



Review

Glycosphingolipid–Protein Interaction in Signal Transduction

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Abstract: Glycosphingolipids (GSLs) are a class of ceramide-based glycolipids essential for embryo development in mammals. The synthesis of specific GSLs depends on the expression of distinctive sets of GSL synthesizing enzymes that is tightly regulated during development. Several reports have described how cell surface receptors can be kept in a resting state or activate alternative signalling events as a consequence of their interaction with GSLs. Specific GSLs, indeed, interface with specific protein domains that are found in signalling molecules and which act as GSL sensors to modify signalling responses. The regulation exerted by GSLs on signal transduction is orthogonal to the ligand–receptor axis, as it usually does not directly interfere with the ligand binding to receptors. Due to their properties of adjustable production and orthogonal action on receptors, GSLs add a new dimension to the control of the signalling in development. GSLs can, indeed, dynamically influence progenitor cell response to morphogenetic stimuli, resulting in alternative differentiation fates. Here, we review the available literature on GSL–protein interactions and their effects on cell signalling and development.

Keywords: glycosphingolipid; signalling; glycan–protein interaction

1. Introduction

Glycosphingolipids (GSLs) are a heterogeneous class of membrane lipids that are constituted by complex glycan moieties linked by a glycosidic bond to a ceramide lipophilic backbone [1]. GSLs have aroused a special interest in the light of their peculiar structures. On the one hand, due to the biophysical properties of their hydrophobic portion, GSLs promote the formation of membrane nanoscopic domains. These domains regulate lateral partitioning of receptors and consequently their activation and recruitment of downstream components of signalling cascades [2–13]. The biophysical properties of GSLs are also important outside the membrane context with simple GSLs contributing to the maintenance of the integrity of the epidermis and of its barrier function [14–16]. On the other hand, GSLs have a surprisingly high number of different glycans constituting their headgroups. Although the biological and evolutionary meaning of this extensive variability is still largely unclear [17], the glycans on GSLs are in a favourable position to interact with the luminal portions of membrane proteins and receptors [18]. Notably, indeed, over the last decades, a number of studies have demonstrated how GSLs influence the behaviour and signalling capability of membrane proteins [2,19–29].

Interestingly, specific GSL headgroups serve as receptors for viruses and microbial virulence factors [30–88] (Table 1), indicating that glycans in GSLs can be recognized with remarkable specificity by dedicated microbial proteins. In fact, a number of studies have demonstrated that GSL–glycan moieties establish interactions with endogenous proteins or glycans located on the plasma membrane of the same or of neighbouring cells. The specificity of these interactions depends on the precise

composition of GSL-glycan sugar residues [20,23,29,89–91]. By this virtue, the glycan portion of the GSLs has the potential to interface with specific plasma membrane proteins to modify their activity [2,19–29] by carbohydrate–carbohydrate or protein–carbohydrate interactions [2,29,92].

Table 1. Glycosphingolipids (GSLs) headgroups serve as receptors for viruses and bacterial toxins.

Virus	Glycosphingolipid Receptors	References
Picornaviridae		
Porcine sapelovirus (PSV)	GD1a	[30]
Caliciviridae		
Human Norovirus (HuNoV)	Type 1, 2, 3 HBGA	[31,32]
Human Norovirus (HuNoV): GIL4 strain	H, B, and A type 1 Lewis b	[33]
Murine Norovirus (MNV): MNV-1 and CR3 strains	GD1a; GT1b	[34,35]
Bovine Norovirus (BoNoV)	HBGA	[36]
Rabbit Hemorrhagic Disease Virus (RHDV)	A and H Type 2 HBGA	[37]
Adenoviridae		
Adenovirus type 37 (Ad37)	GD1a	[38]
Reoviridae		
Reovirus serotype 1 (T1)	GM2	[39]
Porcine Rotavirus: OSU strain	GM3	[40,41]
Porcine Rotavirus: CRW-8 strain	GD1a	[42]
Porcine Rotavirus: TFR-41 strain	Unknown ganglioside	[43]
Simian Rotavirus: SA11 strain	NeuGcGM3, IV ³ NeuAcLc4, GM2, GD1a	[44]
Simian Rhesus Rotavirus: RRV strain	Unknown ganglioside	[43,45]
Bovine Rotavirus: NCVD strain	NeuGcGM3, IV ³ NeuAcLc4, GM2, GD1a	[44,46]
Bovine Rotavirus: UK strain	NeuGcGM3, GM1, GD1a, GM2, IV ³ NeuAcLc4	[43,46]
Human Rotavirus: KU, MO, DS-1 and Wa strains	GM3, GM1	[42,43,47–49]
Polyomaviridae		
Trichodysplasia spinulosa-associated Polyomavirus (TSPyV)	GM1	[50]
Murine Polyomavirus (MPyV)	GD1a, GT1b	[51,52]
Simian Virus 40 (SV40)	GM1	[44,53,54]
BK Virus (BKV)	GD1b, GT1b	[55]
JC Virus (JCV)	GT1b	[56]
Merkel Cell Polyomavirus (MCPyV)	GT1b	[57]
Parvoviridae		
Human Parvovirus B19	Gb4, SSEA-3, SSEA-4, nLc4	[58,59]
Simian Parvovirus	Gb4; Forssmann antigen	[60]
Bovine Adeno-associated Virus (BAAV)	Unknown ganglioside	[61]
Retroviridae		
Human Immunodeficiency Virus (HIV)	Gb3, GM3, GalCer, GD3, SM4 sulfatide	[62–69]
Flaviviridae		
Dengue virus (DENV) type 2	GM3, nLc4	[70–72]
Orthomyxoviridae		
Influenza A virus, subtype H3N2: A/Victoria/3/75 strain	Ganglioside with Neu5Ac α 2-3Gal β 1-4 (Fuc α 1-3)GlcNAc epitope; nLc8, nLc10 and nLc12	[73]
Influenza A virus, subtype H3N2: A/Hiroshima/52/2005 strain		
Poxviridae		
Vaccinia virus (VACV): Western-Reserve strain	SM4 sulfatide	[74,75]
Paramyxoviridae		
Paramyxovirus 1 (Newcastle Disease)	GM3, GM2, GM1, GD1a	[76]
Sendai virus (SV) (murine parainfluenza virus type 1)	GD1a, GQ1b, IV ³ NeuAcLc4, nLC4	[77,78]
Human parainfluenza virus types 1 (HPIV-1)	IV ³ NeuAcLc4, Nlc4	[78]
Human parainfluenza virus types 3 (hPIV-3)		

Table 1. Cont.

Bacterial Toxin	Glycosphingolipid Receptors	References
Cholera toxin <i>Vibrio cholera</i>	GM1	[79,80]
Heat labile toxin 1 <i>Escherichia coli</i>	GM1	[81]
Shiga Toxin <i>Shigella dysenteriae</i>	Gb3	[82,83]
Shiga-like toxins (SLT1 and SLT 2) <i>Escherichia coli</i> (Verotoxins)	Gb4	[84,85]
Tetanus neurotoxin (TeNT) <i>Clostridium tetani</i>	GT1b, GD1b	[86]
Botulinium toxin BoNT <i>Clostridium botulinum</i>	GT1b, GD1a	[87]
Heat labile toxin IIB <i>Escherichia coli</i>	GD1a	[88]

While the mechanistic details of these GSL–protein interactions are often poorly understood, in a small number of cases, the existence of GSL-sensing domains (GSDs) in proteins has been demonstrated [6,29,93,94]. Nonetheless, the basic principles by which GSL-glycans are specifically perceived by GSDs are unknown. Even in the absence of this information, the available data suggest that GSLs constitute a regulatory layer acting orthogonally to the ligand–receptor–transducers module, which allows the dynamic fine-tuning of intracellular signalling. This is of particular interest in cell differentiation events. Indeed, precursor cells for given differentiation lineages might respond differently to morphogenetic stimuli as a consequence of exposing different GSLs on their cell surfaces. As a matter of fact, the GSL-dependent regulation is central for developmental processes as failure to synthesize specific GSLs results in developmental disorders in humans and in tissue specific phenotypes in model organisms [95–101].

In this review, we intend to discuss the role of GSLs as plastic regulators of signal transduction. To this aim, we review some examples of GSL–protein interactions, and discuss their molecular aspects, their impact on the regulation of cell signalling, along with their pathophysiological significance.

2. GSL Synthesis and Turnover

GSL metabolism is accomplished along the endomembrane system [1] (Figure 1). GSL synthesis starts in the endoplasmic reticulum (ER) where a sphingoid base is condensed with an acyl-CoA, to generate Ceramide (Cer) [102,103] (Figure 1). In the ER, Cer can be galactosylated to produce galactosylceramide (GalCer) [104]. GalCer is, then, transported to the Golgi complex where it can be sulphated to produce sulphogalactolipids, or sialylated to produce the GM4 ganglioside [1]. Alternatively, Cer can be conveyed to the Golgi complex via transport vesicles or by the ceramide-transfer protein CERT [105]. CERT transports Cer to the trans-Golgi network (TGN) where it is primarily used for the synthesis of sphingomyelin (SM) [105] (Figure 1). On the contrary, Cer transported to Golgi by vesicular transport is converted to glucosylceramide (GlcCer). GlcCer is synthesized by the addition of a glucose residue to Cer on the cytosolic leaflet of *cis*-Golgi membranes [105,106] and GlcCer is further glycosylated by the activity of glycosyltransferases distributed along Golgi *cisternae*, resulting in more than 300 species of GSLs. These enzymes transfer a specific carbohydrate from the appropriate activated sugar nucleotide (e.g., UDP-Galactose, CMP-sialic acid, UDP-Fucose, etc.) to the non-reducing end of the growing carbohydrate chain linked to GlcCer [107].

Similarly to Cer, GlcCer can be transported through two distinct routes. GlcCer is either transported across the Golgi complex via vesicular trafficking, or, alternatively, it is directly transferred by the lipid-transfer protein FAPP2 to the TGN [108–111] (Figure 1). Irrespective on its mode of transport, GlcCer is translocated to the luminal Golgi/TGN leaflet and undergoes a galactose (Gal) addition and conversion to lactosylceramide (LacCer). Once produced, LacCer cannot be translocated back to the cytosolic leaflet and constitutes the branching point for the synthesis of different GSL metabolic series [1]. These are defined by their internal core carbohydrate sequence [112,113] as ganglio (Gal-(Neu5Acα2-3)-β1-4glc) [114], globo (galα1-4gal) [115], lacto (galβ1-3glcNAcβ1-3gal) [116],

3. GSL-Dependent Regulation of Signal Transduction

Vertebrates possess a vast repertoire of GSLs, which differ according to the sugar composition, the anomeric linkages of the glycosidic bonds, and to the extent of branching of their glycans [1]. Since such complexity represents an exponential leap from the nearest evolutionary neighbour [125], it is tempting to speculate that this led to the emergence of a new level of regulation in vertebrate cells. A number of studies have indeed shown that GSLs regulate cellular signalling pathways by interacting with components of the signal transduction machinery (i.e., hormones, receptors and intracellular transducers). These interactions have different outcomes: (i) allosteric regulation of the protein conformation; (ii) regulation of protein multimerization; (iii) protein segregation to membrane domains; and (iv) clustering of signalling molecules in proximity to their effectors [2–13] (Figure 2).

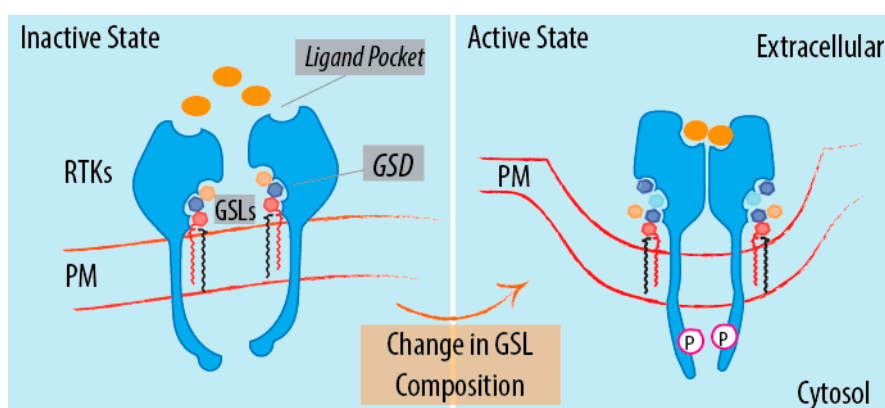


Figure 2. GSL influence on signalling. Schematic representation of the regulatory role of the GSLs on signal transduction. The regulation of RTKs by GSLs is provided as an example to illustrate the signalling role of GSLs. A specific GSL profile on plasma membrane can keep a RTK in an inactive state (**left panel**); A change in GSLs composition can regulate receptor activation/inactivation. GSLs act as allosteric regulators of receptor capability to recognize the ligand, to undergo multimerization and to auto-phosphorylate (**right panel**). GSLs, glycosphingolipids; Orange spheres represent ligands; Colored hexagons represent the different sugar residues of GSL-glycan moieties; Black-Red parallelogram, Cer backbone. Pink circle surrounding P represent phosphate groups; GSD, glycosphingolipid sensing domain; PM, plasma membrane; RTK, receptor tyrosine kinase.

