transport of lipids toward the plasma membrane happens mostly by lateral segregation of lipids from the Golgi membrane and their exclusion from retrograde transport vesicles (van Meer, 1989).

Ceramide is synthesized in the ER and translocates to the Golgi compartment for conversion to more complex sphingolipid species. There are at least two known pathways by which ceramide is transported from the ER to Golgi. The first is mediated by vesicles (Funato and Riezman, 2001). In this system, synthesized ceramide from the ER is packed in a cargo vesicle and delivered by fusing the vesicle membrane to the Golgi membrane. These cargo vesicles preferentially target ceramide molecules to the *cis*-Golgi (Hanada et al., 2003). The second transport pathway (the ATP- and cytosol-dependent pathway) has been described both in yeast (Funato and Riezman, 2001) and in mammalian cell lines (Hanada et al., 2003, 2007; Kawano et al., 2006) and is known to be mediated by ceramide transfer protein (CERT). CERT is a cytoplasmic protein containing a phosphatidylinositol-4-monophosphate-binding domain and a putative domain for catalyzing lipid transfer (START). This protein specifically extracts ceramide from the phospholipid bilayer of the ER membrane and targets it to the SM synthesis site at the Golgi membrane after diffusion through the cytosol. This targeting event is mediated by the PH domain of CERT (Kumagai et al., 2005, 2007). Alternatively, CERT may induce membrane contacts between ER and trans-Golgi (Munro, 2003).

Like ceramide, the glycosphingolipid (GSL) GalCer is synthesized in the lumen of ER, and is a substrate for the synthesis of sulfatide in the lumen of Golgi by CST. This indicates that GalCer must be transported to the Golgi for sulfatide synthesis. It has been reported from in vitro experiments that GalCer translocates from the luminal to the cytosolic face of the ER following its synthesis (Burger et al., 1996). In addition, a GSL transfer protein has been described, which is capable of transferring both GlcCer and GalCer from donor to target membranes, in vitro (Sasaki and Demel, 1985; Sasaki, 1990). This suggests that the transport of GalCer from ER to Golgi might be mediated by those proteins. Except GalCer and ceramide that are synthesized in the ER and transported to the plasma membrane both by vesicular and monomeric transport, SM and the complex GSLs are synthesized in the Golgi lumen and have no access to the monomeric transport (Nilsson and Dallner, 1977; Brown et al., 1993; Burger et al., 1996). In this case, the transport toward the plasma membrane from the Golgi lumen happens by incorporation into anterograde vesicles and exclusion from retrograde vesicles (reviewed by van Meer, 1989; Sprong et al., 2001). This means that in the Golgi lumen, sphingolipids are subjected to lateral segregation from other membrane lipids such as PtdCho. In addition, elaborate studies on lipid transport using Madin-Darby Canine Kidney (MDCK) cells have been performed to elucidate the mechanisms by which lipids are transported in polarized cells such as OL.

3.1 Lipid Transport in Polarized Cells

The plasma membrane of polarized cells is divided into two specific compartments: the apical and basolateral membrane compartments. These compartments are distinct from each other by their specific protein and lipid composition. For example, the apical domain displays a twofold higher level of GSLs with a significantly lower level of phospholipids. Such a typical lipid composition is found in OLs (Stoffel and Bosio, 1997). To build up such unique compartments, lipids and proteins have to be sorted and transported to the appropriate plasma membrane compartment. The apical transport of GSLs in polarized cells such as MDCK occurs by direct transport from the TGN. In such a transport mechanism, GSLs are first sorted in

the TGN by association with protein to form a GSL-enriched domain (Brown and Rose, 1992), or GSL raft (Simons and Ikonen, 1997). The budding of these GSL-enriched domains will give rise to vesicles, which will be transported to the apical compartment (Matlin and Simons, 1984; Misek et al., 1984; Pfeiffer et al., 1985; Simons and Wandinger-Ness, 1990; Zegers and Hoekstra, 1997; Chang et al., 2006). In such a transport pathway, the glycosylphosphatidylinositol (GPI)-anchored proteins associate with sphingolipids and cholesterol in the TGN (Lisanti and Rodriguez-Boulan, 1990; Muñiz and Riezman, 2000). Alternatively, the apical delivery of lipids can be made by an indirect pathway in polarized cells. The lipid–protein complex formed in the TGN is first transported to the basolateral domain of the plasma membrane where it is endocytosed and transcytosed to the apical surface (Nyasae et al., 2003; Polishchuk et al., 2004). The similarity between the lipid composition of the myelin sheath and the apical and basolateral membrane domain in polarized cells (Stoffel and Bosio, 1997) suggests that apical and basolateral intracellular transport of lipids may also occur in OL.

4 Role of Lipids in the Regulation of Protein Sorting and Transport

4.1 Fatty Acids and the Regulation of Myelin Protein Sorting and Transport

Membrane proteins enter the membrane environment through transportation from their site of synthesis. Following synthesis, proteins are subjected to various posttranslational modifications that include acylation. Cysteinyl-palmitoylation is the major, dynamic posttranslational lipid modification of proteins that appears necessary to direct them to cholesterol/sphingolipid-rich microdomains (rafts) in the plasma membrane (Mumby, 1997; Paterson, 2002; Smotrys and Linder, 2004). In most cases, palmitoylation is the signal for membrane attachment of proteins that have been previously myristoylated at an N-terminal glycine residue or prenylated at the C-terminus (Mumby, 1997; Paterson, 2002; Smotrys and Linder, 2004). In CNS myelin, Src-family tyrosine kinase is palmitoylated in this way, but proteolipid protein is acylated at multiple sites (Bizzozero and Good, 1991). Recently, it has been demonstrated that palmitoylation is the sorting determinant of PLP/DM20 for transport to the myelin membrane and that the N-terminal 13 amino acids, which are palmitoylated at 3 cysteine sites, were sufficient to target PLP/DM20 to the myelin-like membrane in vitro (Schneider et al., 2005). Moreover, the fatty acid chain length of sphingolipids in yeast was found to be crucial for membrane delivery of protein cargo. These findings support the idea that lipids are not playing only a structural role by separating the extracellular from the intracellular compartment, but are more deeply implicated in the regulation of cellular physiology such as sorting and transport.

4.2 Sphingolipids/Cholesterol and the Regulation of Intracellular Transport

In membranes, sphingolipids appear organized in clusters or domains called rafts. These domains are formed in the TGN by self-association of newly synthesized sphingolipids/cholesterol and proteins (Simons and Ikonen, 2000). By this association, sphingolipid/cholesterol/proteins are packed into vesicles and delivered to the plasma membrane. The raft formation is the process by which most plasma membrane-associated proteins are sorted and transported. Hence, by recruiting proteins to the raft, sphingolipids regulate the transport of proteins that lack plasma membrane-targeting signals. This hypothesis is confirmed by numerous experiments that used different cell lines. Evidence for the role of sphingolipids and cholesterol in intracellular trafficking has been demonstrated in neuronal cells that also display a polarized trafficking mechanism (Ledesma et al., 1998). Neuronal inhibition of sphingolipid synthesis affects the sorting and transport of Thy-1 protein to the axon, implicating sphingolipids directly to the axonal sorting mechanism. Cholesterol, a component of the raft domain has also been suggested to play a role in the intracellular delivery of proteins. By culturing MDCK cell depleted of LDL, the principal source of cholesterol, the trafficking of gD1-DAF (GPI-anchored protein that preferentially associates to rafts in the TGN) was inhibited (Hannan and Edidin, 1996). In OL, proteolipid protein associates with the CHAPS-insoluble membrane fraction after leaving the ER, but before exiting the Golgi, suggesting that myelin lipids

and proteins assemble in the Golgi complex before transport to the myelin sheath (Krämer-Albers et al., 2006). Moreover, the binding of PLP/DM20 to cholesterol suggests that cholesterol is required for the sorting and transport of PLP/DM20 to the myelin sheath (Simons et al., 2000). In addition, the myelin and lymphocyte protein (MAL) is a tetraspan raft-associated proteolipid predominantly expressed by OLs and Schwann cells. MAL is synthesized in the ER and transported to the plasma membrane most likely by vesicular delivery (Zacchetti et al., 1995; Kim and Pfeiffer, 1999). To date, it is still unclear how MAL transport to the myelin sheath is regulated. However, it is evident that MAL is redistributed in the endosome–lysosome compartment in sulfatide-storing kidney cells (Saravanan et al., 2004). This finding points in the direction of sulfatide as a regulator of the transport of MAL to the myelin sheath.

5 Role of Lipids in the Biogenesis and Maintenance of Myelin

The biogenesis of myelin by Schwann cells or OLs requires the coordinate synthesis, transport, and integration of large quantities of specific proteins and lipids into the organized multilamellar structure (Morell and Ousley, 1994). The dry mass of myelin is characterized by a high proportion of lipid (70–85%), and consequently, a low proportion of protein (15–30%) (*Table 10-1*; Quarles et al., 2006). In comparison to myelin, most biological membranes display a high protein-to-lipid ratio, with identical lipid species. This suggests that there are no myelin-specific lipids; rather, there are myelin-enriched lipids. Galactolipids fall in this category and constitute 27–30% of the total myelin lipids (Norton and Cammer, 1984). Because of the enrichment of galactolipids (especially GalCer and sulfatide) in OLs and myelin of all mammals, it had been speculated that they would be essential for the formation of the myelin sheath, but in fact, that does not appear to be the case. Specifically, mice lacking CGT that do not synthesize GalCer, sulfatide, monogalactosyldiacylglycerol (MGDG), GM4, and seminolipid (*Table 10-2*; Bosio et al., 1996; Coetzee et al., 1996) are surprisingly able to synthesize the myelin membrane, which exhibits the characteristic ultrastructure of compact myelin, including the major dense line and intraperiod line both in the CNS and PNS (*Figure 10-1*). Similarly, mice lacking CST that do not synthesize sulfatide, seminolipid, LacCer sulfate, and sulfated gangliosides (*Table 10-2*) are also able to synthesize a compact myelin membrane (Honke et al., 2002). Nevertheless, careful analysis of myelin from these mice indicates the presence of substantial alteration of the myelin structure at the paranodal junction observed in the CNS (Dupree et al., 1999; Marcus et al., 2000; Ishibashi et al., 2002; Marcus and Popko, 2002; Marcus et al., 2002; Rasband et al.,

Table 10-2
Lipids based comparison between CGT- and CST-null mutant mice

Galactolipids	Synthesis compartment	CGT-null	CST-null
GalCer	ER	–	+
Sulfatide (SM4)	Golgi	–	–
MDGD	ER	–	+
GM4	Golgi	–	+
OH-GlcCer	Golgi	+	0
GalEAG[a]	ER	–	+
Seminolipid	Golgi	–	–
LacCer-sulfate (SM3)	Golgi	–	–
SM2a	Golgi	+	–
SM1a	Golgi	+	–
SB1a	Golgi	+	–

(–) means that the lipid is lost

(+) means that the lipid is present

(0) means that the lipid does not normally exist in myelin

[a]Galactosylalkylacylglycerol

2003) and PNS (Hoshi et al., 2007). The disruption of the paranodes in the CNS of galactolipid mutant mice is probably due to defects in the clustering of nodal, paranodal, and juxtaparanodal proteins such as sodium and potassium channels (Dupree et al., 1999; Ishibashi et al., 2002), neurofascin-155 (Dupree et al., 1999; Schaffer et al., 2004), caspr, and paranodin (Dupree et al., 1999). Further, CGT- or CST-null mice display significant alterations of the myelin sheath and axon in adulthood (Stoffel and Bosio, 1997; Coetzee et al., 1998; Dupree et al., 1998, 2005; Marcus et al., 2006). These findings suggest that GalCer and sulfatide are required for the stabilization and maintenance of myelin. However, to draw such a conclusion it will be important to segregate the roles played by sulfatide and GalCer and the other glycolipids also missing in the mutants.

Beside galactolipids, the other abundant lipids of the myelin membrane are cholesterol and phospholipids. Based on weight, the level of cholesterol in myelin is comparable to that of galactolipids, and the total phospholipids are the most abundant (*Table 10-1*). On a molar basis, in contrast, CNS myelin preparations contain more cholesterol than any other lipid classes (Morell, 1984; Morell and Jurevics, 1996). In addition to being the most abundant myelin lipid species, cholesterol is known to be implicated in the regulation of some cellular physiology such as membrane structure, thickness, fluidity (Ohvo-Rekila et al., 2002), and to limit ion leakage through the membrane (Haines, 2001). Together with other lipids, cholesterol participates in the formation and stabilization of lipid microdomains that serve as platforms for protein sorting and signal transduction (Simons and Toomre, 2000; van Meer and Lisman, 2002; van Meer and Vaz, 2005). Moreover, cholesterol defines the biophysical properties of all cell membranes. By compacting phospholipids, it may reduce membrane fluidity, and defines the functional properties of membrane-resident proteins, such as ion channels and transmitter receptors (Burger et al., 2000). Most brain cholesterol is unesterified and primarily localized within the myelin sheath. However, cholesterol seems to be required for myelination. This idea is supported by the fact that the CNS white matter of mice with OL-specific disruption of cholesterol biosynthesis is severely hypomyelinated and the mice develop ataxia and tremor. Examination of OLs by TUNEL experiment shows no signs of cell death, indicating that OL apoptosis was not the reason for hypomyelination (Saher et al., 2005). Moreover, numerous studies using murine (Quan et al., 2003) and human (Thelen et al., 2006) brains have demonstrated that the level of cholesterol rises during development and declines with aging. The increase of cholesterol during development was significantly higher in the brain stem and spinal cord, two regions of the CNS known to contain high amounts of myelin. These data strongly support the hypothesis that cholesterol synthesis is critical for myelin biogenesis.

In addition to major lipid component, the myelin membrane also contains minor components such as gangliosides, which represent 0.1–0.7% of total myelin lipid (Suzuki et al., 1967; Ledeen et al., 1980; Ccochran et al., 1982; Quarles et al., 2006). Besides GM4, the monosialoganglioside GM1 represents the most abundant ganglioside species in the myelin membrane. Numerous studies have demonstrated that myelin basic protein could interact directly with gangliosides such as GM1, GM4, and GD1b, at least in vitro (Yohe et al., 1983; Ong and Yu, 1984). In addition, GM1, GT1b, GD1a, and GD1b modulate protein phosphorylation in myelin, and in contrast, completely inhibit the phosphorylation of the 18.5-kDa MBP isoform (Chan, 1987). More importantly, the ganglioside content of myelin increases during the maturation of the myelin sheath and may reflect myelination.

To determine if ganglioside accumulation is a crucial factor in myelinogenesis, genetically engineered mice with defects in enzymes catalyzing specific biosynthetic pathways have been generated (*Figure 10-3*). Analysis of these mutant animals has provided strong insights for the role of gangliosides in myelinogenesis. Mice lacking specific ganglioside series develop normal myelin (Horinouchi et al., 1995; Furukawa et al., 2001; Kolter et al., 2002). In contrast, gangliosides are required for growth and myelin maintenance. This affirmation is strongly supported by the results from analysis of mice lacking all or a number of ganglioside series. Mice deficient in ceramide glucosyltransferase (*Figure 10-3a*) begin to die as early as embryonic day 7.5, indicating that GlcCer and higher-order glycolipids are important for development, but it is important to know that accumulation of ceramide in such an animal could also lead to deleterious effects. As shown in *Figure 10-3*, GalNAcT-null (*Figure 10-3d*) animals lacking the major gangliosides GM2, GD2, GM1a, GD1b, GD1a, GT1a, GT1b, and GQ1b, and SialT2-null (*Figure 10-3c*) animals lacking GD3, GD2, GD1b, GT1b, and GQ1b, displayed only a subtle impairment of brain function that included demyelination (Sheikh et al., 1999; Chiavegatto et al., 2000). Double mutant mouse deficient in both

GalNacT and SialT1 synthesize only LacCer and SM3 as major brain gangliosides. These mice display a striking vacuolar pathology in the white matter of the CNS with axonal degeneration and perturbed axo–glial interaction (Yamashita et al., 2005). Moreover, double mutant mice that lack both GalNacT and SialT2 and express only GM3 as major brain gangliosides display a disrupted paranodal axo–glial junction (Susuki et al, 2007a). Similar paranodal abnormalities were observed in the PNS of SialT2 mutant mice lacking the b-series gangliosides, but with increased levels of GM1 and GD1a (Okada et al., 2002). These findings suggest a role for gangliosides in the maintenance of the CNS myelin.

6 Role of Lipids in the Regulation of Oligodendrocyte Physiology

6.1 Fatty Acids

As one of the fattiest tissues in the body, the brain needs fats (together with glucose) for energy, structure, and maintenance of its normal function. In the CNS and PNS cells, as well as in the other cells of living organisms, fatty acids (FAs) are key components of phospholipids and sphingolipids, which are the most abundant components of the cellular membranes and myelin. They are commonly classified as saturated, monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) (Agostoni and Bruzzese, 1992; Millar and Kunst, 1997). According to the hydrocarbon chain length, FAs are also classified as short-chain, long-chain fatty acids (LFAs), and very long chain FAs (VLCFAs). The richest source of saturated and monounsaturated FAs in the brains of most animals is myelin (Bourre and Baumann, 1980; for review, see Poulos, 1995). FAs are known to play a major role in the regulation of myelin thickness, myelin structure, and compaction (Bourre et al., 1978a, b). In addition, the observation of deficiencies in PUFAs in MS patients has led to attempts to influence the disease course by dietary uptake, particularly by increased intake of specific PUFAs of n-3 and n-6 series (Borlak and Welch, 1994; Mayer, 1999). Moreover, dietary supplementation of gamma-linolenic acid (18:3n-6) ameliorates the course of both acute and chronic experimental autoimmune encephalomyelitis (EAE: Harbige et al., 2000), and FAs from n-6 series improve biochemical parameters and cognitive functions in rats with EAE (Yehuda et al., 1997). PUFAs in these cases might influence the disease course by repairing the myelin sheath or stimulating OL progenitor differentiation. This idea is supported by findings that MBP and PLP mRNA levels were reduced in pups nursed by mothers that were fed a fat-free diet, and this effect was reversed by feeding the mother with a corn-oil-based diet rich in PUFAs (DeWille and Farmer, 1992). In addition, supplementing primary cultured OLs with PUFAs results in an increase of CNP, MBP, and PLP expression (van Meeteren et al., 2006). The stimulating effect of PUFAs on the differentiation of OL progenitors might be mediated through the thromboxane receptor, since it has been recently demonstrated that the metabolites of arachidonic acid, eicosanoids (e.g., thromboxane A2 and prostaglandins) are produced in cells following the action of cellular stimuli that activate phospholipases A2 and C, leading to the liberation of membrane-esterified arachidonic acid. Free arachidonic acid is first metabolized by the cyclooxygenases (COX-1 or COX-2) and then by terminal prostaglandin synthases to produce the prostaglandins (PGD2, PGE2, PGF2a, PGI2) and thromboxane A2 (TXA2). Arachidonic acid metabolites have been implicated in apoptosis and neurodegeneration (Brault et al., 2004; Farooqui et al., 2004). On the other hand, one of these eicosanoids, TXA2, has been implicated in the proliferation of OL progenitors and survival of mature OLs through its interaction with TXA2 receptors (Lin et al., 2005; Ramamurthy et al., 2006) present in myelinated fibers of the optic nerve and striatum (Borg et al., 1994). In addition, it is also likely that PUFAs might modulate the expression of OL-specific genes through syntaxin-3, since it is evidenced that PUFAs of n-3 and n-6 series promote membrane growth in PC12 cells in vitro by acting on syntaxin-3 (Darios and Davletov, 2006).