One paradigmatic example is that of the epidermal growth factor receptor (EGFR) [2,126,127]. EGFR was reported to be activated/inactivated depending on the GSLs composition of the membrane in which it resides. Epidermal Growth Factor (EGF) upon binding to EGFR stimulates the transition of the receptor from the inactive monomeric to an active homodimeric form. This event triggers the intrinsic receptor-associated tyrosine kinase activity, leading to receptor auto-phosphorylation [2,126,127] and to the activation of a signalling cascade that promotes cell proliferation. In 1986, Bremer et al. reported that EGFR auto-phosphorylation is inhibited by the exogenous addition of the GM3 ganglioside [126]. More recent studies approached the molecular details of this effect by in vitro studies using the purified receptor reconstituted in liposomes [2]. In spite of the absence of a known GSD, EGFR was reported to establish two different lateral interactions with GM3, having functional implications on the behaviour of the receptor. One is a protein–carbohydrate interaction involving the terminal *N*-acetylneuraminic acid of the GM3 and a lysine (Lys-642) localized in proximity to the transmembrane domain of EGFR. Through this interaction GM3 maintains the EGFR in its resting state preventing receptor dimerization and activation in absence of the ligand [2]. GM3 interacts with the EGFR also by a carbohydrate–carbohydrate interaction involving the sialylated Gal of GM3 and the terminal *N*-acetylglucosamine residues on EGFR *N*-glycans. In addition, this interaction, although weaker, was demonstrated to inhibit EGFR auto-phosphorylation and activation [128].

Apart from GM3, other gangliosides sharing the same glycan core structure as GM3 (i.e., GM1, GD1a and GT1b) have inhibitory effects on EGFR signalling [2,129]. On the contrary, the neutral GSL Gb4 exerts the opposite effect on EGFR [20]. Gb4, indeed, interacts directly with EGFR, potentiates its auto-phosphorylation capability and activates the phosphorylation of the downstream cascade components, ERK1/2. Of note, other neutral GSLs (i.e., LacCer and Gb3) do not have the same effect [20], indicating that GSL-mediated regulation depends on specific glycan configurations. Similar results were reported showing that gangliosides regulate different receptor tyrosine kinases like the fibroblast growth factor receptor [130–132], neurotrophin receptors [24,133,134], hepatocyte growth factor receptor [135–137], platelet-derived growth factor receptor [23,138,139], vascular endothelial growth factor receptor [140,141], and insulin receptor [27,142,143] (Figure 2). In some of these instances, it has been proposed that mono-sialylated gangliosides act as negative regulators while poly-sialylated gangliosides would activate the RTK signalling activity. Conflicting data have, nevertheless, challenged this interpretation suggesting that the regulatory function of gangliosides is not simply attributable to their sialic acid content [144]. Among these GSL–protein interactions, two are of special interest for their physiological importance: that of GM1 with neural tropomyosin receptor kinase A (TrkA), and that of GM3 with insulin receptor.

Nerve growth factor similarly to EGF activates its specific receptors (i.e., TrkA) by inducing dimerization and autophosphorylation. Already in the 1990s, seminal studies reported that the ganglioside GM1 specifically interacts with TrkA [24] to promote its dimerization [145] and consequent activation [24,133]. While the mechanistic aspects of GM1 interaction with TrkA are unknown, the interaction requires the glycosylation of the receptor [146] and its clustering with GM1 in membrane microdomains [147]. The neurotrophic effect of GM1 has been confirmed in neurodegenerative disease models like in Huntington disease mice where GM1 metabolism was compromised and GM1 administration ameliorated the disease symptomatology [148,149]. Of clinical relevance is also the interaction of GM3 with the insulin receptor [27,150]. Here an inhibitory effect of GM3 on insulin signalling has been reported both in cell systems [27] and in mice where depletion on the GM3 synthesizing enzyme leads to increased insulin sensitivity [98]. Following on this experimental evidence, studies aimed at evaluating the possible association between GM3 ganglioside and diabetes/metabolic syndrome in humans have observed that high circulating levels of GM3 represent a risk factor for the development of insulin resistance [151,152].

A different example of how GSL-glycans regulate receptor function is the regulation of the Fas (CD95) receptor by Gb3 globoside. Here, Fas was found to bear a GSD that interacts specifically with Gb3 and LacCer but not with Gb4 or gangliosides [29]. The Fas-GSL interaction has important functional consequences as the GSD of Fas defines its internalization route, as well as the signalling outcome upon activation by the ligand. When paired to Gb3, the ligand-bound Fas is internalized by clathrin-dependent endocytosis. This results in the transduction of a cell death signal deriving from caspase-8 cascade activation. The disruption of the lipid–receptor interaction induces Fas to be internalized by ezrin-mediated endocytosis and to activate a pro-growth signalling through MAPK cascade signalling [29].

Another example of GSL regulation is that of mammalian Notch ligand Delta-like 1 (Dll1), where the GSD is present in the ligand itself [93]. The Notch signalling relies on a complex network where activation is regulated by both the signal-emitting and signal-receiving cell. Thus, first endocytosis and recycling of Dll1 are required to produce the activated form of the ligand and to enrich its surface level on the signal-emitting cells. Then, upon ligand–receptor interaction and endocytosis of this complex, a proteolytic cleavage of the receptor occurs causing the activation of the kinase signalling in the signal-receiving cells [93]. In co-culture assays, mutations in the key residues of the Dll1-GSD result in the rapid inactivation of Dll1 by degradation and inability to activate Notch signalling. Consistent with this finding, the authors also showed that inhibition of GSL production in the signal-emitting cells resulted in impaired Notch activation [9,93]. Thus, GSL–Dll1 interaction is required for a proper Notch signalling function. Indeed GSLs might either act as a docking platform to concentrate Dll1 to

membrane microdomains specifically devoted to the Notch signalling, or increase Dll1 affinity for the Notch receptor [9,93].

Further complexity to the GSL-dependent signalling regulation is added by the heterogeneity in the ceramide backbones to which glycan moieties are bound [153,154]. With this respect many studies have underlined the involvement of cholesterol and GSL-hydrophobic portion in the formation of ordered membrane nanodomains, which can drive the clustering and distribution of receptors and non-receptor proteins (as reviewed in [155]). The GSL-ceramide backbone is also responsible of the direct interaction with transducer molecules. The physical linkage between GSL-ceramide backbone and some signalling mediators might be due to their post-translational fatty acylation, or to the presence of lipophilic protein domains able to mediate interaction with GSL-hydrophobic portion (as reviewed in [156,157]).

4. GSL-Sensing Domains (GSDs) as Sensors of GSLs

One of the first GSD to be discovered was that in the V3 loop of the gp120 glycoprotein of Human Immunodeficiency Virus 1 (HIV-1) [158]. HIV uses the GSLs as alternative receptors to infect cells that do not express the canonical receptor CD4 [159]. Several GSLs are, indeed, recognized by gp120, including GalCer, 3'-sulphogalactosylceramide (SGC), GM3 ganglioside and Gb3 globoside [63–65,68,160]. The GSL binding site in gp120, consists of the amino-acidic sequence XXXGPGRAFXXX [161], which has been exploited as a molecular template for the discovery of endogenous proteins containing similar GSDs. Interestingly, gp120-type sequences have been identified in soluble proteins (i.e., synucleins and galectins [6,162]) as well as in transmembrane receptors. A gp120-type GSD is, indeed, present in the extracellular portion of the TNF α receptors super-family. For instance, the GSD molecular organization of the CD95 receptor consists of a hairpin structure containing two aromatic residues (Phe-133 and Phe-134) at the turn that strongly interact with LacCer and Gb3 but weakly with GD3 and Gb4. On the other hand, SM that lacks a sugar headgroup does not show any interaction [29]. A similar GSD has been found in the serotonin 1a receptor extracellular domain, with the LNKWTLGQVTC motif conserved in the whole serotonin receptor family. Interestingly, this specific sequence contains all the GSD hallmarks with basic (Lys-101), aromatic (Trp-102), and turn-inducing residues (Gly-105) [163].

The gp120-type GSDs, indeed, share common structural features. The gp120 GSD motif is nested within two α -helices and the central phenylalanine residue mediates the docking to a specific sugar ring in the GSL glycan portion [158]. Similarly gp120-type GSDs consist of a hairpin structure exposing an aromatic residue to the solvent, which plays a prominent role in protein-sugar interaction [29,163]. Carbohydrate-aromatic interactions usually occur at a number of axial CH groups located on the same face of the furanoses and/or pyranoses cyclic structure forming a planar apolar surface and the Pi electron density of the planar aromatic ring (CH:Pi stacking interactions). Replacing any of these aromatic residues with alanine results in decreased binding affinity [164]. The key sugar residue showing highest affinity to the aromatic amino acids is the Gal of the basic GSL core Cer-Glc β 1-4Gal motif present in most GSLs [164] (Figure 3). Nonetheless, the GSD of the gp120 V3 loop binds with greater affinity to Gb3 than GM3 ganglioside (both the GSL share the same "Cer-Glc β 1-4Gal" core), suggesting that molecular bonds not restricted to the common GSL core structure participate in the interaction (Figure 3).

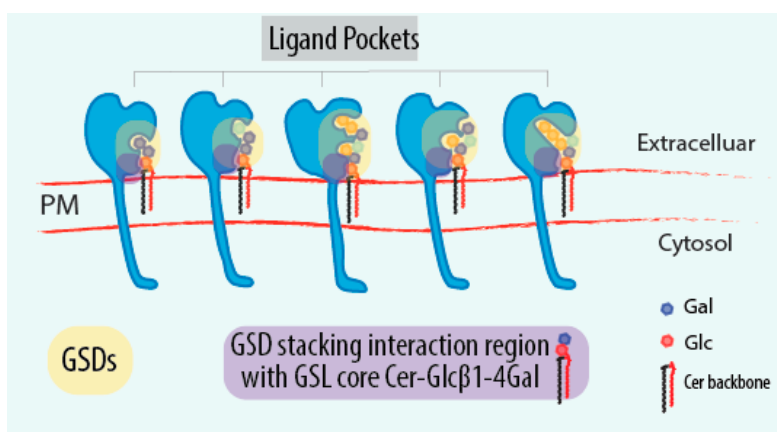


Figure 3. GSDs as sensors of the GSL-glycocode. Schematic representation of receptors containing different GSDs that recognize specific GSL-glycan portions. In light orange are highlighted GSDs in the receptors. In violet are indicated the conserved GSD portions recognizing the basic GSL core motif Cer-Glc β 1-4Gal present in the most GSLs. Coloured hexagons represent the different sugar residues of GSL-glycan moieties. Blue hexagon, Gal residue; Red hexagon, Glc residue; Black-Red parallelogram, Cer backbone; GSD, glycosphingolipid sensing domain; PM, plasma membrane.