6.2 Sphingolipids and the Regulation of Oligodendrocyte Lineage Progression

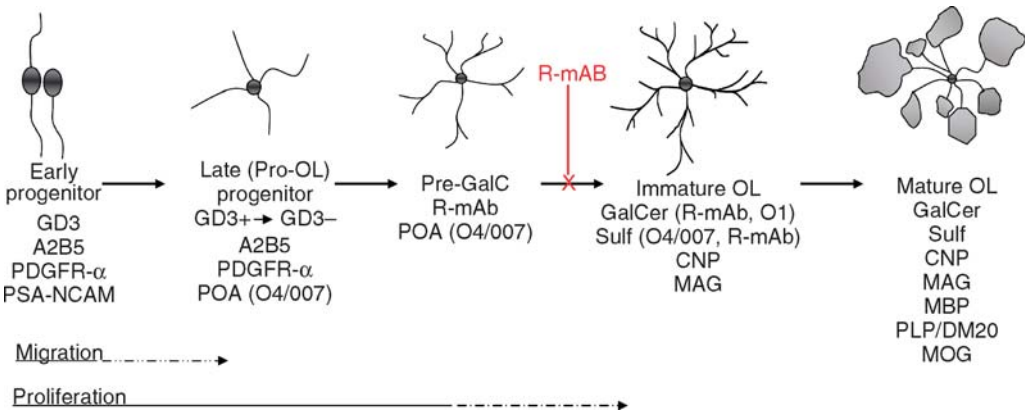
The normal timing of OL differentiation can be reconstituted in cultures of postnatal rat brain. This requires that OL progenitor cells (OPCs) are stimulated to proliferate by platelet-derived growth factor

(PDGF: Raff et al., 1988). It also requires the presence of hydrophobic signals such as thyroid hormone (TH) or retinoic acid (RA) (Barres et al., 1994). Clonal analyses in such cultures show that the progeny of an individual OPC stops dividing and differentiates at about the same time, even if separated and cultured in different microwells, indicating that an intrinsic timing mechanism operates in OPCs to limit their proliferation and initiate differentiation after a certain period of time or number of cell divisions (Temple and Raff, 1986; Barres et al., 1994). Among the molecules that can modulate the differentiation of OPCs in vitro and in vivo are lipids, particularly sphingolipids. The development of antibodies against GSLs has been a critical tool to identify specific stages of OL lineage progression both in vitro and in vivo. The early progenitor stage is characterized by the expression of specific antigens such as PDGF alpha-receptor, A2B5 and GD3 ganglioside, and by uni- or bipolar morphology. The next stage is the late progenitor or pro-oligodendroblast (Pro-OL) stage, which is identified by the expression of an unidentified sulfated antigen called Pro-OL antigen (POA: Gard and Pfeiffer, 1990; Knapp, 1991; Bansal et al., 1992), which reacts with the monoclonal antibodies O4 and A007 (these antibodies also recognizes sulfatide and seminolipids on differentiated OL). At the Pro-OL stage of lineage progression, sulfatide is not synthesized (Bansal and Pfeiffer, 1994a; Bansal et al., 1992). Further, inhibition of sulfation both in vivo (Bansal et al., 1999; Hirahara et al., 2004) and in vitro (Bansal and Pfeiffer, 1994a) completely eliminates immunoreactivity of Pro-OLs with O4/A007, confirming that POA is a sulfated antigen. Cells at this stage are morphologically characterized by additional primary processes and are still proliferative. The decision to stop proliferating and start differentiating is made at the pre-GalCer stage, characterized by immunoreactivity with the monoclonal antibody R-mAb that recognizes both sulfatide and GalCer (Ranscht et al., 1982; Bansal et al., 1989). Although cells at this stage are R-mAb-positive, the immunoreactivity with O1 (a monoclonal antibody that reacts with GalCer but not sulfatide) is still negative. This is a highly transient stage and it is possible that R-mAb could be recognizing an antigen other than sulfatide at this stage.

Terminal differentiation of OL starts as cells exit the pre-GalCer stage and is characterized by a change in the morphology and a dramatic increase in secondary processes. Biochemically, this stage of OL lineage progression is characterized by the appearance of GalCer, sulfatide, CNP, and MAG on the OL membrane (Pfeiffer et al., 1993). This stage can be identified by immunostaining with O1, O4, and R-mAb, which specifically binds to the major GSLs, GalCer, and sulfatide (Bansal et al., 1989; Bansal and Pfeiffer, 1992) (*Figure 10-6*). As cells exit from the immature OL stage of the lineage progression, they start to synthesize markers for mature OL such as MBP, PLP, and MOG. Among these proteins, MBP is the only protein that is required for synthesis of the myelin sheath (Peterson and Bray, 1984).

Figure 10-6

Schematic representation of the oligodendrocyte developmental pathway. Each stage of the lineage is characterized by a change in morphology, migratory and proliferative capacity, and the expression of specific protein and lipid markers (Adapted from Pfeiffer et al., 1993; Taylor et al., 2004)



More than a decade ago, it was proposed that GalCer and sulfatide regulate OL lineage progression. The implication of GalCer and sulfatide in the regulation of OL physiology was observed in vitro. When late progenitors were exposed to anti-GalCer/sulfatide (R-mAb) or anti-sulfatide (O4), their terminal differentiation was reversibly blocked (Bansal and Pfeiffer, 1989; Bansal et al., 1999). Since the inhibition of OL lineage progression at the Pro-OL stage was not observed with anti-GalCer (O1) or other anti-lipids antibodies such as anti-human natural killer-1 (HNK-1), or anti-cholesterol, it was concluded that sulfatide is the glycosphingolipid that regulated the terminal differentiation during OL lineage progression (Bansal and Pfeiffer, 1989; Bansal et al., 1999). The generation of mice lacking sulfatide and GalCer together (Bosio et al., 1996; Coetzee et al., 1996) or sulfatide alone (Honke et al., 2002) has been an important contribution in understanding the effect of GalCer and sulfatide in the development of OL in vivo. In the absence of GalCer and sulfatide together (Bansal et al., 1999) or sulfatide alone (Hirahara et al., 2004), the terminal differentiation of OL is enhanced, indicating that sulfatide, but not GalCer, plays a key role in the entry of OL progenitors into terminal differentiation.

Unfortunately, the mechanism by which GSLs mediate the regulation of OL development has not yet been definitively identified. However, pioneering studies indicate that exposure of OL cultures to anti-GalCer followed by crosslinking of the complex GalCer/anti-GalCer induces the translocation of membrane surface GalCer to the internal MBP domain, disruption of microtubules and microfilaments within the myelin sheath, and influx of extracellular calcium (Dyer and Benjamins, 1988). Further, treatment of mature OLs with O4 or RmAb leads to process retraction and upon crosslinking of the complex O4/sulfatide or RmAb/GalCer/sulfatide with secondary antibodies results in a dramatic hyperphosphorylation of MAPK; in contrast, crosslinking of the complex O1/GalCer had no effect on the phosphorylation state of MAPK (Bansal and Pfeiffer, 1994b; Stockdale Ngamli-Fewon, and Pfeiffer, unpublished observation). Taken together, these findings indicate that GSLs can act as receptors that mediate signal transduction. Moreover, anti-GalCer and antisulfatide together induce dysmyelination in vitro (Rosenbluth and Moon, 2003) while antisulfatide alone induces demyelination in vivo (Rosenbluth et al., 2003).

6.3 Gangliosides and Oligodendrocyte Physiology

Gangliosides are sialic acid-containing GSLs that are known to modulate the activity of a number of receptor tyrosine kinases, including the insulin receptor (Allende and Proia, 2002). For example, the tyrosine kinase activity of the epidermal growth factor receptor can be enhanced or repressed by gangliosides GD1a or GM3, respectively (Bremer et al., 1986; Liu et al., 2004). In addition, the activities of the platelet-derived growth factor receptor and the nerve growth factor receptor TrkA are negatively regulated by overexpression of GM1 (Mitsuda et al., 2002; Nishio et al., 2005). In both cases GM1 appears to act by displacing the PDGF receptor or TrkA from lipid rafts to the nonraft compartment (Allende and Proia, 2002; Pike, 2003; Ikonen and Vainio, 2005).

GM1, GD1a, GD1b, and GT1b are the most abundant gangliosides in the adult mammalian nervous system (Yu et al., 1989). The neurobiological roles of the major nervous system gangliosides are not completely defined. However, the gangliosides GT3 and O-acetyl GT3 are surface antigens that are expressed at the early stage of OL lineage progression and immunoreact with the monoclonal antibody A2B5 (*Figure 10-6*; Farrer and Quarles, 1999). Together with GD3 and GD1a, A2B5-labeled gangliosides are the most abundant gangliosides expressed in early progenitor cells as assessed by double immunostaining with NG2 in the human brain (Marconi et al., 2005). As OLs differentiate, these ganglioside epitopes disappear from the membrane surface and the immature stage shows no immunoreactivity. Marconi et al. (2005) also showed that GD2 is preferentially expressed by mature OLs in the human adult brain. In the peripheral nervous system (PNS), the most abundant gangliosides are GM3, GD3, and sialosylneolactotetraosylceramide [NeuAc(α2-3 or α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer] also known as sialosylparaglobo-side. At the early stage of Schwann cell development GM3 and GD3 with 50 and 18 mol%, respectively, are the most abundant gangliosides of the PNS, but the amount of these lipids decreases as Schwann cells mature and myelinate (Chou et al., 1982). In contrast, the amount of sialosylparagloboside did not change with PNS development. The particularity of the PNS gangliosides is the presence of VLCFAs in the sialosylparaglobosides

compared with the CNS gangliosides, which did not contain VLCFAs. Another important difference between PNS and CNS gangliosides is the presence of GlcNAc in the neolactosyl series in the PNS instead of GalNAc in the gangliosyl series of the CNS (Chou et al., 1982; Ogawa-Goto and Abe, 1998).