In addition, some bacteria are able to exert cytotoxicity by using GSLs as a receptor for the entry of bacterial toxins into cells (Table 1). One of the best-characterized GSL-toxin interactions is that of Shiga toxin B subunit (ShTxB) with the Gb3 globoside [82]. As for the gp120-type of GSL–GSD interactions, in this case, the stacking interaction also involves aromatic residues and the Gal residues of Gb3 [165]. ShTxB is composed of five identical monomers, each of which has three Gb3-binding sites not all equally important for the binding. The first and third Gb3-binding sites are mainly involved in the interaction with the receptor [166]. Indeed, both these binding sites have key aromatic amino acids, Phe-30 in the first case, and the Trp-34 in the second, involved in the establishment of stacking interactions with the central and the terminal Gal residues of Gb3 respectively [165]. In addition, the ShTxB-GSD was used as a template to identify ShTx-type GSDs in endogenous proteins. Verotoxin B subunit (VTB), a Shiga-like toxin, was used for comparison in a multiple sequence alignment with the corresponding sequences in the extracellular domain of CD19 B cells receptor. When the CD19 primary sequence was aligned with a consensus sequence obtained from the VT1, VT2, and VT2e B-subunits, it showed overall 50% of identity. Moreover, high percentages of identity with CD19 were found in the region of the toxin where the Gb3-binding domain is located [167]. Interestingly the Glu-16, and Asp-17 residues present in the GSDs of VTB-subunits and potentially able to establish multiple hydrogen bonds with polar residue of the Gb3-trisaccharide were conserved also in the primary sequence of the CD19 receptor at the position 30 and 32 respectively. In addition, the aromatic residues Phe-30 and Trp-34 present in all the three VTB-subunits (as in ShTxB) and involved in the hydrophobic stacking interactions, are conserved in the CD19 receptor at the position 122 and 124 [167]. These considerations suggest the existence of a GSD in the CD19 extracellular domain and indicate a possible regulatory function for Gb3 on CD19 function. Nevertheless, the 3D structure of CD19 extracellular domain has not been solved and thus the existence of a GSD is not proven.

An interesting notion deriving from the reported data is that the Gal residue in the GSL core structure is involved in a default interaction with GSDs, while specificity is dictated by the surrounding sugar configuration (Figure 3). As we reviewed earlier [168], Gal is the most frequently found sugar in GSL glycans. Gal is also the most represented residue at even positions with other residues frequently found at odd positions when considering the occurrence of different residues along the reducing/non-reducing synthetic axis. Interestingly, when we measured the amount of the theoretical information content associated with the GSL glycan chain, we found that to anti-correlate with the position occupied by Gal with odd-positioned residues being extremely more

information-rich than even-positioned ones. This makes us speculate that Gal residues are involved in the establishment of default interactions with GSDs while the other intercalating sugar residues determine binding specificity.

5. GSL Regulation in Development

Individual cells can survive and grow in the complete absence of GSLs while mammals require GSLs to complete their embryonic development [95,112,169], suggesting that GSLs play fundamental roles in multicellularity and development [18]. Accordingly the GSL composition of cells changes during differentiation, as a direct consequence of a change in the expression of specific GSL synthesizing enzymes [18,170–172]. The transcriptional programmes responsible for these changes are unknown, nonetheless GSLs are directly involved in the regulation of the differentiation processes. Thus knock out animal models for given GSLs synthesizing enzymes display specific developmental defects [168].

A prototypical case is that of neurogenesis, where a switch in GSL synthesis from globo and lacto series GSLs (synthesized by stem cells), toward the production of ganglio series GSLs at the stage of neuronal progenitor cells has been reported [18]. A further metabolic shift has been described in the transition from neuronal progenitor cells, that produce simple gangliosides such as GM3 and GD3, to mature neurons or glial cells showing an increased synthesis of more complex gangliosides such as GM1a, GD1a, GD1b and GT1b [173]. The role of GSLs in neural differentiation has been demonstrated by manipulating the GSL composition in various neuronal cell lines in culture and in animal models. According to these studies, GM3 and GD3 in NPCs contribute to β 1-integrin expression to promote cell proliferation and self-renewal [174]. This creates a niche of precursors constantly sustaining adult neurogenesis [173]. Conversely, GM1 and GT1b promote neuronal differentiation and dendrite generation. GM1 and GT1b, indeed, by enhancing NGF-induced dimerization of TrkA and its phosphorylation promote the entry of neuronal progenitor cells into a postmitotic stage, and thus neuronal maturation [173]. Interestingly GM1, probably by this signalling, induces an epigenetic remodelling at level of the GM2/GD2 synthase promoter, increasing GM2/GD2 synthase expression and GM2 production. GM2 is then, directly converted into GM1 by GM1 synthase [175]. By this circuit GM1 establishes a positive feedback loop to promote neuronal maturation and sustain its own synthesis and that of GM1-derived gangliosides in the brain.

These data suggest that brain-enriched gangliosides, probably by their regulatory function on signalling, modulate neuronal function and contribute to neuronal development by influencing the epigenetic state of the cell. By this mechanism gangliosides like GM1 have the potential to promote the expression of differentiation genes and thus to favour cell commitment to specific differentiation fates. This regulatory layer adds to known mechanisms of tissue patterning such as morphogen gradients that induce the differentiation of specific cell types in a distinct spatial order [176] (Figure 4). In this respect, GSL composition might determine the sensitivity of groups of cells, not necessarily located in a specific embryonic district to several morphogens. By these qualities, GSLs would provide the cells with the capability to stably maintain or modify their identity. On the other hand, the GSL dependent regulatory mechanism should also confer plasticity allowing cells to respond to sudden changes in the environment by remodelling their GSL composition without affecting the expression of the receptor pools on the plasma membrane (Figure 4).

Besides, the regulation of other enzymes of the sphingolipid metabolism like for example the ceramide synthases influence the GSL composition by the production of ceramides of different chain lengths [177]. Indeed, these ceramides are afterward incorporated into GlcCer, the precursor of most GSLs. The mechanisms that regulate the action of the six different known ceramide synthases go from transcriptional and post-translational regulation to altering enzyme activity by dimerization [177].

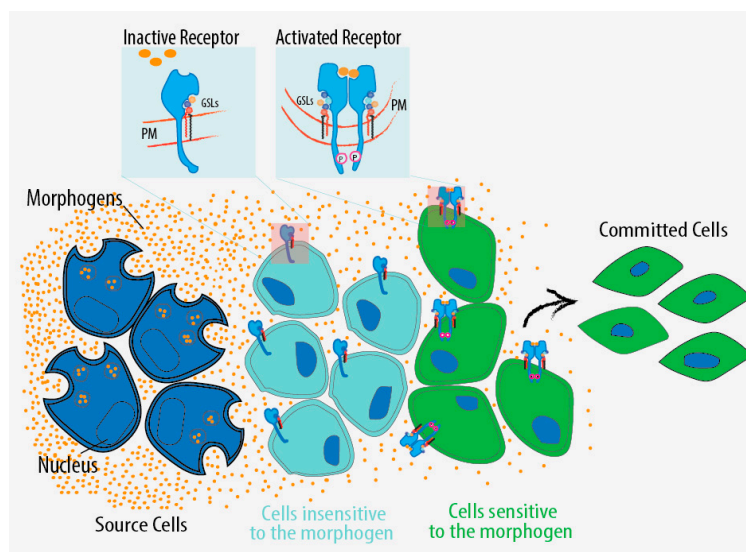


Figure 4. GSL regulation of signalling in development. Schematic representation of GSL-dependent regulation affecting the environmental sensing of the cell and the commitment to alternative differentiation fates. This regulatory layer might act in parallel to known mechanisms of tissue patterning such as morphogen gradients that induce the differentiation of specific cell types in a distinct spatial order. The same differentiation factor might be invisible to some cells and at the same time perceived by others depending on the fact that the target receptor is in a dormant or active state in relation to the GSL composition on the plasma membrane. Orange spheres represent morphogens; PM, plasma membrane.

6. Open Questions

This review focuses on GSL–protein interactions and on their possible biological outcomes by reporting some examples where they have been studied thoroughly. Nevertheless, the extent of GSL involvement in cell regulation is not yet fully addressed. This knowledge gap derives from the lack of technologies that has hampered investigators to easily assess quantitatively and qualitatively the cellular GSL composition in physiopathological conditions and to reveal protein–GSL interactions. Thus, although GSDs have been recognized to bind GSL-glycan portions with different affinities, the basic principles accounting for specificity of these interactions are still far from being understood. As a consequence, future developments in the field will probably derive from solving the following issues.

6.1. What Are the Molecular Rules Driving GSL Sensing?

Glycan sequences in GSLs can be seen as a biological “language”, used by cells to specify their identities in multicellular contexts. To date, the rules determining how the information contained in GSL “words” is read are not understood. GSDs might act as sensors for specific GSLs. GSDs physically interact with GSL sugar residues by protein–carbohydrate and carbohydrate–carbohydrate interactions to sense the changes in the GSL composition, and consequently modify the activity of the protein in which they are embedded [29,163]. An important contribution to clarify details of the GSLs–GSD interactions has been provided by the studies on viral/bacterial GSDs [158,165]. Nevertheless, more effort is required to systematically identify new GSDs in the whole proteome, and to learn about their biochemical features. Indeed, to date it is unknown how many GSD types exist, and how they are structured. Moreover, further bioinformatics and biochemical studies are required to understand the role of GSL-glycan moieties in GSL–protein interactions. Finally, structural and dynamics data on GSD/GSL complexes are required to understand how each sugar residue interfaces with the surrounding amino acid counterpart. An interesting tool to study protein–lipid interactions and

its dynamics in the bilayer context is the electron paramagnetic resonance (EPR) spectroscopy. EPR measures the magnetic moment of unpaired electrons contained in spin-labels (usually nitroxyl groups) that are synthetically introduced in the lipid of interest [178]. Spin-labelled lipids show changes in their EPR spectra when in proximity of interacting proteins. These spectral changes can be exploited to infer the stoichiometry, strength and the time scale of a specific protein–lipid interaction [179]. While EPR has been widely used to study the bilayer properties influenced by GSLs [180,181] few studies have directly approached GSL–protein interaction by the use of EPR [182] thus leaving space for future investigation.

6.2. Which Are the Targets of GSL-Dependent Regulation?

GSLs exert their biological functions by binding to specific proteins and regulating their activities. The list of proteins interacting with specific GSLs is slowly but steadily growing and comprises a number of plasma membrane receptors as well as tetraspanins, integrins, and caveolins [18]. The functional outcomes of these interactions have been defined in some cases and include both activatory and repressive regulations. Nevertheless the number of reported GSL–protein interactions is limited. As a result, to date, we lack an estimation of the fraction of the proteome that interacts with specific GSLs and of protein domains/motifs involved in these interactions. GSL–protein interaction studies have suffered from the absence of methods to systematically approach this issue. Indeed, large-scale screenings based on the identification of GSL–protein interactions have yet not been performed. Probably, the most promising approach to tackle this issue is based on photochemistry. By using a photo-activable analogue of the GSLs, containing a photo-activable diazirine group, GSLs can be covalently crosslinked to neighbouring/interacting proteins by UV irradiation [183]. Recent developments of this technique have made available bi-functional sphingolipid analogues, where in addition to the photo-activable diazirine group a terminal alkyne moiety was included in the sphingosine structure that allows tagging of protein–lipid complexes by click chemistry [184]. Thus, by the cycloaddition of a fluorophore-azide to the terminal alkine group of the bi-functional sphingolipid, it is now possible to visualize the lipid–protein crosslinked complexes. Alternatively, the cycloaddition of a tag-azide group (e.g., biotin-azide) allows the immunoprecipitation and the identification by mass spectrometry of the lipid–protein crosslinked complexes. By the use of this technique it will be possible in a near future to systematically reveal GSL–protein interactions. The scale up of this approach will possibly lead to the construction of a map of the GSL-interactome, which might be used to understand the GSDs–GSL recognition code.

6.3. What Is the Role of the Hydrophobic GSL Portion in the Regulation of Signal Transduction?

While not discussed in this review the role of GSL hydrophobic portion in the signalling control is not of minor importance. Indeed, GSL-ceramides act as organizers of membrane domains by inducing heterogeneous membrane partitioning and specialization of functional membrane domains. In addition, here, there are still many aspects to be clarified regarding how protein binding specificity is conferred by parameters such as the length of the acyl chains of the ceramide, the degree of unsaturation and how these features can specifically modulate signal transduction. The combination of all these parameters yields a further level of complexity [154], which constitute an additional information reservoir being perceived by direct protein interactors, and involved in the GSL-dependent fine tuning of cell signalling.