The implication of gangliosides in the regulation of cellular physiology has been studied mostly in nonglial cells. However, it has been shown that exogenous GM3 enhances differentiation of OLs (Yim et al., 1994), indicative of its role in OL differentiation. Numerous studies have reported the involvement of gangliosides in the long-term stabilization of axon and myelin contacts through the interaction of GD1a/GT1b with MAG both in the CNS and PNS (Sheikh et al., 1999; Pan et al., 2005). In vitro studies have demonstrated that gangliosides are functional nerve cell ligands for MAG (Vyas et al., 2002), and that binding of MAG to gangliosides leads to signal transduction by inducing the translocation of p75NTR to lipid rafts (Fujitani et al., 2005). Moreover, the ganglioside GM1, which is not the binding partner of the myelin MAG protein, is implicated in the stabilization of the paranodal axo–glial junctions and ion channel clusters in myelinated nerve fibers both in the CNS and PNS (Susuki et al., 2007a, b). These findings suggest a functional role for gangliosides in the development and maintenance of the CNS.

7 Conclusion

During the last two decades, lipids have attracted widespread attention due to the appreciation that this class of molecules has a major impact on various biological processes. Lipids are the major components in the cell membrane of all organisms and are synthesized at different membrane compartments and then transported to the plasma membrane. Together with membrane-associated proteins, lipids build up the plasma membrane and act as barriers that separate the extracellular from the intracellular compartment. In addition to their structural role at the plasma membrane, lipids are being assigned a broad range of new functions, including regulation of cell growth and differentiation, signal transduction, regulation of intracellular trafficking, and apoptosis. Future avenues of research are likely to be directed toward a better understanding of these new functions of lipids. Lipids have been originally described as cornerstones in the field of neurochemistry and myelin biology. Gene-targeting studies have shown that most of the lipids present in the myelin sheath are needed for myelin stabilization and maintenance, although these are not critical for initial myelin formation, with the exception of cholesterol. In this chapter, we emphasized areas of particular promise in myelin lipidomics that include analysis of mechanisms by which lipids regulate myelin biogenesis, protein sorting, transport, and OL physiology. For further background, we refer the reader to our recent comprehensive chapter on myelin lipids (Taylor et al., 2004).

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References

Alderson NL, Rembiesa BM, Walla MD, Bielawska A, Bielawski J, et al. 2004. The human *FA2H* gene encodes a fatty acid 2-hydroxylase. J Biol Chem 279: 48562-48568.

Allende ML, Proia RL. 2002. Lubricating cell signaling pathways with gangliosides. Curr Opin Struct Biol 12: 587-592.

Andrieu-Abadie N, Levade T. 2002. Sphingomyelin hydrolysis during apoptosis. Biochim Biophys Acta 1585: 126-134.

Anitei M, Pfeiffer SE. 2006. Myelin biogenesis: Sorting out protein trafficking. Curr Biol 16: 418-421.

Arthur G, Page L. 1991. Synthesis of phosphatidylethanolamine and ethanolamine plasmalogen by CDP-ethanolamine and decarboxylase pathways in rat heart, kidney and liver. Biochem J 273: 121-125.

Bakovic M, Fullerton MD, Michel V. 2007. Metabolic and molecular aspects of ethanolamine phospholipid

biosynthesis: The role of CTP:phosphoethanolamine cytidylyltransferase (Pcyt2). Biochem Cell Biol 85: 283-300.

Bansal R, Pfeiffer SE. 1989. Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. Proc Natl Acad Sci USA 86: 6181-6185.

Bansal R, Pfeiffer SE. 1992. A novel stage in the oligodendrocyte lineage defined by reactivity of progenitors with R-mAb prior to O1 anti-galactocerebroside. J Neurosci Res 32: 309-316.

Bansal R, Warrington AE, Gard AL, Ranscht B, Pfeiffer SE. 1989. Multiple and novel specificity's of monoclonal antibodies O1, O4 and R-mAb used in the analysis of oligodendrocyte development. J Neurosci Res 24: 548-557.

Bansal R, Stefansson K, Pfeiffer SE. 1992. Proligodendroblast antigen (POA), a developmental antigen expressed by A007/O4-positive oligodendrocyte progenitors prior to the appearance of sulfatide and galactocerebroside. J Neurochem 58: 2221-2229.

Bansal R, Pfeiffer SE. 1994a. Inhibition of protein and lipid sulfation in oligodendrocytes blocks biological responses to FGF-2 and retards cytoarchitectural maturation, but not developmental lineage progression. Dev Biol 162: 511-524.

Bansal R, Pfeiffer SE. 1994b. 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 12: 173-179.

Bansal R, Winkler S, Bheddah S. 1999. Negative regulation of oligodendrocyte differentiation by galactosphingolipids. J Neurosci 19: 7913-7924.

Barres BA, Lazar MA, Raff MC. 1994. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120: 1097-1108.

Basu S, Kaufman B, Roseman S. 1973. Enzymatic synthesis of glucocerebroside by a glucosyltransferase from embryonic chicken brain. J Biol Chem 248: 1388-1394.

Bizzorero OA, Good LK. 1991. Rapid metabolism of fatty acids covalently bound to myelin proteolipid protein. J Biol Chem 266: 17092-17098.

Becker I, Wang-Eckhardt L, Yaghootfam A, Gieselmann V, Eckhardt M. 2007. Differential expression of (dihydro)ceramide synthases in mouse brain: Oligodendrocyte-specific expression of CerS2/Lass. Histochem Cell Biol Histochem Cell Biol. 129: 233-241.

Bektas M, Spiegel S. 2004. Glycosphingolipids and cell death. Glycoconj J 20: 39-47.

Benjamins JA, Smith ME. 1984. Metabolism of myelin. Myelin. Morell P, editor. New York: Plenum; pp. 225-258.

Borg C, Lim CT, Yeomans DC, Dieter JP, Komiotis D, et al. 1994. Purification of rat brain, rabbit aorta, and human platelet thromboxane A_2/prostaglandin H2 receptors by immunoaffinity chromatography employing anti-peptide and anti-receptor antibodies. J Biol Chem 269: 6109-6116.

Borlak JT, Welch VA. 1994. Health implications of fatty acids. Arzneimittelforschung 44: 976-981.

Bosio A, Binczek E, Stoffel W. 1996. Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc Natl Acad Sci USA 93: 13280-13285.

Bourre JM, Bornhofen JH, Araoz CA, Daudu O, Baumann NA. 1978a. Pelizaeus–Merzbacher disease: Brain lipid and fatty acid composition. J Neurochem 30: 719-727.

Bourre JM, Jacque C, Nguyen-Legros J, Bornhofen JH, Araoz CA, et al. 1978b. Pelizaeus-Merzbacher disease: Biochemical analysis of isolated myelin (electron-microscopy; protein, lipid and unsubstituted fatty acids analysis). Eur Neurol 17: 317-326.

Bourre JM, Baumann N. 1980. Elongation of fatty acids in the nervous system. Ann Nutr Aliment 34: 401-413.

Brault S, Martinez-Bermudez AK, Roberts J, Cui QL, Fragoso G, et al. 2004. Cytotoxicity of the E2-isoprostane 15-E2T-IsoP on oligodendrocyte progenitors. Free Radic Biol Med 37: 358-366.

Bremer EG, Schlessinger J, Hakomori SI. 1986. Ganglioside-mediated modulation of cell growth: Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor. J Biol Chem 261: 2434-2440.

Brose N, Rosenmund C. 2002. Move over protein kinase C, you've got company: Alternative cellular effectors of diacylglycerol and phorbol esters. J Cell Sci 115: 4399-4411.

Brown DA, Rose JK. 1992. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 68: 533-544.

Brown MC, Besio-Moreno M, Bongarzone ER, Cohen PD, Soto EF, et al. 1993. Vesicular transport of myelin proteolipid and cerebroside sulfates to the myelin membrane. J Neurosci Res 35: 402-408.

Burger KN, Bijl van der P, van Meer G. 1996. Topology of sphingolipid galactosyltransferases in ER and Golgi: Transbilayer movement of monohexosyl sphingolipids is required for higher glycosphingolipid biosynthesis. J Cell Biol 133: 15-28.

Burger K, Gimpl G, Fahrenholz F. 2000. Regulation of receptor function by cholesterol. Cell Mol Life Sci 57: 1577-1592.

Carre A, Graf C, Stora S, Mechtcheriakova D, Csonga R, et al. 2004. Ceramide kinase targeting and activity determined by its N-terminal pleckstrin homology domain. Biochem Biophys Res Commun 324: 1215-1219.

Chan KFJ. 1987. Ganglioside-modulated protein phosphorylation in myelin. J Biol Chem 262: 2415-2422.

Chang MC, Wisco D, Ewers H, Norden C, Winckler B. 2006. Inhibition of sphingolipid synthesis affects kinetics but not fidelity of L1/NgCAM transport along direct but

not transcytotic axonal pathways. Mol Cell Neurosci 31: 525-538.

Chiavegatto S, Sun J, Nelson RJ, Schnaar RL. 2000. A functional role for complex gangliosides: Motor deficits in GM2/GD2 synthase knockout mice. Exp Neurol 166: 227-234.

Chou KH, Nolan CE, Jungalwala FB. 1982. Composition and metabolism of gangliosides in rat peripheral nervous system during development. J Neurochem 39: 1547-1558.

Ccochran FB, Yu RK, Ando S, Ledeen RW. 1982. Myelin gangliosides in vertebrates. J Neurochem 39: 773-779.

Coetzee T, Fujita N, Dupree J, Shi R, Blight A, et al. 1996. Myelination in the absence of galactocerebroside and sulfatide: Normal structure with abnormal function and regional instability. Cell 86: 209-219.

Coetzee T, Dupree JL, Popko B. 1998. Demyelination and altered expression of myelin-associated glycoprotein isoforms in the central nervous system of galactolipid-deficient mice. J Neurosci Res 54: 613-622.

Cooke FT. 2004. Phosphoinositides: Older than we first thought? Curr Biol 14: 762-764.

D'Angelo G, Polishchuk E, Di Tullio G, Santoro M, Di Campli A, et al. 2007. Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. Nature 449: 62-67.

Daniotti JL, Zurita AR, Trindade VM, Maccioni HJ. 2002. GD3 expression in CHO-K1 cells increases growth rate, induces morphological changes, and affects cell-substrate interactions. Neurochem Res 27: 1421-1429.

Darios F, Davletov B. 2006. Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 440: 813-817.

DeWille JW, Farmer SJ. 1992. Postnatal dietary fat influences mRNAS involved in myelination. Dev Neurosci 14: 61-68.

Dupree JL, Suzuki K, Popko B. 1998. Galactolipids in the formation and function of the myelin sheath. Microsc Res Tech 41: 431-440.

Dupree JL, Girault JA, Popko B. 1999. Axo-glial interactions regulate the localization of axonal paranodal proteins. J Cell Biol 147: 1145-1152.

Dupree JL, Mason JL, Marcus JR, Stull M, Levinson R, et al. 2005. Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. Neuron Glia Biol 1: 1-14.

Dyer CA, Benjamins JA. 1988. Redistribution and internalization of antibodies to galactocerebroside by oligodendroglia. J Neurosci 8: 883-891.

Eckford PD, Sharom FJ. 2005. The reconstituted P-glycoprotein multidrug transporter is a flippase for glucosylceramide and other simple glycosphingolipids. Biochem J 389: 517-526.

Eckhardt M, Yaghootfam A, Fewou SN, Zoller I, Gieselmann V. 2005. A mammalian fatty acid hydroxylase responsible for the formation of alpha-hydroxylated galactosylceramide in myelin. Biochem J 388: 245-254.

Furukawa K, Takamiya K. Okada M, Inoue M, Fukumoto S, 2001. Novel function of complex carbohydrates elucidated by the mutant of glycosyltransferase genes. Biochem Biophys Acta 1525: 1-12.

Farooqui AA, Horrocks LA, Farooqui T. 2000. Glycerophospholipids in brain: Their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem Phys Lipids 106: 1-29.

Farooqui AA, Ong WY, Horrocks LA. 2004. Biochemical aspects of neurodegeneration in human brain: Involvement of neural membrane phospholipids and phospholipases A_2. Neurochem Res 29: 1961-1977.

Farrer RG, Quarles RH. 1999. GT3 and its O-acetylated derivative are the principal A2B5-reactive gangliosides in cultured O2A lineage cells and are down-regulated along with O-acetyl GD3 during differentiation to oligodendrocytes. J Neurosci Res 57: 371-380.

Fishman PH, McFarland VW, Morat PT, Brady RO. 1972. Ganglioside biosynthesis in mouse cells. Glycosyltransferase activities in normal and virally-transformed lines. Biochem Biophys Res Commun48: 48-57.

Fujitani M, Kawai H, Proia RL, Atsunori Kashiwagi A, Yasuda H, et al. 2005. Binding of soluble myelin-associated glycoprotein to specific gangliosides induces the association of p75NTR to lipid rafts and signal transduction. J Neurochem 94: 15-21.

Fullekrug J, Simons K. 2004. Lipid rafts and apical membrane traffic. Ann N Y Acad Sci 1014: 164-169.

Funato K, Riezman H. 2001. Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. J Cell Biol 155: 949-959.

Futerman AH, Hannun YA. 2004. The complex life of simple sphingolipids. EMBO 5: 777-782.

Futerman AH, Pagano RE. 1991. Determination of the intracellular sites and topology of glucosylceramide synthesis in rat liver. Biochem J 280: 295-302.

Futerman AH, Stieger B, Hubbard AL, Pagano RE. 1990. Sphingomyelin synthesis in rat liver occurs predominantly at the cis and medial cisternae of the Golgi apparatus. J Biol Chem 265: 8650-8657.

Giraudo CG, Marcioni HJ. 2003. Ganglioside glycosyltransferases organize in distinct multienzyme complexes in CHO-K1 Cells. J Biol Chem. 278: 40262-40271.

Gard AL, Pfeiffer SE. 1990. Two proliferative stages of the oligodendrocyte lineage (A2B5 + O4- and O4 + GalC-) under different mitogenic control. Neuron 5: 615-625.

Giraudo CG, Rosales Fritz VM, Maccioni HJ. 1999. GA2/GM2/GD2 synthase localizes to the trans-golgi network of CHO-K1 cells. Biochem J 342: 633-640.

Giraudo CG, Daniotti JL, Maccioni HJ. 2001. Physical and functional association of glycolipid *N*-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus. Proc Natl Acad Sci USA 98: 1625-1630.

Goldstein JL, Brown MS. 1990. Regulation of the mevalonate pathway. Nature 343: 425-430.

Gomez-Munoz A. 2004. Ceramide-1-phosphate: A novel regulator of cell activation. FEBS Lett 562: 5-10.

Gomez-Munoz A, Duffy PA, Martin A, O'Brien L, Byun HS, et al. 1995. Short-chain ceramide-1-phosphates are novel stimulators of DNA synthesis and cell division: Antagonism by cell-permeable ceramides. Mol Pharmacol 47: 833-839.

Gomez-Munoz A, Kong JY, Parhar K, Wang SW, Gangoiti P, et al. 2005. Ceramide-1-phosphate promotes cell survival through activation of the phosphatidylinositol 3-kinase/protein kinase B pathway. FEBS Lett 579: 3744-3750.

Haines TH. 2001. Do sterols reduce proton and sodium leaks through lipid bilayers? Prog Lipid Res 40: 299-324.

Halter D, Neumann S, van Dijk SM, Wolthoorn J, de Maziere AM, et al. 2007. Pre- and post-Golgi translocation of glucosylceramide in glycosphingolipid synthesis. J Cell Biol 179: 101-115.

Horinouchi H, Erlich S, Perl DP, Ferlinz K, Bisgaier CL, Sandhoff K, Desnick RJ, Stewart CL, Schuchman EH. 1995. Acid sphingomyelinase deficient mice: A model of types A and B Niemand-Pick disease. Nat Genet 10: 288-293.

Hanada K, Hara T, Nishijima M. 2000. Purification of the serine palmitoyltransferase complex responsible for sphingoid base synthesis by using affinity peptide chromatography techniques. J Biol Chem 275: 8409-8415.

Hanada K, Hara T, Fukasawa M, Yamaji A, Umeda M, et al. 1998. Mammalian cell mutants resistant to a sphingomyelin-directed cytolysin. Genetic and biochemical evidence for complex formation of the LCB1 protein with the LCB2 protein for serine palmitoyltransferase. J Biol Chem 273: 33787-33794.

Hanada K, Hara T, Nishijima M, Kuge O, Dickson RC, et al. 1997. A mammalian homolog of the yeast LCB1 encodes a component of serine palmitoyltransferase, the enzyme catalyzing the first step in sphingolipid synthesis. J Biol Chem 272: 32108-32114.

Hanada K, Kumagai K, Tomishige N, Kawano M. 2007. CERT and intracellular trafficking of ceramide. Biochim Biophys Acta 1771: 644-653.

Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, et al. 2003. Molecular machinery for non-vesicular trafficking of ceramide. Nature 426: 803-809.

Hannan LA, Edidin M. 1996. Traffic, polarity, and detergent solubility of a glycosylphosphatidylinositol-anchored protein after LDL-deprivation of MDCK cells. J Cell Biol 133: 1265-1276.

Harbige LS, Layward L, Morris-Downes MM, Dumonde DC, Amor S. 2000. The protective effects of omega-6 fatty acids in experimental autoimmune encephalomyelitis (EAE) in relation to transforming growth factor-beta 1 (TGF-beta1) up-regulation and increased prostaglandin E2 (PGE2) production. Clin Exp Immunol 122: 445-452.

Hirahara Y, Bansal R, Honke K, Ikenaka K, Wada Y. 2004. Sulfatide is a negative regulator of oligodendrocyte differentiation: Development in sulfatide-null mice. Glia 45: 269-277.

Hoekstra D, Maier O, Wouden van der JM, Slimane TA, van IJzendoorn SC. 2003. Membrane dynamics and cell polarity: The role of sphingolipids. J Lipid Res 44: 869-877.

Holthuis JCM, Pomosrski T, Raggers RJ, Sprong H, van Meer G. 2001. The organizing potential sphingolipids in intracellular membrane transport. Physiol Rev 81: 1689-1723.

Honke K, Hirahara Y, Dupree J, Suzuki K, Popko B, et al. 2002. Paranodal junction formation and spermatogenesis require sulfoglycolipids. Proc Natl Acad Sci USA 99: 4227-4232.

Honke K, Tsuda M, Hirahara Y, Ishii A, Makita A, et al. 1997. Molecular cloning and expression of cDNA encoding human 3′-phosphoadenylylsulfate: Galactosylceramide 3′-sulfotransferase. J Biol Chem 272: 4864-4868.

Honke K, Yamane M, Ishii A, Kobayashi T, Makita A. 1996. Purication and characterization of 3′-phosphoadenosine-5′-phosphosulfate: GalCer sulfotransferase from human renal cancer cells. J Biochem (Tokyo) 119: 421-427.

Hoshi T, Suzuki A, Hayashi S, Tohyama K, Hayashi A, et al. 2007. Nodal protrusions, increased Schmidt-Lanterman incisures, and paranodal disorganization are characteristic features of sulfatide-deficient peripheral nerves. Glia 55: 584-594.

Huitema K, van den Dikkenberg J, Brouwers JFHM, Holthuis JCM. 2004. Identification of a family of animal sphingomyelin synthases. EMBO J 23: 33-44.

Ichikawa S, Sakiyama H, Suzuki G, Hidari KI, Hirabayashi Y. 1996. Ecpression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first step of glycosphingolipid synthesis. Proc Natl Acad Sci USA 93: 4638-4643.

Ikonen E, Vainio S. 2005. Lipid microdomains and insulin resistance: Is there a connection? Sci STKE 268: 3.