6.4. How Is GSL Metabolism Regulated?

Since GSLs act on a regulatory level integrating with the classical ligand–receptor module, aberrations in GSL composition result in signalling defects, which can be corrected by appropriately manipulating the GSL metabolism. This requires a deep knowledge on the spatiotemporal regulation of the GSL synthesis, which is influenced by multiple parameters. Importantly, the transcriptional programs regulating the expression of specific GSL glycosyltransferases, their physical interactions

and sub-Golgi compartmentalization, as well as the accessibility to their substrates are parameters that we still have not completely unravelled. Thus, although the GSL metabolism has been satisfactorily described, we have little clue of how GSL synthesis is controlled. Future studies focused on the regulation of GSL metabolism, should provide a more systematic view on the GSL role in signalling and organism pathophysiology.

6.5. Which Is the Role of the GSL-Dependent Regulation in Development?

Once bound to their interacting partners, GSLs modulate their activities. By the virtue of this property, GSLs impact on cell signalling and ultimately on cellular transcriptional programmes. Nevertheless a comprehensive picture of the impact of GSL composition on cell signalling and transcriptional regulation is lacking. Moreover, although GSLs are widely recognized regulators of developmental processes, only a modest number of GSLs have been studied in any real detail, thus leaving the understanding of the specific roles of most GSLs in driving differentiation processes to future research.

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References

1. Merrill, A.H., Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem. Rev.* **2011**, *111*, 6387–6422. [[CrossRef](#)] [[PubMed](#)]
2. Coskun, U.; Grzybek, M.; Drechsel, D.; Simons, K. Regulation of human EGF receptor by lipids. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9044–9048. [[CrossRef](#)] [[PubMed](#)]
3. Vyas, K.A.; Patel, H.V.; Vyas, A.A.; Schnaar, R.L. Segregation of gangliosides GM1 and GD3 on cell membranes, isolated membrane rafts, and defined supported lipid monolayers. *Biol. Chem.* **2001**, *382*, 241–250. [[CrossRef](#)] [[PubMed](#)]
4. Kiyokawa, E.; Baba, T.; Otsuka, N.; Makino, A.; Ohno, S.; Kobayashi, T. Spatial and functional heterogeneity of sphingolipid-rich membrane domains. *J. Biol. Chem.* **2005**, *280*, 24072–24084. [[CrossRef](#)] [[PubMed](#)]
5. Lindquist, S.; Karitkina, D.; Langnaese, K.; Posevitz-Fejfar, A.; Schraven, B.; Xavier, R.; Seed, B.; Lindquist, J.A. Phosphoprotein associated with glycosphingolipid-enriched microdomains differentially modulates SRC kinase activity in brain maturation. *PLoS ONE* **2011**, *6*, e23978. [[CrossRef](#)] [[PubMed](#)]
6. Ideo, H.; Seko, A.; Ishizuka, I.; Yamashita, K. The N-terminal carbohydrate recognition domain of galectin-8 recognizes specific glycosphingolipids with high affinity. *Glycobiology* **2003**, *13*, 713–723. [[CrossRef](#)] [[PubMed](#)]
7. Sprong, H.; Degroote, S.; Nilsson, T.; Kawakita, M.; Ishida, N.; van der Sluijs, P.; van Meer, G. Association of the Golgi UDP-galactose transporter with UDP-galactose:ceramide galactosyltransferase allows UDP-galactose import in the endoplasmic reticulum. *Mol. Biol. Cell* **2003**, *14*, 3482–3493. [[CrossRef](#)] [[PubMed](#)]
8. Schulze, H.; Sandhoff, K. Lysosomal lipid storage diseases. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*. [[CrossRef](#)] [[PubMed](#)]
9. Hamel, S.; Fantini, J.; Schweisguth, F. Notch ligand activity is modulated by glycosphingolipid membrane composition in *Drosophila melanogaster*. *J. Cell Biol.* **2010**, *188*, 581–594. [[CrossRef](#)] [[PubMed](#)]
10. Minoguchi, K.; Swaim, W.D.; Berenstein, E.H.; Siraganian, R.P. Src family tyrosine kinase p53/56lyn, a serine kinase and Fc epsilon RI associate with α -galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL-2H3 cells. *J. Biol. Chem.* **1994**, *269*, 5249–5254. [[PubMed](#)]
11. Kasahara, K.; Watanabe, Y.; Yamamoto, T.; Sanai, Y. Association of Src family tyrosine kinase Lyn with ganglioside GD3 in rat brain: Possible regulation of Lyn by glycosphingolipid in caveolae-like domains. *J. Biol. Chem.* **1997**, *272*, 29947–29953. [[CrossRef](#)] [[PubMed](#)]

12. Yamamura, S.; Handa, K.; Hakomori, S. A close association of GM3 with c-Src and Rho in GM3-enriched microdomains at the B16 melanoma cell surface membrane: A preliminary note. *Biochem. Biophys. Res. Commun.* **1997**, *236*, 218–222. [[CrossRef](#)] [[PubMed](#)]
13. Iwabuchi, K.; Yamamura, S.; Prinetti, A.; Handa, K.; Hakomori, S. GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells. *J. Biol. Chem.* **1998**, *273*, 9130–9138. [[CrossRef](#)] [[PubMed](#)]
14. Holleran, W.M.; Takagi, Y.; Menon, G.K.; Legler, G.; Feingold, K.R.; Elias, P.M. Processing of epidermal glucosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J. Clin. Investig.* **1993**, *91*, 1656–1664. [[CrossRef](#)] [[PubMed](#)]
15. Holleran, W.M.; Ginns, E.I.; Menon, G.K.; Grundmann, J.U.; Fartasch, M.; McKinney, C.E.; Elias, P.M.; Sidransky, E. Consequences of β -glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J. Clin. Investig.* **1994**, *93*, 1756–1764. [[CrossRef](#)] [[PubMed](#)]
16. Jennemann, R.; Sandhoff, R.; Langbein, L.; Kaden, S.; Rothermel, U.; Gallala, H.; Sandhoff, K.; Wiegandt, H.; Gröne, H.J. Integrity and barrier function of the epidermis critically depend on glucosylceramide synthesis. *J. Biol. Chem.* **2007**, *282*, 3083–3094. [[CrossRef](#)] [[PubMed](#)]
17. Lingwood, C.A. Glycosphingolipid functions. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*. [[CrossRef](#)]
18. Hakomori, S.I. Structure and function of glycosphingolipids and sphingolipids: Recollections and future trends. *Biochim. Biophys. Acta* **2008**, *1780*, 325–346. [[CrossRef](#)] [[PubMed](#)]
19. Mirkin, B.L.; Clark, S.H.; Zhang, C. Inhibition of human neuroblastoma cell proliferation and EGF receptor phosphorylation by gangliosides GM1, GM3, GD1A and GT1B. *Cell Prolif.* **2002**, *35*, 105–115. [[CrossRef](#)] [[PubMed](#)]
20. Park, S.Y.; Kwak, C.Y.; Shayman, J.A.; Kim, J.H. Globoside promotes activation of ERK by interaction with the epidermal growth factor receptor. *Biochim. Biophys. Acta* **2012**, *1820*, 1141–1148. [[CrossRef](#)] [[PubMed](#)]
21. Guan, F.; Handa, K.; Hakomori, S.I. Regulation of epidermal growth factor receptor through interaction of ganglioside GM3 with GlcNAc of N-linked glycan of the receptor: Demonstration in Id1D cells. *Neurochem. Res.* **2011**, *36*, 1645–1653. [[CrossRef](#)] [[PubMed](#)]
22. Bremer, E.G.; Hakomori, S. GM3 ganglioside induces hamster fibroblast growth inhibition in chemically-defined medium: Ganglioside may regulate growth factor receptor function. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 711–718. [[CrossRef](#)]
23. Bremer, E.G.; Hakomori, S.; Bowen-Pope, D.F.; Raines, E.; Ross, R. Ganglioside-mediated modulation of cell growth, growth factor binding, and receptor phosphorylation. *J. Biol. Chem.* **1984**, *259*, 6818–6825. [[PubMed](#)]
24. Mutoh, T.; Tokuda, A.; Miyadai, T.; Hamaguchi, M.; Fujiki, N. Ganglioside GM1 binds to the Trk protein and regulates receptor function. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 5087–5091. [[CrossRef](#)] [[PubMed](#)]
25. Kimura, M.; Hidari, K.I.; Suzuki, T.; Miyamoto, D.; Suzuki, Y. Engagement of endogenous ganglioside GM1a induces tyrosine phosphorylation involved in neuron-like differentiation of PC12 cells. *Glycobiology* **2001**, *11*, 335–343. [[CrossRef](#)] [[PubMed](#)]
26. Kabayama, K.; Sato, T.; Saito, K.; Loberto, N.; Prinetti, A.; Sonnino, S.; Kinjo, M.; Igarashi, Y.; Inokuchi, J. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13678–13683. [[CrossRef](#)] [[PubMed](#)]
27. Tagami, S.; Inokuchi, J.; Kabayama, K.; Yoshimura, H.; Kitamura, F.; Uemura, S.; Ogawa, C.; Ishii, A.; Saito, M.; Ohtsuka, Y.; et al. Ganglioside GM3 participates in the pathological conditions of insulin resistance. *J. Biol. Chem.* **2002**, *277*, 3085–3092. [[CrossRef](#)] [[PubMed](#)]
28. Kondo, Y.; Ikeda, K.; Tokuda, N.; Nishitani, C.; Ohto, U.; Akashi-Takamura, S.; Ito, Y.; Uchikawa, M.; Kuroki, Y.; Taguchi, R.; et al. TLR4-MD-2 complex is negatively regulated by an endogenous ligand, globotetraosylceramide. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 4714–4719. [[CrossRef](#)] [[PubMed](#)]
29. Chakrabandhu, K.; Huault, S.; Garmy, N.; Fantini, J.; Stebe, E.; Mailfert, S.; Marguet, D.; Hueber, A.O. The extracellular glycosphingolipid-binding motif of Fas defines its internalization route, mode and outcome of signals upon activation by ligand. *Cell Death Differ.* **2008**, *15*, 1824–1837. [[CrossRef](#)] [[PubMed](#)]
30. Kim, D.S.; Son, K.Y.; Koo, K.M.; Kim, J.Y.; Alfajaro, M.M.; Park, J.G.; Hosmillo, M.; Soliman, M.; Baek, Y.B.; Cho, E.H.; et al. Porcine sapelovirus uses α 2,3-linked sialic acid on GD1a ganglioside as a receptor. *J. Virol.* **2016**, *90*, 4067–4077. [[CrossRef](#)] [[PubMed](#)]