Ishibashi T, Dupree JL, Ikenaka K, Hirahara Y, Honke K, et al. 2002. A myelin galactolipid, sulfatide, is essential for maintenance of ion channels on myelinated axon but not essential for initial cluster formation. J Neurosci 22: 6507-6514.

Istvan ES, Palnitkar M, Buchanan SK, Deisenhofer J. 2000. Crystal structure of the catalytic portion of human HMG-CoA reductase: Insights into regulation of activity and catalysis. EMBO J 19: 819-830.

Jeckel D, Karrenbauer A, Birk R, Schmidt RR, Wieland F. 1990. Sphingomyelin is synthesized in the cis Golgi. FEBS Lett 261: 155-157.

Jeckel D, Karrenbauer A, Burger KN, van Meer G, Wieland F. 1992. Glucosylceramide is synthesized at the cytosolic surface of various Golgi subfractions. J Cell Biol 117: 259-267.

Kaufman B, Basu S, Roseman S. 1968. Enzymatic synthesis of disialogangliosides from monosialogangliosides by sialyltransferases from embryonic chicken brain. J Biol Chem 243: 5804-5807.

Kaufman B, Basu S, Roseman S. 1966. Studies on the biosynthesis of gamgliosides. Inborn Disorders of Sphingolipid Metabolism. Aronson SM, Volk BW, editors. Pergamon; New York: pp. 193-213.

Kawano M, Kumagai K, Nishijima M, Hanada K. 2006. Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT. J Biol Chem 281: 30279-30288.

Keenan TW, Morre DJ, Basu S. 1974. Ganglioside biosynthesis. Concentration of glycosphingolipid glycosyltransferases in Golgi apparatus from rat liver. J Biol Chem 249: 310-315.

Kent C. 1995. Eukaryotic phospholipid biosynthesis. Annu Rev Biochem 64: 315-343.

Kihara A, Igarashi Y. 2004. FVT-1 is a mammalian 3-ketodihydrosphingosine reductase with an active site that faces the cytosolic side of the endoplasmic reticulum membrane. J Biol Chem 279: 49243-49250.

Kim T, Pfeiffer SE. 1999. Myelin glycosphingolipid/cholesterol-enriched microdomains selectively sequester the non-compact myelin proteins CNP MOG. J Neurocytol 28: 281-293.

Knapp PE. 1991. Studies of glial lineage and proliferation in vitro using an early marker for committed oligodendrocytes. J Neurosci Res 30: 336-345.

Kolesnick R. 2002. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. J Clin Invest 110: 3-8.

Kolter T, Proia RL, Sandhoff K. 2002. Combinatorial ganglioside biosynthesis. J Biol Chem 277: 25859-25862.

Kovacs WJ, Faust PL, Keller GA, Krisans SK. 2001. Purification of brain peroxisomes and localization of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Eur J Biochem 268: 4850-4859.

Krämer-Albers EM, Gehrig-Burger K, Thiele C, Trotter J, Nave KA. 2006. Perturbed interactions of mutant proteolipid protein/DM20 with cholesterol and lipid rafts in oligodendroglia: Implications for dysmyelination in spastic paraplegia. J Neurosci 26: 11743-11752.

Kuge O, Nishijima M. 1997. Phosphatidylserine synthase I and II of mammalian cells. Biochim Biophys Acta 1348: 151-156.

Kuge O, Saito K, Nishijima M. 1997. Cloning of a Chinese hamster ovary (CHO) cDNA encoding phosphatidylserine synthase (PSS) II, overexpression of which suppresses the phosphatidylserine biosynthetic defect of a PSS I-lacking mutant of CHO-K1 cells. J Biol Chem 272: 19133-19139.

Kumagai K, Yasuda S, Okemoto K, Nishijima M, Kobayashi S, et al. 2005. CERT mediates intermembrane transfer of various molecular species of ceramides. J Biol Chem 280: 6488-6495.

Kumagai K, Kawano M, Shinkai-Ouchi F, Nishijima M, Hanada K. 2007. Interorganelle trafficking of ceramide is regulated by phosphorylation-dependent cooperativity between the PH and START Domains of CERT. J Biol Chem 282: 17758-17766.

Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA. 1999. Golgi structure in three dimensions: Functional insights from the normal rat kidney cell. J Cell Biol 144: 1135-1149.

Lala P, Ito S, Lingwood CA. 2000. Retroviral transfection of Madin-Darby canine kidney cells with human MDR1 results in a major increase in globotriaosylceramide and 10(5)- to 10(6)-fold increased cell sensitivity to verocytotoxin. Role of *p*-glycoprotein in glycolipid synthesis. J Biol Chem 275: 6246-6251.

Ledeen RW, Cochran FB, Yu RK, Samuels FG. 1980. Gangliosides of the CNS myelin membrane. Adv Exp Med Biol 125: 167-176.

Ledesma MD, Simons K, Dotti CG. 1998. Neuronal polarity: Essential role of protein-lipid complexes in axonal sorting. Proc Natl Acad Sci USA 95: 3966-3971.

Lee JT, Xu J, Lee JM, Ku G, Han X, et al. 2004. Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. J Cell Biol 164: 123-131.

Lin X, Ramamurthy SK, Le Breton GC. 2005. Thromboxane A receptor-mediated cell proliferation, survival and gene expression in oligodendrocytes. J Neurochem 93: 257-268.

Lisanti MP, Rodriguez-Boulan E. 1990. Glycophospholipid membrane anchoring provides clues to the mechanism of protein sorting in polarized epithelial cells. TIBS 15: 113-118.

Liu Y, Li R, Ladisch S. 2004. Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. J Biol Chem 279: 36481-36489.

Lloyd KO, Furukawa K. 1998. Biosynthesis and functions of gangliosides: Recent advances. Glycoconj J 15: 627-636.

Maccioni HJ, Daniotti JL, Martina JA. 1999. Organization of ganglioside synthesis in the Golgi apparatus. Biochim Biophys Acta 1437: 101-118.

Marconi S, De Toni L, Lovato L, Tedeschi L, Gaetti L, et al. 2005. Expression of gangliosides on glial and neuronal cells

in normal and pathological adult human brain. J Neuroimmunol 170: 115-121.

Marcus J, Dupree JL, Popko B. 2000. Effects of galactlipid elimination on oligodendrocyte development and myelination. Glia 30:319-328.

Marcus J, Popko B. 2002. Galactolipids are molecular determinants of myelin development and axo-glial organization. Biochim Biophys Acta 1573: 406-413.

Marcus J, Dupree JL, Popko B. 2002. Myelin-associated glycoprotein and myelin galactolipids stabilize developing axo-glial interactions. J Cell Biol 156: 567-577.

Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, et al. 2006. Sulfatide is essential for the maintenance of CNS myelin and axon structure. Glia 53: 372-381.

Matlin KS, Simons K. 1984. Sorting of an apical plasma membrane glycoprotein occurs before it reaches the cell surface in cultured epithelial cells. J Cell Biol 99: 2131-2139.

Mayer M. 1999. Essential fatty acids and related molecular and cellular mechanisms in multiple sclerosis: New looks at old concepts. Folia Biol (Praha) 45: 133-141.

Merrill AH Jr, Jones DD. 1990. An update of the enzymology and regulation of sphingomyelin metabolism. Biochim Biophys Acta 1044: 1-12.

Millar AA, Kunst L. 1997. Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. Plant J 12: 121-131.

Miller MA, Kent C. 1986. Characterization of the pathway for phosphatidylethanolamine biosynthesis in Chinese hamster ovary mutant and parental cell lines. J Biol Chem 261: 9753-9761.

Misek DE, Bard E, Rodriguez-Boulan E. 1984. Biogenesis of epithelial cell polarity: Intracellular sorting and vectorial exocytosis of an apical plasma membrane glycoprotein. Cell 39: 537-546.

Mitsuda T, Furukawa K, Fukumoto S, Miyazaki H, Urano T, et al. 2002. Overexpression of ganglioside GM1 results in the dispersion of platelet-derived growth factor receptor from glycolipid-enriched microdomains and in the suppression of cell growth signals. J Biol Chem 277: 11239-11246.

Mizutani Y, Kihara A, Igarashi Y. 2005. Mammalian Lass6 and its related family members regulate synthesis of specific ceramides. Biochem J 390: 263-271.

Mizutani Y, Kihara A, Igarashi Y. 2006. LASS3 (longevity assurance homologue 3) is a mainly testis-specific (dihydro)ceramide synthase with relatively broad substrate specificity. Biochem J 398: 531-538.

Morell P, Ousley AH. 1994. Metabolic turnover of myelin glycerophospholipids. Neurochem Res 19: 967-974.

Morell P, Jurevics H. 1996. Origin of cholesterol in myelin. Neurochem Res 21: 4634-4670.

Morell P, Quarles R, Norton WT. 1994. Myelin formation, structure, and biochemistry. Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. Siegel GJ, Agranoff BW, Albers RW, Molinoff PB, editors. Raven; New York: pp. 117-143.

Morell P. 1984. A correlative synopsis of the leukodystrophies. Neuropediatrics 15 Suppl: 62-65.

Mumby S, Margarson M, Quinlan GJ, Evans TW, Gutteridge JM. 1997. Is bleomycin-detectable iron present in the plasma of patients with septic shock? Intensive Care Med 23: 635-639.

Muñiz M, Riezman H. 2000. Intracellular transport of GPI-anchored proteins. EMBO J 19: 10-15.

Munro S. 2003. Cell biology: Earthworms and lipid couriers. Nature 426: 775-776.

Nagai K, Tadano-Aritomi K, Iida-Tanaka N, Yoshizawa H, Ishizuka I. 2005. Metabolism of sulfolipids in isolated renal tubules from rat. Comp Biochem Physiol B Biochem Mol Biol 140: 487-495.

Nakanishi M, Goldstein JL, Brown MS. 1988. Multivalent control of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mevalonate-derived product inhibits translation of mRNA and accelerates degradation of enzyme. J Biol Chem 263: 8929-8937.