31. Rydell, G.E.; Dahlin, A.B.; Hook, F.; Larson, G. QCM-D studies of human norovirus VLPs binding to glycosphingolipids in supported lipid bilayers reveal strain-specific characteristics. *Glycobiology* **2009**, *19*, 1176–1184. [[CrossRef](#)] [[PubMed](#)]
32. Le Pendu, J.; Ruvoen-Clouet, N.; Kindberg, E.; Svensson, L. Mendelian resistance to human norovirus infections. *Semin. Immunol.* **2006**, *18*, 375–386. [[CrossRef](#)] [[PubMed](#)]
33. Rydell, G.E.; Svensson, L.; Larson, G.; Johannes, L.; Romer, W. Human GII.4 norovirus VLP induces membrane invaginations on giant unilamellar vesicles containing secretor gene dependent α 1,2-fucosylated glycosphingolipids. *Biochim. Biophys. Acta* **2013**, *1828*, 1840–1845. [[CrossRef](#)] [[PubMed](#)]
34. Taube, S.; Perry, J.W.; Yetming, K.; Patel, S.P.; Auble, H.; Shu, L.; Nawar, H.F.; Lee, C.H.; Connell, T.D.; Shayman, J.A.; et al. Ganglioside-linked terminal sialic acid moieties on murine macrophages function as attachment receptors for murine noroviruses. *J. Virol.* **2009**, *83*, 4092–4101. [[CrossRef](#)] [[PubMed](#)]
35. Taube, S.; Perry, J.W.; McGreevy, E.; Yetming, K.; Perkins, C.; Henderson, K.; Wobus, C.E. Murine noroviruses bind glycolipid and glycoprotein attachment receptors in a strain-dependent manner. *J. Virol.* **2012**, *86*, 5584–5593. [[CrossRef](#)] [[PubMed](#)]
36. Zakhour, M.; Ruvoen-Clouet, N.; Charpilienne, A.; Langpap, B.; Poncet, D.; Peters, T.; Bovin, N.; Le Pendu, J. The α Gal epitope of the histo-blood group antigen family is a ligand for bovine norovirus Newbury2 expected to prevent cross-species transmission. *PLoS Pathog.* **2009**, *5*, e1000504. [[CrossRef](#)] [[PubMed](#)]
37. Ruvoen-Clouet, N.; Ganiere, J.P.; Andre-Fontaine, G.; Blanchard, D.; Le Pendu, J. Binding of rabbit hemorrhagic disease virus to antigens of the ABH histo-blood group family. *J. Virol.* **2000**, *74*, 11950–11954. [[CrossRef](#)] [[PubMed](#)]
38. Nilsson, E.C.; Storm, R.J.; Bauer, J.; Johansson, S.M.; Lookene, A.; Angstrom, J.; Hedenstrom, M.; Eriksson, T.L.; Frangmyr, L.; Rinaldi, S.; et al. The GD1a glycan is a cellular receptor for adenoviruses causing epidemic keratoconjunctivitis. *Nat. Med.* **2011**, *17*, 105–109. [[CrossRef](#)] [[PubMed](#)]
39. Stencel-Baerenwald, J.; Reiss, K.; Blaum, B.S.; Colvin, D.; Li, X.N.; Abel, T.; Boyd, K.; Stehle, T.; Dermody, T.S. Glycan engagement dictates hydrocephalus induction by serotype 1 reovirus. *mBio* **2015**, *6*, e02356. [[CrossRef](#)] [[PubMed](#)]
40. Rolsma, M.D.; Gelberg, H.B.; Kuhlenschmidt, M.S. Assay for evaluation of rotavirus-cell interactions: Identification of an enterocyte ganglioside fraction that mediates group A porcine rotavirus recognition. *J. Virol.* **1994**, *68*, 258–268. [[PubMed](#)]
41. Rolsma, M.D.; Kuhlenschmidt, T.B.; Gelberg, H.B.; Kuhlenschmidt, M.S. Structure and function of a ganglioside receptor for porcine rotavirus. *J. Virol.* **1998**, *72*, 9079–9091. [[PubMed](#)]
42. Haselhorst, T.; Fleming, F.E.; Dyason, J.C.; Hartnell, R.D.; Yu, X.; Holloway, G.; Santegoets, K.; Kiefel, M.J.; Blanchard, H.; Coulson, B.S.; et al. Sialic acid dependence in rotavirus host cell invasion. *Nat. Chem. Biol.* **2009**, *5*, 91–93. [[CrossRef](#)] [[PubMed](#)]
43. Martinez, M.A.; Lopez, S.; Arias, C.F.; Isa, P. Gangliosides have a functional role during rotavirus cell entry. *J. Virol.* **2013**, *87*, 1115–1122. [[CrossRef](#)] [[PubMed](#)]
44. Delorme, C.; Brussow, H.; Sidoti, J.; Roche, N.; Karlsson, K.A.; Neeser, J.R.; Teneberg, S. Glycosphingolipid binding specificities of rotavirus: Identification of a sialic acid-binding epitope. *J. Virol.* **2001**, *75*, 2276–2287. [[CrossRef](#)] [[PubMed](#)]
45. Dormitzer, P.R.; Sun, Z.Y.; Blixt, O.; Paulson, J.C.; Wagner, G.; Harrison, S.C. Specificity and affinity of sialic acid binding by the rhesus rotavirus VP8* core. *J. Virol.* **2002**, *76*, 10512–10517. [[CrossRef](#)] [[PubMed](#)]
46. Fukudome, K.; Yoshie, O.; Konno, T. Comparison of human, simian, and bovine rotaviruses for requirement of sialic acid in hemagglutination and cell adsorption. *Virology* **1989**, *172*, 196–205. [[CrossRef](#)]
47. Guo, C.T.; Nakagomi, O.; Mochizuki, M.; Ishida, H.; Kiso, M.; Ohta, Y.; Suzuki, T.; Miyamoto, D.; Hidari, K.I.; Suzuki, Y. Ganglioside GM1a on the cell surface is involved in the infection by human rotavirus KUN and MO strains. *J. Biochem.* **1999**, *126*, 683–688. [[CrossRef](#)] [[PubMed](#)]
48. Fleming, F.E.; Bohm, R.; Dang, V.T.; Holloway, G.; Haselhorst, T.; Madge, P.D.; Deveryshetty, J.; Yu, X.; Blanchard, H.; von Itzstein, M.; et al. Relative roles of GM1 ganglioside, N-acylneuraminic acids, and α 2 β 1 integrin in mediating rotavirus infection. *J. Virol.* **2014**, *88*, 4558–4571. [[CrossRef](#)] [[PubMed](#)]
49. Yu, X.; Coulson, B.S.; Fleming, F.E.; Dyason, J.C.; von Itzstein, M.; Blanchard, H. Novel structural insights into rotavirus recognition of ganglioside glycan receptors. *J. Mol. Biol.* **2011**, *413*, 929–939. [[CrossRef](#)] [[PubMed](#)]

50. Stroh, L.J.; Gee, G.V.; Blaum, B.S.; Dugan, A.S.; Feltkamp, M.C.; Atwood, W.J.; Stehle, T. Trichodysplasia spinulosa-associated polyomavirus uses a displaced binding site on VP1 to engage sialylated glycolipids. *PLoS Pathog.* **2015**, *11*, e1005112. [[CrossRef](#)] [[PubMed](#)]
51. Tsai, B.; Gilbert, J.M.; Stehle, T.; Lencer, W.; Benjamin, T.L.; Rapoport, T.A. Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J.* **2003**, *22*, 4346–4355. [[CrossRef](#)] [[PubMed](#)]
52. You, J.; O'Hara, S.D.; Velupillai, P.; Castle, S.; Levery, S.; Garcea, R.L.; Benjamin, T. Ganglioside and non-ganglioside mediated host responses to the mouse polyomavirus. *PLoS Pathog.* **2015**, *11*, e1005175. [[CrossRef](#)] [[PubMed](#)]
53. Ewers, H.; Romer, W.; Smith, A.E.; Bacia, K.; Dmitrieff, S.; Chai, W.; Mancini, R.; Kartenbeck, J.; Chambon, V.; Berland, L.; et al. GM1 structure determines SV40-induced membrane invagination and infection. *Nat. Cell Biol.* **2010**, *12*, 11–18. [[CrossRef](#)] [[PubMed](#)]
54. Neu, U.; Woellner, K.; Gauglitz, G.; Stehle, T. Structural basis of GM1 ganglioside recognition by simian virus 40. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5219–5224. [[CrossRef](#)] [[PubMed](#)]
55. Low, J.A.; Magnuson, B.; Tsai, B.; Imperiale, M.J. Identification of gangliosides GD1b and GT1b as receptors for BK virus. *J. Virol.* **2006**, *80*, 1361–1366. [[CrossRef](#)] [[PubMed](#)]
56. Komagome, R.; Sawa, H.; Suzuki, T.; Suzuki, Y.; Tanaka, S.; Atwood, W.J.; Nagashima, K. Oligosaccharides as receptors for JC virus. *J. Virol.* **2002**, *76*, 12992–13000. [[CrossRef](#)] [[PubMed](#)]
57. Erickson, K.D.; Garcea, R.L.; Tsai, B. Ganglioside GT1b is a putative host cell receptor for the Merkel cell polyomavirus. *J. Virol.* **2009**, *83*, 10275–10279. [[CrossRef](#)] [[PubMed](#)]
58. Brown, K.E.; Anderson, S.M.; Young, N.S. Erythrocyte P antigen: Cellular receptor for B19 parvovirus. *Science* **1993**, *262*, 114–117. [[CrossRef](#)] [[PubMed](#)]
59. Nasir, W.; Nilsson, J.; Olofsson, S.; Bally, M.; Rydell, G.E. Parvovirus B19 VLP recognizes globoside in supported lipid bilayers. *Virology* **2014**, *456–457*, 364–369. [[CrossRef](#)] [[PubMed](#)]
60. Brown, K.E.; Liu, Z.; Gallinella, G.; Wong, S.; Mills, I.P.; O'Sullivan, M.G. Simian parvovirus infection: A potential zoonosis. *J. Infect. Dis.* **2004**, *190*, 1900–1907. [[CrossRef](#)] [[PubMed](#)]
61. Schmidt, M.; Chiorini, J.A. Gangliosides are essential for bovine adeno-associated virus entry. *J. Virol.* **2006**, *80*, 5516–5522. [[CrossRef](#)] [[PubMed](#)]
62. Hammache, D.; Pieroni, G.; Yahi, N.; Delezay, O.; Koch, N.; Lafont, H.; Tamalet, C.; Fantini, J. Specific interaction of HIV-1 and HIV-2 surface envelope glycoproteins with monolayers of galactosylceramide and ganglioside GM3. *J. Biol. Chem.* **1998**, *273*, 7967–7971. [[CrossRef](#)] [[PubMed](#)]
63. Hammache, D.; Yahi, N.; Pieroni, G.; Ariasi, F.; Tamalet, C.; Fantini, J. Sequential interaction of CD4 and HIV-1 gp120 with a reconstituted membrane patch of ganglioside GM3: Implications for the role of glycolipids as potential HIV-1 fusion cofactors. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 117–122. [[CrossRef](#)] [[PubMed](#)]
64. Hammache, D.; Yahi, N.; Maresca, M.; Pieroni, G.; Fantini, J. Human erythrocyte glycosphingolipids as alternative cofactors for human immunodeficiency virus type 1 (HIV-1) entry: Evidence for CD4-induced interactions between HIV-1 gp120 and reconstituted membrane microdomains of glycosphingolipids (Gb3 and GM3). *J. Virol.* **1999**, *73*, 5244–5248. [[PubMed](#)]
65. Hug, P.; Lin, H.M.; Korte, T.; Xiao, X.; Dimitrov, D.S.; Wang, J.M.; Puri, A.; Blumenthal, R. Glycosphingolipids promote entry of a broad range of human immunodeficiency virus type 1 isolates into cell lines expressing CD4, CXCR4, and/or CCR5. *J. Virol.* **2000**, *74*, 6377–6385. [[CrossRef](#)] [[PubMed](#)]
66. Bhat, S.; Spitalnik, S.L.; Gonzalez-Scarano, F.; Silberberg, D.H. Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 7131–7134. [[CrossRef](#)] [[PubMed](#)]
67. Bhat, S.; Mettus, R.V.; Reddy, E.P.; Ugen, K.E.; Srikanthan, V.; Williams, W.V.; Weiner, D.B. The galactosyl ceramide/sulfatide receptor binding region of HIV-1 gp120 maps to amino acids 206–275. *AIDS Res. Hum. Retrovir.* **1993**, *9*, 175–181. [[CrossRef](#)] [[PubMed](#)]
68. Rawat, S.S.; Johnson, B.T.; Puri, A. Sphingolipids: Modulators of HIV-1 infection and pathogenesis. *Biosci. Rep.* **2005**, *25*, 329–343. [[CrossRef](#)] [[PubMed](#)]
69. Cook, D.G.; Fantini, J.; Spitalnik, S.L.; Gonzalez-Scarano, F. Binding of human immunodeficiency virus type I (HIV-1) gp120 to galactosylceramide (GalCer): Relationship to the V3 loop. *Virology* **1994**, *201*, 206–214. [[CrossRef](#)] [[PubMed](#)]
70. Wang, K.; Wang, J.; Sun, T.; Bian, G.; Pan, W.; Feng, T.; Wang, P.; Li, Y.; Dai, J. Glycosphingolipid GM3 is Indispensable for Dengue Virus Genome Replication. *Int. J. Biol. Sci.* **2016**, *12*, 872–883. [[CrossRef](#)] [[PubMed](#)]

71. Hidari, K.I.; Abe, T.; Suzuki, T. Carbohydrate-related inhibitors of dengue virus entry. *Viruses* **2013**, *5*, 605–618. [[CrossRef](#)] [[PubMed](#)]
72. Wichit, S.; Jittmittraphap, A.; Hidari, K.I.; Thaisomboonsuk, B.; Petmitr, S.; Ubol, S.; Aoki, C.; Itonori, S.; Morita, K.; Suzuki, T.; et al. Dengue virus type 2 recognizes the carbohydrate moiety of neutral glycosphingolipids in mammalian and mosquito cells. *Microbiol. Immunol.* **2011**, *55*, 135–140. [[CrossRef](#)] [[PubMed](#)]
73. Meisen, I.; Dzudzek, T.; Ehrhardt, C.; Ludwig, S.; Mormann, M.; Rosenbruck, R.; Lumen, R.; Kniep, B.; Karch, H.; Muthing, J. The human H3N2 influenza viruses A/Victoria/3/75 and A/Hiroshima/52/2005 preferentially bind to α 2–3-sialylated monosialogangliosides with fucosylated poly-*N*-acetylactosaminyl chains. *Glycobiology* **2012**, *22*, 1055–1076. [[CrossRef](#)] [[PubMed](#)]
74. Perino, J.; Crouzier, D.; Spehner, D.; Debouzy, J.C.; Garin, D.; Crance, J.M.; Favier, A.L. Lung surfactant DPPG phospholipid inhibits vaccinia virus infection. *Antivir. Res.* **2011**, *89*, 89–97. [[CrossRef](#)] [[PubMed](#)]
75. Perino, J.; Foo, C.H.; Spehner, D.; Cohen, G.H.; Eisenberg, R.J.; Crance, J.M.; Favier, A.L. Role of sulfatide in vaccinia virus infection. *Biol. Cell* **2011**, *103*, 319–331. [[CrossRef](#)] [[PubMed](#)]
76. Ferreira, L.; Villar, E.; Munoz-Barroso, I. Gangliosides and *N*-glycoproteins function as Newcastle disease virus receptors. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 2344–2356. [[CrossRef](#)] [[PubMed](#)]
77. Epand, R.M.; Nir, S.; Parolin, M.; Flanagan, T.D. The role of the ganglioside GD1a as a receptor for Sendai virus. *Biochemistry* **1995**, *34*, 1084–1089. [[CrossRef](#)] [[PubMed](#)]
78. Suzuki, T.; Portner, A.; Scroggs, R.A.; Uchikawa, M.; Koyama, N.; Matsuo, K.; Suzuki, Y.; Takimoto, T. Receptor specificities of human respiroviruses. *J. Virol.* **2001**, *75*, 4604–4613. [[CrossRef](#)] [[PubMed](#)]
79. Jobling, M.G.; Holmes, R.K. Mutational analysis of ganglioside G_{M1}-binding ability, pentamer formation, and epitopes of cholera toxin B (CTB) subunits and CTB/heat-labile enterotoxin B subunit chimeras. *Infect. Immun.* **2002**, *70*, 1260–1271. [[CrossRef](#)] [[PubMed](#)]
80. Heyningen, S.V. Cholera toxin: Interaction of subunits with ganglioside GM1. *Science* **1974**, *183*, 656–657. [[CrossRef](#)]
81. Lencer, W.I.; Saslowsky, D. Raft trafficking of AB5 subunit bacterial toxins. *Biochim. Biophys. Acta* **2005**, *1746*, 314–321. [[CrossRef](#)] [[PubMed](#)]
82. Jacewicz, M.; Clausen, H.; Nudelman, E.; Donohue-Rolfe, A.; Keusch, G.T. Pathogenesis of shigella diarrhea. XI. Isolation of a shigella toxin-binding glycolipid from rabbit jejunum and HeLa cells and its identification as globotriaosylceramide. *J. Exp. Med.* **1986**, *163*, 1391–1404. [[CrossRef](#)] [[PubMed](#)]
83. Lindberg, A.A.; Brown, J.E.; Stromberg, N.; Westling-Ryd, M.; Schultz, J.E.; Karlsson, K.A. Identification of the carbohydrate receptor for Shiga toxin produced by *Shigella dysenteriae* type 1. *J. Biol. Chem.* **1987**, *262*, 1779–1785. [[PubMed](#)]
84. Keusch, G.T.; Jacewicz, M.; Acheson, D.W.; Donohue-Rolfe, A.; Kane, A.V.; McCluer, R.H. Globotriaosylceramide, Gb3, is an alternative functional receptor for Shiga-like toxin 2e. *Infect. Immun.* **1995**, *63*, 1138–1141. [[PubMed](#)]
85. Okuda, T.; Tokuda, N.; Numata, S.; Ito, M.; Ohta, M.; Kawamura, K.; Wiels, J.; Urano, T.; Tajima, O.; Furukawa, K.; et al. Targeted disruption of Gb3/CD77 synthase gene resulted in the complete deletion of globo-series glycosphingolipids and loss of sensitivity to verotoxins. *J. Biol. Chem.* **2006**, *281*, 10230–10235. [[CrossRef](#)] [[PubMed](#)]
86. Brunger, A.T.; Rummel, A. Receptor and substrate interactions of clostridial neurotoxins. *Toxicon* **2009**, *54*, 550–560. [[CrossRef](#)] [[PubMed](#)]
87. Nakamura, K.; Kohda, T.; Shibata, Y.; Tsukamoto, K.; Arimitsu, H.; Hayashi, M.; Mukamoto, M.; Sasakawa, N.; Kozaki, S. Unique biological activity of botulinum D/C mosaic neurotoxin in murine species. *Infect. Immun.* **2012**, *80*, 2886–2893. [[CrossRef](#)] [[PubMed](#)]
88. Fukuta, S.; Magnani, J.L.; Twiddy, E.M.; Holmes, R.K.; Ginsburg, V. Comparison of the carbohydrate-binding specificities of cholera toxin and *Escherichia coli* heat-labile enterotoxins LTh-I, LT-IIa, and LT-IIb. *Infect. Immun.* **1988**, *56*, 1748–1753. [[PubMed](#)]
89. Nakayama, H.; Ogawa, H.; Takamori, K.; Iwabuchi, K. GSL-enriched membrane microdomains in innate immune responses. *Arch. Immunol. Ther. Exp.* **2013**, *61*, 217–228. [[CrossRef](#)] [[PubMed](#)]
90. Duan, J.; Zhang, J.; Zhao, Y.; Yang, F.; Zhang, X. Ganglioside GM2 modulates the erythrocyte Ca²⁺-ATPase through its binding to the calmodulin-binding domain and its 'receptor'. *Arch. Biochem. Biophys.* **2006**, *454*, 155–159. [[CrossRef](#)] [[PubMed](#)]

91. Boscher, C.; Zheng, Y.Z.; Lakshminarayan, R.; Johannes, L.; Dennis, J.W.; Foster, L.J.; Nabi, I.R. Galectin-3 protein regulates mobility of N-cadherin and GM1 ganglioside at cell-cell junctions of mammary carcinoma cells. *J. Biol. Chem.* **2012**, *287*, 32940–32952. [[CrossRef](#)] [[PubMed](#)]
92. Handa, K.; Hakomori, S.I. Carbohydrate to carbohydrate interaction in development process and cancer progression. *Glycoconj. J.* **2012**, *29*, 627–637. [[CrossRef](#)] [[PubMed](#)]
93. Heuss, S.F.; Tarantino, N.; Fantini, J.; Ndiaye-Lobry, D.; Moretti, J.; Israel, A.; Logeat, F. A glycosphingolipid binding domain controls trafficking and activity of the mammalian notch ligand delta-like 1. *PLoS ONE* **2013**, *8*, e74392. [[CrossRef](#)] [[PubMed](#)]
94. Singh, P.; Paila, Y.D.; Chattopadhyay, A. Role of glycosphingolipids in the function of human serotonin(1)A receptors. *J. Neurochem.* **2012**, *123*, 716–724. [[CrossRef](#)] [[PubMed](#)]
95. Yamashita, T.; Wada, R.; Sasaki, T.; Deng, C.; Bierfreund, U.; Sandhoff, K.; Proia, R.L. A vital role for glycosphingolipid synthesis during development and differentiation. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9142–9147. [[CrossRef](#)] [[PubMed](#)]
96. Yamashita, T.; Wu, Y.P.; Sandhoff, R.; Werth, N.; Mizukami, H.; Ellis, J.M.; Dupree, J.L.; Geyer, R.; Sandhoff, K.; Proia, R.L. Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glia interactions. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2725–2730. [[CrossRef](#)] [[PubMed](#)]
97. Kumagai, T.; Tanaka, M.; Yokoyama, M.; Sato, T.; Shinkai, T.; Furukawa, K. Early lethality of β -1,4-galactosyltransferase V-mutant mice by growth retardation. *Biochem. Biophys. Res. Commun.* **2009**, *379*, 456–459. [[CrossRef](#)] [[PubMed](#)]
98. Yamashita, T.; Hashiramoto, A.; Haluzik, M.; Mizukami, H.; Beck, S.; Norton, A.; Kono, M.; Tsuji, S.; Daniotti, J.L.; Werth, N.; et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3445–3449. [[CrossRef](#)] [[PubMed](#)]
99. Takamiya, K.; Yamamoto, A.; Furukawa, K.; Yamashiro, S.; Shin, M.; Okada, M.; Fukumoto, S.; Haraguchi, M.; Takeda, N.; Fujimura, K.; et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10662–10667. [[CrossRef](#)] [[PubMed](#)]
100. Sheikh, K.A.; Sun, J.; Liu, Y.; Kawai, H.; Crawford, T.O.; Proia, R.L.; Griffin, J.W.; Schnaar, R.L. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7532–7537. [[CrossRef](#)] [[PubMed](#)]
101. Wu, G.; Lu, Z.H.; Kulkarni, N.; Amin, R.; Ledeen, R.W. Mice lacking major brain gangliosides develop parkinsonism. *Neurochem. Res.* **2011**, *36*, 1706–1714. [[CrossRef](#)] [[PubMed](#)]
102. Linn, S.C.; Kim, H.S.; Keane, E.M.; Andras, L.M.; Wang, E.; Merrill, A.H., Jr. Regulation of de novo sphingolipid biosynthesis and the toxic consequences of its disruption. *Biochem. Soc. Trans.* **2001**, *29*, 831–835. [[CrossRef](#)] [[PubMed](#)]
103. Mullen, T.D.; Hannun, Y.A.; Obeid, L.M. Ceramide synthases at the centre of sphingolipid metabolism and biology. *Biochem. J.* **2012**, *441*, 789–802. [[CrossRef](#)] [[PubMed](#)]
104. Holthuis, J.C.; Pomorski, T.; Raggars, R.J.; Sprong, H.; van Meer, G. The organizing potential of sphingolipids in intracellular membrane transport. *Physiol. Rev.* **2001**, *81*, 1689–1723. [[PubMed](#)]
105. Hanada, K.; Kumagai, K.; Yasuda, S.; Miura, Y.; Kawano, M.; Fukasawa, M.; Nishijima, M. Molecular machinery for non-vesicular trafficking of ceramide. *Nature* **2003**, *426*, 803–809. [[CrossRef](#)] [[PubMed](#)]
106. Funakoshi, T.; Yasuda, S.; Fukasawa, M.; Nishijima, M.; Hanada, K. Reconstitution of ATP- and cytosol-dependent transport of de novo synthesized ceramide to the site of sphingomyelin synthesis in semi-intact cells. *J. Biol. Chem.* **2000**, *275*, 29938–29945. [[CrossRef](#)] [[PubMed](#)]
107. Maccioni, H.J.; Quiroga, R.; Ferrari, M.L. Cellular and molecular biology of glycosphingolipid glycosylation. *J. Neurochem.* **2011**, *117*, 589–602. [[CrossRef](#)] [[PubMed](#)]
108. Buton, X.; Herve, P.; Kubelt, J.; Tannert, A.; Burger, K.N.; Fellmann, P.; Muller, P.; Herrmann, A.; Seigneuret, M.; Devaux, P.F. Transbilayer movement of monohexosylsphingolipids in endoplasmic reticulum and Golgi membranes. *Biochemistry* **2002**, *41*, 13106–13115. [[CrossRef](#)] [[PubMed](#)]
109. D'Angelo, G.; Polishchuk, E.; di Tullio, G.; Santoro, M.; Fi Campli, A.; Godi, A.; West, G.; Bielawski, J.; Chuang, C.C.; van der Spoel, A.C.; et al. Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. *Nature* **2007**, *449*, 62–67. [[CrossRef](#)] [[PubMed](#)]