Nakayama J, Fukuda MN, Hirabayashi Y, Kanamori A, Sasaki K, et al. 1996. Expression cloning of a human GT3 synthase. GD3 and GT3 are synthesized by a single enzyme. J Biol Chem 271: 3684-3691.

Nicholson KM, Quinn DM, Kellett GL, Warr JR. 1999. Preferential killing of multidrug-resistant KB cells by inhibitors of glucosylceramide synthase. Br J Cancer 81: 423-430.

Nilsson OS, Dallner G. 1977. Enzyme and phospholipid asymmetry in liver microsomal membranes. J Cell Biol 72: 568-583.

Nishijima M, Kuge O, Akamatsu Y. 1986. Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. I. Inhibition of de novo phosphatidylserine biosynthesis by exogenous phosphatidylserine and its efficient incorporation. J Biol Chem 261: 5784-5789.

Nishio M, Tajima O, Furukawa K, Urano T, Furukawa K. 2005. Over-expression of GM1 enhances cell proliferation with epidermal growth factor without affecting the receptor localization in the microdomain in PC12 cells. Int J Oncol 26: 191-199.

Norton W, Cammer W. 1984. Isolation and characterization of myelin. Myelin, 2nd edition. Morell P, editor. New York: Plenum; 147-195.

Nyasae LK, Hubbard AL, Tuma PL. 2003. Transcytotic efflux from early endosomes is dependent on cholesterol and glycosphingolipids in polarized hepatic cells. Mol Biol Cell 14: 2689-2705.

Ogawa-Goto K, Abe T. 1998. Gangliosides and glycosphingolipids of peripheral nervous system myelins – a minireview. Neurochem Res 23: 305-310.

Ohvo-Rekila H, Ramstedt B, Leppimaki P, Slotte JP. 2002. Cholesterol interactions with phospholipids in membranes. Prog Lipid Res 41: 66-97.

Okada M, Itoh M, Haraguchi M, Okajima T, Inoue M, et al. 2002. b-Series ganglioside deficiency exhibits no definite changes in the neurogenesis and the sensitivity to Fas-mediated apoptosis but impairs regeneration of the lesioned hypoglossal nerve. J Biol Chem 277: 1633-1636.

Omkumar RV, Darnay BG, Rodwell VW. 1994. Modulation of Syrian hamster 3-hydroxy-3-methylglutaryl-CoA reductase activity by phosphorylation. Role of serine 871. J Biol Chem 269: 6810-6814.

Ong RL, Yu RK. 1984. Interaction of ganglioside GM1 and myelin basic protein studied by carbon-13 and proton nuclear magnetic resonance spectroscopy. J Neurosci Res 12: 377-393.

Pan B, Fromholt SE, Hess EJ, Crawford TO, Griffin JW, Sheikh KA, Schnaar RL. 2005. Myelin-associated glycoprotein and complementary axonal ligands, gangliosides, mediate axon stability in the CNS and PNS: Neuropathology and behavioral deficits in single- and double-null mice. Exp Neurol 195: 208-217.

Paterson LJ, Weselake RJ, Mir PS, Mir Z. 2002. Positional distribution of CLA in TAG of lamb tissues. Lipids 37: 605-611.

Paul P, Kamisaka Y, Marks DL, Pagano RE. 1996. Purification and characterization of UDP-glucose: Ceramide glucosyltransferase from rat liver Golgi membranes. J Biol Chem 271: 2287-2293.

Percy AK, Moore JF, Carson MA, Waechter CJ. 1983. Characterization of brain phosphatidylserine decarboxylase: Localization in the mitochondrial inner membrane. Arch Biochem Biophys 223: 484-494.

Peterson AC, Bray GM. 1984. Hypomyelination in the peripheral nervous system of shiverer mice and in shiverer in equilibrium normal chimaera. J Comp Neurol 227: 348-356.

Pewzner-Jung Y, Ben-Dor S, Futerman AH. 2006. When do Lasses (longevity assurance genes) become CerS (ceramide synthases): Insights into the regulation of ceramide synthesis. J Biol Chem 281: 25001-25005.

Pfeiffer SE, Fuller SD, Simons K. 1985. Intracellular sorting and basolateral appearance of the G protein of vesicular stomatitis virus in Madin-Darby canine kidney cells. J Cell Biol 101: 470-476.

Pfeiffer SE, Warrington AE, Bansal R. 1993. The oligodendrocyte and its many cellular processes. Trends Cell Biol 3: 191-197.

Pike LJ. 2003. Lipid rafts: Bringing order to chaos. J Lipid Res 44: 655-667.

Polishchuk R, Di Pentima A, Lippincott-Schwartz J. 2004. Delivery of raft-associated, GPI-anchored proteins to the apical surface of polarized MDCK cells by a transcytotic pathway. Nat Cell Biol 6: 297-307.

Poulos A. 1995. Very long chain fatty acids in higher animal. Lipids 30: 1-14.

Quarles RH, Macklin WB, Morell P. 2006. Myelin formation, structure, and biochemistry. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 7th edition. Siegel GJ, Albers RW, Brady ST, Price D, editors. Academic Press Elsevier; New York: pp. 51-71.

Quan G, Xie C, Dietschy JM, Turley SD. 2003. Ontogenesis and regulation of Cholesterol metabolism in the central nervous system of mouse. Brain Res Dev Brain Res 146: 87-98.

Raff MC, Lillien LE, Richardson WD, Burne JF, Noble MD. 1988. Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. Nature 333: 562-565.

Ramamurthy S, Mir F, Gould RM, Le Breton GC. 2006. Characterization of thromboxane A2 receptor signaling in developing rat oligodendrocytes: Nuclear receptor localization and stimulation of myelin basic protein expression. J Neurosci Res 84: 1402-1414.

Ranscht B, Clapshaw PA, Price J, Noble M, Seifert W. 1982. Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. Proc Natl Acad Sci USA 79: 2709-2713.

Rasband MN, Taylor CM, Bansal R. 2003. Paranodal transverse bands are required for maintenance but not initiation of Nav1.6 sodium channel clustering in CNS optic nerve axons. Glia 44: 173-182.

Reinhart MP, Billheimer JT, Faust JR, Gaylor JL. 1987. Subcellular localization of the enzymes of cholesterol biosynthesis and metabolism in rat liver. J Biol Chem 262: 9649-9655.

Roseman S. 1970. Synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. Chem Phys Lipids 5: 270-297.

Rosenbluth J, Moon D. 2003. Dysmyelination induced in vitro by IgM antisulfatide and antigalactocerebroside monoclonal antibodies. J Neurosci Res 71: 104-109.

Rosenbluth J, Schiff R, Liang WL, Dou W. 2003. Antibody-mediated CNS demyelination II. Focal spinal cord lesions induced by implantation of an IgM antisulfatide-secreting hybridoma. J Neurocytol 32: 265-276.

Ross BM, Moszczynska A, Blusztajn JK, Sherwin A, Lozano A, Kish SJ. 1997. Phospholipid biosynthetic enzymes in human brain. Lipids 32: 351-358.

Sadeghlar F, Sandhoff K, van Echten-Deckert G. 2000. Cell type specific localization of sphingomyelin biosynthesis. FEBS Lett 478: 9-12.

Saher G, Brugger B, Lappe-Siefke C, Mobius W, Tozawa R, et al. 2005. High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8: 468-475.

Saito K, Kuge O, Akamatsu Y, Nishijima M. 1996. Immunochemical identification of the pssA gene product as phosphatidylserine synthase I of Chinese hamster ovary cells. FEBS Lett 395: 262-266.

Sandhoff K, van Echten G. 1993. Ganglioside metabolism–topology and regulation. Adv Lipid Res 26: 119-142.

Saravanan K, Schaeren-Wiemers N, Klein D, Sandhoff R, Schwarz A, et al. 2004. Specific downregulation and mistargeting of the lipid raft-associated protein MAL in a glycolipid storage disorder. Neurobiol Dis 16: 396-406.

Sasaki T. 1990. Glycolipid transfer protein and intracellular traffic of glucosylceramide. Experientia 46: 611-616.

Sasaki T, Demel RA. 1985. Net mass transfer of galactosylceramide facilitated by glycolipid transfer protein from pig brain: A monolayer study. Biochemistry 24: 1079-1083.

Schaeren-Wiemers N, van der Bijl P, Schwab ME. 1995. The UDP-galactose: Ceramide galactosyltransferase: Expression pattern in oligodendrocytes and Schwann cells during myelination and substrate preference for hydroxyceramide. J Neurochem 65: 2267-2278.

Schneider A, Laender H, Schulz G, Wolburg H, Nave KA, et al. 2005. Palmitoylation is a sorting determinant for transport to the myelin membrane. J Cell Sci 118: 2415-2423.

Scurlock B, Dawson G. 1999. Differential responses of oligodendrocytes to tumor necrosis factor and other pro-apoptotic agents: Role of ceramide in apoptosis. J Neurosci Res 55: 514-522.

Sheikh KA, Sun J, Lui Y, Kawai H, Crawford TO, et al. 1999. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proc Natl Acad Sci USA 96: 7532-7537.

Simons K, van Meer G. 1988. Lipid sorting in epithelial cells. Biochemistry 27: 6197-6202.

Simons K, Wandinger-Ness A. 1990. Polarized sorting in epithelia. Cell 62: 207-210.

Simons K, Ikonen E. 1997. Functional rafts in cell membranes. Nature 387: 569-572.

Simons K, Ikonen E. 2000. How cells handle cholesterol. Science 290: 1721-1726.

Simons K, Toomre D. 2000. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1: 31-39.

Simons M, Krämer EM, Thiele C, Stoffel W, Trotter J. 2000. Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. J Cell Biol 151: 143-154.

Smotrys JE, Linder ME 2004. Palmitoylation of intracellular signaling proteins: Regulation and function. Annu Rev Biochem 73: 559-587.