110. Halter, D.; Neumann, S.; van Dijk, S.M.; Wolthoorn, J.; de Maziere, A.M.; Vieira, O.V.; Mattjus, P.; Klumperman, J.; van Meer, G.; Sprong, H. Pre- and post-Golgi translocation of glucosylceramide in glycosphingolipid synthesis. *J. Cell Biol.* **2007**, *179*, 101–115. [[CrossRef](#)] [[PubMed](#)]
111. D'Angelo, G.; Uemura, T.; Chuang, C.C.; Polishchuk, E.; Santoro, M.; Ohvo-Rekila, H.; Sato, T.; di Tullio, G.; Varriale, A.; D'Auria, S.; et al. Vesicular and non-vesicular transport feed distinct glycosylation pathways in the Golgi. *Nature* **2013**, *501*, 116–120. [[CrossRef](#)] [[PubMed](#)]
112. Kumagai, T.; Sato, T.; Natsuka, S.; Kobayashi, Y.; Zhou, D.; Shinkai, T.; Hayakawa, S.; Furukawa, K. Involvement of murine β -1,4-galactosyltransferase V in lactosylceramide biosynthesis. *Glycoconj. J.* **2010**, *27*, 685–695. [[CrossRef](#)] [[PubMed](#)]
113. Nomura, T.; Takizawa, M.; Aoki, J.; Arai, H.; Inoue, K.; Wakisaka, E.; Yoshizuka, N.; Imokawa, G.; Dohmae, N.; Takio, K.; et al. Purification, cDNA cloning, and expression of UDP-Gal: Glucosylceramide β -1,4-galactosyltransferase from rat brain. *J. Biol. Chem.* **1998**, *273*, 13570–13577. [[CrossRef](#)] [[PubMed](#)]
114. Ishii, A.; Ohta, M.; Watanabe, Y.; Matsuda, K.; Ishiyama, K.; Sakoe, K.; Nakamura, M.; Inokuchi, J.; Sanai, Y.; Saito, M. Expression cloning and functional characterization of human cDNA for ganglioside GM3 synthase. *J. Biol. Chem.* **1998**, *273*, 31652–31655. [[CrossRef](#)] [[PubMed](#)]
115. Kojima, Y.; Fukumoto, S.; Furukawa, K.; Okajima, T.; Wiels, J.; Yokoyama, K.; Suzuki, Y.; Urano, T.; Ohta, M.; Furukawa, K. Molecular cloning of globotriaosylceramide/CD77 synthase, a glycosyltransferase that initiates the synthesis of globo series glycosphingolipids. *J. Biol. Chem.* **2000**, *275*, 15152–15156. [[CrossRef](#)] [[PubMed](#)]
116. Biellmann, F.; Hulsmeier, A.J.; Zhou, D.; Cinelli, P.; Hennet, T. The Lc3-synthase gene B3gnt5 is essential to pre-implantation development of the murine embryo. *BMC Dev. Biol.* **2008**, *8*. [[CrossRef](#)] [[PubMed](#)]
117. Nagata, Y.; Yamashiro, S.; Yodoi, J.; Lloyd, K.O.; Shiku, H.; Furukawa, K. Expression cloning of β 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J. Biol. Chem.* **1992**, *267*, 12082–12089. [[PubMed](#)]
118. Hidari, J.K.; Ichikawa, S.; Furukawa, K.; Yamasaki, M.; Hirabayashi, Y. β 1–4N-acetylgalactosaminyltransferase can synthesize both asialoglycosphingolipid GM2 and glycosphingolipid GM2 in vitro and in vivo: Isolation and characterization of a β 1–4N-acetylgalactosaminyltransferase cDNA clone from rat ascites hepatoma cell line AH7974F. *Biochem. J.* **1994**, *303*, 957–965. [[PubMed](#)]
119. Maccioni, H.J.; Quiroga, R.; Spessott, W. Organization of the synthesis of glycolipid oligosaccharides in the Golgi complex. *FEBS Lett.* **2011**, *585*, 1691–1698. [[CrossRef](#)] [[PubMed](#)]
120. Uliana, A.S.; Crespo, P.M.; Martina, J.A.; Daniotti, J.L.; Maccioni, H.J. Modulation of GalT1 and SialT1 sub-Golgi localization by SialT2 expression reveals an organellar level of glycolipid synthesis control. *J. Biol. Chem.* **2006**, *281*, 32852–32860. [[CrossRef](#)] [[PubMed](#)]
121. Aureli, M.; Loberto, N.; Chigorno, V.; Prinetti, A.; Sonnino, S. Remodeling of sphingolipids by plasma membrane associated enzymes. *Neurochem. Res.* **2011**, *36*, 1636–1644. [[CrossRef](#)] [[PubMed](#)]
122. Kitatani, K.; Idkowiak-Baldys, J.; Hannun, Y.A. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal.* **2008**, *20*, 1010–1018. [[CrossRef](#)] [[PubMed](#)]
123. Ginzburg, L.; Kacher, Y.; Futerman, A.H. The pathogenesis of glycosphingolipid storage disorders. *Semin. Cell Dev. Biol.* **2004**, *15*, 417–431. [[CrossRef](#)] [[PubMed](#)]
124. Brunngraber, E.G.; Berra, B.; Zambotti, V. Altered levels of tissue glycoproteins, gangliosides, glycosaminoglycans and lipids in Niemann–Pick's disease. *Clin. Chim. Acta* **1973**, *48*, 173–181. [[CrossRef](#)]
125. Fahy, E.; Subramaniam, S.; Murphy, R.C.; Nishijima, M.; Raetz, C.R.; Shimizu, T.; Spener, F.; van Meer, G.; Wakelam, M.J.; Dennis, E.A. Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* **2009**, *50*, S9–S14. [[CrossRef](#)] [[PubMed](#)]
126. Bremer, E.G.; Schlessinger, J.; Hakomori, S. Ganglioside-mediated modulation of cell growth. Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **1986**, *261*, 2434–2440. [[PubMed](#)]
127. Hanai, N.; Nores, G.A.; MacLeod, C.; Torres-Mendez, C.R.; Hakomori, S. Ganglioside-mediated modulation of cell growth: Specific effects of GM3 and lyso-GM3 in tyrosine phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **1988**, *263*, 10915–10921. [[PubMed](#)]
128. Kawashima, N.; Yoon, S.J.; Itoh, K.; Nakayama, K. Tyrosine kinase activity of epidermal growth factor receptor is regulated by GM3 binding through carbohydrate to carbohydrate interactions. *J. Biol. Chem.* **2009**, *284*, 6147–6155. [[CrossRef](#)] [[PubMed](#)]

129. Liu, Y.; Li, R.; Ladisch, S. Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. *J. Biol. Chem.* **2004**, *279*, 36481–36489. [[CrossRef](#)] [[PubMed](#)]
130. Meuillet, E.; Cremel, G.; Dreyfus, H.; Hicks, D. Differential modulation of basic fibroblast and epidermal growth factor receptor activation by ganglioside GM3 in cultured retinal Muller glia. *Glia* **1996**, *17*, 206–216. [[CrossRef](#)]
131. Toledo, M.S.; Suzuki, E.; Handa, K.; Hakomori, S. Cell growth regulation through GM3-enriched microdomain (glycosynapse) in human lung embryonal fibroblast WI38 and its oncogenic transformant VA13. *J. Biol. Chem.* **2004**, *279*, 34655–34664. [[CrossRef](#)] [[PubMed](#)]
132. Toledo, M.S.; Suzuki, E.; Handa, K.; Hakomori, S. Effect of ganglioside and tetraspanins in microdomains on interaction of integrins with fibroblast growth factor receptor. *J. Biol. Chem.* **2005**, *280*, 16227–16234. [[CrossRef](#)] [[PubMed](#)]
133. Rabin, S.J.; Mocchetti, I. GM1 ganglioside activates the high-affinity nerve growth factor receptor trkA. *J. Neurochem.* **1995**, *65*, 347–354. [[CrossRef](#)] [[PubMed](#)]
134. Duchemin, A.M.; Ren, Q.; Mo, L.; Neff, N.H.; Hadjiconstantinou, M. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. *J. Neurochem.* **2002**, *81*, 696–707. [[CrossRef](#)] [[PubMed](#)]
135. Cazet, A.; Lefebvre, J.; Adriaenssens, E.; Julien, S.; Bobowski, M.; Grigoriadis, A.; Tutt, A.; Tulasne, D.; le Bourhis, X.; Delannoy, P. GD₃ synthase expression enhances proliferation and tumor growth of MDA-MB-231 breast cancer cells through c-Met activation. *Mol. Cancer Res.* **2010**, *8*, 1526–1535. [[CrossRef](#)] [[PubMed](#)]
136. Cazet, A.; Groux-Degroote, S.; Teylaert, B.; Kwon, K.M.; Lehoux, S.; Slomianny, C.; Kim, C.H.; le Bourhis, X.; Delannoy, P. GD₃ synthase overexpression enhances proliferation and migration of MDA-MB-231 breast cancer cells. *Biol. Chem.* **2009**, *390*, 601–609. [[CrossRef](#)] [[PubMed](#)]
137. Cazet, A.; Bobowski, M.; Rombouts, Y.; Lefebvre, J.; Steenackers, A.; Popa, I.; Guerardel, Y.; le Bourhis, X.; Tulasne, D.; Delannoy, P. The ganglioside GD₂ induces the constitutive activation of c-Met in MDA-MB-231 breast cancer cells expressing the GD₃ synthase. *Glycobiology* **2012**, *22*, 806–816. [[CrossRef](#)] [[PubMed](#)]
138. Yates, A.J.; Saqr, H.E.; van Brocklyn, J. Ganglioside modulation of the PDGF receptor. A model for ganglioside functions. *J. Neurooncol.* **1995**, *24*, 65–73. [[CrossRef](#)] [[PubMed](#)]
139. Hynds, D.L.; Summers, M.; van Brocklyn, J.; O'Dorisio, M.S.; Yates, A.J. Gangliosides inhibit platelet-derived growth factor-stimulated growth, receptor phosphorylation, and dimerization in neuroblastoma SH-SY5Y cells. *J. Neurochem.* **1995**, *65*, 2251–2258. [[CrossRef](#)] [[PubMed](#)]
140. Lang, Z.; Guerrero, M.; Li, R.; Ladisch, S. Ganglioside GD1a enhances VEGF-induced endothelial cell proliferation and migration. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 1031–1037. [[CrossRef](#)] [[PubMed](#)]
141. Chung, T.W.; Kim, S.J.; Choi, H.J.; Kim, K.J.; Kim, M.J.; Kim, S.H.; Lee, H.J.; Ko, J.H.; Lee, Y.C.; Suzuki, A.; et al. Ganglioside GM3 inhibits VEGF/VEGFR-2-mediated angiogenesis: Direct interaction of GM3 with VEGFR-2. *Glycobiology* **2009**, *19*, 229–239. [[CrossRef](#)] [[PubMed](#)]
142. Kabayama, K.; Sato, T.; Kitamura, F.; Uemura, S.; Kang, B.W.; Igarashi, Y.; Inokuchi, J. TNF α -induced insulin resistance in adipocytes as a membrane microdomain disorder: Involvement of ganglioside GM3. *Glycobiology* **2005**, *15*, 21–29. [[CrossRef](#)] [[PubMed](#)]
143. Sasaki, A.; Hata, K.; Suzuki, S.; Sawada, M.; Wada, T.; Yamaguchi, K.; Obinata, M.; Tateno, H.; Suzuki, H.; Miyagi, T. Overexpression of plasma membrane-associated sialidase attenuates insulin signaling in transgenic mice. *J. Biol. Chem.* **2003**, *278*, 27896–27902. [[CrossRef](#)] [[PubMed](#)]
144. Kaucic, K.; Liu, Y.; Ladisch, S. Modulation of growth factor signaling by gangliosides: Positive or negative? *Methods Enzymol.* **2006**, *417*, 168–185. [[PubMed](#)]
145. Farooqui, T.; Franklin, T.; Pearl, D.K.; Yates, A.J. Ganglioside GM1 enhances induction by nerve growth factor of a putative dimer of TrkA. *J. Neurochem.* **1997**, *68*, 2348–2355. [[CrossRef](#)] [[PubMed](#)]
146. Mutoh, T.; Hamano, T.; Tokuda, A.; Kuriyama, M. Unglycosylated Trk protein does not co-localize nor associate with ganglioside GM1 in stable clone of PC12 cells overexpressing Trk (PCTrk cells). *Glycoconj. J.* **2000**, *17*, 233–237. [[CrossRef](#)] [[PubMed](#)]
147. Ichikawa, N.; Iwabuchi, K.; Kurihara, H.; Ishii, K.; Kobayashi, T.; Sasaki, T.; Hattori, N.; Mizuno, Y.; Hozumi, K.; Yamada, Y.; et al. Binding of laminin-1 to monosialoganglioside GM1 in lipid rafts is crucial for neurite outgrowth. *J. Cell Sci.* **2009**, *122*, 289–299. [[CrossRef](#)] [[PubMed](#)]

148. Maglione, V.; Marchi, P.; Di Pardo, A.; Lingrell, S.; Horkey, M.; Tidmarsh, E.; Sipione, S. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. *J. Neurosci.* **2010**, *30*, 4072–4080. [[CrossRef](#)] [[PubMed](#)]
149. Di Pardo, A.; Maglione, V.; Alpaugh, M.; Horkey, M.; Atwal, R.S.; Sassone, J.; Ciammola, A.; Steffan, J.S.; Fouad, K.; Truant, R.; et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 3528–3533. [[CrossRef](#)] [[PubMed](#)]
150. Nojiri, H.; Stroud, M.; Hakomori, S. A specific type of ganglioside as a modulator of insulin-dependent cell growth and insulin receptor tyrosine kinase activity: Possible association of ganglioside-induced inhibition of insulin receptor function and monocytic differentiation induction in HL-60 cells. *J. Biol. Chem.* **1991**, *266*, 4531–4537. [[PubMed](#)]
151. Sato, T.; Nihei, Y.; Nagafuku, M.; Tagami, S.; Chin, R.; Kawamura, M.; Miyazaki, S.; Suzuki, M.; Sugahara, S.; Takahashi, Y.; et al. Circulating levels of ganglioside GM3 in metabolic syndrome: A pilot study. *Obes. Res. Clin. Pract.* **2008**, *2*. [[CrossRef](#)] [[PubMed](#)]
152. Veillon, L.; Go, S.; Matsuyama, W.; Suzuki, A.; Nagasaki, M.; Yatomi, Y.; Inokuchi, J. Identification of ganglioside GM3 molecular species in human serum associated with risk factors of metabolic syndrome. *PLoS ONE* **2015**, *10*, e0129645. [[CrossRef](#)] [[PubMed](#)]
153. Merrill, A.H., Jr.; Stokes, T.H.; Momin, A.; Park, H.; Portz, B.J.; Kelly, S.; Wang, E.; Sullards, M.C.; Wang, M.D. Sphingolipidomics: A valuable tool for understanding the roles of sphingolipids in biology and disease. *J. Lipid Res.* **2009**, *50*, S97–S102. [[CrossRef](#)] [[PubMed](#)]
154. Hannun, Y.A.; Obeid, L.M. Many ceramides. *J. Biol. Chem.* **2011**, *286*, 27855–27862. [[CrossRef](#)] [[PubMed](#)]
155. Simons, K.; Ikonen, E. Functional rafts in cell membranes. *Nature* **1997**, *387*, 569–572. [[CrossRef](#)] [[PubMed](#)]
156. Hakomori, S.; Handa, K.; Iwabuchi, K.; Yamamura, S.; Prinetti, A. New insights in glycosphingolipid function: “glycosignaling domain”, a cell surface assembly of glycosphingolipids with signal transducer molecules, involved in cell adhesion coupled with signaling. *Glycobiology* **1998**, *8*, xi–xix. [[CrossRef](#)] [[PubMed](#)]
157. Regina Todeschini, A.; Hakomori, S.I. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *Biochim. Biophys. Acta* **2008**, *1780*, 421–433. [[CrossRef](#)] [[PubMed](#)]
158. Mahfoud, R.; Garmy, N.; Maresca, M.; Yah, N.; Puigserver, A.; Fantini, J. Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. *J. Biol. Chem.* **2002**, *277*, 11292–11296. [[CrossRef](#)] [[PubMed](#)]
159. Lingwood, C.A.; Branch, D.R. The role of glycosphingolipids in HIV/AIDS. *Discov. Med.* **2011**, *11*, 303–313. [[PubMed](#)]
160. Nehete, P.N.; Vela, E.M.; Hossain, M.M.; Sarkar, A.K.; Yah, N.; Fantini, J.; Sastry, K.J. A post-CD4-binding step involving interaction of the V3 region of viral gp120 with host cell surface glycosphingolipids is common to entry and infection by diverse HIV-1 strains. *Antivir. Res.* **2002**, *56*, 233–251. [[CrossRef](#)]
161. Delezay, O.; Hammache, D.; Fantini, J.; Yah, N. SPC3, a V3 loop-derived synthetic peptide inhibitor of HIV-1 infection, binds to cell surface glycosphingolipids. *Biochemistry* **1996**, *35*, 15663–15671. [[CrossRef](#)] [[PubMed](#)]
162. Fantini, J.; Yah, N. Molecular basis for the glycosphingolipid-binding specificity of α -synuclein: Key role of tyrosine 39 in membrane insertion. *J. Mol. Biol.* **2011**, *408*, 654–669. [[CrossRef](#)] [[PubMed](#)]
163. Chattopadhyay, A.; Paila, Y.D.; Shrivastava, S.; Tiwari, S.; Singh, P.; Fantini, J. Sphingolipid-binding domain in the serotonin_{1A} receptor. *Adv. Exp. Med. Biol.* **2012**, *749*, 279–293. [[PubMed](#)]
164. Maresca, M.; Derghal, A.; Caravagna, C.; Dudin, S.; Fantini, J. Controlled aggregation of adenine by sugars: Physicochemical studies, molecular modelling simulations of sugar-aromatic CH- π stacking interactions, and biological significance. *Phys. Chem. Chem. Phys.* **2008**, *10*, 2792–2800. [[CrossRef](#)] [[PubMed](#)]
165. Soltys, A.M.; MacKenzie, C.R.; Wolski, V.M.; Hiramata, T.; Kitov, P.I.; Bundle, D.R.; Brunton, J.L. A mutational analysis of the globotriaosylceramide-binding sites of verotoxin VT1. *J. Biol. Chem.* **2002**, *277*, 5351–5359. [[CrossRef](#)] [[PubMed](#)]
166. Ling, H.; Boodhoo, A.; Hazes, B.; Cummings, M.D.; Armstrong, G.D.; Brunton, J.L.; Read, R.J. Structure of the shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb3. *Biochemistry* **1998**, *37*, 1777–1788. [[CrossRef](#)] [[PubMed](#)]

167. Maloney, M.D.; Lingwood, C.A. CD19 has a potential CD77 (globotriaosyl ceramide)-binding site with sequence similarity to verotoxin B-subunits: Implications of molecular mimicry for B cell adhesion and enterohemorrhagic *Escherichia coli* pathogenesis. *J. Exp. Med.* **1994**, *180*, 191–201. [[CrossRef](#)] [[PubMed](#)]
168. D'Angelo, G.; Capasso, S.; Sticco, L.; Russo, D. Glycosphingolipids: Synthesis and functions. *FEBS J.* **2013**, *280*, 6338–6353. [[CrossRef](#)] [[PubMed](#)]
169. Ichikawa, S.; Nakajo, N.; Sakiyama, H.; Hirabayashi, Y. A mouse B16 melanoma mutant deficient in glycolipids. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2703–2707. [[CrossRef](#)] [[PubMed](#)]
170. Liang, Y.J.; Kuo, H.H.; Lin, C.H.; Chen, Y.Y.; Yang, B.C.; Cheng, Y.Y.; Yu, A.L.; Khoo, K.H.; Yu, J. Switching of the core structures of glycosphingolipids from globo- and lacto- to ganglio-series upon human embryonic stem cell differentiation. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 22564–22569. [[CrossRef](#)] [[PubMed](#)]
171. Park, H.; Haynes, C.A.; Nairn, A.V.; Kulik, M.; Dalton, S.; Moremen, K.; Merrill, A.H., Jr. Transcript profiling and lipidomic analysis of ceramide subspecies in mouse embryonic stem cells and embryoid bodies. *J. Lipid Res.* **2010**, *51*, 480–489. [[CrossRef](#)] [[PubMed](#)]
172. Liang, Y.J.; Yang, B.C.; Chen, J.M.; Lin, Y.H.; Huang, C.L.; Cheng, Y.Y.; Hsu, C.Y.; Khoo, K.H.; Shen, C.N.; Yu, J. Changes in glycosphingolipid composition during differentiation of human embryonic stem cells to ectodermal or endodermal lineages. *Stem Cells* **2011**, *29*, 1995–2004. [[CrossRef](#)] [[PubMed](#)]
173. Yu, R.K.; Tsai, Y.T.; Ariga, T. Functional roles of gangliosides in neurodevelopment: An overview of recent advances. *Neurochem. Res.* **2012**, *37*, 1230–1244. [[CrossRef](#)] [[PubMed](#)]
174. Suzuki, Y.; Yanagisawa, M.; Yagi, H.; Nakatani, Y.; Yu, R.K. Involvement of β 1-integrin up-regulation in basic fibroblast growth factor- and epidermal growth factor-induced proliferation of mouse neuroepithelial cells. *J. Biol. Chem.* **2010**, *285*, 18443–18451. [[CrossRef](#)] [[PubMed](#)]
175. Tsai, Y.T.; Yu, R.K. Epigenetic activation of mouse ganglioside synthase genes: Implications for neurogenesis. *J. Neurochem.* **2014**, *128*, 101–110. [[CrossRef](#)] [[PubMed](#)]
176. Gurdon, J.B.; Bourillot, P.Y. Morphogen gradient interpretation. *Nature* **2001**, *413*, 797–803. [[CrossRef](#)] [[PubMed](#)]
177. Wegner, M.S.; Schiffmann, S.; Parnham, M.J.; Geisslinger, G.; Grosch, S. The enigma of ceramide synthase regulation in mammalian cells. *Prog. Lipid Res.* **2016**, *63*, 93–119. [[CrossRef](#)] [[PubMed](#)]
178. Borbat, P.P.; Costa-Filho, A.J.; Earle, K.A.; Moscicki, J.K.; Freed, J.H. Electron spin resonance in studies of membranes and proteins. *Science* **2001**, *291*, 266–269. [[CrossRef](#)] [[PubMed](#)]
179. Marsh, D.; Horvath, L.I. Structure, dynamics and composition of the lipid-protein interface: Perspectives from spin-labelling. *Biochim. Biophys. Acta* **1998**, *1376*, 267–296. [[CrossRef](#)]
180. Ionova, I.V.; Livshits, V.A.; Marsh, D. Phase diagram of ternary cholesterol/palmitoylsphingomyelin/palmitoyl-oleoyl-phosphatidylcholine mixtures: Spin-label EPR study of lipid-raft formation. *Biophys. J.* **2012**, *102*, 1856–1865. [[CrossRef](#)] [[PubMed](#)]
181. Feng, J.; Lindahl, P.A. Effect of sodium sulfide on Ni-containing carbon monoxide dehydrogenases. *J. Am. Chem. Soc.* **2004**, *126*, 9094–9100. [[CrossRef](#)] [[PubMed](#)]
182. Esmann, M.; Marsh, D.; Schwarzmann, G.; Sandhoff, K. Ganglioside-protein interactions: Spin-label electron spin resonance studies with (Na⁺, K⁺)-ATPase membranes. *Biochemistry* **1988**, *27*, 2398–2403.
183. Haberkant, P.; Schmitt, O.; Contreras, F.X.; Thiele, C.; Hanada, K.; Sprong, H.; Reinhard, C.; Wieland, F.T.; Brugger, B. Protein-sphingolipid interactions within cellular membranes. *J. Lipid Res.* **2008**, *49*, 251–262. [[CrossRef](#)] [[PubMed](#)]
184. Haberkant, P.; Stein, F.; Hoglinger, D.; Gerl, M.J.; Brugger, B.; van Veldhoven, P.P.; Krijgsveld, J.; Gavin, A.C.; Schultz, C. Bifunctional sphingosine for cell-based analysis of protein-sphingolipid interactions. *ACS Chem. Biol.* **2016**, *11*, 222–230. [[CrossRef](#)] [[PubMed](#)]