Sprong H, Degroote S, Claessens T, van Drunen J, Oorschot V, et al. 2001. Glycosphingolipids are required for sorting melanosomal proteins in the Golgi complex. J Cell Biol 155: 369-380.

Sprong H, Kruithof B, Leijendekker R, Slot JW, van Meer G, et al. 1998. UDP-galactose: Ceramide galactosyltransferase is a class I integral membrane protein of the endoplasmic reticulum. J Biol Chem 273: 25880-25888.

Stegemeyer HR, Stegemeyer H. 2004. Finally, I propose the medullary substance to be named myelin. Dtsch Med Wochenschr 129: 2784-2787.

Stoffel W, Bosio A. 1997. Myelin glycolipids and their functions. Curr Opin Neurobiol 7: 654-661.

Stone SJ, Cui Z, Vance JE. 1998. Cloning and expression of mouse liver phosphatidylserine synthase-1 cDNA. Overexpression in rat hepatoma cells inhibits the CDP-ethanolamine pathway for phosphatidylethanolamine biosynthesis. J Biol Chem 273: 7293-7302.

Stone SJ, Vance JE. 2000. Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated membranes. J Biol Chem 275: 34534-34540.

Susuki K, Baba H, Tohyama K, Kanai K, Kuwabara S, et al. 2007a. Gangliosides contribute to stability of paranodal junctions and ion channel clusters in myelinated nerve fibers. Glia 55: 746-757.

Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, et al. 2007b. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci 27: 3956-3967.

Suzuki K, Poduslo SE, Norton WT. 1967. Gangliosides in the myelin fraction of developing rats. Biochim Biophys Acta 144: 375-381.

Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, et al. 1996. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proc Natl Acad Sci USA 93: 10662-10667.

Taylor CM, Marta CB, Bansal R, Pfeiffer ES. 2004. The transport, assembly, and function of myelin lipids. Myelin Biology and Disorders, 1st edition. Lazzarini RA, Griffin JW, Lassman H, Nave KA, Miller RH, et al., editors. New York: Lazzarini; pp. 55–88.

Temple S, Raff MC. 1986. Clonal analysis of oligodendrocyte development in culture: Evidence for a developmental clock that counts cell divisions. Cell 44: 773-779.

Thelen KM, Falkai P, Bayer TA, Lutjohann D. 2006. Cholesterol synthesis rate in human hippocampus declines with aging. Neurosci Lett 403: 15-19.

Uliana AS, Crespo PM, Martina JA, Daniotti JL, Maccioni HJ. 2006a. Modulation of GalT1 and SialT1 sub-Golgi localization by SialT2 expression reveals an organellar level

of glycolipid synthesis control. J Biol Chem 281: 32852-32860.

Uliana AS, Giraudo CG, Maccioni HJ. 2006b. Cytoplasmic tails of SialT2 and GalNAcT impose their respective proximal and distal Golgi localization. Traffic 7: 604-612.

Ullman MD, Radin NS. 1974. The enzymatic formation of sphingomyelin from ceramide and lecithin in mouse liver. J Biol Chem 249: 1506-1512.

van Echten G, Sandhoff K. 1993. Ganglioside metabolism. Enzymology, topology, and regulation. J Biol Chem 268: 5341-5344.

van Golde LM, Raben J, Batenburg JJ, Fleischer B, Zambrano F, et al. 1974. Biosynthesis of lipids in Golgi complex and other subcellular fractions from rat liver. Biochim Biophys Acta 360: 179-192.

van Helvoort A, van't Hof W, Ritsema T, Sandra A, van Meer G. 1994. Conversion of diacylglycerol to phosphatidylcholine on the basolateral surface of epithelial (Madin-Darby canine kidney) cells. Evidence for the reverse action of a sphingomyelin synthase. J Biol Chem 269: 1763-1769.

van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, et al. 1996. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 87: 507-517.

van Meer G. 1989. Lipid traffic in animal cells. Annu Rev Cell Biol 5: 247-275.

van Meer G, Holthuis JC. 2000. Sphingolipid transport in eukaryotic cells. Biochim Biophys Acta 1486: 145-170.

van Meer G, Lisman Q. 2002. Sphingolipid transport: Rafts and translocators. J Biol Chem 277: 25855-25858.

van Meer G, Vaz WL. 2005. Membrane curvature sorts lipids. Stabilized lipid rafts in membrane transport. EMBO Rep 6: 418-419.

van Meeteren ME, Baron W, Beermann C, Dijkstra CD, van Tol EA. 2006. Polyunsaturated fatty acid supplementation stimulates differentiation of oligodendroglia cells. Dev Neurosci 28: 196-208.

Van Overloop H, Gijsbers S, Van Veldhoven PP. 2006. Further characterization of mammalian ceramide kinase: Substrate delivery and (stereo) specificity, tissue distribution, and subcellular localization studies. J Lipid Res 47: 268-283.

Vance DE. 1990. Boehringer Mannheim Award lecture. Phosphatidylcholine metabolism: Masochistic enzymology, metabolic regulation, and lipoprotein assembly. Biochem Cell Biol 68: 1151-1165.

Vance DE. 1996. Glycerolipid biosynthesis in eukaryotes. Biochemistry of Lipids, Lipoproteins, and Membranes. Vance DE, Vance J, editors. Amsterdam: Elsevier; pp. 153-181.

Veldman RJ, Klappe K, Hinrichs J, Hummel I, Schaaf van der G, et al. 2002. Altered sphingolipid metabolism in multidrug-resistant ovarian cancer cells is due to uncoupling of glycolipid biosynthesis in the Golgi apparatus. FASEB J 16: 1111-1113.

Verkade P, Simons K. 1997. Robert Feulgen Lecture 1997. Lipid microdomains and membrane trafficking in mammalian cells. Histochem Cell Biol 108: 211-220.

Vermeulen PS, Geelen MJH, Tijburg LBM, van Golde LMG. 1997. The CDP-ethanolamine pathway in mammalian cells. Advances in Lipobiology. Gross RW, editor. J.A.I. Press; Greenwich, USA: pp. 287-322.

Virchow R. 1854. Über das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den tierischen Geweben. Virchows Arch Pathol Anat 6: 562-572.

Voelker DR, Kennedy EP. 1982. Cellular and enzymic synthesis of sphingomyelin. Biochemistry 21: 2753-2759.

Voelker DR. 1985. Disruption of phosphatidylserine translocation to the mitochondria in baby hamster kidney cells. J Biol Chem 260: 14671-14676.

Voelker DR, Frazier JL. 1986. Isolation and characterization of a Chinese hamster ovary cell line requiring ethanolamine or phosphatidylserine for growth and exhibiting defective phosphatidylserine synthase activity. J Biol Chem 261: 1002-1008.

Vos JP, de Haas CGM, van Golde LMG, Lopes-Cardozo M. 1997. Relationships between phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin metabolism in cultured oligodendrocytes. J Neurochem 68: 1252-1260.

Vyas AA, Patel HV, Fromholt SE, Heffer-Lauc M, Vyas KA, et al. 2002. Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc Natl Acad Sci USA 99: 8412-8417.

Weiss B, Stoffel W. 1997. Human and murine serine-palmitoyl-CoA transferase–cloning, expression and characterization of the key enzyme in sphingolipid synthesis. Eur J Biochem 249: 239-247.

Yamashiro S, Haraguchi M, Furukawa K, Takamiya K, Yamamoto A, et al. 1995. Substrate specificity of beta 1,4-*N*-acetylgalactosaminyltransferase in vitro and in cDNA-transfected cells. GM2/GD2 synthase efficiently generates asialo-GM2 in certain cells. J Biol Chem 270: 6149-6155.

Yamaoka S, Miyaji M, Kitano T, Umehara H, Okazaki T. 2004. Expression cloning of a human cDNA restoring sphingomyelin synthesis and cell growth in sphingomyelin synthase-defective lymphoid cells. J Biol Chem 279: 18688-18693.

Yamashita T, Wu YP, Sandhoff R, Werth N, Mizukami H, et al. 2005. Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glial interactions. Proc Natl Acad Sci USA 102: 2725-2730.

Yasuda S, Nishijima M, Hanada K. 2003. Localization, topology, and function of the LCB1 subunit of serine palmitoyltransferase in mammalian cells. J Biol Chem 278: 4176-4183.

Yavin E, Zeigler BP. 1977. Regulation of phospholipids metabolism in differentiating cells from rat brain cerebral hemispheres in culture. J Biol Chem 252: 260-267.

Yehuda S, Rabinovitz S, Mostofsky DI, Huberman M, Sredni B. 1997. Essential fatty acid preparation improves biochemical and cognitive functions in experimental allergic encephalomyelitis rats. Eur J Pharmacol 328: 23-29.

Yim SH, Farrer RG, Hammer JA, Yavin E, Quarles RH. 1994. Differentiation of oligodendrocytes cultured from developing rat brain is enhanced by exogenous GM3 gangliosides. J Neurosci Res 38: 268-281.

Yohe HC, Jacobson RI, Yu RK. 1983. Ganglioside-basic protein interaction: Protection of gangliosides against neuraminidase action. J Neurosci Res 9: 401-412.

Yu RK, Macala LJ, Farooq M, Sbaschnig-Agler M, Norton WT, et al. 1989. Ganglioside and lipid composition of bulk-isolated rat and bovine oligodendroglia. J Neurosci Res 23: 136-141.

Zacchetti D, Peranen J, Murata M, Fiedler K, Simons K. 1995. VIP17/MAL, a proteolipid in apical transport vesicles. FEBS Lett 377: 465-469.

Zborowski J, Dygas A, Wojtczak L. 1983. Phosphatidylserine decarboxylase is located on the external side of the inner mitochondrial membrane. FEBS Lett 157: 179-182.

Zegers MM, Hoekstra D. 1997. Sphingolipid transport to the apical plasma membrane domain in human hepatoma cells is controlled by PKC and PKA activity: A correlation with cell polarity in HepG2 cells. J Cell Biol 138: 307-321.